Sarcopenia in Older People

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Dedication

I dedicate this thesis to my loving and devoted family:
my loving parents, Lee Ching, Isabelle and Isaac, who
have always been there with patience beyond words.

Special dedication is also extended to my supervisor,
my mentor and my dear friend, Renuka Visvanathan

Above all, this thesis is dedicated to our God Most High,
who is the strength and the rock for me.

The glory of young men is their strength,
gray hair the splendor of the old. **Proverbs 20:29**

Commit to the Lord whatever you do,
and he will establish your plans. **Proverbs 16:3**

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Publications and Presentations

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Yu S, Appleton S, Adams R, Chapman I, Wittert G, Visvanathan T, Visvanathan R. (2014).An anthropometric prediction equation for appendicular skeletal muscle mass in combination with a measure of muscle performance to screen for sarcopenia in primary and aged care. *J Am Med Dir Assoc.* In Press 2014.Accepted 1st July 2014.1st ranked clinical journal in geriatrics and gerontology. Impact factor 5.30.

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Abstract presentations

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National conferences, Poster presentation

Yu S, Appleton S, Adams R, Chapman I, Wittert G, Visvanathan T, Visvanathan R. (2014). The impact of low muscle mass definition on the prevalence of sarcopenia in older Australians.*Australasian Journal on Ageing.*33 (Sppl 1):69.

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Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signed …….

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Abstract

Under-nutrition and weight loss in older people remain poorly recognized and so are undermanaged. Those at nutritional risk, and especially those losing weight, experience a loss of muscle mass referred to as *sarcopenia*, which is related to many different adverse health outcomes, including falls and increased risk of fracture.

Although research into the condition has gained momentum over the last two decades, especially for those aged eighty years and older, research has predominately been conducted overseas. In Australia, very few studies have investigated the prevalence of sarcopenia in our older population. Local evidence is required in order to inform Australian policy makers and the health and aged care sector. Furthermore, in spite of the increasing call for appreciation, screening and early diagnosis of the condition, there is no consensus as to a preferred screening method. Without acceptable clinical screening tools, identification of sarcopenia continues to be problematic. It is therefore important to develop a simple clinical test to facilitate early detection in primary or aged care settings as part of continuing and increasing Australian research into sarcopenia. Additionally, whilst appetite loss is known to be a contributing factor, the relationship between inflammation and appetite loss in healthy individuals with no recent history of weight loss is unclear.

The aims of this thesis were therefore: (1) to identify the prevalence of sarcopenia in primary care; (2) to develop and validate simple anthropometric prediction equations (PE) for lean body mass (LBM) and appendicular skeletal muscle mass (ASM); (3) to determine the performance of the ASM PE compared to dual absorptiometry x-ray assessment (DXA) of ASM in combination with grip strength; and (4) to explore the association between cytokines and appetite in a healthy population.

Research from this doctoral thesis has confirmed that sarcopenia is common in community dwelling older Australians and increases with age. Anthropometric prediction equations for LBM and ASM were developed and validated: *LBM= 22.932326 + 0.684668 (weight) - 1.137156 (BMI) -0.009213 (age) + 9.940015 (if male)* and *ASM= 10.047427 + 0.353307 (weight) - 0.621112 (BMI) - 0.022741 (age) + 5.096201 (if male).* Cut-offs for low muscle mass for use in Australia was also developed.

The use of ASM PE for the identification of low muscle mass, in combination with a measure of low muscle function, such as grip strength, performs well as a 'rule out' screening test for sarcopenia when compared to the diagnostic test of ASM assessed using DXA in combination with low grip strength. At the same time, appetite was found to be negatively associated with serum levels of pro-inflammatory IL-1ß and positively associated with serum levels of anti-inflammatory cytokine IL-10 in apparently healthy people with no recent weight loss.

Research from this doctoral thesis has contributed to increased awareness that sarcopenia is common and this will aid early intervention. At the same time, a clinical screening tool to support the early diagnosis of sarcopenia was developed.

Sarcopenia in Older People

Chapter 1

Introduction: Under-nutrition and sarcopenia

The population of developed countries is ageing. People are living longer in both developed and developing countries. In 2007, people aged 65 years and over made up 13% (2.6 million) of the Australian population (ABS, 2008), a number that is projected to increase to 27-31% (7 – 9 million) by 2056 (ABS, 2008). At the same time, the proportion of people aged 85 years and over is expected to increase rapidly, rising from 1.5% (300 000) of the population currently to 6-8% (1.6 – 2.7 million) by 2056 (2008). The cost to the community will be both social and monetary. It is logical, therefore, that healthy ageing should be one of the areas of research at the forefront of research agendas and health policies in all countries experiencing the ageing of their populations.

In Australia alone, between 2012 and 2013, the total health and residential aged care expenditure was estimated to be around $115 billion dollars, and projection on health spending by 2050 is estimated at an additional $200 billion (Goss, 2008). Age-related health conditions, therefore, require increased attention in order to identify optimal prevention and management strategies, which will, in turn reduce both the direct and indirect costs of disease to individuals and society.

1.1 Under-nutrition

Good nutrition is an important component of healthy ageing. Unfortunately, poor nutritional health is all too common among older individuals for a variety of physiological reasons associated with ageing, including loss of appetite and issues related to access to food and food preparation. In spite of multiple adverse health outcomes from under-nutrition, the problem is often overlooked or under-emphasised in older people for a variety of reasons.

Confirming the widespread nature of the problem, the research team of which I am a member has been able to report that almost 5% of community dwelling, older, domiciliary care recipients in South Australia are malnourished and a further 40% are at nutritional risk (Visvanathan et al., 2003). Furthermore, the team noted that the prevalence of under-nutrition in one sub-acute hospital was 43.1% (Visvanathan et al., 2004). With increasing frailty, the risk of under-nutrition increases, and in one study up to 90% of nursing home residents were classified as either at-risk of under-nutrition or under-nourished (Pauly et al., 2007).

1.1.1 Risks of under-nutrition

Under-nutrition is known to be associated with increased risk of infections arising from immune dysfunction, deep vein thrombosis as a result of reduced mobility, pressure ulcers, falls, peri-operative mortality and hospitalization (Omran and Morley, 2000b, Visvanathan et al., 2003). For these reasons, among others, under-nutrition is associated with reduced longevity (Flegal et al., 2005), and our research team has previously demonstrated that undernourished, community dwelling older South Australians are more likely to be admitted to hospital (45.2% vs. 29.1%, p-value=0.021) and spend more than four weeks in hospital (16.1% vs. 4.7%, p-value=0.012) than appropriately nourished older individuals (Visvanathan et al., 2003). Under-nourished patients following rehabilitation have poorer outcomes upon discharge than nourished older patients (50% vs.21.6%, p-value=0.017) and are more likely to be readmitted to hospital or residential care (Visvanathan et al., 2004). Poor diet has such a negative effect on the quality of life of older people, in fact, that under-nutrition should be identified early and treated (Visvanathan et al., 2004).

Under-nutrition is also costly, not only to the individual, but also to society. In 2005, the New Health Economic Report from The British Association for Parenteral & Enteral Nutrition (BAPEN) advised that under-nutrition cost the United Kingdom more than £7.3 billion each year – double the projected £3.5 billion cost of obesity (M. Elia et al., 2005). The bulk of the cost was attributed to the hospital treatment (~£3.8 billion) and residential care management of under-nourished older people (~£2.6 billion) (M. Elia et al., 2005).

Under-nutrition leads to unintentional weight loss, which in turn results in loss of muscle mass. Loss of muscle mass, including loss of strength, is termed *sarcopenia*, as described by Irwin Rosenberg in 1989. It has been reported that muscle mass declines at a rate of 1-2% annually after the age of 50, with a decline of muscle strength at a rate of 1.5%. After the age of 60, the rate of decline in muscle mass accelerates up to as much as 3% per year (Abellan van Kan, 2009). The effect of sarcopenia can be so severe that it interferes with a person's ability to manage even simple daily tasks (Lynch, 2004). It is also associated with increased risk of falls and healthcare costs (Janssen et al., 2004b, Landi et al., 2012), as well as a general decline in functional performance, disability and increased mortality (Landi et al., 2011, Abellan van Kan, 2009). With the realization among health providers that frailty is pervasive and its consequences debilitating and expensive, sarcopenia is beginning to receive attention worldwide.

1.1.2 The identification and management of sarcopenia

Although weakness and loss of muscle mass would appear to be obvious symptoms of sarcopenia, debate is ongoing on many issues relating to the definition, diagnosis and optimal management of the condition (Cruz-Jentoft et al., 2010, Fielding et al., 2011, Morley et al., 2011, Muscaritoli et al., 2010, Walston, 2012, Cederholm et al., 2013).

In the Australian context, there remains a paucity of information on the prevalence of sarcopenia in community dwelling older people, especially using agreed upon definitions, such as those outlined by the European Working Group on Sarcopenia in Older People (EWGSOP) (Cruz-Jentoft et al., 2010).

Although experts have been calling for early identification of sarcopenia in clinical practice, there is currently a lack of clinical tools to support screening programs. Currently, it is expected that following a measure of muscle performance (e.g. gait speed), all those considered at-risk must have a dual absorptiometry x-ray assessment (DXA) to confirm the diagnosis (Cruz-Jentoft et al., 2010). However, not all older people can easily access DXA. For those living in rural areas, who are home bound or living in residential aged care, access to DXA can at times be a challenge. It is therefore important that clinicians are equipped with practical and easy to use screening tools to ensure that only those who are likely to actually have sarcopenia go on to have a DXA in order to confirm the clinician's diagnosis.

Furthermore, there may be an association between appetite loss and future weight loss. A low score on the Simplified Nutrition Assessment Questionnaire (SNAQ) is associated with future weight loss (Wilson, 2007, Wilson et al., 2005). It is also known that weight loss, as well as inflammation, is associated with a loss of muscle mass (Boirie, 2009). It is therefore possible that there may be a relationship between inflammation and reduced appetite that occurs much earlier in the course of the ageing process, even in those who are apparently very healthy, but this has not been extensively explored, although this relationship has been observed in a frail, older population (Bruunsgaard and Pedersen, 2003).

1.2 Research aims of this thesis

This doctoral thesis focuses on sarcopenia in the Australian context. The research aims were to:

- determine the prevalence rate based on the various methods to define sarcopenia as proposed by European Working Group on Sarcopenia in Older People (EWGSOP)
- determine the best method to identify cutoff values for low muscle mass
- develop and validate simple anthropometric prediction equation for lean body mass (LBM) and appendicular skeletal muscle mass (ASM)

- investigate the performance of the developed anthropometric prediction equation of ASM against DXA assessment of ASM when used in combination with grip strength
- explore the relationship between cytokines and appetite.

1.3 Research cohort

The research reported in this thesis utilized data from three different South Australian population cohorts.

1.3.1 Cytokine, Adiposity, Sarcopenia and Ageing (CASA) study

A 'healthier' cohort of 195 healthy subjects (aged 18-83 years) was specifically recruited to form the Cytokine, Adiposity, Sarcopenia and Ageing (CASA) study cohort for the purpose of supporting the research reported in Chapters 3-5 and 7. The recruitment method was similar to that previously used for the two existing larger longitudinal studies described later. Briefly, there were two phases to the recruitment.

Phase 1: All households in the western region of Adelaide with a telephone number listed in the electronic White Pages were eligible for selection in the study. Selected households were sent an approach letter and brochure informing them about the study. The person who was last to have a birthday and aged 18 years or older was invited to participate in a short telephone interview. Interviews were conducted using computer-assisted telephone interview (CATI) technology.

Selected persons were considered 'non-replaceable'. Hence, if the selected person was not available, interviews were not conducted with alternative household members. A minimum of six telephone calls was made to each household before the selected individual was classified as a non-contact. Selected telephone numbers found to be businesses, hotels, motels, hospitals, nursing homes and other institutions were excluded from the survey. Respondents to the telephone interview were asked a number of health-related and demographic questions. A list of potential participants was then passed on to clinical research staff who then carried out a Phase 2 screening.

Phase 2: Clinical research staff undertook further screening via telephone interviews. The screening involved checking the inclusion and exclusion criteria. The inclusion criteria were being:

- aged 18 and above
- able to comply with study protocol
- stable in terms of weight over the last three months prior to the interview.

The exclusion criteria included being:

- underweight (adults < 70years old, body mass index (BMI) < 20kg/m^2 and in adults ≥ 70 years old, BMI < 22kg/m^2)
- overweight (adults < 70years old, BMI > 28kg/m^2 and in adults ≥ 70 years old, BMI > 35kg/m^2)
- diagnosed with a serious medical illness (see Appendix 1)
- diagnosed with an acute illness in three months prior to the interview or in the two weeks following blood sampling
- unable to stop medications for three days prior to blood sampling
- in receipt of vaccinations
- pregnant.

The eligible participants were sent an information pack about the study, including an appetite questionnaire (Appendix 1). Participants were asked to refrain from smoking, drinking alcohol or performing vigorous exercise in the 24 hours before their clinic appointment. Consent was obtained from participants when the participant attended the clinic.

During the clinic visit, the following assessments occurred (see Appendix 2):

- body composition – fat mass and lean mass estimation using bio-electrical impedance (The Quantum II BIA Analyzer) and DXA
- blood investigations – full blood investigation, total cholesterol, triglycerides, electrolytes, liver function tests, highly sensitive C-reactive protein (CRP), creatinine kinase and creatine kinase MM (CK-MM)
- plasma for cytokine analysis and future blood analysis was stored in a – 70 degree freezer
- postural blood pressure
- anthropometric measurements – waist and hip circumference
- timed get up and go test
- grip strength assessment
- a measure of appetite – simplified nutrition assessment questionnaires (SNAQ)
- socio-demographic, co-morbidity and medication history.

Following the clinic, summarised results of blood investigations and DEXA scans were sent to the participants. A full copy of blood results was sent to the participant's general practitioner at the same time (Appendix 3). Participants were also contacted two weeks after blood sampling to ensure that no acute illness had occurred in the interim. As the PhD candidate, I organised the participants' consent for enrolment in the study and undertook the necessary clinic based assessments. I was also responsible for summarising the results that were sent to participants. A junior medical doctor (Dr Kamal Esa) provided some support, including obtaining consent and collecting blood. To support the research reported in Chapter 7, I collaborated with a researcher from the Commonwealth Scientific and Industrial Research Organization (CSIRO) and participated in the conduct of the cytokine analysis. I was responsible for data entry, as well as ensuring data integrity. Further details about the characteristics of this cohort are provided in Chapter 7.

1.3.2 North West Adelaide Health Study (NWAHS)

The North West Adelaide Health Study (NWAHS) is an existing representative, population-based, community-dwelling South Australian cohort of 4060 men and women, aged 18 years and over(Grant et al., 2009). This sample was drawn from a region that represents half of the metropolitan area of Adelaide and one third of the South Australian population (Grant et al., 2009). This cohort was recruited in order to investigate effective strategies for prevention, early detection and management of chronic conditions (Grant et al., 2009). Further details about this cohort are provided in Appendix 4. The investigation of this cohort is described in Chapters 3 to 6.

1.3.3 Florey Adelaide Male Ageing Study (FAMAS)

The Florey Adelaide Male Ageing Study (FAMAS) involves a male only cohort, with identical recruiting methods and similar demographics to the participants in the NWAHS. The male subjects are between 35 and 80 years of age. Further details about this cohort are provided in Appendix 5. The investigation of this cohort is described in Chapters 3 to 6.

1.4 The organization of the thesis

The remaining chapters of this thesis are ordered as follows:

- **Chapter 2** is a review of sarcopenia with an in-depth focus on the definition and prevalence of sarcopenia, as well as methods to screen and assess for sarcopenia. The consequences, pathophysiology and treatment of sarcopenia are also discussed. The chapter is a reproduction of an article currently in press.

Yu S, Umapathysivam K, Visvanathan R. (2014). Sarcopenia in older people. *Int J Evid Based Healthc.* Accepted 7th July 2014. In Press 2014.

- **Chapter 3** reports on the gender specific cut-offs for low skeletal muscle mass using the three different methods as outlined by the European Working Group on Sarcopenia in Older People (EWGSOP). Also, the prevalence of sarcopenia in community dwelling, older (aged 65 years and older) Australians, using these three different methods is reported. The chapter is a reproduction of a published article [see Appendix 7].

- **Chapter 4** presents the prediction equations (PEs) for lean body mass (LBM) developed, including some with biochemical variables. The best performing PE were then validated with a different cohort to identify the preferred PE for use in Australia. The chapter is a reproduction of a published article [see Appendix 8].

- **Chapter 5** reports on the development and validation of an anthropometric PE for appendicular skeletal muscle mass (ASM). The chapter is a reproduction of a published article [Appendix 9].

- **Chapter 6** discusses the diagnostic accuracy of PE derived ASM compared to DXA derived ASM in diagnosing low muscle mass. An assessment of the diagnostic accuracy of PE derived ASM, when used in combination with low grip strength to diagnose sarcopenia, is also presented. The chapter is a reproduction of an article in press.

  Yu S, Appleton S, Adams R, Chapman I, Wittert G, Visvanathan T, Visvanathan R. (2014).An anthropometric prediction equation for appendicular skeletal muscle mass in combination with a measure of muscle performance to screen for sarcopenia in primary and aged care. *J Am Med Dir Assoc.* Accepted 1st July 2014. In Press 2014. 1st ranked clinical journal in geriatrics and gerontology. Impact factor 5.30.

- **Chapter 7** explores the association between cytokines and appetite in a healthy population without weight loss to better understand contributing factors to the future development of sarcopenia. The chapter is a reproduction of a published article [see Appendix 11].

- **Chapter 8** discusses the research findings through this PhD, and provides an outline of possible future research directions that could be pursued.

Chapter 2

Sarcopenia in older people

Summary

Sarcopenia, the age-associated loss of muscle mass and strength, is increasing in Western societies, such as Australia, which have large and growing older populations, leading to an increase in interest among medical researchers because of the associated poor health outcomes. Adverse health consequences include general frailty, falls and loss of independence, which reduce quality of life for the individual and increase health costs for the community. Increasingly, clinicians are being encouraged to screen for sarcopenia, and there have been recent international attempts to come to a consensus with regards to a definition of the condition. Screening pathways are being investigated, and it is believed that early detection will allow for early intervention, initiatives which are discussed in this review.

As with most conditions in older age, there are many environmental and medical factors that can contribute to the development and worsening of sarcopenia, and it is important that, where possible, these contributing factors be addressed. Pharmaceutical treatment strategies are under development with some early promise and there is the possibility of clinical trials in the near future. Currently, nutritional supplementation and physical therapy are the strategies advocated for the management of sarcopenia once it is diagnosed.

This chapter provides for an overview of the literature in relation to sarcopenia in older people with a focus on defining prevalence, consequences, screening and treatment. The chapter is currently in press with the *International Journal of Evidence-Based Healthcare.* The statement of authorship can be found in Appendix 6.

2.1 Introduction

With normal ageing, physiological changes in body composition are observed. In general, body weight increases and peaks at the age of 65 years in women and 54 years in men (Steen, 1988, Omran and Morley, 2000a). The weight gain is predominantly as a result of gain in fat mass(Hughes et al., 2002), which tends to be distributed viscerally in both genders (Beaufrere and Morio, 2000). There is also a decrease in the adipose tissue thickness in the arms and legs (Borkan et al., 1985). The decline of muscle mass is approximately 8% per decade from the age of 50 years until the age of 70 years (Grimby and Saltin, 1983). After 70 years, weight loss with concomitant muscle mass loss is more common, reaching rates of 15% per decade (Grimby and Saltin, 1983, Omran and Morley, 2000a). Irwin Rosenberg proposed the term *sarcopenia* in 1989, to describe the age-related loss of muscle mass observed with ageing (Rosenberg, 1997, Roubenoff, 2000a). Taken from Greek, sarcopenia means 'poverty of flesh' (Rosenberg, 1997, Roubenoff, 2000a).

2.2 Defining sarcopenia

Over the last decade, refining the definition of sarcopenia has led to significant variation in the meaning (Abellan van Kan et al., 2009). Initially, researchers focused on the loss of muscle mass or muscle strength or physical function individually rather than in combination. Furthermore, various measurements of muscle mass were referred to in the literature, including the use of terms such as *lean body mass* (LBM) and *appendicular skeletal muscle mass* (ASM). However, in recent years, there have been attempts to standardize the definition of sarcopenia internationally. In keeping with this, since 2009, there have been six international efforts at reaching consensus (detailed in Table 2.1).

Whilst there remains great variability as to how best to assess muscle mass and what cut-offs should be used to define low muscle mass, there appears to be a general consensus that gait speed is likely to be the most practical method by which to assess muscle performance in clinical practice. However, there remains some ambiguity on the best cut-off for gait speed, with the preferred cut-off either <0.8metre/second (m/s) or <1m/s.

Table 2.1 The various consensus diagnostic criteria for sarcopenia

Study group	Definition	Criteria/Cut-off points
ESPEN special interest group(Muscaritoli et al., 2010)	Loss of muscle mass and strength	*Criteria 1*:Low muscle mass >2SD below mean of the younger adults
		Criteria 2: Low gait speed <0.8m/s in 4m
		Diagnosis based on presence of both criteria
European Working Group on Sarcopenia in Older People(Cruz-Jentoft et al., 2010)	Sarcopenia is a syndrome characterized by progressive loss of skeletal muscle mass and strength with a risk of adverse outcomes such physical disability and mortality	*Criteria 1*: Low muscle mass DXA >2SD below mean of the younger adults(Baumgartner et al., 1998): o Men<7.26 kg/m^2 o Women <5.5 kg/m^2 Lowest 20% of the distribution of appendicular skeletal mass (ASM) in a normative population (aged 65 years and older)(Newman et al., 2003): o Men<7.23 kg/m^2 o Women <5.67 kg/m^2 Lowest 20% distribution of the residual of ASM adjusting for height and fat mass(Newman et al., 2003) o Men<2.29 o Women: <1.73 BIA >2SD below mean (SMI) of the younger adults(Chien et al., 2008): o Men<8.87 kg/m^2 o Women<6.42 kg/m^2
		Criteria 2: Low grip strength(Lauretani et al., 2003) o Men: <30 kg o Women: <20 kg
		Criteria 3: Low physical function o Short Performance Battery (SPPB)≤8(Guralnik et al., 2000) o Gait speed <0.8 m/s(Lauretani et al., 2003)
		Diagnosis based on presence of criteria 1 plus criteria 2 or 3

Study group	Definition	Criteria/Cut-off points
International Working Group on Sarcopenia(Fielding et al., 2011) Target at risk group: ▪ Noted decline in function, strength, 'health status' ▪ Self-reported mobility-related difficulty ▪ History of recurrent falls ▪ Recent unintentional weight loss (>5%) ▪ Post-hospitalization ▪ Other chronic conditions	Age-associated loss of skeletal muscle mass and function	If patient unable to walk –consider sarcopenia and need DXA to confirm low muscle mass, otherwise
		Criteria 1:Low physical function Walk Speed <1.0m/s, then DXA
		Criteria 2: Low muscle mass Low muscle mass: Appendicular fat free mass (AFFM) to height squared or whole body fat free mass to height. Recommended AFFM cut-offs for sarcopenia: o Men<7.23kg/m^2 o Women<5.67 kg/m^2
		Diagnosis based on the presence of both criteria
Society of Sarcopenia, Cachexia and Wasting Disorders(Morley et al., 2011) Screen at risk group: ▪ 60 years and over with falls ▪ Decreased walking speed ▪ Recent hospitalization ▪ Prolonged bed rest ▪ Problem raising from a chair ▪ Need to use assistive device for walking	Sarcopenia with limited mobility – loss of muscle mass whose walking speed is ≤1m/s or who walks <400m during 6 minutes walk test	*Criteria 1*:Low physical function Slow walk speed o ≤1m/s or <400m during 6 minutes walk
		Criteria 2: Low muscle mass Low muscle mass o >2 SD below younger people between 20-30 years.
		Diagnosis based on the presence of both criteria.

Study group	Definition	Criteria/Cut-off points
Asian Working Group for Sarcopenia (Chen et al., 2014)	Low muscle mass and low muscle strength or low physical performance (walk speed). Recommended measuring both muscle strength and physical performance as screening test.	*Criteria 1*: Low muscle mass: appendicular skeletal muscle mass/height^2>2SD below mean of the younger adults DXA o Men <7.0 kg/m^2 o Women <5.4 kg/m^2 BIA o Men <7.0 kg/m^2 o Women <5.7 kg/m^2
		Criteria 2: Low grip strength: lowest 20th percentile of the study population(Lauretani et al., 2003) o Men: <26 kg o Women: <18 kg
		Criteria 3: Low physical performance o Gait speed ≤0.8 m/s
		Diagnosis based on presence of criteria 1 plus criteria 2 or 3
Foundation for the National Institutes of Health (FNIH) Sarcopenia Project (Studenski et al., 2014, Dam et al., 2014a)	Two possible FNIH definitions: (i) clinically relevant weakness and low lean mass (low grip strength + low ALM_{BMI}) or; ii) clinically relevant slowness with weakness and low lean mass (slow gait speed + low grip strength + low ALM_{BMI}).	*Criteria 1:* Appendicular lean body mass (ALM) Recommended: ALM_{BMI} o Men: <0.789 o Women: <0.512 *Criteria 2:* Weakness Recommended: grip strength o Men: <26 kg o Women: <16 kg *Criteria 3:* Low gait speed o Gait speed ≤0.8m/s

DXA – dual energy X-ray absorptiometry; BIA – bioelectrical impedance analysis; BMI – body mass index; ALM_{BMI}- appendicular lean body mass adjusted for BMI

2.3 The prevalence of sarcopenia

The prevalence of sarcopenia varies depending on the age group, settings and definitions or cut-offs used (Table 2.2) (Bijlsma et al., 2012). The majority of recent studies investigating the prevalence of sarcopenia using a combination of low muscle mass and low muscle function (strength or performance) have been in Europe and Asia and in the community setting (Scott et al., 2013a, Patel et al., 2013, Patil et al., 2012, Lee et al., 2013, Yoshida et al., 2014). The prevalence of sarcopenia increases with age, as seen with the Taiwanese and Belgian studies, which included a large number of subjects aged 80 years and older (Lin et al., 2013, Lee et al., 2013, Legrand et al., 2013). Sarcopenia is more prevalent in the frail. The prevalence of sarcopenia is lower in studies which focus on healthier subjects, as seen with the Finnish study (Patil et al., 2012). In the Finnish study, only women living independently with a history of one fall in the preceding year were included (Patil et al., 2012). The study excluded women who were older than 81 years, had evidence of cognitive impairment or decline in the basic physical activities of daily living. These stringent criteria resulted in fewer frail individuals being investigated, which likely contributed to the very low prevalence of sarcopenia reported in this study (Patil et al., 2012). There has only been one study on the prevalence of sarcopenia in residential care, and the reported prevalence was very high and in keeping with the fact that these residents usually are older and frailer, and exhibit multiple co-morbidities (Landi et al., 2011).

2.4 The clinical consequences of sarcopenia

Much like osteoporosis predicts the future risk of fracture, sarcopenia is a powerful predictor of future disability (Fielding et al., 2011). In fact, sarcopenia may contribute to falls, increasing the risk of fracture (Landi et al., 2012, Cederholm et al., 2013). Several epidemiological studies have documented associations between low muscle mass and future functional decline and physical disability(Janssen et al., 2002). Furthermore, as noted, sarcopenia has also been linked to higher hospitalization rates, increased morbidity and mortality (Landi et al., 2012, Landi et al., 2011). Sarcopenia may also be associated with metabolic and cardiovascular diseases, such as diabetes, dyslipidaemia and hypertension.

Results are, however, mixed and, therefore, not conclusive (Karakelides and Nair, 2005). For example, one study found that obese post-menopausal women without sarcopenia had a more favourable lipid profile than those with sarcopenia (Aubertin-Leheudre et al., 2006). However, sarcopenia was found not to be a risk factor for the development of cardiovascular diseases at eight years in another study (Stephen and Janssen, 2009).

Table 2.2 Studies of sarcopenia

Study	Sarcopenia Definition and Relevant cutoffs	Subject Characteristics (SD- standard deviation)	Setting	Prevalence
Scott et al.(Scott et al., 2013b) (2013) Men and Women Australia	Definition: low muscle mass and low grip strength Muscle Mass (BIA) Cut-offs: lowest 20% of predictive population o Men: <7.09 kg/m^2 o Women: <5.91 kg/m^2 Grip Strength (handgrip strength); Cut-offs: o Men: <28.8 kg o Women: <18.2 kg	Age range: 50 -79 years o Men=352, mean age 61.7 (SD7.1) years o Women=329, mean age 61.0 (SD6.8) years	Community dwelling	5%
Legrand et al(Legrand et al., 2013) (2013) Men and Women Belgium	Definition: low muscle mass and low grip strength Muscle Mass (BIA) Cut-offs: lowest 20% of predictive population o Men: <8.87 kg/m^2 o Women: <6.42 kg/m^2 Grip Strength (handgrip strength); Cut-offs: o Men: <30.0 kg o Women: <20.0 kg Physical Performance (Walking Speed); Cut-offs: o <0.8 m/s	Age ≥80 years o Men=103, mean age 84.6 (SD 3.4) years o Women=185, mean age 85.0 (SD 3.8) years	Community dwelling	12.5% o Men: 8.0% o Women: 4.5%
Patel et al(Patel et al., 2013) (2013) Men only UK	According to EWGSOP algorithm: Presence of Low muscle mass plus either low grip strength or low physical performance Muscle Mass (DXA) Cut-offs: Lowest third of lean mass o Men: not stated Grip strength (handgrip strength); Cut-offs: o Men: <30 kg Physical Performance (Walking Speed); Cut-offs: o ≤0.8 m/s	Mean: 72.5 (SD 2.5) years Range: 68.3-77.4 years o Men=103	Community dwelling	Men: 6.8%

Study	Sarcopenia Definition and Relevant cutoffs	Subject Characteristics (SD- standard deviation)	Setting	Prevalence
Volpato et al(Volpato et al., 2013) (2013) Men and Women Italy	According to EWGSOP algorithm: Presence of low muscle mass plus either low grip strength or low physical performance Muscle Mass (BIA); Cut-offs o Men: 8.87 kg/m^2 o Women: 6.42 kg/m^2 Grip Strength (hand held dynamometer); Cut-offs: o Based on BMI method as shown in EWGSOP Physical Performance (Walking Speed); Cut-offs: o <0.8 m/s	Age ≥65 years mean age 77.1 (SD5.5) years o Men=250 o Women=288	Community dwelling	Overall 10.2% o Men:4.9% o Women: 9.4%
Patil et al(Patil et al., 2012) (2012) Women only Finland	According to EWGSOP algorithm: Presence of Low muscle mass plus either low grip strength or low physical performance Muscle Mass (DXA) - SMI Cut-offs: o Women: 5.5 kg/m^2 Grip Strength (handgrip strength) o Women: <20 kg Physical Performance (Walking Speed); Cut-offs: o <0.8 m/s	Age range: 70 – 81 years o Women=409	Community dwelling	o Women: 0.9%
Lin et al (Lin et al., 2013) (2013) Men and Women Taiwan	According to EWGSOP algorithm: Presence of Low muscle mass plus either low grip strength or low physical performance Muscle Mass (DXA) Cut-offs: 2 SD below younger reference o Men: <7.26 kg/m^2 o Women: <5.5 kg/m^2 Grip Strength (handgrip strength) Cut-offs: lowest quintile o Did not specify the cut-offs Physical Performance (Walking Speed) Cut-offs: slowest quintile o Did not specify the cut-offs	Age range: 65-98 years o Men=354 o Women=407	Community dwelling, metropolitan area.	Overall: 13.0% o Men: 13.3% o Women: 12.8%

Study	Sarcopenia Definition and Relevant cutoffs	Subject Characteristics (SD- standard deviation)	Setting	Prevalence
Lee et al (Lee et al., 2013) (2013) Men and Women Taiwan	According to EWGSOP algorithm: Presence of Low muscle mass plus either low grip strength or low physical performance Muscle Mass (DXA) Cut-offs: lower 20th percentile of the mean of younger reference group o Men: <7.27 kg/m^2 o Women: <5.44 kg/m^2 Grip Strength (handgrip strength); Cut-offs: o Men: <22.4 kg o Women: <14.3 kg Physical Performance (Walking Speed); Cut-offs: o ≤0.8 m/s	Age ≥65 years, mean age 73.7 (SD 5.6)years, n=386 Men=223, mean age 74.4 (SD 6.1) years Women=163, mean age 72.8 (SD 4.9) years	Community dwelling	Overall: 7.8% o Men: 10.8% o Women: 3.7%
Yoshida et al (Yoshida et al., 2014) (2014) Men and Women Japan	According to EWGSOP algorithm: Presence of Low muscle mass plus either low grip strength or low physical performance Muscle Mass (BIA) Cut-offs: lowest 20% of predictive population o Men: <7.09 kg/m^2 o Women: <5.91 kg/m^2 Grip Strength (handgrip strength); Cut-offs: o Men: <28.8 kg o Women: <18.2 kg Physical Performance (Walking Speed); Cut-offs: o ≤0.8 m/s	Age ≥65 years o Men=2343, mean age 72.2 (SD 5.5) years o Women=2468,mean age 72.1 (SD 5.7) years	Community dwelling	Overall: 7.5% o Men:8.8% o Women: 7.4%
Landi et al (Landi et al., 2011) (2012) Italy Men and Women	According to EWGSOP algorithm: Presence of Low muscle mass plus either low grip strength or low physical performance Muscle Mass (BIA) Cut-offs: lowest 20% of predictive population o Men: <8.87 kg/m^2 o Women: <6.42 kg/m^2 Grip Strength (handgrip strength) Cut-offs: o Men: <30.0 kg o Women: <20.0 kg Physical Performance (Walking Speed) Cut-offs: o ≤0.8 m/s	≥70 years Mean age: 84.1 (SD 6.9) o Men=31 o Women=91	Nursing home	Overall: 32.8% o Men: 15.6% o Women:17.2%

Study	Sarcopenia Definition and Relevant cutoffs	Subject Characteristics (SD- standard deviation)	Setting	Prevalence
Smoliner et al (Smoliner et al., 2014) (2014) Men and Women Germany	According to EWGSOP algorithm: Presence of Low muscle mass plus either low grip strength or low physical performance Muscle Mass (BIA) Cut-offs: lowest 20% of predictive population o Men: <8.87 kg/m^2 o Women: <6.42 kg/m^2 Grip Strength (handgrip strength); Cut-offs: o Men: <30.0 kg o Women: <20.0 kg Physical Performance (Short Physical Performance Battery); Cut-offs: o ≤8	Age range: 80-88 years Mean age: 82.8 (SD 5.9) years o Men=59 o Women=139	Hospital Geriatric Ward	Overall: 25.3% o Men: 15.2% o Women: 10.1%

The direct healthcare cost resulting from sarcopenia has been reported to approximate US$18.5 billion, a cost comparable with osteoporosis (Janssen et al., 2004b). It has been projected in Australia that health spending by 2050 will cost an additional $200 billion and so any potential cost savings, for example, through the better management of sarcopenia, would likely be very beneficial to society as a whole (ABS, 2013). For example, in a United States study in 2000 by Jansen et al., it was estimated that a 10% reduction in the prevalence of sarcopenia would result in a savings of up to $1.1billion per year (Janssen et al., 2004b). Although there has been no specific research pertaining to sarcopenia and health costs in Australia, it is highly probable that preventing sarcopenia will similarly result in cost-savings. Early identification of sarcopenia, allowing for prevention and treatment, will eventually reduce undesirable and costly health consequences, thus delivering savings while helping older people achieve healthy ageing.

2.5 Screening for sarcopenia

Sarcopenia, like many other health issues, is asymptomatic at the early stages, when intervention can prevent downstream adverse health outcomes. Screening for sarcopenia is currently not a routine part of clinical practice, however, in part because of the lack of availability of appropriate screening strategies. An ideal screening test should be cheap, acceptable and easily implementable in clinical practice (Grimes and Schulz, 2002) without requiring additional training. Once at risk individuals are recognized and their condition confirmed, intervention could begin before symptoms appeared. In this section, we discuss four possible screening methods that have recently been proposed.

The European Working Group on Sarcopenia in Older People (EWGSOP) in their consensus document outlined an algorithm to aid the screening and diagnosis of sarcopenia (Cruz-Jentoft et al., 2010). Gait speed was proposed as the first step in the screening process. A cut-off of ≤0.8m/s was proposed as an indication of risk. Those with gait speeds of ≤0.8m/s would then undergo a second performance assessment, such as grip strength (Cruz-Jentoft et al., 2010). Those meeting the criteria for low grip strength would finally be assessed by dual energy x-ray absorptiometry (DXA) to confirm the presence or absence of sarcopenia (Cruz-Jentoft et al., 2010). As grip strength is currently not routinely performed in primary or aged care settings, then it is less likely that this would be undertaken as part of a screening process, but more so as part of a diagnostic process.

Two more recent studies have proposed screening methods for low muscle mass and sarcopenia. Goodman et al. describes a clinical screening grid with age and body mass index (BMI) as variables for identifying low muscle mass (Goodman et al., 2013). In men, the model sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are 81.2%, 66.2%, 58.5% and 86% respectively. For women, the sensitivity is reported at 90.6%, specificity 66.2%, PPV 54.7% and NPV 94% (Goodman et al., 2013). It would appear that this model is best used as a 'rule out' screening test with those identified as at-risk undergoing further assessment to confirm or 'rule out' the presence of sarcopenia.

Ishii and colleagues, on the other hand, developed a prediction model for sarcopenia, based on three variables: age, grip strength and calf circumference (Ishii et al., 2014). They developed a chart that allowed for the estimation of the probability of sarcopenia. For example, a sum score of 105 for men and 120 for women was proposed to give the optimal sensitivity and specificity. With this cut-off value, the sensitivity, specificity, PPV and NPV were 84.9%, 88.2%, 54.4% and 97.2% for men and 75.5%, 92.0%, 72.8% and 93.0% for women(Ishii et al., 2014). Calf circumference and grip strength are not routinely measured in clinical practice and the use of grip strength, especially, might be a barrier to screening.

More recently, Malmstrom et al. have developed a Slowness, Assistance walking, Rising from chair, Climbing stairs, and Falls (SARC-F) questionnaire as a rapid screening tool for sarcopenia (Malmstrom and Morley, 2013). The questionnaire consists of five functional domains: strength, walking, chair rise, stair climbing and postural stability (i.e. falls). With each domain, a score of zero indicates independence whilst a score of 2 indicates dependence. A score of ≥ 4 is said to be predictive of sarcopenia (Malmstrom and Morley, 2013).

2.6 Assessing sarcopenia

The diagnosis of sarcopenia requires an accurate assessment of muscle function and muscle mass (Safer et al., 2013).

2.6.1 Muscle function assessment

As discussed earlier, it appears that the most practical method for assessing muscle performance in clinical practice is likely to be through an assessment of gait speed. This can be achieved through a

walk test over a predefined distance where gait speed is estimated or, alternatively, it can also be estimated during the walking aspect of the timed up-and-go (TUG) test commonly used to assess gait and function as part of a comprehensive geriatric assessment.

Walk test. Gait speed is assessed as part of a six meter timed walk test. A distance of 10 meters in length must firstly be identified. Markers should be placed at 0, 2, 8 and 10 metres. The older person is then asked to walk the 10 metres, and the time taken between the two and eight metre marks is estimated. Gait speed is measured over the six metres at metres per second (m/s). It has been well established that slower gait speed in older people is associated with a higher risk of developing activity-of-daily-living disability (Vermeulen et al., 2011).

Timed up-and-go test. With the TUG test, the older person sitting in an armchair is asked to stand and walk as quickly as possible to a marked position three metres away, turn around and return to a sitting position in the chair. The activity is timed in seconds to produce the TUG measurements. Used commonly in geriatric practice, this test is correlated with the Berg Balance Scale (r=-0.81), gait speed (r=-0.61) and the Barthel Index of Activities of Daily Living (r=-0.78) (Podsiadlo and Richardson, 1991).

Hand grip strength. Hand grip strength (HGS) is frequently used in research studies to assess muscle performance (Cruz-Jentoft et al., 2010). HGS can be measured quantitatively in Kg using a hand dynamometer. There are many types of hand dynamometers but the Jamar hand dynamometer is frequently used in research, as it is reported to have an excellent test-retest reproducibility and inter-rater reliability (Roberts et al., 2011). In the case where subjects have severe arthritis, the Martin vigorimeter (which uses rubber balls in three different sizes) may be a suitable alternative (Cooper et al., 2013). The American Society of Hand Therapists (ASHT) recommends that older subjects be seated with their elbow flexed at 90° putting the forearm in a neutral position with the wrist between 0-30° of dorsiflexion (Roberts et al., 2011). The subject is then encouraged to squeeze the dynamometer as hard as possible for approximately five seconds without moving the rest of their body. Three measurements are obtained and usually the final grip strength reported is an average of the three measurements (Roberts et al., 2011).

2.6.2 Muscle mass assessment

Older techniques, such as isotope dilution, in vivo neutron and measurement of potassium-40 isotope, underwater weighing and urine metabolites are rarely used today, especially in clinical practice (Cooper et al., 2013). These methods are expensive, invasive and involve radiation (Cooper et al., 2013). Table 2.3 provides a summary of the advantages and disadvantages of the common methods used to assess muscle mass in research and clinical practice.

Table 2.3 Methods to assess muscle mass

	Methods To Muscle Mass	BIA	DXA	CT	MRI	aPE
Advantages	Low cost	X	X			X
	Portable	X				X
	Cross-sectional measurement of lean mass in specific parts of body			X	X	
	Muscle quality assessment			X	X	
	Widely available		X			X
	Estimates of lean mass for entire body or specific parts of it		X			
Disadvantages	High cost			X	X	
	Limited accuracy	X				X
	Affected by hydration status	X				
	Exposure to radiation		X	X		
	Technically difficult to perform		X	X	X	
	Requires equipment	X	X	X	X	
	No specific information about body parts (e.g. limbs)	X				X
	Lack of access to outer metropolitan and rural areas		X	X	X	
	Difficulty in accessing for patients with reduced mobility and/or home bound		X	X	X	

BIA-Bioelectrical Impedance Analysis; DXA-Dual-Energy X-Ray absorptiometry; CT-Computed Tomography; MRI-Magnetic resonance imaging; aPE-Anthropometry Prediction equation

Adapted from Pahor et al(Pahor et al., 2009) and Cooper et al(Cooper et al., 2012a)

DXA is recommended as the preferred method for assessing muscle mass in clinical practice, as well as in research (Cruz-Jentoft et al., 2010), because of its relatively low cost (Mijnarends et al., 2013). DXA measures body composition in three different areas and is able to provide information not only on bone mass (i.e. used to diagnose osteoporosis), but also on fat mass and lean mass (Heymsfield et al., 1990). The measurement of lean mass by DXA has been validated against underwater weighing, computed tomography (CT) and magnetic resonance imaging (MRI) when it comes to assessing lean mass (Mijnarends et al., 2013, Haarbo et al., 1991, Tothill, 1995).

Appendicular skeletal muscle mass (ASM) when measured by DXA is defined as the sum of four limbs, excluding the fat and bone (Heymsfield et al., 1990). DXA, however, is unable to provide information on intramuscular or visceral fat, which account for approximately 15% of observed

muscle mass (Plank, 2005). CT and MRI, on the other hand, are able to provide additional information about intramuscular fat, are validated tools with good accuracy, but are more expensive (Cooper et al., 2013). Compared to DXA, both CT and MRI are better at detecting smaller changes in the muscle mass (Delmonico et al., 2008). However, CT exposes patients to large doses of radiation, which limits its utility (Cooper et al., 2013). Many older people also find MRI machines quite claustrophobic and those with pacemakers cannot be investigated using a MRI (Sierra and Machado, 2008, McIsaac et al., 1998).

Bioelectrical impedance analysis (BIA) has gained some popularity due to its portability. However, for accuracy, it does require the purchase of a device and some training as to how to use it. Most importantly, there are significant differences in the estimation of fat free mass (FFM) and between the BIA and DXA (Mijnarends et al., 2013). Furthermore, the accuracy of this method is influenced by the state of hydration and preceding exercise. This method also cannot be used with patients with a cardiac pacemaker (Kyle et al., 2004).

Anthropometric methods may be simpler but may lack precision and are difficult to generalize across different ethnic groups. Nevertheless, they may be useful as part of a screening process given that they can be easily performed in primary, as well as aged care settings. Prediction equations for ASM, including simple anthropometric variables, have recently been developed, including one by our research group (Chapter 5), and these are described in Table 2.4. Skinfold (measured by a calliper) thickness is used to estimate lean mass. Ageing, however, is associated with loss of subcutaneous tissue, which may affect the accuracy of such measurements in older people (Omran and Morley, 2000a).

Table 2.4 Simple anthropometric prediction equations for appendicular skeletal muscle mass (ASM), which have been validated against dual absorptiometry x-ray (DXA)

Study	Development Population	Number	Criterion Measure	Predictor Variables	Equations	R^2	SEE
Baumgartner et al (Baumgartner et al., 1998) 1998 USA Community cohort Men and Women	Not clearly defined	Total=149	DXA; Lunar DPX	Gender Weight Height Hip circumference Grip Strength	ASM (kg) = 0.2487 (weight) + 0.0483 (height) -0.1584 (hip circumference) + 0.0732 (grip strength) +2.5843 (sex) + 5.8828	0.91	1.58
Tanko et al (Tanko et al., 2002) 2002 Denmark Community Women only	18-85 years Mean age: Given in different age group. Ranging from 25.7 (SD 2.5) years for 18-29 years to 72.2 (SD 3.4) in those aged >70	Total=754	DXA;QDR4500 AHologic	Age Weight Height	ASM = −13.3-0.05 (age) + 0.11 (weight) + 16.1* (height)	0.58	0.70
Wen et al (Wen et al., 2011) 2011 China Community cohort Men and Women	18-69 years Mean age: ▪ M=39.3 (SD14.5) ▪ F=41.1 (SD14.1)	Total=763 ▪ M=345 ▪ F=418	DXA;QDR 4500 A, Hologic	ULL CAG CTG CCG Weight Height Gender Age	ASM= $(0.000123\times ULL\times CAG^2)+(0.00002739\times TL\times CTG^2)+(0.0000269\times CL\times CCG^2)-(3.11\times gender+0.128\times weight)+(0.082\times height)-(0.029\times age)-1.769$ ASM= $height\times(0.001509\times CAG^2+0.0008555\times CTG^2)+(0.0007709\times CCG^2)-(4.044\times gender)+(0.149\times weight)-(0.038\times age)+12.246$ ASM = (0.193 × weight) + (0.107 × height) – (4.157 ×gender) – (0.037 × age) – 2.631	0.93 0.92 0.90	1.33 1.44 1.63

Study	Development Population	Number	Criterion Measure	Predictor Variables	Equations	R^2	SEE
Visvanathan et al (Visvanathan et al., 2012) **2012** Australia Community cohort Men and Women	18-83 years Mean age: ▪ M=48.0 (SD 17.0) ▪ F=52.7 (SD 14.0)	Total=195 ▪ M=78 ▪ F=117	DXA;Lunar Prodigy	Weight BMI Age Gender	ASM= 10.047427 +0.353307 (weight) -0.621112 (BMI) - 0.022741 (age) +5.096201 (if male)	0.91	1.87
Kulkarni et al (Kulkarni et al., 2013) **2013** India Community cohort Men and Women	18-79 years Mean age: ▪ M=30.1 (SD 14.7) ▪ F=34.7 (SD 14.4)	Total=1332 ▪ M=851 ▪ F=481	DXA;Hologic Discovery A or Hologic 4500W	Age Weight Height Arm circumference Hip circumference Calf circumference CAMA	M=ASM=-13.432 - (0.0445 × age) + (0.200 × weight) + (0.140 × height) F=ASM= -9.852 - (0.028× age) + (0.170 × weight) + (0.102 × height) M=ASM= -12.81 - (0.029 × age) + (0.211 × weight) + (0.153 × height) + (0.255 × calf circumference) + (0.141 × arm circumference) - (0.178 × hip circumference) F=ASM= -2.658 - (0.023 × age) + (0.244 × weight) + (0.082 × height) + (0.087 × calf circumference) - (0.058 × arm circumference) - (0.102 ×hip circumference) M=ASM= - 16.270 - (0.037 × age) + (0.143 × weight) + (0.159 ×height) + (0.087 × CAMA)	0.78 0.82 0.82 0.84 00.82 00.83	1.28 1.05 1.17 1.01 11.18 11.02

Study	Development Population	Number	Criterion Measure	Predictor Variables	Equations	R^2	SEE
					F=ASM= - 10.818 - (0.027 × age) + (0.142 × weight) + (0.109 × height) + (0.051 × CAMA)	0.86	1.02
					M=ASM= -0.996 - (0.023 × age) + (0.274 × weight) + (0.090 × height) + (0.223 × calf circumference) + (0.143 × arm circumference) - (0.104 ×hip circumference) - (3.163 × logarithm of sum of 4skinfolds)	0.86	0.94
					F=ASM= 1.609 - (0.021 × age) + (0.250 × weight) + (0.070 × height) + (0.098 × calf circumference) + (0.027 × arm circumference) - (0.085 × hip circumference) - (1.821 × logarithm of sum of 4skinfolds)		
Pereira et al (Pereira et al., 2013) **2013** Brazil Community cohort Men and Women	No range was given Mean age: ▪ M=67.3 (SD 6.24) ▪ F=34.7 (SD 14.4)	Total=234	DXA;Lunar Prodigy Advance	Age Weight BMI Right forearm perimeter Squared right forearm perimeter Hip perimeter	ASM = 5.843 + 0.309 (weight)-0.376 (BMI) ASM = 4.150 + 0.251 (weight)-0.411 (BMI) + 0.011 $(PANTd)^2$ ASM= 4.087 + 0.255 (weight)-0.371 (BMI) + 0.011 $(PANTd)^2$-0.035 (TS) ASM= 7.944 + 0.244 (weight) + 0.010 (AGE)-0.145 (PH) + 0.230 (PANTd)	0.68 0.73 0.77 0.68	1.33 1.21 1.14 1.34

Study	Development Population	Number	Criterion Measure	Predictor Variables	Equations	R^2	SEE
				Thigh skinfold Thigh muscle circumference	ASM = 5.927 + 0.2399 (weight) + 0.0119 (AGE)-0.121 (PH) + 0.272 (PANTd)-0.033 (TS) ASM= 2.855 + 0.298 (weight) + 0.019 (AGE)-0.082 (PH) + 0.400 (PANTd)-0.332 (BMI) ASM= 11.631 + 0.256 (weight)-0.141 (PH) + 0.036 (TMC) ASM = 3.971 + 0.292 (weight)-0.328 (BMI) + 0.397 (PANTd)-0.078 (PH) ASM= 8.527 + 0.230 (PANTd)-0.142 (PH) + 0.241 (weight) ASM= 6.575 + 0.272 (PANTd)-0.117 (PH) + 0.236 (weight)-0.033 (TS)	0.70 0.75 0.68 0.75 0.68 0.70	1.29 1.17 1.32 1.17 1.33 1.28
Lera et al (Lera et al., 2014) 2014 Chile Community cohort Men and Women	≥60 years Mean age:68 (SD 5.2)	Total=616 ▪ M=218 ▪ F=398	DXA;Lunar Prodigy	Age Gender Knee height Calf circumference Grip strength Hip circumference	ASM=0.107 (weight) +0.251 (knee height in cm) +0.197 (calf circumference) + 0.047 (grip strength) – 0.034 (hip circumference) + 3.417 (men) – 0.02 (age) – 7.646	0.89	Not reported

M=male; F=female; ULL=Upper limb length; CAG_2=corrected arm girth; CTG=corrected thigh girth; CCG=corrected calf girth; TL=thigh length; CAMA=corrected arm muscle area (in cm); BMI=body mass index; PANTd=right forearm perimeter (cm); $PANTd^2$=squared right forearm perimeter (cm); PH=hip perimeter (cm); TS=thigh Skinfold; TMC=thigh muscle circumference

2.7 Pathophysiology of sarcopenia

The pathophysiology of sarcopenia remains poorly understood. Multiple factors contribute to the loss of muscle mass and these not only include cellular and tissue changes, but also environmental and behavioral factors (Table 2.5) (Boirie, 2009). It is very important to identify reversible factors and institute treatment, as these reversible factors must be remediated if sarcopenia is to be treated adequately and the cycle of functional and health decline halted and reversed.

Table 2.5 Factors contributing to sarcopenia in older people

Potentially treatable
Social factors
Lack of access to transport
Social isolation, living alone
Abuse – elderly
Poverty, food insecurity
Failure to provide for ethnic food preference
Inability to prepare and cook meals or to feed self
Inability to shop
Alcoholism
Sedentary lifestyle
Reduced protein intake
Medical
Thyroid problem
Cardiac failure
Gastrointestinal disease affecting absorption or intake such as vomiting, diarrheoa
Mood – depression, paranoia
Medications/polypharmacy*
Sensory deprivation – vision/hearing
Oral problem i.e. poorly-fitting denture
Swallowing problem/dysphagia, thickened diet
Poorly managed pain or constipation
Hormonal abnormalities – Low Vitamin D, insulin resistance
More difficult to treat
Medical factors
Loss of taste and smell, restricted diets
Cognition – dementia
Catabolism
Gastritis
Cancer

*Medications that cause nausea/vomiting (antibiotics/opiates), anorexia (antibiotics/digoxin), early satiety (anticholinergic drug), reduced feeding ability (such as sedatives/psychotropics), dysphagia (NSAIDs), constipation (opiates/diurectics), diarrhea (laxatives/antibiotics), hypermetabolism (thyroxine)

- Age-related factors leading to sarcopenia have been reported to act through several pathways, including impairment of homeostasis, apoptosis and mitochondrial dysfunction (Boirie, 2009, Cruz-Jentoft et al., 2010). It is worth noting that many changes threaten muscle integrity because of their inter-relatedness:
- The regeneration of skeletal muscle stem cells in older people appears to be slower than regeneration seen in younger people (Collins-Hooper et al., 2012). This may be due to the slower migration of stem cells into areas of regeneration as a result of reduced integrin expression (Collins-Hooper et al., 2012). Integrin is a trans-membrane receptor that mediates the attachment between a cell and its surroundings (Collins-Hooper et al., 2012).
- At the molecular level, dysfunctional mitochondrial biogenesis, or the production of new organelles in the mitochondria, impairs skeletal muscle performance and contributes to muscle atrophy (Joseph et al., 2012).
- Sarcopenia is associated with a reduction in the number of motor neuron units, but enlargement of motor neuron units appear to preserve physical performance (Drey et al., 2014).
- There is, furthermore, a disproportionate decrease in protein synthesis with increasing age, with a preferential loss of type 2 fibers (Chai et al., 2011, Fielding et al., 2011). These fast-twitch fibers are recruited for very short duration, high intensity bursts of power. Their loss is thought to be one reason for the loss of agility and walking speed in older people (i.e. reduced physical performance) (Fielding et al., 2011).
- Of particular interest is the theory that the ageing of body cells, including those of the musculo-skeletal system, is strongly influenced by increased levels of inflammatory activity in the body and, therefore, protein catabolism (Bruunsgaard and Pedersen, 2003). Chronic exposure to low-grade inflammation contributes to the development of sarcopenia and to the anorexia of ageing, by promoting loss of appetite, lower nutrient intake and weight loss (Chapman, 2007).
- IL-1ß, TNF-α and IL-6 have been shown to variably suppress appetite both centrally and peripherally(Buchanan and Johnson, 2007), and increased levels of circulating C-reactive protein (CRP), interleukin-6 (IL-6) and TNF-α are also associated with a steeper decline in muscle strength, walking ability and physical disability (Ferrucci et al., 2002, Schaap et al., 2009).
- Reduced oral intake of nutrition leads to weight loss, resulting in undesirable muscle mass loss (Chapman, 2007).

- The existence of co-morbidities, such as renal or cardiac failure, only act to accelerate the loss of muscle mass and the worsening of sarcopenia, possibly as a result of the increased inflammation (Bruunsgaard and Pedersen, 2003) associated with disease conditions. It follows that adequate treatment of co-morbidities, thus indirectly reducing inflammation, or strategies that directly reduce inflammation, may therefore contribute to the prevention of further loss of muscle mass.
- Several hormonal changes have been linked to reductions in muscle mass and strength. Low serum testosterone, for example, is associated with low muscle strength (Morley and Perry, 2000). A meta-analyses of trials on androgen replacement suggests that testosterone supplementation results in improved muscle strength, especially in those who are testosterone deficient (Ottenbacher et al., 2006). The need for androgen therapy would need to be balanced by the potential adverse effects of testosterone supplementation (Basaria et al., 2010).
- It has also been suggested that growth hormone (GH) plays a role in the development of sarcopenia. However, the extent of its contribution in the etiology is less clear and not supported by treatment studies, with replacement of GH failing to improve either strength or muscle mass (Yarasheski et al., 1995, Taaffe et al., 1994).

2.8 Treating sarcopenia

Sarcopenia should be recognized as both 'reversible' and 'treatable' (Visvanathan and Chapman, 2010, Rolland et al., 2008, Roubenoff, 2000b). Current prevention strategies are aimed at halting the loss of muscle mass and strength well before weakness, poor physical performance, impaired mobility and disability become apparent (Abellan van Kan et al., 2009). Treatment, on the other hand, is aimed at improving or reversing symptoms and signs that are already present (Abellan van Kan et al., 2009). To date, there are very few pharmacological intervention strategies that have been proven to be effective, and there have been no intervention trials demonstrating effective prevention or treatment strategies, in part because of issues in relation to the definitions of sarcopenia, as noted earlier (Morley, 2008).

Attention to nutrition, including the nutrition supplementation (e.g., protein, vitamin D), along with physical activity remains the mainstay of treatment where sarcopenia or risk is identified (Rolland et al., 2008, Visvanathan and Chapman, 2010, Roubenoff, 2000b, Morley, 2008) and are discussed in greater detail here.

2.8.1 Physical activity

In prescribing an exercise program, it is important that factors such as motivation, co-morbidities, social circumstances and finances are taken into account (Chao et al., 2000). Clearly, musculoskeletal pain may limit the older person's ability to participate in exercise, and where possible medical practitioners should adequately treat pain. Any exercise program must be enjoyable, relevant, safe, effective and realistic for older people (Chao et al., 2000).

Physical activity and exercise. Physical activity (PA), the contraction of skeletal muscle to produce movement, increases energy expenditure (Montero-Fernandez and Serra-Rexach, 2013). It is measured as the *metabolic equivalent of tasks* (MET), or the *physiological energy cost* (oxygen consumption) required to perform a task in reference to the resting metabolic rate (one MET=3.5ml O_2/kg/min) (Montero-Fernandez and Serra-Rexach, 2013). MET can be used to classify light intensity activities (<3 MET, e.g. sleeping, watching television and very slow walking (2.7km/h)), moderate intensity activities (3-6 MET, e.g. bicycling, calisthenics and home exercise) and vigorous intensity activities (>6 MET, e.g. jogging, rope jumping, heavy calisthenics such as push-ups and sit-ups)(Haskell et al., 2007). Exercise is a form of PA that consists of structured, planned and repetitive movements with the aim of improving physical fitness (Montero-Fernandez and Serra-Rexach, 2013). Emerging evidence suggests that endurance training and resistance have beneficial effects on muscle mass and strength and this is discussed in a little bit more detail here (Visvanathan and Chapman, 2010).

Aerobic/endurance exercise. Aerobic exercise (AE) involves structured physical activity in a way that utilizes oxygen to meet energy demands (Haskell et al., 2007). When this is done over a prolonged period of time, it improves cardiovascular health, even in older individuals with multiple co-morbidities (Haskell et al., 2007, Coggan et al., 1992). AE improves not only muscle quality and function but also contributes to neuro-adaptation (Rolland et al., 2008). However, AE does not prevent aged-related loss of muscle mass and strength to the same extent as resistance exercise (Visvanathan and Chapman, 2010, Rolland et al., 2008). Examples of AE are swimming, jogging, dancing, cycling, brisk walking and water aerobic.

Performed regularly, AE can increase VO_2 max by about 10-25% (Lemura et al., 2000). The American Heart Association and American College of Sports Medicine (ACSM) have recommended 30-40 minutes per day of ***moderate*** intensity aerobic activity, five days per week or three times per week of ***vigorous*** intensity AE for 20-30 minutes. However, achieving this can be challenging for frail older people. In one study, a modified form of ***lower*** intensity AE

characterized by 30 minutes, twice per week, with incremental exercises over a period of nine weeks, attenuated the aged-related decline in aerobic capacity (Lepretre et al., 2009). Reassuringly, even low intensity and less frequent, exercise is of benefit and it has been suggested that a combination of aerobic and progressive resistance exercise for older people is desirable (Visvanathan and Chapman, 2010).

Progressive resistance exercise. With progressive resistance exercise (PRE), the older person is encouraged to work their muscles against a resistant force, progressively increasing the resistance, as the individual becomes stronger (Montero-Fernandez and Serra-Rexach, 2013). Positive effects of PRE on muscle protein synthesis (Yarasheski et al., 1999), muscle mass and strength have been noted (Jozsi et al., 1999, Fiatarone et al., 1994), even in frail older people (Schulte and Yarasheski, 2001). Older people can use free weights, such as dumbbells, or work on resistance machines in the gym (Visvanathan and Chapman, 2010). They can also use their own body weight and focus on bent-knee sit-ups, squats or chin-ups (Visvanathan and Chapman, 2010).

PRE in general is well tolerated by older people, even those in residential care and can be chair-based (Visvanathan and Chapman, 2010). PRE can attenuate the development of sarcopenia in several ways and these include improved muscle mass and strength, improved balance and improved flexibility (Orr et al., 2008). It is recommended that PRE should be performed on two non-consecutive days per week where8-10 exercises consisting of up to 10-15 repetitions are undertaken (Nelson et al., 2007). Also, the exercise should involve the use of major muscle groups with two minutes rest between exercise sets (Nelson et al., 2007).

2.8.2 Vitamin D supplementation

Low serum vitamin D levels are associated with reduced strength (Bischoff Ferrari, 2009). It has also been demonstrated that a dose-response relationship exists between serum vitamin D levels and muscle health (Bischoff Ferrari, 2009). It has been demonstrated that in older people, low levels of vitamin D are associated with an increased risk of sarcopenia after adjusting for physical activity, season, serum creatinine, chronic diseases, smoking and body mass index (Wicherts et al., 2007). Factors such as inadequate dietary intake, lack of sun exposure and co-morbidity, such as renal disease (Visvanathan and Chapman, 2010) contribute to inadequate levels of vitamin D. If it is found that serum levels of the vitamin are too low, the vitamin must be replaced. Suggested replenishment dosages range from 700 to 1000 IU per day. For example, 800 IU/day has been shown to be beneficial in reducing the risk of falls (Bischoff et al., 2003).

2.8.3 Nutrition

Weight stability prevents unnecessary loss of muscle mass, and is one of the goals of treatment and prevention of sarcopenia. Body weight and body mass index (BMI) increase with age, peak at around 50-60 years and decline thereafter (Flegal et al., 2002). As already discussed, weight loss is inadvertently accompanied by the loss of muscle mass (Visvanathan and Chapman, 2010). It is important to note that as individuals age, a healthy BMI is higher, between 22 and 27kg/m^2, whereas in younger people, it is between 18.5 and 25kg/m^2 (Heiat et al., 2001). In general, older people should be encouraged to maintain their weight and obese individuals should only be encouraged to lose weight where the weight is affecting their musculoskeletal health or mobility (Visvanathan and Chapman, 2010). Where weight loss is recommended, the weight loss program should be individually tailored and closely monitored with the aim of preserving muscle mass. A combination of reduced caloric intake, but with adequate protein, vitamins, mineral supplementation and exercise is likely to be necessary (Waters et al., 2013, Visvanathan and Chapman, 2010).

While over-nutrition could be an issue, many older people are actually not meeting the recommended daily dietary allowance (RDA). They consume small portions of main meals and fail to snack in between due to earlier satiety and reduced appetite associated with the anorexia of ageing (Visvanathan and Chapman, 2010). In Australia, the current daily RDA for protein is 0.75g/kg/day (Truswell et al., 1991). However, even this is thought to be not enough, and recently experts have called for increased protein intake for older people, as there is age-related resistance to the anabolic effects of proteins (Bauer et al., 2013). A recent international position paper from the International Study Group review on Dietary Protein Needs with Aging (PROT-AGE study group), recommends that the average daily protein intake should be between1.0 and 1.2 g/kg/day for the majority of older people (Bauer et al., 2013). For those with renal dysfunction, i.e. glomerular filtration rate [GFR] (Bauer et al., 2013) <60 ml/min/1.73m^2, a lower amount of daily protein is recommended (Bauer et al., 2013). This group suggests a protein intake of 0.8g/kg/day where there is moderate kidney disease (GFR between 30 to 60 ml/min/1.73m^2), for example. Those with severe renal dysfunction (i.e. GFR<30) and not on dialysis would need to further limit their protein intake to between 0.6 and 0.8 g/kg/daily (Bauer et al., 2013). A higher protein intake of up to 1.5 g/kg/day is considered allowable for patients on dialysis, be it hemodialysis or peritoneal dialysis (Bauer et al., 2013).

Specific feeding strategies to optimize protein utilization are also important aspects of protein supplementation, but it is not absolutely clear which strategies work best (Bauer et al., 2013). Studies have shown that the anabolic effect of protein is most pronounced when the protein is provided with a meal (Bauer et al., 2013). Furthermore, protein supplementation should be spread evenly across the day between breakfast, lunch and dinner to optimize protein synthesis (Bauer et al., 2013). Some researchers, on the other hand, have suggested that pulse feeding (i.e. main protein intake at midday vs. spread feeding throughout the day) might also be effective (Bouillanne et al., 2012). There is also suggestion that the consumption of protein immediately after resistance exercise enhances muscle mass and strength in older men (Esmarck et al., 2001).

The types of protein ingested may also affect protein anabolism. There are two major sources of protein: *whey protein*, which is a 'fast' protein, and *casein protein*, which is a 'slow' protein (Bauer et al., 2013). It has been proposed that the 'fast' protein is more effective at limiting protein loss in older people compared to the 'slow' protein (Bauer et al., 2013). It is also thought that 'fast' protein is more effective at building muscle stores than 'slow' protein, but to date there have been no conclusive findings on this topic that would allow for firm recommendations (Bauer et al., 2013).

Essential amino acids (EAA) are also said to be important stimulants of muscle protein anabolism in older adults. A small study examining the effect of EAA showed evidence of a small (4%) increase in lean body mass but no change in muscle strength (Dillon et al., 2009) when compared to a baseline (before the use of EAA). In one study, co-ingestion of leucine, a type of EAA with a bolus dietary protein, increased muscle protein synthesis in older men (Wall et al., 2012).

Two other dietary supplements have been noted as having a positive effect on muscle mass and strength, especially when combined with exercise, but are the subject to further research. Creatine is a major component of muscle stores, and studies have suggested that creatine supplementation may increase the concentration of creatine in skeletal muscle, promoting an increase in strength and mass (Cooper et al., 2012b, Brose et al., 2003, Candow and Chilibeck, 2007). Beta-hydroxy-beta-methylbutyrate (ß-HMB) is a metabolite of the EAA leucine and early studies suggest that it might potentially increase muscle mass, as well as reduce muscle breakdown (Fitschen et al., 2012).

2.8.4 Other pharmacological treatments for sarcopenia

Several pharmacological agents are being investigated for the treatment of sarcopenia(Morley, 2008). Examples are angiotensin converting enzyme (ACE) inhibitors, statins, testosterone, growth hormones and estrogen (Chumlea et al., 2011, Onder et al., 2009). Despite some early promising results, however, it is currently difficult to recommend these pharmacological agents as part of routine clinical treatment. Studies to date have not only been small in size, but have at times provided conflicting results. Larger and longer-term studies are required (Onder et al., 2009).

2.9 Conclusion

Sarcopenia is common and contributes to poor health outcomes. It is increasingly prevalent in the community as the number of older Australians grows, and is inevitably going to have an impact on the health system, economy and society at large. Screening for sarcopenia will allow earlier diagnosis and treatment, and it is anticipated that the result will be a healthier citizenry who experience less functional decline and require less hospitalization.

Multiple factors contribute to the development of sarcopenia and treatment will require a thorough assessment of contributing factors followed by remediation of reversible factors where possible. Current research indicates that, once recognized by screening, sarcopenia can be treated beneficially with exercise and nutritional supplements.

The impact of low muscle mass definition on the prevalence of sarcopenia in older Australians

Summary

Sarcopenia is a health condition characterised by low muscle mass and low muscle function. To date, there has only been one Australian study reporting on the prevalence of sarcopenia in Australia (Table 2.2) incorporating both low muscle mass and low grip strength. Furthermore, the EWGSOP has suggested three different methods of deriving gender specific cut-offs for low muscle mass, none of which has been investigated in the Australian context. The aim of the study discussed in this chapter was to establish cut-offs for low muscle mass using three published methods and to compare the prevalence of sarcopenia in older Australians.

Gender specific cut-off levels were identified for low muscle mass using three different methods. Low grip strength was determined using established cut-offs of <30kg for men and <20kg for women to estimate the prevalence of sarcopenia.

This research identified the following gender specific cut-off levels for low muscle mass: a) <6.89kg/m^2 for men and <4.32kg/m^2 for women, < 2 standard deviation (SD) of a young reference population; b)<7.36kg/m2for men and <5.81kg/m2 for women from the lowest 20% percentile of the older group; and c) <-2.15 for men and <-1.42 for women from the lowest 20% of the residuals of linear regressions of appendicular skeletal mass, adjusted for fat mass and height. Prevalence of sarcopenia in older (65 years and older) people by these three methods for men was 2.5%, 6.2% and 6.4% and for women 0.3%, 9.3% and 8.5% respectively.

The research demonstrated that sarcopenia is common. However, consensus on the best method to confirm low muscle mass is still required. Research from this chapter (Appendix 7 – including the statement of authorship) has formed the basis of a research paper that has now been published following peer review in *BioMed Research International*.

3.1 Introduction

Sarcopenia commonly affects older people and is characterized by loss of both muscle mass and strength (Cruz-Jentoft et al., Baumgartner et al., 1998). Sarcopenia is associated with disability, a loss of independence and reduced quality of life (Clark and Manini, 2010). In one American study, sarcopenia and its consequences were estimated to cost the US healthcare system US$18 billion (Janssen et al., 2004b). Sarcopenia is a costly and growing issue in all healthcare systems, especially in countries with ageing populations (Baumgartner et al., 2004, Janssen et al., 2004b).

The European Working Group on Sarcopenia in Older People (EWGSOP) has recently defined sarcopenia as a combination of both low muscle mass and low muscle function (Cruz-Jentoft et al.). Grip strength is one method to assess muscle function (Cruz-Jentoft et al.). Low grip strength cut-offs of <30kg for men and <20kg for women are recommended and derived from receiver operating characteristic (ROC) curves predicting walking speeds slower than 0.8m/s (Lauretani et al., 2003).

Appendicular skeletal muscle mass (ASM) is commonly assessed using dual absorptiometry x-ray assessment (DXA). The EWGSOP identifies three different methods to define low muscle mass (Cruz-Jentoft et al., 2010). With the oldest method, gender specific cut-off values for low muscle mass are derived from a younger reference group (< 2 standard deviation, age 18-40 years) and cut-off values of <7.26kg/m^2 for men and <5.50kg/m^2 for women were reported in the original paper (Baumgartner et al., 1998). With the second method, cut-off points for low muscle mass are derived from gender-specific lowest 20% of a predictive population, thus circumventing the need for a younger reference group (Newman et al., 2003). Cut-off points similar to those identified by the Baumgartner and colleagues have been reported, i.e., < 7.23 kg/m^2 for men and <5.67 kg/m^2 for women (Newman et al., 2003, Delmonico et al., 2007). The third method adjusts for fat mass and is derived from the gender-specific lowest 20% of the distribution of residuals of the linear regression on appendicular lean mass adjusted for fat mass and height and cut offs of <-2.29 kg for men and <-1.73 kg for women are reported (Newman et al., 2003).

To date, there have only been three studies in Australia investigating the prevalence of low muscle mass, and only one of these has reported on the prevalence of sarcopenia (i.e. low muscle mass and low muscle strength) in the community (Scott et al., 2013a, Woods et al., 2011, Hairi et al., 2010). Scott et al reported a 5% prevalence of sarcopenia in those aged 50-79 years and using the lowest 20% distribution of the predictive population identified the cut-off points for both low muscle mass

and low grip strength (Scott et al., 2014). In a second Australian study, cut-off points of <4.85 kg/m^{2}derived from a young reference group were used to establish that 3.2% of older women residing in low level aged care had sarcopenia (Woods et al., 2011). The third Australian study examined the prevalence of low ASM in older (≥70 years) men living in the community using linear regression and the gender specific lowest 20% method and reported a prevalence rate ranging from 15% in those aged 70 to 74 years, to 26% for those aged 80-84 years and increasing to 45% for those aged 85-89 years (Hairi et al., 2010).

To date, no study in Australia has examined the prevalence of sarcopenia in both men and women and compared the methods for identifying low muscle mass. The aims of the current study were to firstly establish gender specific cut-off points for low skeletal muscle mass using each of the three methods identified by the EWGSOP, and then report the prevalence of sarcopenia in older (aged 65 years and older) Australians living in the community.

3.2 Method

3.2.1 Study cohorts

Three cohorts were investigated in this study: Cytokine, Adiposity, Sarcopenia and Ageing Study (CASA), the North West Adelaide Health Study (NWAHS), and the Florey Adelaide Male Ageing Study (FAMAS) (Grant et al., 2006, Martin et al., 2007b, Dent et al., 2012). The three cohorts were combined to derive two broad cohorts: a younger reference population (aged 18-40 years; CASA and FAMAS) and an older group (aged ≥65; FAMAS and NWAHS) (see Figure 3.1). For the purposes of the current study, only those participants with a complete set of information on weight, height, grip strength and DXA were included in the analysis.

The methodology of recruitment was similar for all three cohorts and has been described in detail elsewhere (Martin et al., 2007b, Grant et al., 2006, Dent et al., 2012). Ethical approval was obtained from the Central Northern Adelaide Health Service Ethics of Human Research Committee. All participants in the three cohort studies provided written, informed consent. Briefly, all households in the northern and western region of Adelaide with a telephone number listed in the electronic White Pages were eligible for selection, and once selected were sent an approach letter and brochure informing them about the study. The person in the house who was last to have a birthday and was aged 18 years or older was invited to participate in a short telephone interview. Interviews were conducted using computer-assisted telephone interview (CATI) technology.

Selected persons were deemed "non-replaceable" and if the selected person was not available, interviews were not conducted with alternative household members. Up to six telephone calls were made to each household before the selected individual was classified as non-contactable. During the telephone interviews, potential participants were asked a number of health-related and demographic questions. Following the recruitment interview, respondents were invited to make an appointment to attend the clinic for a biomedical examination and investigations.

NWAHS: 4060 adults were included in the baseline biomedical examination between December 1999 and July 2003. 3566 participants attended the follow-up (median four years) between May 2004 and February 2006. Of these, a total of 1553 participants aged 65 years and older (men= 724, women=829) were included in the analysis (Grant et al., 2006).

FAMAS: 1195 community dwelling men aged between 35 and 80 years from the North West regions of Adelaide were recruited between August 2002 and April 2005. Of these, 295 men were aged 65 years and older (Martin et al., 2007b).

CASA: Healthy subjects aged 18 to 83 years (n=195) were recruited from the western suburbs of Adelaide (2005 – mid-2007). In this study, the aim was to recruit a 'healthier' population and so there were additional criteria. To participate in this study, subjects had to be 18 years and older, able to comply with the study protocol and be weight stable over the preceding three months.

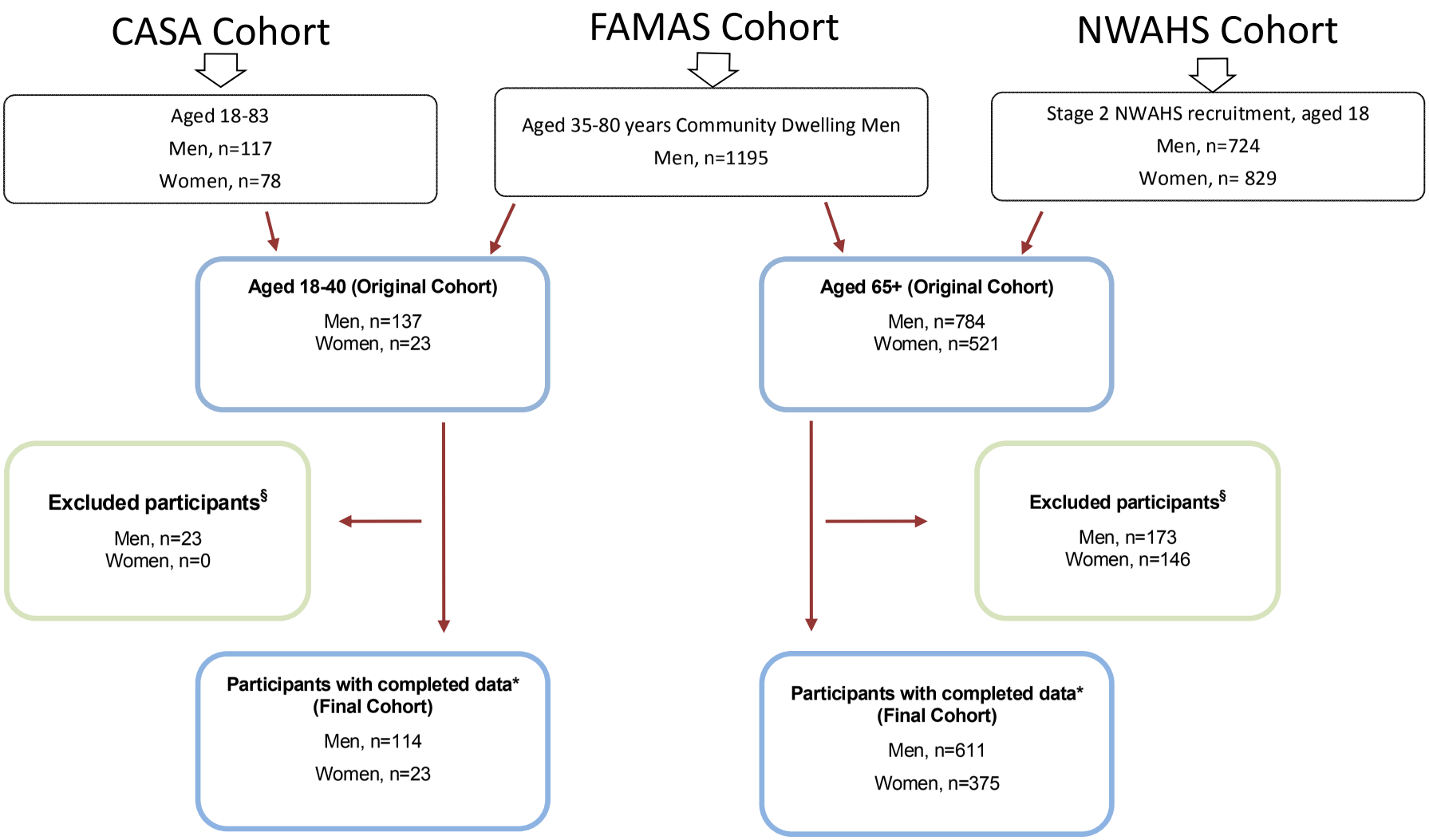
Those with a serious medical illness, inflammatory disease, an acute illness in the previous three months or in the two weeks following blood sampling, unable to stop medications for three days prior to blood sampling, being in receipt of vaccinations and pregnant were excluded from the study (Dent et al., 2012).

3.2.2 Measurements

Anthropometry: Height (m) was measured with shoes off to the nearest 0.1cm. Weight (kg) was measured wearing light clothing to the nearest 0.1kg. Body mass index (BMI, weight/height^2) was calculated. Three measurements of the waist and hip were taken and the mean for each was calculated (Grant et al., 2006).

Grip strength: Grip strength (kg) was measured three times with each dominant hand using a grip dynamometer (Lafayette Instrument Company, IN, USA [CASA and NWAHS], Smedley, Chicago, IL [FAMAS]) while subjects were sitting with their arm supported by a horizontal surface. The mean of the three readings was used in the current study (Grant et al., 2009).

Dual Energy X-ray Absorptiometry (DXA): Appendicular skeletal muscle mass (ASM) in this study was defined as the sum of lean soft-tissue masses for arms and legs, assuming that all non-fat and non-bone tissue is skeletal muscle. Skeletal muscle index (SMI) is ASM divided by height^2. **CASA:** ASM was determined using a Lunar PRODIGY whole-body scanner (GE Medical Systems, Madison, WI) in conjunction with Encore 2002 software. **NWAHS and FAMAS:** A Lunar PRODIGY scanner (GE Medical Systems, Madison, WI) in conjunction with Encore 2002 software and a DPX+ (GE Medical Systems, Madison, WI) scanner in conjunction with LUNAR software version 4.7e were used. Cross-calibration analysis reported no significant differences between the two machines (Mazess and Barden, 2000).



* completed data=those with completed measurements of DXA, grip strength and anthropometric measurements (weight and height)
§ missing either one or more of the components of DXA, grip strength and anthropometric measurements

Figure 3.1 Cohorts combined to develop the younger reference (aged 18-<40) and older study group (aged 65+)

3.2.3 Statistical analysis

SPSS 19 for Windows software (SPSS, Inc., Chicago, IL) was used for statistical analysis. Descriptive data was expressed as mean ± standard deviation (SD). Independent two-sample t-test was used to assess the mean difference in the characteristics variables between men and women. Low muscle mass was identified using three different methods: a) the Baumgartner's method whereby, cut-off values of < 2 standard deviation (SD) of a young reference population; b) the 20% gender specific method where cut-offs were derived for the lowest 20% of the older study population and; c) the linear regression method where the lowest 20% of residual of the linear regression models of ASM adjusting for fat mass and height in men and women were applied to the older study population to derive cut-points. As walk speed was not available within the NWAHS cohort, grip strength was used to determine muscle performance and cut-offs of <30kg for men and <20kg for women were applied (Lauretani et al., 2003). $P<0.05$ was considered statistically significant.

3.3 Results

The flow diagram in Figure 3.1 illustrates the path to establishing the two study populations from the three cohorts. For the young reference group, from the CASA and FAMAS cohort, there were a total of 137 men and 23 women aged 18-40 years. Of these, 23 men were excluded because of insufficient data. There were no statistically significant differences between the original and final cohorts in terms of age (35.7±4.9 vs. 35.5±5.3 years, p=0.75), weight (88.0±16.3 vs. 87.7±15.9 kg, p=0.99), height (1.8±0.1 vs. 1.8±0.1m, p=0.98), BMI (27.9±4.6 vs. 27.8±4.6 kg/m^2, p=0.98), %fat (26.7±8.5 vs. 26.7±8.5%, p=0.97), ASM (28.6±4.3 vs. 28.6±4.3 kg, p=0.83), SMI (9.1±1.1 vs. 9.1±1.1kg/m^2, p=0.85) and grip strength (52.2±10.8 vs. 51.6±11.1, p=0.68).

For the older group, from the FAMAS and NWAHS cohorts, there were 784 men and 521 women (Figure 3.1). 173 men and 146 women were excluded because of incomplete data. Consequently, the final cohort consisted of 611 men and 375 women. Women in the original cohort were significantly older than the women in the final cohort (74.0±6.3 vs. 73.2±6.0 years, p=0.05). No age difference was noted for men (73.0±6.0 vs. 72.7±5.7 years, p=0.30). There were no statistically significant differences between the original and final cohort in terms of weight (81.8±13.6 vs. 81.8±13.3 kg, p=0.96), height (1.7±0.1 vs. 1.7±0.1 years, p=0.85), BMI (27.9±4.3 vs. 27.9±4.2 kg/m^2, p=0.88), %fat (28.6±6.9 vs.

28.6±6.9 %, p=0.95), ASM (23.9±3.3 vs. 24.0±3.2 kg, p=0.92), SMI (8.2±0.9 vs. 8.2±0.9 kg/m^2, p=0.94) and grip strength (37.2±8.9 vs. 37.6±8.9 kg, p=0.37).

Table 3.1 shows the characteristics of participants in the final cohort aged 18-40 and aged 65 years and older. Comparing men to women in the younger reference group, the men were significantly older (35.5±5.3 vs. 31.2±7.3 years, p=0.01), heavier (87.7±15.9 vs. 69.3±15.3 kg, p<0.001), taller (1.8±0.1 vs. 1.7±0.1 m, p<0.001), had higher BMI (27.8±4.6 vs. 25.5±5.5 kg/m^2, p=0.03) and SMI (9.1±1.1 vs. 6.7±1.2 kg/m^2, p<0.001) than the women. Similar to the younger population group, older men were significantly heavier (81.8±13.3 vs. 69.4±12.4, p<0.001), taller (1.7±0.1 vs. 1.6±0.1m, p<0.001) with higher values for ASM (24.0±3.2 vs. 16.1±2.4 kg, p<0.001) and SMI (8.2±0.9 vs. 6.4±0.8 kg/m^2, p<0.001) than women. Interestingly, there was no difference in the BMI (27.9±4.2 vs. 27.8±4.7 kg/m^2, p=0.79) between the older men and women. The spread of various chronic conditions were shown in Table 3.1, with higher prevalence of chronic conditions amongst the older population compared with the younger population.

In men, low grip strength (Table 3.2) was noted in approximately 14% of men aged between 65 and less than 80 years and almost half of men aged 80 years and older. A higher proportion of women (i.e. 33.5%) between 65 years and less than 80 years had low grip strength compared to men. Similarly, 63% of women aged 80 years and older had low grip strength and this was higher in proportion within the same age group of men.

The cut-off points (Table 3.2) for low muscle mass were established at:

- a) <6.89 kg/m^2 for men and <4.32 kg/m^2 for women using the Baumgartner method
- b) <7.36 kg/$m^2$for men and <5.81 kg/m^2 for women using the 20% gender-specific method
- c) <-2.15 for men and <-1.42 for women using the linear regression method.
  The linear regression model was ASM (kg) = -18.24 + 23.09 x height (m) + 0.11 x total fat mass for men and ASM (kg) = -15.84 + 18.18 x height (m) + 0.11 x total fat mass for women.

Table 3.1: Characteristics of subjects from the younger reference group and older adults (aged ≥ 65) in the NWAHS and FAMAS included in the analysis

Characteristics	Younger Reference Population, 18+<40 years (FAMAS and CASA)			Older Study Population, 65+ years (NWAHS and FAMAS)		
	Men (n=117) Mean (SD)	**Women (n=23)** Mean (SD)	**P values**	**Men (n=611)** Mean (SD)	**Women (n=375)** Mean (SD)	**P values**
Age (SD), years	35.5 (5.3)	31.2 (7.3)	0.01	72.7 (5.7)	73.2 (6.0)	0.21
Weight (SD), kg,	87.7 (15.9)	69.3 (15.3)	<0.001	81.8 (13.3)	69.4 (12.4)	<0.001
Height (SD),m	1.8 (0.1)	1.7 (0.1)	<0.001	1.7 (0.1)	1.6 (0.1)	<0.001
BMI (SD), kg/m^2	27.8 (4.6)	25.5 (5.5.)	0.03	27.9 (4.2)	27.8 (4.7)	0.79
% Fat	26.7 (8.5)	29.9 (11.6)	0.22	28.6 (6.9)	40.2 (6.9)	<0.001
ASM (SD), kg	28.6 (4.3)	18.4 (4.1)	<0.001	24.0 (3.2)	16.1 (2.4)	<0.001
SMI (SD), kg/m^2	9.1 (1.1)	6.7 (1.2)	<0.001	8.2 (0.9)	6.4 (0.8)	<0.001
Chronic conditions	**%**	**%**		**%**	**%**	
Cardiovascular Disease	1.7	0.0	0.54	24.1	17.8	0.019
Diabetes	1.7	0.0	0.54	24.4	19.1	0.050
Hypertension	27.6	4.5	0.02	77.3	69.7	0.007
Hypercholesterolemia	44.7	13.6	0.06	31.1	50.3	<0.001
Arthritis	0.9	0.0	0.66	33.7	61.5	<0.001
Number of prescribed medications						
0	92.2	54.5	<0.001	15.1	6.3	0.02
1-3	7.8	45.5		37.1	39.7	
4-6	0.0	0.0		25.8	32.9	
≥7	0.0	0.0		22.0	21.1	

SMI- Skeletal Muscle Index; ASM-Appendicular Skeletal Muscle Mass; BMI – body mass index; SD-standard deviation; NS, not significant (p>0.05); NA-not applicable; Cardiovascular disease - Ischemic heart disease, acute myocardial infarction, stroke, angina; Diabetes - self-reported, Dr diagnosed, FPG>=7.0mmol/L or HbA1c>=6.5%; Hypertension - BP≥140/90 or already on treatment; Hypercholesterolaemia - serum total cholesterol ≥5.5mmol/L; Arthritis - self-reported osteo- or rheumatoid.

Table 3.2: The prevalence of low muscle mass and low grip strength in the North West Adelaide Health Study (NWAHS) and Florey Adelaide Male Ageing Study (FAMAS) based upon dual absorptiometry x-ray assessments of appendicular skeletal muscle mass

	Low grip strength (n%)	**Low SMI (n%)**	**Low SMI (n%)**	**Low SMI (n%)**
	EWGSOP Criteria(Lauretani et al., 2003)	<2 SD below mean of younger reference group (FAMAS and NWAHS) (Table 1)	Gender Specific lowest 20% of study group (FAMAS and NWAHS)	Residuals of linear regression on appendicular lean mass adjusted for fat and height (FAMAS and NWAHS)
NWAHS+FAMAS Men				
cut-offs	<30 Kg	<6.89 Kg/m^2	<7.36 Kg/m^2	< -2.15 Kg
65-<80 (n=540)	78 (14.0)	38 (7.0)	92 (17.0)	101 (18.7)
80+ (n=71) Total 65+ (n=611)	32 (45.1) 110 (18.0)	9 (12.7) 44 (7.2)	29 (40.8) 121 (19.8)	21 (29.6) 122 (20)
NWAHS Female				
cut-offs	<20 Kg	<4.32Kg/m^2	< 5.81 Kg/m^2	<-1.42 Kg
65-<80 (n=313)	105 (33.5)	0 (0)	56 (17.9)	63 (20.1)
80+ (n=62) Total 65+ (n=375)	39 (62.9) 144 (42.5)	1 (1.6) 1 (1.6)	18 (29) 74 (19.7)	12 (19.4) 75 (20)

The prevalence of low muscle mass ranged between 7-18% for men aged between 65 and 80 years but increased to between 12-29.6% for men aged 80 years and older (Table 3.2). However, for women, there was no increase in the reported prevalence with increasing age with the prevalence of low muscle mass ranging from 0-20.1% in those aged between 65 and <80 years and remaining between 1.6-19.4% in those aged 80 years and older. The prevalence reported by the 20% gender-specific method and linear regression methods were similar and much higher than the prevalence reported by the Baumgartner method.

Figure 3.2 shows that the prevalence of sarcopenia was higher in men (7-19.7%) and women (1.6-22.6%) aged 80 years and older compared to men (1.9-5.0%) and women (2.5-7.0%) aged between 65 and < 80 years. The prevalence of sarcopenia in people aged 65 years and older in this study was between 2.5% to 6.4% for men and between 0.3% to 9.3% for women. The overall prevalence of sarcopenia as estimated by the Baumgartner method, the lowest 20% method and the linear regression method was 1.6%, 7.4% and 7.2% respectively.

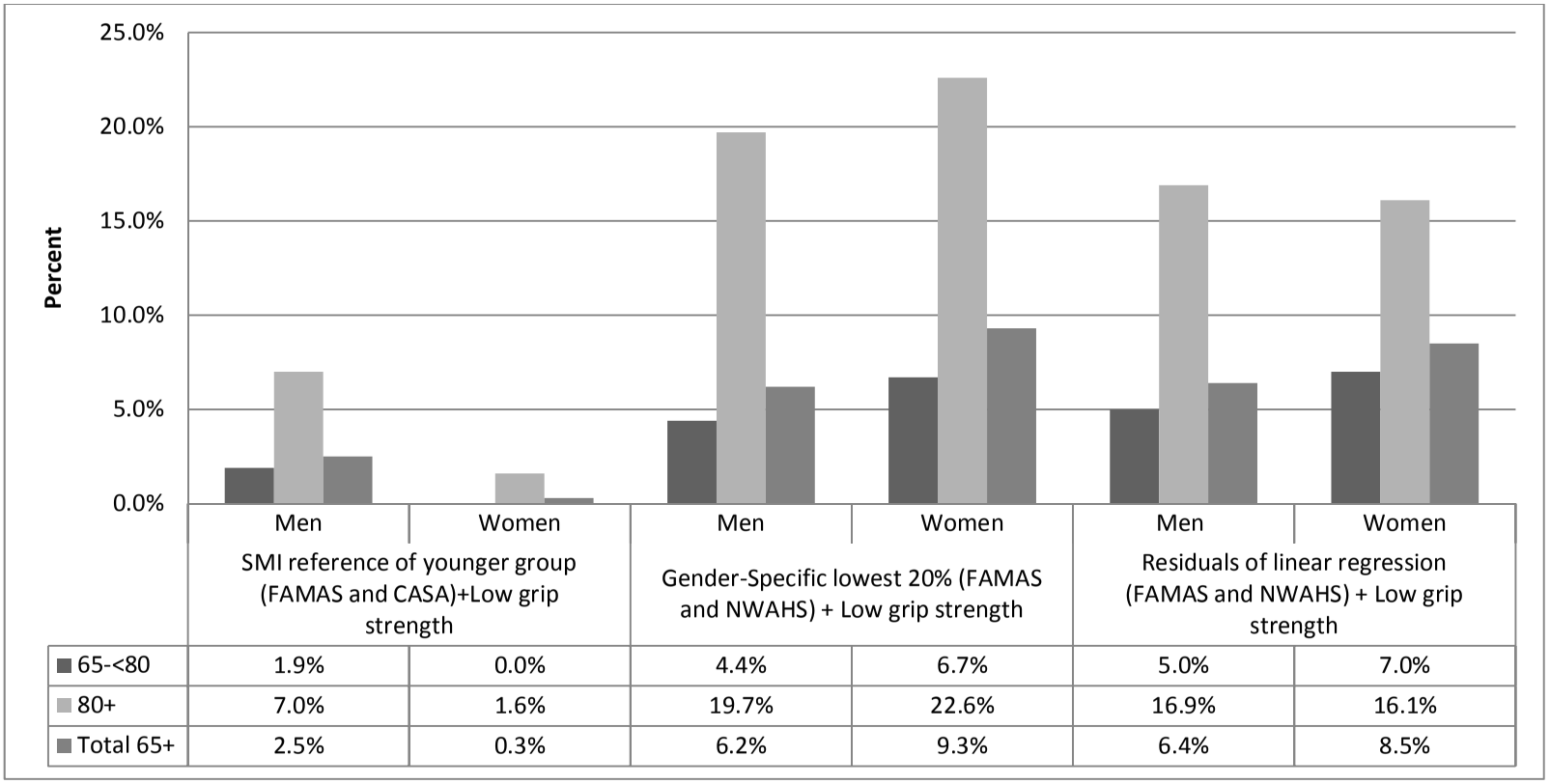
3.4 Discussion

The key finding from this study is that, in combination with grip strength, different methods of determining low muscle mass result in different prevalences being found for sarcopenia. The cut-off points for low muscle mass derived by the gender specific lowest 20% method and the linear regression method yielded similar prevalence rates for low muscle mass and sarcopenia. Also, the cut-offs generated by these two methods, in this study, were similar to those reported by EWGSOP (Cruz-Jentoft et al., 2010). However, the cut-offs derived by the Baumgartner method (<6.89 kg/m^2 for men and <4.32 kg/m^2 for women) in this study were much lower than those previously reported (<7.26 kg/m^2 for men and <5.50 kg/m^2 for women) (Baumgartner et al., 1998).

Our findings of a lower cut-off than that previously reported was similarly noted in an Australian study of women (<4.85 kg/m^2) (Woods et al., 2011). Researchers from Korea have recently reported similar SMI cut-off values (6.58 kg/m^{2}for men and 4.59 kg/m^{2}for women) (Kim et al., 2012). The mean ASM for the younger reference population in this study was lower than that reported in the Baumgartner (28.6 kg vs. 30.6 kg for men and 18.4 kg vs. 20.9 kg) study and this potentially contributed to the difference in the reported cut-off values (Baumgartner et al., 1998). Importantly, the sample size making up the younger reference population in our study was small and so there is a need to derive cut-offs from a larger cohort of younger people before firm conclusions can be reached.

Using the lowest 20% method and the linear regression method to define low muscle mass, the prevalence of sarcopenia reported in this study was approximately 6.2% for men and 9% for women aged 65 years. To the best of our knowledge, there has only been one other Australian study which used the lowest 20% method to define low muscle mass (Scott et al., 2013a). In that study, the overall sarcopenia prevalence rate was 5% (Scott et al., 2013a). In the current study, there was a higher overall prevalence rate at 7.6% most likely because of the older age group in our study population compared with the population in the other Australian study (72.7±5.7 vs. 61.7±7.1 years in men and 73.2±6.0 vs. 61.0±6.8 years in women) (Scott et al., 2013a).

SMI – Skeletal Muscle Index



65-<80	1.9%	0.0%	4.4%	6.7%	5.0%	7.0%
80+	7.0%	1.6%	19.7%	22.6%	16.9%	16.1%
Total 65+	2.5%	0.3%	6.2%	9.3%	6.4%	8.5%

Figure 3.2 **Comparison of prevalence rate of sarcopenia as defined by EWGSOP, by using different methods of SMI cut-points derivation with a low grip strength (<30kg for men and <20kg for women).**

Consistent with other studies, the prevalence of low muscle mass increased with age in men and was higher in those aged 80 years and older compared to those between 65 and < 80 years using all three methods (von Haehling et al., 2010). However, in women, this relationship was not seen with the linear regression method, which also accounts for fat mass. Fat mass reduces with increasing age in women but not in men (Baumgartner et al., 1995). In this study, the prevalence of low grip strength increased with age in both men and women. A greater proportion of women however met the criteria of low grip strength compared to men in older age. It is well known that a decline in sex hormones with increasing age (andropause and menopause) contributes to a decline in strength (Horstman et al., 2012).

Neither the FAMAS nor the NWAHS cohorts included subjects from residential care facilities where the prevalence of sarcopenia is likely higher. The requirement for subjects to attend a hospital-based clinic also made it very likely that frail individuals would be less likely to participate. Therefore, the reported prevalence in this study is likely to be an under-estimate of the true prevalence of sarcopenia in the community. Subjects enrolled in these studies were predominantly Caucasian and so the findings from this study are not generalizable to the wider multi-cultural Australian population. Ethnic specific cut-offs need to be determined and future research, including different ethnic population groups, is important.

3.5 Conclusion

To conclude, the prevalence of sarcopenia varies depending on the method used to estimate the cut-off values for low muscle mass. Therefore, a consensus is required to identify the preferred method to define sarcopenia. This will allow for pooling of research data. However, sarcopenia is common in the community. Given that sarcopenia is linked to morbidity and costs (Janssen et al., 2004b), early recognition and intervention through exercise and nutritional programs may contribute to healthy ageing outcomes and a reduction in health costs (Visvanathan and Chapman, 2010).

Chapter 4

Lean body mass: The development and validation of prediction equations in healthy adults

Summary

There is a loss of lean body mass (LBM) with increasing age. Prior to the effort of the consensus groups in defining sarcopenia, lack of LBM had been used to identify sarcopenia. A low LBM has more recently also been associated with increased adverse effects from prescribed medications, such as chemotherapy. Accurate assessment of LBM may allow for more accurate drug prescribing. The aims of this study were to develop new prediction equations (PEs) for LBM with anthropometric and biochemical variables from a development cohort and then validate the best performing PEs using validation cohorts.

In the current study, PEs were developed using a cohort of 188 healthy subjects and then validated in a convenience cohort of 52 healthy subjects. The best performing anthropometric PE was then compared to published anthropometric PEs in an older (age > 50 years) cohort of 2287 people. Best subset regression analysis was used to derive the PEs. Correlation, Bland-Altman and Sheiner & Beal methods were used to validate and compare the PEs against dual X-ray absorptiometry (DXA)-derived LBM. The PE which included biochemistry variables performed only marginally better than the anthropometric PE. The anthropometric PE on average over-estimated LBM by 0.74 kg in the combined cohort. Across gender (male vs. female), body mass index (< 22, 22-<27, 27-<30 and >30 kg/m2) and age groups (50-64, 65-79 and >80 years), the maximum mean over-estimation of the anthropometric PE was 1.36 kg.

As a result of this research, a new anthropometric PE for LBM has been developed that offers an alternative for clinicians when access to DXA is limited. Further research is required to determine the clinical utility and if it will improve the safety of medication use. The research in this chapter forms the basis of research published in *BMC Pharmacology & Toxicology* (Appendix 8 – including statement of authorship) following peer review.

4.1 Introduction

With increasing age, there is a decline in lean body mass (LBM) and very often an increase in adiposity (Visvanathan and Chapman, 2010). The decline in LBM may also be accompanied by a reduction in physical function and when a pathological threshold is reached, the person is said to have sarcopenia (Cruz-Jentoft et al., 2010). In recent times, sarcopenia has been recognized as an independent predictor of drug related adverse outcomes in the oncology setting where muscle wasting can be common (Parsons et al., Prado et al., 2009). Drug-related adverse effects are defined as medical events related to the use of medication which may result in disability, hospital admissions or death (Nebeker et al., 2004).

In patients with cancer, the use of LBM might be superior to body surface area (BSA) (Prado et al., 2007). For example, in a prospective study of colon cancer patients treated with 5-fluorouracil (5-FU), the incidence of dose limiting toxicity was examined with respect to conventional dosing of 5-FU/m^2 of BSA versus 5-FU/kg of LBM. LBM was a better predictor of toxicity ($p=0.011$) but not BSA (Prado et al., 2007). Similar findings have been reported in other studies (Gusella et al., 2002, Aslani et al., 2000). In anaesthesia, propofol pharmacokinetic parameters scaled linearly to LBM are also said to provide for improved dosing in adults (Coetzee, 2012). Therefore, accurate measurement of LBM may have clinical application in improving drug prescribing safety and efficacy, especially for older people for whom loss of lean mass is common.

A major impediment to the routine clinical use of LBM is the reliance on relatively inaccessible or expensive methods of body composition measurements. Computed tomography (CT), magnetic resonance imaging and dual absorptiometry x-ray (DXA) are used to assess LBM but these methods may be difficult to access in clinical practice (e.g. frail or rural patients) (Coetzee, 2012). Although the bioelectrical impedance analysis (BIA) method is portable, it still requires the purchase of special equipment and its accuracy is also dependent on many other factors, such as state of hydration, food intake and exercise (Kyle et al., 2004).

Total body weight consists of fat mass and fat free mass. Fat free mass (FFM) consists of bone, muscle, vital organs and extracellular fluid. LBM differs from FFM in that lipid in cellular membranes is included in LBM but this accounts for only a small fraction of total body weight (up to 3% in men and 5% in women) (Janmahasatian et al., 2005). In the literature, bone mass has at times been included in LBM and at other times not included (Prado et al., 2009, Mourtzakis et al., 2008).

Anthropometric-based prediction equations (PEs) have been examined as an alternative in measuring LBM in settings where access to these accurate methods is limited. In a very recent study of older (≥70 years) Australian men, FFM as estimated by three PEs, was compared to FFM as estimated by DXA (FFM_{DXA}) (Mitchell et al.). The three PEs were the Heitmann, Janmahasatian and Deurenberg equations as shown below:

Heitmann equation (Heitmann, 1990):

Body fat $(kg)_{male}$= (0.988 x BMI) + (0.242 x weight) + (0.094 x age) - 30.180

Body fat $(kg)_{female}$= (0.988 x BMI) + (0.344 x weight) + (0.094 x age) - 30.180.

Janmahasatian equation(Janmahasatian et al., 2005) :

FFM $(kg)_{female}$= (9270 x weight) / (8780+ (244 x BMI)

FFM $(kg)_{male}$ = (9270 x weight) / (6680 + (216 x BMI)

Deurenberg equation(Deurenberg et al., 1991):

Body fat (%) = (1.2 x BMI) + (0.23 x Age) – (10.8 x Sex) - 5.4

Male = 1, Female = 0

For two of the PEs (Heitmann and Deurenberg equations), FFM was calculated by subtracting fat mass from total body mass. In defining the FFM and LBM, the authors in that study proposed that FFM and LBM could be used interchangeably. Mitchell et al reported that FFM as estimated by Deurenberg equation had the smallest mean difference and overestimated FFM_{DXA} for overweight men but underestimated FFM_{DXA} for all other body mass index (BMI) subgroups (Mitchell et al.). The Heitmann and Janmahasatian equations, on the other hand, overestimated FFM_{DXA} across various BMI categories (Mitchell et al.).

The addition of biochemistry variables might improve the performance of prediction equations but few studies have examined this. Creatine kinase (CK) is found predominantly in skeletal muscle and serum levels were associated with the lean muscle mass (Norton et al., 1985).There has only been one study evaluating the relationship between LBM and plasma creatine kinase activity (CK) and a weak and partial correlation ($r < 0.262$) between log CK and LBM was reported (Swaminathan et al., 1988). Serum albumin has also been reported to reflect protein reserve and lower albumin levels have been shown to be associated with loss of lean mass (Visser et al., 2005).

Therefore, the aims of this study were to develop and validate PEs for LBM with anthropometric and biochemistry variables against DXA.

4.2 Methods

The Central Northern Adelaide Health Service Ethics of Human Research Committee approved this study. All participants provided written informed consent.

4.2.1 Study cohorts

Four study cohorts were investigated in this study: a) the Cytokine, Adiposity, Sarcopenia and Ageing (CASA) cohort; b) the validation cohort (VC); c) the North West Adelaide Health Study (NWAHS) cohort and d) the Florey Adelaide Male Ageing Study (FAMAS) cohort. CASA was used to derive the PEs for LBM which included anthropometric and biochemistry variables. The selected LBM PEs were then validated in a second independent cohort, the VC (n=52). As sarcopenia is more prevalent in older populations, validation of the best performing PE and other published FFM PEs (Heitmann, Janmahasatian and Deurenberg equations) were then undertaken in the larger population representative NWAHS and FAMAS cohorts (n=2287, age ≥ 50 years).

CASA.195 population representative healthy subjects (age 18 to 83 years) were recruited from the western suburbs of Adelaide (Dent E, 2012). The inclusion criteria were: being aged 18 and above, able to comply with study protocol and weight stable over the last three months. We excluded those with a serious medical illness, an acute illness in the past three months or in the two weeks following blood sampling, an inability to stop medications for three days prior to blood sampling, being in receipt of vaccinations and pregnancy. In undertaking the analysis, data from seven subjects were excluded due to haemolysed or insufficient blood samples.

VC. This was a convenience sample of 52 healthy subjects (age 22 – 83 years) recruited through advertisement for another study (Tai et al., 2009). Subjects with known medical illness including gastrointestinal disease or symptoms, significant respiratory, renal or cardiac disease and who were pregnant were excluded from this study.

NWAHS. This is a longitudinal study of community dwelling adults aged 18 years and older. The population which is a representative biomedical cohort of predominantly of mixed European descent has been described in detail previously (Grant et al., 2009). DXA scans were offered to NWAHS participants who were aged ≥50 years at follow up (median time = 4 years). Participants with complete anthropometric and DXA measurements at follow up (2004-06) aged ≥50 were included in this analysis (n= 1575).

FAMAS. This male only cohort has also been described in detail elsewhere (Martin et al., 2007a). The recruitment process was very similar to that used for the NWAHS and so the men in FAMAS were comparable with men in the same age groups from the NWAHS study and of mixed European descent (Grant et al., 2006). DXA measurements at baseline (2002-2005) were obtained on 700 participants aged 50 years and over.

4.2.2 Measurements

Anthropometry. Height (m) was measured without shoes using a wall-mounted SECA stadiometer to the nearest 0.1cm. Weight (kg) was measured wearing light clothing to the nearest 0.1kg (A&D FV platform scales 0.5 – 150 kg). Body mass index (BMI, weight/height^2) was calculated. The healthy BMI for older people is said to be between 22-27 kg/m^2 (Visvanathan, 2007). Caucasians with BMI > 30 kg/m^2 were classified as obese (WHO, 2000).

Dual energy x-ray absorptiometry (DXA). DXA analysis in all cohorts measured three compartments of the total body composition; fat mass, LBM and bone mineral content. For the purpose of this study, LBM refers to soft tissues and muscle mass, but excludes fat and bone mass.

CASA. A Lunar PRODIGY whole-body scanner (GE Medical Systems, Madison, WI), in conjunction with *Encore 2002* software, was used to estimate LBM. The majority of subjects underwent DXA within two hours of attending the morning clinic when blood sampling occurred.

VC. A Norland densitometer XR36 (Norland Medical Systems, Fort Atkinson, Wisconsin, USA), in conjunction with *Illuminatus 4.2.4a* software, was used to estimate LBM. The DXA was performed on a separate study day but within two weeks of blood sampling and, given that the subjects were healthy, it was unlikely that there would have been significant changes in body composition within that time frame. To account for differences between machines, LBM data from the VC had a correction factor applied to convert the data to Lunar equivalent (Maple-Brown et al., 2012b).

NWAHS and **FAMAS**. The fan-beam Lunar PRODIGY (GE Medical Systems, Madison, WI) in conjunction with *Encore 2002* software and a pencil-beam DPX+ (GE Medical Systems, Madison, WI) in conjunction with *LUNAR* software version 4.7e were used. Cross-calibration analysis had been undertaken and no differences between these two densitometers were reported (Mazess and Barden, 2000).

Blood analyses. For both the CASA and VC cohorts, a venous sample was obtained from each participant after an overnight fast. Both cohorts were asked to refrain from smoking, consuming alcohol or vigorous exercise in the 24 hours before the clinic appointment. Final regular medications were taken the day before and the morning dose was held until after venous sampling.

For CASA, the blood was placed in ethylenediaminetetraacetic acid (EDTA) tubes and transported immediately to the Institute for Medical and Veterinary Sciences Laboratories (IMVS) in South Australia for analysis. The blood was centrifuged at 5000 rpm for seven minutes and analyzed immediately at 37°C. For the VC, samples that had been centrifuged and stored at -70°C were transferred to be processed by the IMVS using the same methodology. The measured coefficients of variation (CV) were: alanine transferase (ALT, 1.98%), aspartate transaminase (AST, 2.8%), albumin (2.8%), creatinine (3%), lactate dehydrogenase (LDH, 2.2%), creatinine kinase (CK, 2.2%) and high sensitivity C-reactive protein (hsCRP, 1.4%). A Beckman Coulter AU 2700 was used to perform the blood analysis and the methods, reagents and calibration were as per manufacturer instructions.

4.2.3 Statistical analysis

Demographic characteristics in both groups were expressed as mean ± standard deviation (SD). Independent samples *t* test was used to compare means between the two cohorts. Differences between methods of LBM measurements in the same cohort were examined by paired *t* test. PEs for LBM were developed from CASA where the independent variable was DXA derived LBM.

The initial 10 independent variables were gender, age, weight, height, body mass index, albumin, AST, LDH, CK and hsCRP. The best PEs (as assessed by adjusted R^2: the proportion of the variance of the dependent variable accounted for by the independent variables, and adjusted for the number of independent variables) were developed considering up to six equations with *n* predictors. For each *n*, the PE for validation was selected by considering the adjusted R^2 value and likely clinical utility. In the VC, LBM was calculated from the developed prediction equations (LBM_{PE}) and compared with DXA derived LBM (LBM_{DXA}).

The anthropometric PE was also compared to other known PEs (Janmahasatian et al., 2005, Heitmann, 1990, Deurenberg et al., 1991) in the NWAHS and FAMAS cohorts.

To assess the accuracy and predictive performance of the prediction equations against LBM_{DXA}, a regression analysis as proposed by Lin (Lin, 1989) was undertaken and the concordance correlation

coefficient (ρ_c) was derived. ρ_c measures how much the data deviates from the line of identity representing congruence between the methods. It is a product of the Pearson correlation (ρ) and a bias correction factor (C_b): $\rho_c = \rho\ C_b$ (Chumlea and Baumgartner, 1989).

In addition, to assess the level of agreement between the two methods, Bland-Altman analysis was performed to obtain the 95% limits of agreement (Bland and Altman, 1999). Furthermore, the goodness of fit with root mean square error (RMSE) and bias (mean error [ME]) was also determined. RMSE and ME were calculated according to the method of Sheiner and Beal (Sheiner and Beal, 1981). When the 95% confidence interval of the ME includes 0 (i.e. no error), it indicates that the model is not biased. In this study, mean difference was taken to be the same as ME. This gives an estimation of R^2 and the standard error of the estimate [SEE]. SPSS 11.5 for Windows software (SPSS, Inc., Chicago, IL) and the R statistical language (R Foundation for Statistical Computing, Vienna, Austria) were used for the analyses. $P<0.05$ was considered statistically significant.

4.3 Results

The CASA and VC cohorts were similar in age (CASA mean [SD] 49.2 [17.0] vs. VC 50.6 [15.7] years), but younger than the NWAHS (64.7 [9.84] years) and FAMAS (62.3 [8.2] years) cohorts. The BMI (23.7 [2.3] vs. 26.7 [5.2] kg/m^2) and CK (93.3 [54.7] vs. 114.3 [66.0] U/L), were significantly lower in the VC compared to the CASA. LDH (194.4 [37.8] vs. 175.0 [37.4] U/L) and albumin (40.4 [2.5] vs. 39.1 [3.1] g/L) were significantly higher in the VC compared to the CASA. No significant differences between the two cohorts were noted for hsCRP or LBM. The BMI of subjects in the NWAHS and FAMAS studies were higher at 28.2 [4.8] and 28.6 [4.6] kg/m^2 respectively.

Based on adjusted R^2 and potential clinical utility, the following PEs were selected for further validation in the VC:

LBM_{PE1}= 22.93 + 0.68 (weight) -1.14 (BMI) - 0.01 (age) + 9.94 (if male) SEE=3.61, R^2= 90.7
LBM_{PE2}= 22.06 + 0.67 (weight) - 1.11 (BMI) + 9.76 (if male) + 0.01 (CK) SEE= 3.56, R^2= 91.0
LBM_{PE3}= 21.19 + 0.67 (weight) - 1.04 (BMI) + 9.51 (if male) - 0.56 (CRP) + 0.01 (CK) SEE=3.47, R^2=91.4
LBM_{PE4}= 23.17 + 0.64 (weight) - 0.91 (BMI) + 9.45 (if male) + 0.02 (CK) - 0.58 (CRP) - 0.02 (LDH) SEE=3.38, R^2=91.9

Table 4.1 compares LBM_{PE1-4} to LBM_{DXA} in the VC. LBM predicted by all PEs was highly correlated with LBM_{DXA}. Concordance correlations, a measure of the degree to which the data lie on the line of identity, were all around 0.9 and similar to the Pearsons correlation coefficient. All PEs over-estimated LBM_{DXA}, ranging from 1.9% for PE_1 to 4.1% for PE_4. The limits of agreement were similar for all PEs, approximately ± 15%. With increasing numbers of variables, there were reducing RMSE and mean errors, indicating improving precision and reducing bias. Because of the costs involved with blood investigations and the marginal benefits, only the anthropometric PE_1 was selected for further comparison in the combined NWAHS and FAMAS cohorts (Table 2-4). Furthermore, biochemistry was not readily available from those cohorts.

Table 4.1 Validation of PE LBM in healthy adults from the Cytokine, Adiposity, Sarcopenia and Ageing (CASA) study cohort (n=195) against DXA derived LBM in the validation cohort (n=52).

	Mean (SD), kg	Mean Error (95%CI), kg	P-value for mean error	R	ρ_c (95% CI) [C_b]	95% Limits of Agreement	RMSE (95% CI), kg
Total (n=52)							
LBM_{DXA}	46.2 (9.49)						
LBM_{PE1}	48.1 (8.93)	1.88 (0.79, 2.97)	0.001	0.911*	0.891 (0.820, 0.935) [0.977]	-9.72, 5.96 (-20.7 to 12.6%)	4.32 (2.84, 5.80)
LBM_{PE2}	47.9 (8.95)	1.69 (0.62, 2.75)	0.003	0.915*	0.899 (0.832, 0.940) [0.982]	-9.20, 5.83 (-19.9 to 12.6%)	4.15 (2.70, 5.60)
LBM_{PE3}	47.7 (9.13)	1.50 (0.44 , 2.57)	0.006	0.917*	0.904 (0.840, 0.943) [0.986]	-8.99, 5.98 (-19.5 to 13.0%)	4.07 (2.63, 5.51)
LBM_{PE4}	47.1 (8.96)	0.86 (-0.22, 1.94)	0.114	0.914*	0.908 (0.846, 0.946) [0.994]	-8.44, 6.72 (-18.3 to 14.6%)	3.93 (2.51, 5.35)

* P-value <0.001, R=correlation, SD= Standard Deviation
RMSE=root mean squared prediction error, CI=confidence interval, R=Pearson Correlation Coefficient, C_b= Bias Correction Factor, ρ_c = Concordance Correlation Coefficient

Table 4.2 compares the performance of various PEs, including PE_1, against LBM_{DXA} in the total combined NWAHS and FAMAS cohorts, as well as in the two gender groups, men and women. All PEs over-estimated the LBM_{DXA} in the total group. PE_1 demonstrated a lower mean error and RMSE score than the Heitmann and Janmahasatian equations in the total population, men and women cohorts. The Deurenberg equation performed the best in the total population with the lowest mean error and RMSE. However, when reviewed within gender groups, PE_1 performed better than the Deurenberg equation in women where both equations over-estimated LBM. In men, the Deurenberg equation under-estimated LBM, whilst all other equations over-estimated LBM.

Table 4.2 Performance of the CASA (LBM_{PE1}) and previously published FFM prediction equations in the NWAHS and FAMAS cohorts (age 50 years and over) in the combined cohort and by gender.

	Mean (SD), kg	Mean Error (95%CI), kg	P-value for mean error	R	ρ_c (95% CI) [C_b]	95% Limits of Agreement	RMSE (95% CI),kg
Total (n=2287)							
LBM_{DXA}	50.62 (10.8)						
Heitmann equation	54.30 (10.7)	3.68 (3.53, 3.83)	<0.001	0.940*	0.888 (0.880, 0.896) [0.945]	-3.77, 11.1	5.24 (4.97, 5.51)
Janmahasatian equation	54.23 (11.0)	3.61 (3.46, 3.76)	<0.001	0.943*	0.884 (0.884, 0.899) [0.946]	-3.78, 11.0	5.17 (4.90, 5.44)
Deurenberg equation	50.64 (10.1)	0.02 (-0.14, 0.19)	0.777	0.931*	0.928 (0.923, 0.934) [0.998]	-7.89, 7.93	3.95 (3.70, 4.20)
LBM_{PE1}	51.36 (10.6)	0.74 (0.59, 0.89)	<0.001	0.942*	0.939 (0.934, 0.944) [0.998]	-6.58, 8.06	3.73 (2.48, 4.98)
Men (n= 1436)							
LBM_{DXA}	57.09 (7.50)						
Heitmann equation	60.56 (7.80)	3.46 (3.25. 3.67)	<0.001	0.863*	0.782 (0.764, 0.800) [0.906]	-11.5, 4.57	5.30 (4.93, 5.67)
Janmahasatian equation	61.18 (6.80)	4.09 (3.89, 4.29)	<0.001	0.852*	0.728 (0.707, 0.747) [0.853]	-12.0, 3.82	5.69 (5.32, 6.06)
Deurenberg equation	56.76 (6.80)	- 0.34 (-0.55, - 0.12)	0.002	0.838*	0.834 (0.818, 0.848) [0.995]	-7.92, 8.60	4.14 (3.85, 4.43)
LBM_{PE1}	58.22 (6.11	1.12 (0.92, 1.33)	<0.001	0.851*	0.822 (0.806, 0.837) [0.851]	-6.78, 9.02	4.11 (3.80, 4.42)
Women (n=851)							
LBM_{DXA}	39.70 (5.30)						
Heitmann equation	43.74 (5.55)	4.04 (3.83, 4.26)	<0.001	0.833*	0.651 (0.620, 0.680) [0.782]	-10.3, 2.26	5.12 (4.75, 5.49)
Janmahasatian equation	42.50 (5.39)	2.81 (2.60, 3.01)	<0.001	0.837*	0.722 (0.693, 0.749) [0.872]	-8.91, 3.29	4.14 (3.83, 4.45)
Deurenberg equation	40.32 (4.90)	0.63 (0.39, 0.87)	<0.001	0.759*	0.751 (0.721, 0.779) [0.990]	-7.75, 6.49	3.61 (3.29, 3.93)
LBM_{PE1}	39.78 (5.11)	0.08 (-0.12, 0.28)	0.433	0.835*	0.835 (0.813, 0.854) [0.999]	-5.91, 6.07	2.99 (2.74, 3.24)

Mean Error=DXA-PE; LBM, Lean Body Mass; DXA, Dual X-ray absorptiometry; RMSE, Root Mean Square Error; CI, Confidence interval; SD, Standard Deviation; R, Pearson Correlation; C_b= Bias Correction Factor; ρ_c = Concordance Correlation Coefficient*p value <0.001

Table 4.3 compares the performance of the various PEs across age groups (60- 64, 65-79, ≥80). PE_1 consistently over-estimated LBM_{DXA} across the age groups but performed better (lowest ME, RMSE values and higher concordance correlation coefficient) than the Janmahasatian and Heitmann equations. The Deurenberg equation did not perform as well as PE_1 in the 50-<65 years

age group and the ≥ 80 years age group and over-estimated LBM in the 50-<65 years age group but under-estimated LBM in the other two age groups.

Table 4.3 Performance of the CASA (LBM_{PE1}) and previously published FFM prediction equations in the NWAHS and FAMAS cohorts (age 50 years and over) across various age groupings.

	Mean (SD), kg	Mean Error (95%CI), kg	P-value for mean error	R	ρ_c (95% CI) [C_b]	95% Limits of Agreement	RMSE (95% CI), kg
Age 50-64, years (n=1265)							
LBM_{DXA}	52.27 (11.2)						
Heitmann equation	56.47 (10.8)	4.20 (3.99, 4.40)	<0.001	0.944 *	0.879 (0.868, 0.890) [0.932]	-11.6, 3.23	5.60 (5.26, 5.95)
Janmahasatian equation	55.62 (11.2)	3.35 (3.15, 3.55)	<0.001	0.948 *	0.907 (0.897, 0.915) [0.956]	-10.6, 3.85	4.92 (4.61, 5.23)
Deurenberg equation	53.15 (10.0)	0.87 (0.66, 1.09)	<0.001	0.938 *	0.929 (0.921, 0.936) [0.990]	-8.72, 6.98	4.02 (3.73, 4.31)
LBM_{PE1}	52.77 (10.7)	0.50 (0.30, 0.70)	<0.001	0.948 *	0.946 (0.939, 0.951) [0.998]	-6.68, 7.68	3.62 (3.36, 3.88)
Age 65-79, years (n=882)							
LBM_{DXA}	49.09 (9.91)						
Heitmann equation	52.23 (10.0)	3.14 (2.90, 3.38)	<0.001	0.933 *	0.887 (0.873, 0.899) [0.951]	-10.5, 4.18	4.82 (4.35, 5.29)
Janmahasatian equation	53.03 (10.5)	3.93 (3.69, 4.18)	<0.001	0.933 *	0.862 (0.846, 0.876) [0.925]	-11.5, 3.66	5.46 (4.97, 5.95)
Deurenberg equation	48.19 (9.14)	-0.90 (-1.15, -0.65)	<0.001	0.924 *	0.916 (0.905, 0.926) [0.993]	-6.70, 8.50	3.90 (3.45, 4.35)
LBM_{PE1}	50.20 (10.2)	0.98 (0.73, 1.22)	<0.001	0.929 *	0.925 (0.915, 0.934) [0.995]	-6.57, 8.53	3.90 (3.48, 4.32)
Age ≥80, years (n=140)							
LBM_{DXA}	44.48 (8.64)						
Heitmann equation	46.71 (9.20)	2.23 (1.60, 2.85)	<0.001	0.929 *	0.902 (0.868, 0.928) [0.969]	-9.05, 4.59	4.06 (3.20, 4.92)
Janmahasatian equation	48.46 (10.1)	3.97 (3.29, 4.66)	<0.001	0.936 *	0.850 (0.806, 0.883) [0.906]	-11.4, 3.46	5.43 (4.31, 6.55)
Deurenberg equation	42.46 (8.41)	-2.03 (-2.58, -1.48)	<0.001	0.937 *	0.911 (0.880, 0.934) [0.971]	-3.97, 8.03	3.61 (2.85, 4.37)
LBM_{PE1}	45.84 (9.81)	1.36 (0.80, 1.93)	<0.001	0.941 *	0.923 (0.897, 0.943) [0.981]	-5.39, 8.11	3.63 (2.90, 4.36)

Mean Error=DXA-PE; LBM, Lean Body Mass; DXA, Dual X-ray absorptiometry; RMSE, Root Mean Square Error; CI, Confidence interval; SD, Standard Deviation; R, Pearson Correlation; C_b= Bias Correction Factor; ρ_c = Concordance Correlation Coefficient* p value <0.001

Table 4. 4 compare the performance of the various PEs across various BMI groups. Once again, PE_1 has the smallest ME and RMSE compared with the Janmahasatian and Heitmann equations across all the BMI groups analyzed but all of these consistently over-estimated LBM_{DXA} across the various BMI groups. PE_1, in comparison with the Deurenberg equation has a lower ME and RMSE in the obese BMI (>30 kg/m^2) and underweight BMI (< 22 kg/m^2) groups. Interestingly, the Deurenberg equation has less bias and better precision than PE_1 in predicting LBM_{DXA} in the 22-27kg/m^2 BMI group. The Deurenberg equation overestimated LBM_{DXA} except in the underweight and obese categories.

Table 4.4 Performance of the CASA (LBM_{PE1}) and previously published FFM prediction equations in the NWAHS and FAMAS cohorts (age 50 years and over) across various body mass index groupings.

	Mean (SD), kg	Mean Error (95%CI), kg	P-value for mean error	R	ρ_c (95% CI) [C_b]	95% Limits of Agreement	RMSE (95% CI), kg
BMI<22 kg/m^2 (n=135)							
LBM_{DXA}	42.45 (8.85)						
Heitmann equation	44.85 (7.65)	2.40 (1.85, 2.96)	<0.001	0.932*	0.885 (0.847, 0.914) [0.949]	-4.12, 8.92	4.04 (3.21, 4.87)
Janmahasatian equation	43.72 (9.26)	1.27 (0.77, 1.77)	<0.001	0.946*	0.937 (0.914, 0.955) [0.989]	-4.65, 7.19	3.21 (2.55, 3.87)
Deurenberg equation	41.26 (8.04)	-1.18 (-1.77, -0.60)	<0.001	0.921*	0.909 (0.876, 0.933) [0.986]	-8.04, 5.68	3.62 (2.86, 4.36)
LBM_{PE1}	43.52 (9.04)	1.08 (0.57, 1.59)	<0.001	0.944*	0.937 (0.913, 0.955) [0.993]	-4.92, 7.08	3.18 (2.53, 3.83)
BMI 22-<27 kg/m^2 (n=847)							
LBM_{DXA}	47.45 (9.18)						
Heitmann equation	50.67 (8.67)	3.22 (2.99, 3.44)	<0.001	0.933*	0.874 (0.860, 0.888) [0.938]	-3.42, 9.86	4.62 (4.26,4.98)
Janmahasatian equation	50.81 (9.71)	3.36 (3.13, 3.59)	<0.001	0.937*	0.880 (0.866, 0.893) [0.939]	-3.41, 10.1	4.77 (4.39, 5.15)
Deurenberg equation	47.91 (8.68)	0.45 (0.22, 0.68)	0.001	0.928*	0.925 (0.915, 0.934) [0.997]	-6.42, 7.32	3.46 (3.16, 3.76)
LBM_{PE1}	48.64 (9.45)	1.19 (0.96, 1.41)	<0.001	0.938*	0.930 (0.920, 0.938) [0.992]	-5.41, 7.79	3.51 (3.20, 3.82)

Table 4.4 continued

BMI 27-<30 kg/m^2 (n=596)							
LBM_{DXA}	52.00 (9.83)						
Heitmann equation	55.65 (9.48)	3.65 (3.36, 3.95)	<0.001	0.929*	0.867 (0.847, 0.883) [0.933]	-3.65, 10.9	5.16 (4.69, 5.63)
Janmahasatian equation	56.11 (9.75)	4.12 (3.83, 4.41)	<0.001	0.932*	0.857 (0.837, 0.874) [0.919]	-3.08, 11.3	5.47 (4.97, 5.97)
Deurenberg equation	52.58 (9.23)	0.59 (0.30, 0.88)	<0.001	0.928*	0.925 (0.912, 0.935) [0.996]	-6.72, 7.90	3.70 (3.35, 4.05)
LBM_{PE1}	52.80 (9.69)	0.81 (0.52, 1.09)	<0.001	0.933*	0.929 (0.918, 0.939) [0.997]	-6.37, 7.99	3.67 (3.31, 4.03)
BMI ≥30 kg/m^2 (n=709)							
LBM_{DXA}	54.80 (11.7)						
Heitmann equation	59.30 (11.7)	4.50 (4.19, 4.80)	<0.001	0.937*	0.867 (0.847, 0.883) [0.933]	-3.80, 12.8	6.12 (5.53, 6.71)
Janmahasatian equation	58.93 (11.0)	4.13 (3.83, 4.43)	<0.001	0.937*	0.857 (0.837, 0.974) [0.919]	-4.02, 12.3	5.80 (5.22, 6.38)
Deurenberg equation	54.07 (10.6)	-0.74 (-1.08, -0.39)	<0.001	0.917*	0.925 (0.912, 0.935) [0.996]	-10.0, 8.55	4.70 (4.14, 5.26)
LBM_{PE1}	54.88 (11.3)	0.08 (-0.23, 0.38)	0.628	0.936*	0.929 (0.918, 0.939) [0.997]	-8.15, 8.31	4.11 (3.61, 4.61)

Mean Error=DXA-PE; LBM, Lean Body Mass; DXA, Dual X-ray absorptiometry; RMSE, Root Mean Square Error; CI, Confidence interval; SD, Standard Deviation; R, Pearson Correlation; C_b= Bias Correction Factor; ρ_c = Concordance Correlation Coefficient
* p value <0.001

4.4 Discussion

In this study, prediction equations for LBM were developed and validated. It was hypothesized that the addition of biochemistry variables would result in an improvement in the performance of the PEs and this was seen. However, the improvement was marginal and insufficient to justify the additional costs.

A significant finding from this study was the development of a new anthropometric PE (PE_1) for LBM: *LBM= 22.932326 + 0.684668 (weight) -1.137156 (BMI) -0.009213 (age) + 9.940015 (if male)*. The close approximation to LBM_{DXA} generated by this equation was reflected by its small bias (ME=0.74kg) and precision (RMSE=3.73kg). It overestimated LBM_{DXA} across gender, age and BMI groups. This PE may be useful in care settings where access to DXA may be limited, providing clinicians a practical alternative to assess LBM. Furthermore, it also provides a bedside

option in hospitals for ill and frail patients where transport for DXA assessment may be difficult. Whilst BIA may be a simple technique to be used at the beside, BIA may be affected by clinical factors such as ascites, hydration status, food intake and exercise and cannot be used in older people with pacemakers (Kyle et al., 2004). Skin fold measurements may be a cheaper option but the accuracy is operator dependent and the loss of subcutaneous tissue in older people may also affect accuracy (Omran and Morley, 2000b).

Interestingly, the Deurenberg equation appeared to have less bias with a ME of 0.02 kg, but similar precision, with a RMSE of 3.95 when compared to the newly developed PE. However, across gender, age and BMI groups, it at times over-estimated and at other times under-estimated the LBM_{DXA} (Mitchell et al., 2010). The newly developed PE_1 appeared to have better precision (smaller RMSE) and less bias (lower ME) than the Deurenberg equation only in women and in obese older individuals. In clinical settings where the dose normalization to LBM is required, an overestimation of LBM could potentially lead to higher incidence of dose limiting toxicity.

Sarcopenia has proved to be an important predictor of toxicity in women with metastatic cancer and colon cancer receiving chemotherapy (Prado et al., 2009, Prado et al., 2007), and it is suggested that chemotherapy dose normalization to LBM may reduce the excess toxicity in women. PE_1 in our study potentially offers a more accurate estimation of LBM than the Deurenberg equation in women and obese individuals and may have clinical utility in these two patient population groups.

This study had several limitations. Only 6% of the study population was under-weight with a BMI < 22 kg/m^2 and therefore, it remains important to validate the newly developed PE in an under-weight population where sarcopenia is likely to be common. Furthermore, only Caucasians were studied and therefore generalizing these results to other ethnic communities is not possible, and ethnic specific PEs will need to be developed. Different DXA machines were used in the CASA and VC cohort studies. This may have affected the results as, even in the same person, reported measurements of the same tissue mass can be different with different DXA machines (Tothill and Hannan, 2000). The researchers adjusted for the difference between the machines in the validation aspects of this study, but clearly it would have been preferable to use the same DXA machine in both cohorts. The use of other anthropometry measurements, such as calf or arm circumference may improve the performance of prediction equations and needs to be explored in future studies.

4.5 Conclusion

This study describes the development of a new prediction equation for LBM as estimated by DXA. This new PE consistently over-estimates across gender, age and BMI groups. There remains a need to confirm these findings in older and leaner cohorts, cohorts with diseases (e.g. renal failure), as well as other cohorts with varying ethnicity. The anthropometric PE is an alternative when access to DXA is difficult and this might occur with homebound frail older people, as well as people residing in rural areas. The availability of simple and accurate methods to estimate LBM might be the necessary catalyst required to support better prescribing to limit toxicity in the oncology setting.

Appendicular skeletal muscle mass: Development and validation of anthropometric prediction equations

Summary

Central to making the diagnosis of sarcopenia is the assessment of appendicular skeletal muscle mass (ASM). The objective of this study was to develop and validate novel anthropometric prediction equations (PEs) for ASM that would be useful in primary or aged care.

PEs were developed using best subset regression analysis. The three best performing PEs (PE1, PE2, and PE3) were selected and validated using the Bland-Altman and Sheiner & Beal methods.

188 healthy subjects were involved in the development study. 2275 older (age $\geq$ 50years) subjects were involved in the validation study. ASM was assessed using dual x-ray absorptiometry (DEXA). Weight and height were measured and body mass index (BMI) estimated. A strong correlation between PE derived ASM and the DEXA derived ASM was seen for the three selected PEs.

PE_3: *ASM= 10.047427 + 0.353307 (weight) - 0.621112 (BMI) - 0.022741 (age) + 5.096201 (if male)* performed the best. PE_3 over-estimated ($P<0.001$) ASM by 0.36 kg (95% CI 0.28-0.44 Kg) and the adjusted R^2 was 0.869. The 95% limit of agreement was between -3.5 and 4.74 kg and the standard error of the estimate was 1.95. The root mean square error was 1.91 (95% CI 1.80-2.01). PE_3 also performed the best across the various age (50-65, 65-<80, 80+ years) and weight (BMI <18.5,, 18.5-24.9, 25-29.9, $\geq$30 kg/m^2) groups.

As a result of this research, a new anthropometric PE for ASM has been developed for use in primary or aged care but is specific to Caucasian population groups. The research of this chapter forms the basis of a research paper published in the *Journal of Frailty & Aging* (Appendix 9 – including statement of authorship) following peer review.

5.1 Introduction

A physiological decline in muscle mass averaging about three kilograms per decade is seen from the 4th decade of life (Tzankoff and Norris, 1977). This decline may be accompanied by a gradual reduction in physical function and can become pathological when sufficiently severe resulting in a loss of autonomy, a condition referred to as sarcopenia (Rosenberg, 1997, Janssen et al., 2002).

Sarcopenia is a Greek word which literally means loss of tissue (sarx [flesh] + paenia [loss]), but is now generally taken to mean loss of lean tissue, and particularly skeletal muscle (Rosenberg, 1997). More recently, the European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as not only the presence of low muscle mass but also included low muscle function (Cruz-Jentoft et al.). Similar to what is seen with the diagnosis of osteoporosis, the EWGSOP has defined that skeletal muscle index (SMI= ASM/[height]^2) cut-offs < two standard deviation (SD) below young male and female reference groups (18- <40 years) are required in addition to loss of physical function to define the presence of sarcopenia. In the late 90s, Appendicular Skeletal Muscle Mass (ASM) cut-offs of <7.26kg/m2 for men and <5.5kg/m^2 for women were developed to identify sarcopenia and in that landmark study, >40% of men and women over the age of 80 years were identified as sarcopenic (Gallagher et al., 1997, Baumgartner et al., 1998).

Clearly, it has become important that clinicians are able to easily estimate ASM in clinical practice to identify those at risk of sarcopenia (Cruz-Jentoft et al., 2010). Computed tomography, magnetic resonance imaging and dual absorptiometry x-ray (DXA) are currently the recommended methods to assess ASM in research but may be difficult to access in some clinical settings (e.g. rural regions) as well as burdensome for some patient population groups such as the frail elderly who may be reluctant to attend tertiary centers for ASM assessment *Dual energy x-ray absorptiometry (DXA)*(Lustgarten and Fielding, 2011). Although the bio-electrical impedance analysis method is portable, it still requires the purchase of equipment that is not routinely used in clinical practice. Therefore, anthropometric prediction equations may have a role to play in primary or aged care settings. The aims of this study were to develop anthropometric PEs for ASM using DXA as the reference method and validate these newly developed PEs in South Australian population cohorts.

5.2 Methods

5.2.1 Study cohorts

Three cohorts were investigated in this study: a) the Cytokine, Adiposity, Sarcopenia and Ageing Study (CASA) cohort; b) the North West Adelaide Health Study (NWAHS) cohort; and c) the Florey Adelaide Male Ageing Study (FAMAS) cohort.

CASA: 195 population representative healthy subjects (age 18 to 83 years) were recruited from the western suburbs of Adelaide. The method used is similar to that used for the NWAHS (Grant et al., 2006). In undertaking the analysis, data from seven subjects were excluded due to samples being haemolysed or insufficient. Subjects were selected randomly from the electronic White Pages. Selected households were sent a letter and brochure about the study. The person in the household aged 18 years or over who had most recently had a birthday was eligible to participate in a brief telephone interview. A minimum of six telephone calls was made to each household before an individual was deemed non contactable. Subjects who were able to comply with the study protocol and who reported weight stability over the preceding three months were included in the study. Those with known inflammatory diseases, those who were pregnant and those who had been ill in the preceding three months or in the two weeks following blood sampling, were excluded.

NWAHS: This study cohort has previously been described in detail (Grant et al., 2009). Briefly, NWAHS is a representative biomedical cohort study of subjects of predominantly of mixed European descent, aged at least 18 years. Subjects living in residential care and those who could not attend the clinics or converse in English were excluded. There was under-representation in the younger age groups but over-representation in the older age groups. From December 1999 to July 2003, 4060 adults underwent baseline biomedical examination (69.4% of those completing the initial interview). At follow-up (May 2004 to Feb 2006, median time = 4.0 years), survey data was obtained on 88% (n=3574) and clinic data on 79% (n=3206) using the same method. Of the baseline sample, 100 subjects were deceased, 226 were unable to be contacted, and 160 refused further participation in the study. At follow-up, DXA scans were offered to NWAHS participants who were aged 50 years and over as part of the clinic assessment. DXA measurements were obtained on 1575 participants.

FAMAS: This male only study cohort has been described in detail elsewhere (Martin et al., 2007a). Briefly, 1195 men age between 35 and 80 years from the North West regions of Adelaide were recruited between August 2002 and April 2005 to this longitudinal study. The recruitment process

was very similar to that described for the NWAHS study and so, it was not surprising that the men in FAMAS were comparable with men in the same age groups from the NWAHS study and of mixed European descent (Grant et al., 2006). DXA measurements were obtained on 700 participants aged 50 years and over.

5.2.2 Measurements

Anthropometry: Height (m) was measured with shoes off using a wall-mounted stadiometer to the nearest 0.1cm. Weight (kg) was measured wearing light clothing to the nearest 0.1kg. Body mass index (BMI-weight/height^2) was calculated.

Dual energy x-ray absorptiometry (DXA): The DXA in all cohorts measured three compartments of the total body composition: fat mass, LBM and bone mineral content. In this study, the ASM refers to sum of lean soft-tissue masses for arms and legs. **CASA:** A Lunar PRODIGY whole-body scanner (GE Medical Systems, Madison, WI), in conjunction with Encore 2002 software, was used to estimate ASM. **NWAHS** and **FAMAS:** For both of these cohort studies, the same fan-beam Lunar PRODIGY (GE Medical Systems, Madison, WI) in conjunction with Encore 2002 software (as per the DC) and a pencil-beam DPX+ (GE Medical Systems, Madison, WI) in conjunction with LUNAR software version 4.7e were used. Cross-calibration analysis was undertaken and reported no differences between the two densitometers (Mazess and Barden, 2000).

5.2.3 Ethics

The Central Northern Adelaide Health Service Ethics of Human Research Committee approved this study. All participants provided written informed consent.

5.2.4 Statistical analysis

Demographic characteristics in both groups are expressed as mean ± standard deviation (SD). Differences between methods of ASM measurements in the same cohort were examined by paired *t* test. PEs for ASM were developed from CASA cohort where the independent variable included gender, age, weight, height and body mass index. The best anthropometric PE (as assessed by adjusted R^2: the proportion of the variance of the dependent variable accounted for by the independent variables, and adjusted for the number of independent variables) involving $n = 1, \ldots,$ four predictors was developed by considering all such equations with *n* predictors. For each *n*, the PE for validation was selected by considering the adjusted R^2 value and clinical utility in primary care. The developed PEs were then cross-validated in two combined populations (FAMAS and NWAHS cohorts). ASM was calculated from the developed prediction equations (ASM_{PE}) and compared with DXA derived ASM (ASM_{DXA}).

To assess the accuracy and predictive performance of the prediction equations, the method of Bland-Altman was used to estimate the level of agreement, whereby the difference between the two measurements was plotted against the average of the two measurements(Bland and Altman, 1999). Precision (root mean square error [RMSE]) and bias (mean error [ME]) were calculated according to the method of Sheiner and Beal (Sheiner and Beal, 1981). When the 95% confidence interval of the ME included 0 (i.e. no error), this indicated that the model was not biased. Linear regression analysis was performed using ASM_{PE} to predict ASM_{DXA}**.** This gives an estimation of R^2 and the standard error of the estimate [SEE]. In this study, mean difference was used interchangeably with ME. *SPSS 11.5* for Windows software (SPSS, Inc., Chicago, IL) and R statistical language (R Foundation for Statistical Computing, Vienna, Austria) were used for the analyses. $P<0.05$ was considered statistically significant.

5.3 Results

The mean age for CASA subjects was lower (50.6 $\pm$ 15.7 years) than for subjects from the NWAHS (64.7 $\pm$ 9.84 years) and FAMAS (62.3 $\pm$ 8.2 years) studies. Similarly, the subjects in the CASA (26.7 $\pm$ 5.2 kg/m^2) had lower BMI than the subjects in the NWAHS (28.2 $\pm$ 4.8 kg/m^2) and FAMAS (28.6 $\pm$ 4.6 kg/m^2) studies. Given that the FAMAS (25.8 $\pm$ 3.75 kg/m^2) study was a study of men only, then, as expected, that cohort had higher mean ASM values compared to the NWAHS (20.7 $\pm$5.25 kg) and CASA (21.4 $\pm$ 2.14 kg).

The three selected PEs are presented here:

***PE_1**: ASM= 9.11472 + 0.36992 (weight) - 0.67551 (BMI) + 5.00840 (if male)*
[Standard Error of Estimate (SEE) 1.89; Adjusted R2 (%) 90.4]

***PE_2**: ASM= -27.879919 + 0.129727 (weight) + 22.122674 (height) + 4.980820 (if male)*
[SEE 1.93; Adjusted R2 (%) 90.1]

***PE_3**: ASM= 10.047427 + 0.353307 (weight) - 0.621112 (BMI) - 0.022741 (age) + 5.096201 (if male)*
[SEE 1.87; Adjusted R2 (%) 90.6]

RMSE, ME and SEE measure the degree of error (precision) of the PEs against the reference method (ASM_{DXA}). Lower values of RMSE, ME and SEE reflect a lower error rate and therefore a higher precision of the PE in predicting the ASM_{DXA}. Table 5.1 compares ASM_{PE1-3} to ASM_{DXA}. PE_3 has the lowest SEE, ME and RMSE and therefore appears to be the most precise of the three PEs. For all equations, there was a significant over-estimation (i.e. mean error >0) of ASM when ASM_{PE1-3} were compared to ASM_{DXA} (Tables 1-3). When the performance of the PEs was compared against various older age cohorts [50-<65 vs. 65- <80 vs. $\geq$80] (Table 5.2), PE_3 continued to perform the best across the age cohorts with the lowest SEE, ME and RMSE. The PEs

were also compared across various BMI groupings [<18.5, 18.5-24.9, 25-29.9, ≥30 kg/m^2] (Table 5.3). Once again, PE_3 performed slightly better (lower SEE, ME and RMSE) than $PE_{1\&2}$ across all BMI groupings except for the BMI category <18.5kg/m2 where the sample size was small (n=7).

Table 5.1: Validation of the 3 prediction equations in the North West Adelaide Health Study and Florey Adelaide Male Ageing Study older (age ≥ 50 years) cohorts

NWAHS & FAMAS N=2275	Mean Kg (SD)	Mean Error Kg (95%CI)	P-value for mean error	Adjusted R^2	SEE	95% Limits of Agreement	RMSE (95% CI)
ASM_{DXA}	22.2 (5.39)						
ASM_{PE1}	22.9 (5.49)	0.62 (0.54, 0.71)	<0.001	0.862	2.00	-3.50, 4.74	2.15 (2.04, 2.26)
ASM_{PE2}	22.9 (5.41)	0.67 (0.58, 0.75)	<0.001	0.859	2.02	-3.45, 4.79	2.03 (1.92, 2.14)
ASM_{PE3}	22.6 (5.44)	0.36 (0.28, 0.44)	<0.001	0.869	1.95	-3.63, 4.35	1.91 (1.80, 2.01)

SD- Standard Deviation, CI- Confidence Interval, SEE- Standard Error of the Estimate, RMSE- Root Mean Square Error

Table 5.2: Comparison of the 3 prediction equations in the North West Adelaide Health Study and Florey Adelaide Male Ageing Study across different older (≥50 years) age groups

	Mean Kg (SD)	Mean Error Kg (95%CI)	P-value for mean error	Adjusted R^2	SEE	95% Limits of Agreement	RMSE (95% CI)
Age 50-<65, years (n=1259)							
$ASM_{DXA\ (VC)}$	23.3 (5.61)						
ASM_{PE1}	23.6 (5.53)	0.33 (0.22, 0.44)	<0.001	0.877	1.97	-3.65, 4.31	2.02 (1.87, 2.17)
ASM_{PE2}	23.6 (5.38)	0.37 (0.26, 0.48)	<0.001	0.874	1.99	-3.61. 4.36	2.03 (1.88, 2.18)
ASM_{PE3}	23.4 (5.45)	0.22 (0.11, 0.32)	<0.001	0.879	1.95	-3.70, 4.14	1.97 (1.82, 2.12)
Age 65-<80, years (n=877)							
$ASM_{DXA\ (VC)}$	21.3 (4.82)						
ASM_{PE1}	22.3 (5.29)	0.95 (0.81, 1.09)	<0.001	0.841	1.92	-3.27, 5.15	2.31 (2.12, 2.50)
ASM_{PE2}	22.3 (5.26)	1.01 (0.87, 1.15)	<0.001	0.842	1.92	-3.17, 5.19	2.32 (2.13, 2.51)
ASM_{PE3}	21.9 (5.21)	0.54 (0.41, 0.68)	<0.001	0.846	1.89	-3.55, 4.63	2.11 (1.93, 2.29)
Age ≥80 years (n=139)							
$ASM_{DXA\ (VC)}$	18.9 (4.22)						
ASM_{PE1}	20.1 (5.11)	1.19 (0.87, 1.52)	<0.001	0.863	1.56	-2.72, 5.10	2.28 (1.83, 2.73)
ASM_{PE2}	20.1 (5.27)	1.19 (0.84, 1.53)	<0.001	0.864	1.56	-2.92, 5.30	2.37 (1.92, 2.82)
ASM_{PE3}	19.5 (5.04)	0.54 (0.23, 0.86)	0.001	0.868	1.53	-3.24, 4.32	1.96 (1.58, 2.34)

SD- Standard Deviation, CI- Confidence Interval, SEE- Standard Error of the Estimate, RMSE- Root Mean Square Error

Table 5.3: Comparison of the 3 prediction equations in the North West Adelaide Health Study and Florey Adelaide Male Ageing Study across different body mass index (BMI) groupings.

	Mean Kg (SD)	Mean Error Kg (95%CI)	P-value for mean error	Adjusted R^2	SEE	95% Limits of Agreement	RMSE (95% CI)
BMI <18.5 kg/m^2 (n=7)							
$ASM_{DXA\ (VC)}$	15.4 (4.41)						
ASM_{PE1}	16.5 (4.00)	1.15 (-0.20, 2.49)	0.082	0.871	1.58	-1.76, 4.06	1.76 (0.72, 3.48)
ASM_{PE2}	16.5 (4.66)	1.07 (-0.24, 2.38)	0.092	0.889	1.47	-1.77, 3.91	1.69 (0.12, 3.26)
ASM_{PE3}	16.2 (4.05)	0.79 (-0.63, 2.21)	0.223	0.854	1.68	-2.28, 3.86	1.63 (0.29, 2.97)
BMI 18.5-24.9 kg/m^2 (n=543)							
$ASM_{DXA\ (VC)}$	19.7 (4.54)						
$ASM_{PE1,}$	20.5 (4.86)	0.87 (0.71, 1.03)	<0.001	0.850	1.76	-2.90, 4.64	2.07 (1.86, 2.28)
ASM_{PE2}	20.6 (5.17)	0.92 (0.75, 1.09)	<0.001	0.849	1.77	-3.12, 4.96	2.22 (2.05, 2.47)
ASM_{PE3}	20.2 (4.81)	0.54 (0.38, 0.69)	<0.001	0.858	1.71	-3.09, 4.17	1.89 (1.66, 2.12)
BMI 25-29.9 kg/m^2 (n=1008)							
$ASM_{DXA\ (VC)}$	22.4 (5.02)						
$ASM_{PE1,}$	23.0 (5.03)	0.60 (0.48, 0.73)	<0.001	0.847	1.96	-3.41, 4.61	2.09 (1.92, 2.26)
ASM_{PE2}	23.1 (5.02)	0.63 (0.51, 0.75)	<0.001	0.845	1.98	-3.40, 4.66	2.11 (1.94, 2.28)
ASM_{PE3}	22.7 (4.98)	0.33 (0.21, 0.45)	<0.001	0.854	1.92	-3.56, 4.22	1.97 (1.89, 2.05)
BMI $\geq$30 kg/m^2 (n=717)							
$ASM_{DXA\ (VC)}$	24.0 (5.70)						
$ASM_{PE1,}$	24.4 (5.93)	0.46 (0.29, 0.62)	<0.001	0.858	2.15	-4.02, 4.94	2.29 (2.07, 2.51)
ASM_{PE2}	24.5 (5.48)	0.52 (0.68, 0.36)	<0.001	0.859	2.14	-3.77, 4.81	2.20 (1.98, 2.42)
ASM_{PE3}	24.2 (5.84)	0.28 (0.12, 0.44)	0.001	0.862	2.12	-4.09, 4.65	2.20 (1.98, 2.42)

SD- Standard Deviation, CI- Confidence Interval, SEE- Standard Error of the Estimate, RMSE- Root Mean Square Error

5.4 Discussion

This study reports on the development of three anthropometric prediction equations (PEs) for appendicular skeletal muscle mass (ASM) with DXA as the reference method and including common variables measured in clinical practice such as age, gender, weight, height and body mass index. The PEs were validated in older (age 50+), population representative combined cohorts of 2275 men and women in total. The main conclusion was that the following prediction equation performed the best when compared across various older age and BMI groups: *PE_3: ASM=*

10.047427 + 0.353307 (weight) - 0.621112 (BMI) - 0.022741 (age) + 5.096201 (if male). This PE will be useful in primary care and aged care settings where access to alternate methods such as DXA and BIA is limited, for example, in rural regions and where patients may be too frail to attend hospital centers.

The findings from this study are consistent with those recently reported by a Chinese research group, but different, in that this PE has been developed for use in Caucasian populations and validated in a large cohort, including older (45% ≥ 65 years) people where sarcopenia is more prevalent (Wen et al., 2011). Wen et. al, in their paper studied 729 individuals (age 18-69 years, mean age men 39 and women 41) and these subjects were randomized to either a development cohort and a validation cohort (Wen et al., 2011). Additionally, our research group found that PE_3 with BMI included in addition to weight, age and gender performed better than the PEs with weight, height, age and gender only as variables. Similar to a previous study, the Chinese research group has proposed that equations with limb lengths and circumferences as additional variables may perform better and this requires further exploration in Caucasian and older population groups (Lee et al., 2000).

It has been reported that squaring appendicular lean tissue circumferences creates a lean tissue area estimate and that by adding the product of the summed estimate of appendicular lean tissue areas and height, the total muscle mass may be estimated (Lee et al., 2000). To the best of our knowledge, Baumgartner et. al. first developed a PE for ASM in the late 1990s and this PE was used to determine the prevalence of sarcopenia in New Mexico (Baumgartner et al., 1998). Baumgartner and colleagues included hip circumference and grip strength as variables (Baumgartner et al., 1998, Wen et al., 2011). Unfortunately, the inclusion of grip strength is likely to limit the use of the PE in primary or aged care as dynamometers are not routinely available in these clinical settings. However, greater accuracy of estimation may be of benefit in research practice and low grip strength is a criteria than can also be used in conjunction with low ASM to confirm the diagnosis of sarcopenia (Cruz-Jentoft et al., 2010).

A major strength of this study was the fact that the PEs were initially developed in a population representative and healthy cohort and subsequently validated in large population representative cohorts of older people. The study methodologies for the three cohort studies were similar and the DXA machines used were comparable. However, sarcopenia is most prevalent in under-weight and older people. Only small numbers of people aged 80 years or over (n=139) or people with BMIs

less than 22kg/m2 (n=132) had DXA assessments in these epidemiological cohorts and this provides some support to the notion that alternate methods of body composition assessments are required for frail and older population groups as they may not wish to travel to hospitals for DXA assessment. It will be very important for PE_3 to be further validated in the underweight and very old, especially those who are home or institution bound the population group this PE is targeted towards. Ethnic specific PEs will need to be developed to assess ASM in different ethnic groups.

5.5 Conclusion

This paper reports on a novel, anthropometric PE to assess ASM, which has application in the primary care and aged care settings. Combined with a physical function measure, such as walk speed, this PE will contribute to the diagnosis of sarcopenia, allowing for early identification and management of at-risk individuals in these care settings (Cruz-Jentoft et al.). The next step is to validate this PE in a larger group of older (mean age > 80 years) and underweight (BMI<22kg/m^2) people and explore the benefits of additional variables such as limb lengths or circumferences.

An anthropometric prediction equation for appendicular skeletal muscle mass in combination with a measure of muscle performance to screen for sarcopenia in primary and aged care

Summary

Unfortunately, there is currently no accepted practical screening tool for sarcopenia, which is an important first step in early diagnosis. Chapter 5 explained how the research team had developed an anthropometric prediction equation (PE). Chapter 6 provides a comparison of the accuracy of the anthropometric prediction equation (PE) to dual absorptiometry x-ray (DXA) for predicting low muscle mass and sarcopenia.

This study included men and women aged 65 years and older living in the community. Gender-specific low muscle mass cut-offs were identified using the lowest 20% of the skeletal muscle index (SMI) where muscle mass was determined using PE in 611 men and 375 women aged 65 years and older. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PE-derived low muscle mass were compared to DXA-derived low muscle mass. The cohort was randomized into a development and validation group to identify various cut-offs for low muscle mass calculated using the PE method and test the equation's performance against the DXA method.

The PE cut-off for low muscle mass was <8.05 Kg/m^2 in men and <5.35 Kg/m^2 in women. On validation of various cut-offs with improving sensitivity values from 70 to 97%, specificity increased from 45.5% to 85.7%, PPV increased from 31.3% to 56.9% and NPV increased from 93.0% to 98.6% in men. In women, specificity improved from 42% to 72%, PPV reduced from 56.9% to 31.3% and NPV improved from 93.0% to 98.6%. When the PE method was combined with a measure of muscle performance, a similar pattern of performance was observed.

This research suggests that when combined with a measure of muscle function to create a screening tool the PE performs as a 'rule out' test with high sensitivity and NPV values. The research described in Chapter 6 forms the basis of a research paper that has been peer reviewed and accepted for publication by the *Journal of the American Medical Directors Association*. Statement of authorship can be found in Appendix 10.

6.1 Introduction

Sarcopenia, or age-related muscle loss, is not just a serious condition in itself, but it is associated with many adverse health consequences (Janssen et al., 2004b). Studies have shown that deterioration in muscle mass quantity, as well as quality, is associated with an overall functional decline, reduced quality of life, falls, loss of independence and mortality (Scott et al., 2013b, Clark and Manini, 2010, Arango-Lopera et al., 2013). The direct health care cost arising from sarcopenia in the USA was reported to be $18.5 billion in 2000. With the population ageing, these costs are increasing (Janssen et al., 2004b).

Our group has recently confirmed that approximately 20% of men and women aged 80 years and older, living in the community in South Australia, have sarcopenia (Yu et al., 2014a). Early identification of sarcopenia will allow for early intervention, which in turn could prevent the downward spiral of decline in function and well-being seen with the development and worsening of the condition (Visvanathan and Chapman, 2010).

The diagnosis of sarcopenia is made when low muscle mass is accompanied by low muscle function, which is either manifests as low muscle strength or low physical performance or both (Cruz-Jentoft et al., 2010). Measuring grip strength via a dynamometer or determining walk speed are measures of muscle performance. On the other hand, determining muscle mass is more complex and usually requires the use of dual-energy X-ray absorptiometry (DXA), which includes a trip to a health facility. If the individual with suspected sarcopenia is home bound, in a nursing home or living in a rural area, it is unlikely that they will have easy access to DXA assessment.

We recently developed and validated an anthropometric prediction equation (PE) for appendicular skeletal muscle mass (ASM as discussed further in Methods) (Visvanathan et al., 2012). We propose that the ASM as derived from PE adjusted for height squared (ASM/height^2) when combined with a measure of muscle performance could form a screening method for sarcopenia applicable to primary and aged care settings (Visvanathan et al., 2012).

The aims of the current study therefore, were primarily to evaluate the performance (sensitivity, specificity, positive predictive value [PPV] and negative predictive value [NPV]) of the newly developed anthropometric PE for ASM (ASM_{PE}) in detecting low muscle mass compared to the detection of low muscle mass by DXA (ASM_{DXA}). Also, further analysis was undertaken to identify the best cut-off to enable the PE to be applied as a ‘rule out’ screen.

6.2 Methods

The study had ethics approval from the Central Northern Adelaide Health Service Ethics of Human Research Committee. Informed consent was obtained from all participants.

6.2.3 Participants

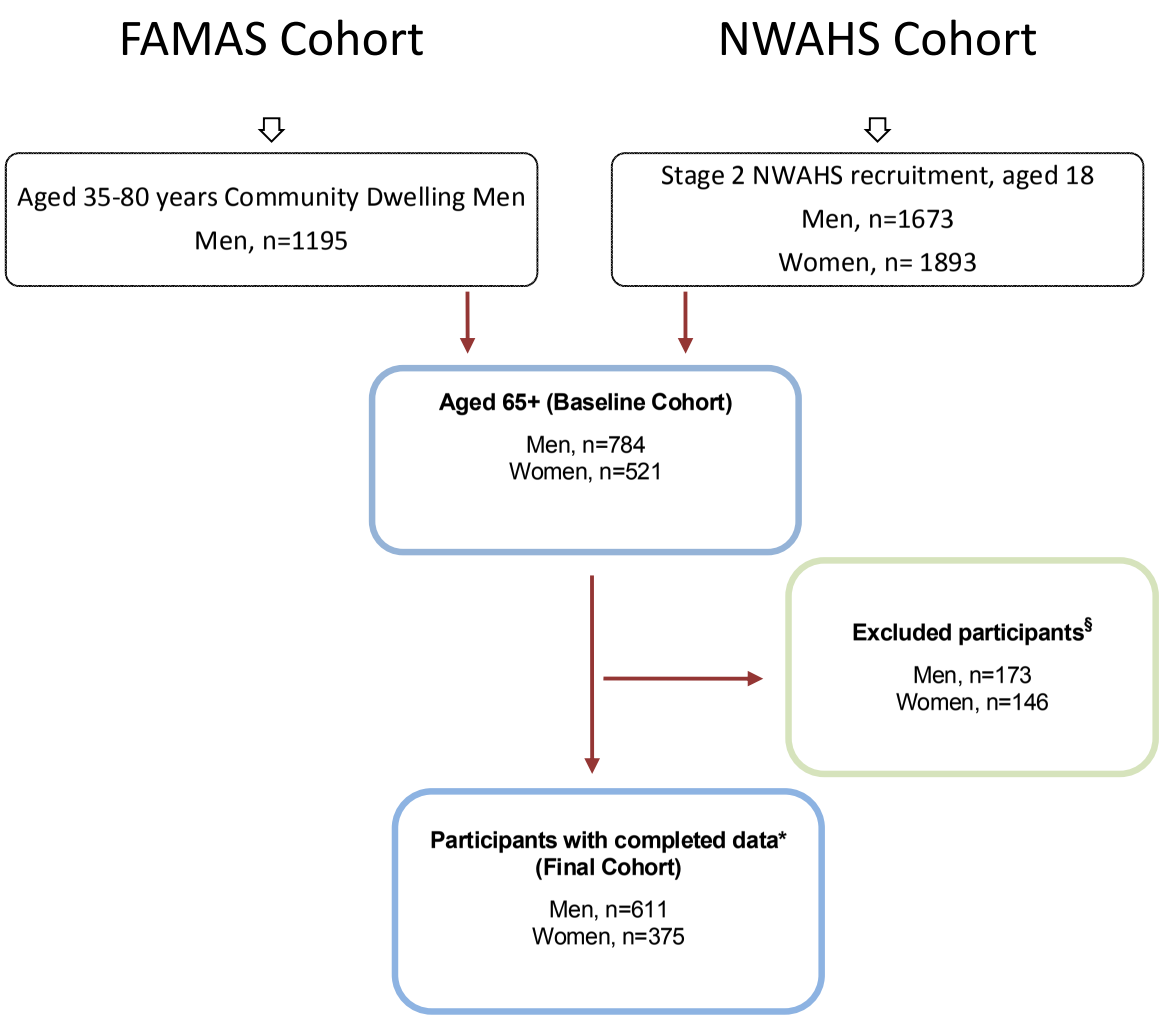
Two cohorts (Figure 6.1) of subjects aged 65 years and older were investigated in this study: The North West Adelaide Health Study (NWAHS), and the Florey Adelaide Male Ageing Study (FAMAS). The characteristics of these cohorts have been described in detail elsewhere and similar recruitment methods were used in both studies (Grant et al., 2006, Martin et al., 2007b).

NWAHS: Randomly selected adults aged 18 years and over from the north-west region of Adelaide were included in this longitudinal study (Grant et al., 2006). 4060 adults were included in the baseline biomedical examination (stage 1) between December 1999 and July 2003. Follow-up occurred at a median of four years and 3566 individuals participated between May 2004 and February 2006. A total of 730 participants aged 65 years and older (men= 355, women=375) who had all the required data were included in the final analysis.

FAMAS: This is a longitudinal study of men only from the north-western region of Adelaide. 1195 community dwelling men aged between 35 and 80 years were recruited between August 2002 and April 2005 (Martin et al., 2007b). In the current study, 256 men aged 65 years and older with the required data were included in the current study.

6.2.4 Measurement of ASM and low muscle mass cut-offs

The assessment of appendicular skeletal muscle mass was undertaken using DXA and a PE. With both the NWAHS and FAMAS cohorts, a *Lunar PRODIGY* scanner (GE Medical Systems, Madison, WI) in conjunction with *Encore 2002* software and a *DPX+* (GE Medical Systems, Madison, WI) scanner in conjunction with *LUNAR* software version 4.7e were used. No significant differences between the two machines were noted through cross-calibration analysis (Mazess and Barden, 2000). Height (m) was measured to the nearest 0.1cm without shoes. Weight (kg) was measured to the nearest 0.1kg while the participants were wearing light clothing. Body mass index (BMI, weight/height^2) was calculated.



* completed data=those with completed measurements of DXA, grip strength and anthropometric measurements (weight and height)
§ missing either one or more of the components of DXA, grip strength and anthropometric measurements

Figure 6.1 **Cohorts combined to develop the older study group (aged 65+)**

With the DXA assessment, ASM was defined as the sum of lean soft tissue masses for arms and legs, assuming that all non-fat and non-bone tissue was skeletal muscle. ASM was adjusted to height in meters squared to yield skeletal muscle index (SMI=ASM/height^2, kg/m^2) (Heymsfield et al., 1990). In the current study, low muscle mass was defined as values below a predetermined SMI cut-off value (Yu et al., 2014a). We have previously estimated cut-offs for low muscle mass using the lowest 20% of the SMI as estimated by DXA (SMI_{DXA}). These are <7.36 kg/m^2 for men and <5.81 kg/m^2 for women (Yu et al., 2014a).

In the current study, low muscle mass values for ASM were also determined by applying the PE our research group had developed and then adjusting to height in meters squared. The following anthropometric PE was applied to the NWAHS and FAMAS cohort to estimate ASM_{PE}: 10.05 + 0.35 (weight) - 0.62 (BMI) - 0.02 (age) + 5.10 (if male) [SEE 1.87; Adjusted R2 (%) 90.6] (Visvanathan et al., 2012).Best subset regression analysis was used to derive this PE. When previously validated, the PE derived ASM was shown to strongly correlate with DXA derived ASM (adjusted R^2=0.869). When compared with DXA-derived measurements, the PE over-estimated ASM by 0.36kg (95% CI 0.28-0.44kg). The 95% limit of agreement was between -3.5 and 4.74kg and the standard error of the estimate was 1.95. The root mean square error for the PE was 1.91 (95% CI1.80-2.01) (Visvanathan et al., 2012).

Table 6.1 The performance of the prediction equation (PE) compared to dual absorptiometry x-ray (DXA) in determining low muscle mass in men and women aged 65 years and older.

	Low Muscle Mass	Sarcopenia
MEN (n=611)	SMI cut-offs< 8.05kg/m^2	SMI cut-off <8.05kg/m^2 and grip strength <30kg
Sensitivity, % (CI)	59.5 (50.2 – 68.2)	57.5 (41.0-72.6)
Specificity, % (CI)	89.8 (86.7 – 92.3)	99.5 (98.3 – 99.9)
PPV, % (CI)	59.0 (49.7 – 67.7)	88.5 (68.7 – 97.0)
NPV, % (CI)	90.0 (86.9 – 92.4)	97.1(95.3– 98.2)
WOMEN (n=375)	SMI cut-off < 5.35kg/m^2	SMI cut-off < 5.35kg/m^2 and grip strength <20kg
Sensitivity, %	45.9 (34.4 – 57.9)	57.1 (39.5 – 73.2)
Specificity, %	87.0 (82.6 – 90.5)	94.7 (91.6 – 96.7)
PPV, %	46.6 (35.0 – 58.6)	52.6 (36.0 – 68.7)
NPV, %	86.8 (82.3 – 90.3)	95.5 (92.6 – 97.4)

6.2.5 Measurement of muscle strength

A grip dynamometer (Lafayette Instrument Company, IN, USA [NWAHS], Smedley, Chicago, IL [FAMAS]) was used to assess grip strength (kg), with subjects sitting with their arm supported by a horizontal surface. Maximum grip strength equalled the mean of three readings from the dominant hand(Grant et al., 2009). Low muscle strength was defined as <30kg for men and <20kg for women (Lauretani et al., 2003). The cut-off values corresponded with low walking speeds of slower than 0.8m/s (Lauretani et al., 2003).

6.2.6 Statistical analysis

All analyses were stratified by gender. Independent sample t-tests were used to compare the differences between men and women in terms of age and body composition. The physical characteristics in both groups were expressed as mean ± standard deviation (SD). *SPSS19* for Windows software (SPSS, Inc., Chicago, IL) was used for conducting the analyses. $P<0.05$ was considered statistically significant.

We investigated the discriminatory power of the PE method compared to DXA in detecting low muscle mass using the area under the curve (AUC) of the receiver operating characteristics (ROC) curve. Plotting the sensitivity against 1 minus the specificity of each possible PE cut-off, the ROC curve illustrated the performance of the proposed PE test. The AUC of the ROC curve is a measure of how well the PE method can identify low muscle mass as defined by DXA. In general, a value approaching 1.0 suggests a high sensitivity and high specificity – a perfect test. An AUC below 0.5 indicates no discriminatory power. An AUC between 0.5 to 1.0 is typical of a clinical test (sufficient at 0.6 to very good at greater than 0.8) (Park et al., 2004).

To assess the diagnostic accuracy of the PE-derived low muscle mass against the DXA-derived low muscle mass (reference standard) and sarcopenia, contingency tables were constructed using combined cohorts. The discriminative ability of PE was quantified as follows: sensitivity (proportion of actual positives who are correctly identified by the tool, ‘true positive’); specificity (proportion of actual negatives who are correctly identified as negative by the tool, ‘true negative’); positive predictive value (the probability of having the condition and having a positive test result); and negative predictive value (the probability of not having the condition and having a negative test result) (Florkowski, 2008). The diagnostic accuracy was first determined using the cut-off values as derived from a PE using the lowest 20% of SMI_{PE} (Table 6.1).

Single hold out cross validations were used to establish the performance of alternative cut-offs in the PE compared to DXA for low muscle mass. The combined cohort was randomly divided into two groups: a development group (70% of the total gender specific group) and a validation cohort (30% of the total gender specific group). From the development group, we derived new cut-off values for low muscle mass using the coordinate of the ROC curve (taken from the PE-derived muscle mass against the DXA-derived low muscle mass). The performance of these new cut-offs was assessed by applying the PE and new cut-offs in the validation group and comparing results to DXA-derived low muscle mass. Cross-validation was used to reduce problems of over-fitting seen when testing a model's performance on the same dataset that was used to develop the model. Results seen in the validation group should therefore more accurately generalize to other independent populations.

Cut-off values with higher sensitivity at 5% increments from 70% to 95% are available in Table 6.2. The cut-offs within the validation group were used to investigate the diagnostic accuracy of, firstly, low muscle mass alone and then low muscle mass in combination with low grip strength (Table 6.2). A high sensitivity test was applied to correctly identify individuals with low muscle mass. However, a high specificity is important in minimizing the unnecessary need for more expensive investigations that might also cause inconvenience to patients. A 'rule out' screening test would demonstrate a high sensitivity and high NPV whilst a 'rule in' screening test would have a high specificity and high PPV (Florkowski, 2008).

Table 6.2 Exploration of the performance of the PE against DXA using various cut-offs for low muscle mass identified in the development cohort and then validating the performance of the PE against DXA to detect low muscle mass and sarcopenia in the validation cohort.

Development Group (coordinates of curve)			Validation Group Low Muscle Mass				PE Based approach to diagnose Sarcopenia (SMI + Low Grip Strength)			
Low Muscle Mass Cut-off Values, kg/m^2	**Sensitivity %**	**Specificity %**	**Sensitivity % [CI]**	**Specificity% [CI]**	**PPV % [CI]**	**NPV % [CI]**	**Sensitivity % [CI]**	**Specificity % [CI]**	**PPV% [CI]**	**NPV % [CI]**
MEN (Development group, n=418 and Validation group, n=193)										
8.09	70.7	89.1	74.4 [57.6 - 86.4]	85.7 [79.0 – 90.6]	56.9 [42.3 – 70.4]	93.0 [87.1 – 96.4]	76.5 [49.8–92.2]	98.9 [95.5-99.8]	86.7 [58.4–97.7]	97.8 [94.0-99.3]
8.16	75.6	85.5	82.1 [65.9 – 91.9]	83.8 [76.8 – 89.0]	56.1 [42.4 – 69.0]	94.9 [89.3 – 97.7]	82.4 [55.8-95.3]	98.3 [94.7-99.6]	82.4 [55.8-95.3]	98.3 [94.7-99.6]
8.28	80.5	77.3	89.7 [74.8 – 96.7]	74.7 [66.9 – 81.2]	47.3 [35.7 – 59.2]	96.6 [91.1 – 98.9]	88.2 [62.3-97.9]	95.5 [90.9-97.9]	65.2 [42.8-82.8]	98.8 [95.4-99.8]
8.39	85.4	68.9	92.3 [78.0 – 98.0]	64.3 [56.1 – 71.7]	39.6 [29.6 – 50.4]	97.1 [91.0 – 99.2]	94.1 [69.2-99.7]	91.5 [86.1-95.0]	51.6 [33.3-69.4]	99.4 [96.1-100.0]
8.46	90.2	61.6	92.3 [78.0 – 98.0]	59.1 [50.9 – 66.9]	36.4 [27.1 – 46.7]	96.8 [90.3 – 99.2]	94.1 [69.2-99.7]	91.5 [86.1-95.0]	51.6 [33.3-69.4]	99.4 [96.1-100.0]
8.57	95.1	49.8	97.4 [84.9 – 99.9]	45.5 [37.5 – 53.7]	31.1 [23.2 – 40.3]	98.6 [91.3 – 99.9]	100.0 [77.1-100.0]	89.8 [84.1-93.7]	48.6 [31.7-65.7]	100.0 [97.0-100.0]
WOMEN (Development group, n=268 and Validation group, n=107)										
5.56	71.7	70.7	61.9 [38.7 – 81.0]	72.1 [61.2 – 81.0]	35.1 [20.7 – 52.6]	88.6 [78.2 – 94.6]	57.1 [20.2-88.2]	94.0 [86.9-97.5]	40.0 [13.7-72.6]	96.9 [90.6-99.2]
5.59	75.5	68.8	66.7 [43.1 – 84.5]	70.2 [57.6 – 77.9]	34.2 [20.6 – 50.7]	89.4 [78.8 – 95.3]	57.1 [20.2-88.2]	93.0 [85.6-96.9]	36.4 [12.4-68.4]	96.9 [90.5-99.2]
5.63	81.1	65.1	71.4 [47.7 – 87.8]	66.3 [55.2 – 75.9]	34.1 [20.9 – 50.0]	90.5 [79.8 – 96.1]	71.4 [30.3-94.9]	92.0 [84.4-96.2]	38.5 [15.1-67.7]	97.9 [91.8-99.6]
5.65	86.8	63.7	71.4 [47.7 – 87.8]	64.0 [52.8 – 73.8]	32.6 [20.0 – 48.1]	91.7 [79.1 – 95.9]	71.4 [30.3-94.9]	91.0 [83.2-95.5]	35.7 [14.0-64.4]	97.8 [91.7-99.6]
5.69	90.6	58.1	81.0 [57.4 – 93.7]	62.8 [51.6 – 72.8]	34.7 [22.1 – 49.7]	93.1 [82.5 – 97.8]	71.4 [30.3-94.9]	91.0 [83.2-95.5]	35.7 [14.0-64.4]	97.8 [91.7-99.6]
5.97	96.2	36.1	100.0 [80.8 – 100.0]	41.7 [31.5 – 53.0]	29.6 [19.6 – 41.8]	100.0 [88.0 – 100.0]	100.0 [56.1-100.0]	83.0 [73.9-89.5]	29.2 [13.4-51.2]	100.0 [94.5-100.0]

6.3 Results

There were 784 men and 521 women aged 65 years and older in the baseline cohort (Figure 6.1). 173 men and 146 women were excluded due to insufficient or incomplete datasets, giving a final number of 611 men and 375 women for the final cohort. Comparing the demographic and body composition characteristics between the baseline and final cohort, the only difference was that women in the baseline cohort were older than the women in the final cohort (74.0±6.3 vs. 73.2±6.0 years, p=0.05) (Yu et al., 2014a). The mean age of the participants in the final cohort was 72.7 ± 5.7 years in men and 73.2 ± 6.0 years in women. There was no difference in the BMI (27.9±4.2 vs. 27.8±4.7 kg/m^2, p=0.79) between the men and women(Yu et al., 2014a). The men were significantly heavier (87.7±15.9 vs. 69.3±15.3 kg, p<0.001) and taller (1.8±0.1 vs. 1.7±0.1 m, p<0.001) and had higher ASM (24.0±3.2 vs. 16.1±2.4 kg, p<0.001) than the women(Yu et al., 2014a).

In terms of general characteristics of the recruited population (final cohort), there was no significant age difference between men and women (72.7±5.7 vs. 73.2±6.0 years, p=0.21). Men were significantly heavier (81.8±13.3 vs. 69.4±12.4 kg, p<0.001) and taller (1.7±0.1 vs. 1.6±0.1m, p<0.001), with higher values for ASM (24.0±3.2 vs. 16.1±2.4 kg, p<0.001) and SMI (8.2±0.9 vs. 6.4±0.8 kg/m^2, p<0.001) than women. There was no difference in the BMI (27.9±4.2 vs. 27.8±4.7 kg/m^2, p=0.79) between the older men and women.

The low muscle mass cut-off values using the lowest 20% of the SMI_{PE} were <8.05 kg/m^2 for men and <5.35 kg/m^2 for women. The area under the curve (AUC) for SMI_{PE} compared to SMI_{DXA} in identifying low muscle mass was 0.854 (CI-0.816, 0.891) for men and 0.791 (CI-0.738, 0.843) for women, indicating very good discriminatory power for the PE. The performance of the PE in predicting low muscle mass compared to DXA is detailed in Table 6.1. Essentially, the PE had very high specificity and negative predictive values, above 85% in both men and women. The sensitivity and PPV values were lower at approximately 60% for men and 46% for women. Combining low muscle mass as predicted by the PE and low grip strength (sarcopenia), a similar observation was made. The specificity and NPV were above 90% in both men and women. The sensitivity was 57% for women and 63% for men. The PPV was 52% in women, but higher at 89% in men.

Using the coordinates of the ROC curve, alternative low muscle mass cut-offs in the development cohort with higher sensitivity values were identified and recorded at 5% increments from 70% to

95% (Table 6.2). With increasing sensitivity, the specificity reduced from 89% to 50%. On further validation, the sensitivity and specificity for the various low muscle mass cut-offs in men ranged between 74% and 97% and between 85% and 45%, respectively. For women, the sensitivity and specificity ranged between 62% to 100% and between 72% to 42%, respectively. With higher cut-off values, the NPV reached 100%, whilst the sensitivity was almost 96% in both genders. The PPV, on the other hand, was low for all cut-offs ranging between 57% and 31%. A similar pattern of results was noted when low grip strength was added.

When using grip strength as an initial screening step (Figure 6.2a), 986 people would require grip strength to be measured; 254 people would then need a DXA assessment; and finally 73 people would be diagnosed with sarcopenia. Given that weight and height rather than grip strength or walking speed are more frequently completed in a primary or aged care currently, applying the PE prior to measuring grip strength might be logistically practical to support screening (Figure 6.2b). Using this method 104 fewer DXAs would be required; fewer people would need to have a grip strength measurement (i.e. n=472); 82.2%of sarcopenia would be detected; but 17.8%of sarcopenia cases would be missed. Measuring grip strength as a first step for 986 individuals (Figure 6.2c) prior to applying the PE did not change the final outcome in terms of the number of people requiring DXA assessment or the number of people with sarcopenia being missed.

(a)

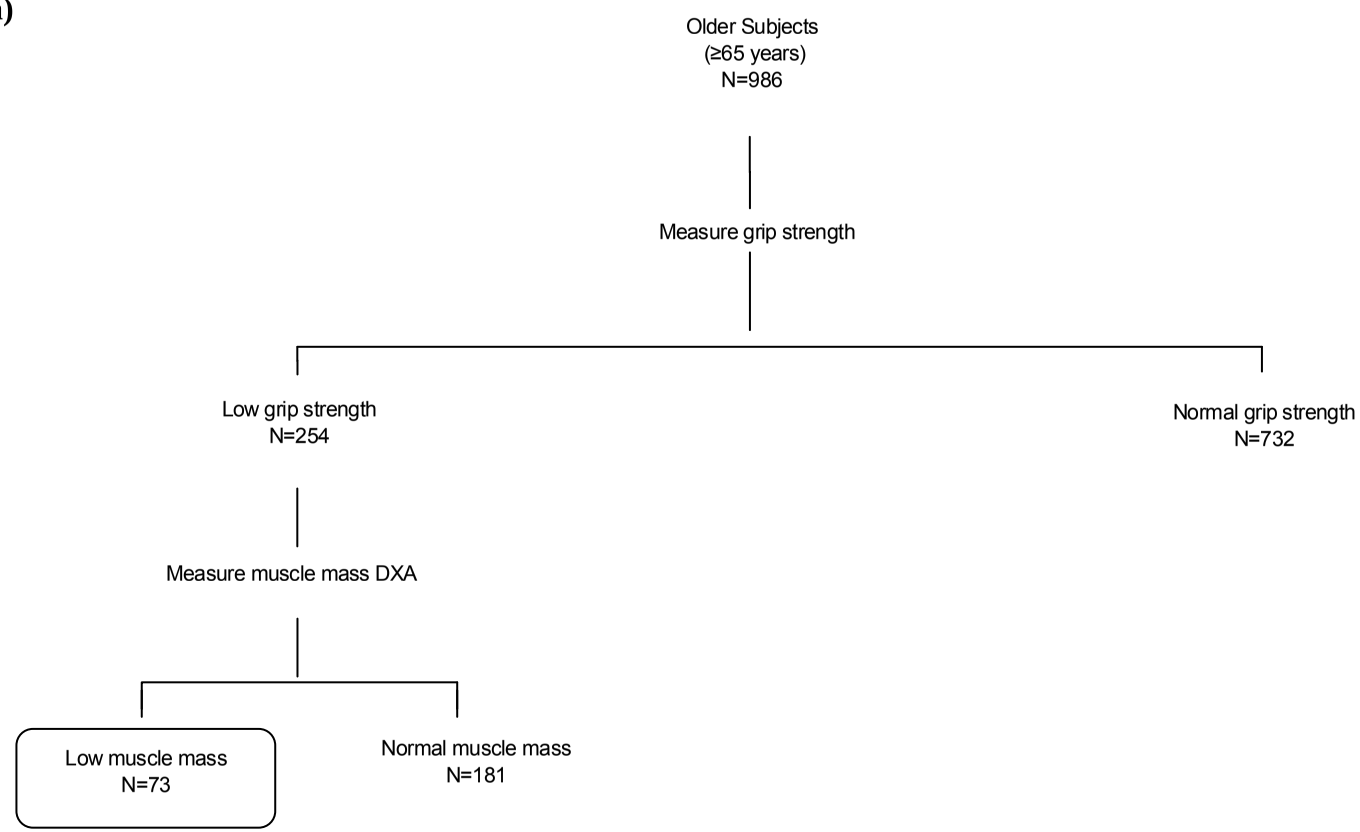
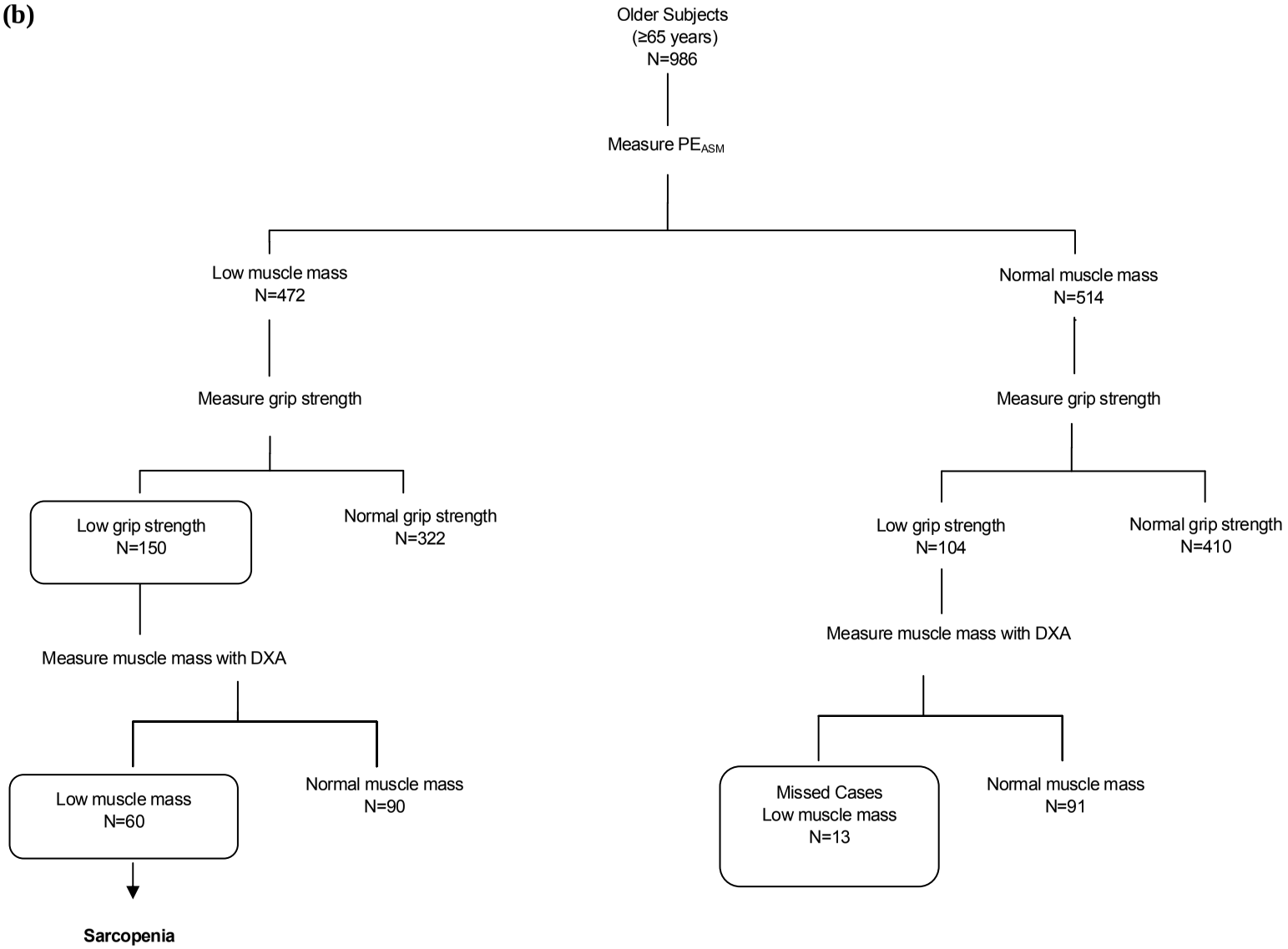
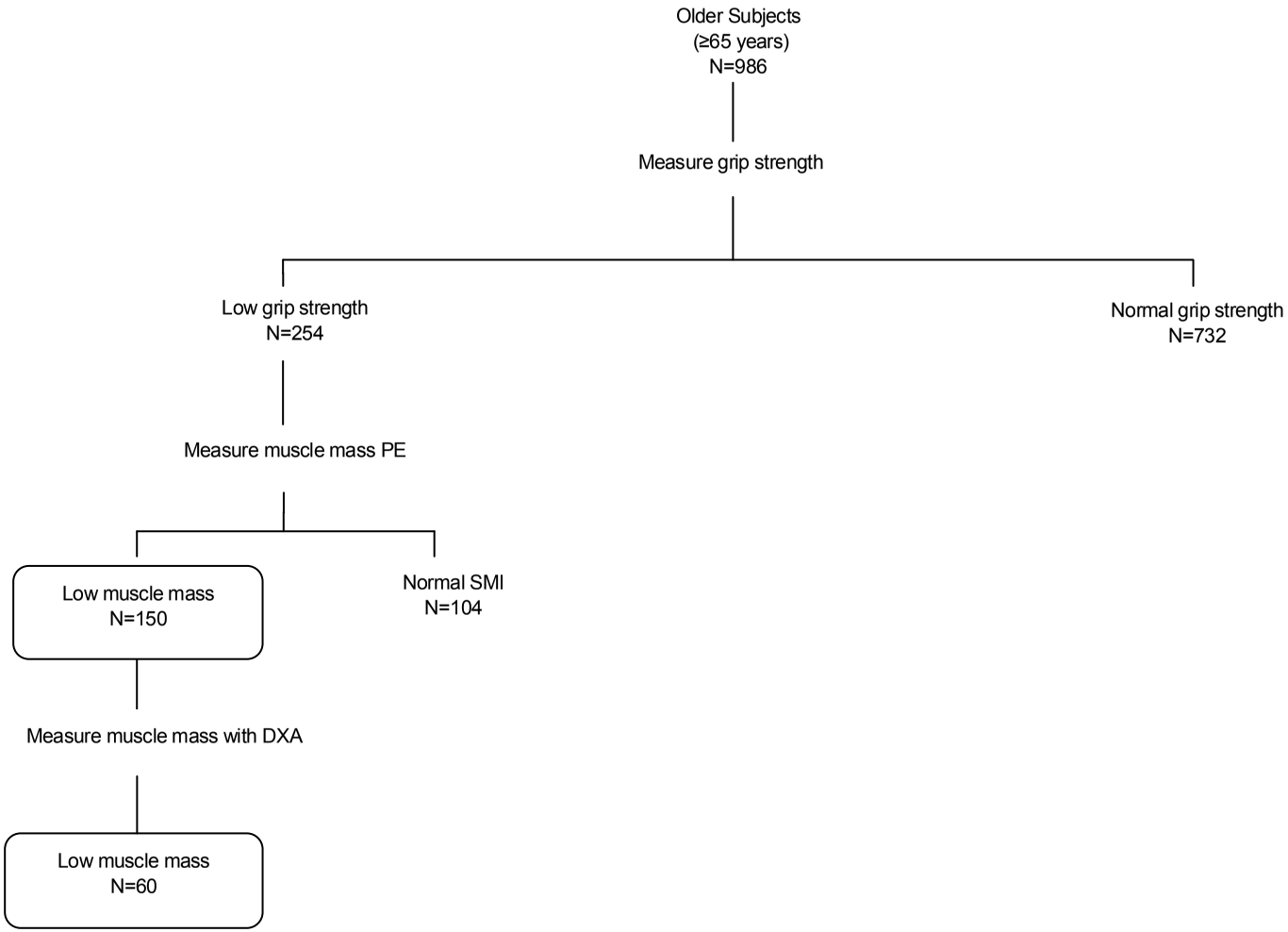


Figure 6.2 **The prevalence of sarcopenia using two different algorithms in primary care. (a) The algorithm based on the muscle strength as an initial step in screening for sarcopenia. (b) The algorithm using the anthropometric PE as an initial screen (low muscle mass cut-offs of <8.28 kg/m^{2}for men and <5.97kg/m^2 for women). (c) The algorithm using muscle strength as initial screening followed by use of anthropometric PE (low muscle mass cut-offs of <8.28 kg/m^{2}for men and <5.97kg/m^2 for women).**

(b)


Older Subjects
(≥65 years)
N=986
Measure PE_{ASM}
Low muscle mass
N=472
Normal muscle mass
N=514
Measure grip strength
Measure grip strength
Low grip strength
N=150
Normal grip strength
N=322
Low grip strength
N=104
Normal grip strength
N=410
Measure muscle mass with DXA
Measure muscle mass with DXA
Low muscle mass
N=60
Normal muscle mass
N=90
Missed Cases
Low muscle mass
N=13
Normal muscle mass
N=91
Sarcopenia

(c)


Older Subjects
(≥65 years)
N=986
Measure grip strength
Low grip strength
N=254
Normal grip strength
N=732
Measure muscle mass PE
Low muscle mass
N=150
Normal SMI
N=104
Measure muscle mass with DXA
Low muscle mass
N=60

6.4 Discussion

The key finding from this study is that low muscle mass as derived from anthropometric PE, when coupled with a measure to assess reduced performance is best used as a 'rule out' screening test for sarcopenia (Figure 6.2). The screening test when applied in clinical practice may reduce the number of the more costly DXA assessments undertaken. The availability of this PE method may also support the implementation of a screening program for sarcopenia in primary and aged care settings. Although we demonstrated that the PE method had good discriminatory power in predicting low muscle mass when compared to a DXA assessment of low muscle mass, wide confidence intervals were noted and so research with larger cohorts would be ideal.

The decision as to the best cut-off value for low muscle mass when applying the PE method may require balancing the cost of DXA assessment (including inconvenience) against the risk of missing the diagnosis of sarcopenia. For example, as demonstrated in Figure 6.2, using the muscle mass cut-off value of $<8.28 kg/m^2$ for men and $<5.97 kg/m^2$ for women when using the anthropometric PE to measure muscle mass, fewer DXA assessments were required, but the risk of missing incidents of sarcopenia was about 17%. Clearly, higher cut-off values for low muscle mass will reduce the risk of missing a case of sarcopenia, but increase the financial cost, given that more DXAs will be required.

Goodman and colleagues have recently published a screening grid for low muscle mass by age and body mass index (Goodman et al., 2013). In their study, low muscle mass was defined as one standard deviation below the mean of a younger reference population (adults aged 20 to 40 years) which is different to the less than two standard deviation used in this study and recommended by the EWSGOP (Cruz-Jentoft et al., 2010). The Goodman study reported an AUC of 0.88, which was slightly higher than the AUC in our study of 0.85 in men and 0.79 in women. The sensitivity, specificity and NPV values reported in our study were comparable to those reported by Goodman et al. (Goodman et al., 2013). Similar to our study, low PPV were also noted in the Goodman study (Goodman et al., 2013).The likely reason for low PPV is because of the low overall prevalence of low muscle mass in the community. It would therefore be important to further investigate the performance of the PE screening method in population groups where the prevalence of low muscle mass is higher, such as in the residential aged care and hospital setting.

A Japanese group recently developed a chart where the probability of sarcopenia is derived from age, grip strength and calf circumference (Ishii et al., 2014). Using this model, the study reported

sensitivity and specificity values of 84.9% and 88.2% in men and 75.5% and 92% for women, respectively. Our anthropometric PE-based approach to diagnosing sarcopenia appears comparable. Similar to the current study, a high NPV and low PPV were also noted in a study by Ishii et.al. (Ishii et al., 2014). Grip strength is currently not routinely measured in primary or aged care as it requires the use of a hand dynamometer. This may be viewed as a barrier to screening. It is more likely that clinicians in primary or aged care will be more comfortable with the use of an anthropometric PE combined with a measurement of walk speed to screen for sarcopenia. Further research is required to confirm the performance of the PE in combination with gait speed in screening for sarcopenia.

A limitation of the current study was that neither the FAMAS nor the NWAHS cohorts included individuals living in residential care facilities or in hospitals. The prevalence of sarcopenia in these population cohorts is likely higher than in the general population, and so the performance of the PE method could be different. Further investigation is required therefore to develop a more in depth understanding of the capacities of the new method. In addition, the current study included predominantly Caucasian subjects. Research including other ethnic population groups would ensure the generalizability of research findings to clinical practice. Gait speed was not assessed in the NWAHS and research including gait speed is required.

6.5 Conclusion

In conclusion, a screening method incorporating the use of an anthropometric PE, together with a performance measure as part of a 'rule out' screening test, is proposed for use in primary and aged care. It is anticipated that the availability of this simple screening tool will encourage the implementation of screening programs for sarcopenia in primary and aged care. Screening followed by confirmatory diagnosis of sarcopenia with DXA would support early treatment. At a minimum, dietary and physical activity advice could be provided with confidence by general practitioners.

Inflammatory cytokines and appetite in healthy people

Summary

Inflammation has been associated with reduced appetite and body composition changes in populations with established diseases. However, it is not known if an association exists between appetite, body composition and inflammation in healthy people.

Chapter 7 describes the association of appetite with markers of inflammation and body composition, data from the Cytokines, Adiposity, Sarcopenia and Ageing (CASA) study.

The study was conducted in the Western suburbs of Adelaide, South Australia with 180 participants aged from 18 to 82 years. Body composition was measured by both dual x-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA). Appetite was assessed by the Simplified Nutritional Appetite Questionnaire (SNAQ). Circulating cytokine concentrations were measured.

Multiple regression analysis showed appetite scores were increased in non-smokers (P = 0.031) and men (P = 0.024), negatively associated with serum levels of the pro-inflammatory IL-1β (β coefficient = - 0.379, P= 0.007), and positively associated with serum levels of the anti-inflammatory cytokine IL-10 (βcoefficient = 0.25, P = 0.010). There was no association between appetite and body composition.

This research suggests that appetite loss may reflect background inflammation even in apparently healthy people, and probably occurs before consequent changes in body composition. Further explorations of longer term appetite changes with respect to inflammation and body composition changes are needed. This research forms the basis of a research paper peer reviewed and published in *The Journal of Aging Research & Clinical Practice*. 2012;1 (1):40-3 (Appendix 11 – including statement of authorship).

7.1 Introduction

Under-nutrition is common among older people, even in developed countries (Westergren et al., 2009, Gomez Ramos et al., 2005, Guigoz, 2006, Visvanathan et al., 2003) and is associated with serious consequences, including more frequent and prolonged hospital admissions (Cansado et al., 2009), increased infection risk (Paillaud et al., 2005), functional decline (Oliveira et al., 2009) and reduced life expectancy (Guigoz, 2006). It is important to identify factors that might predict those older people more likely to lose weight and become under-nourished, so prevention and early treatment measures can be implemented.

Multiple methods have been used to define and diagnose under-nutrition in older people, but features commonly seen in this condition are weight loss (particularly muscle loss), reduced body weight, reduced appetite and sometimes cachexia (Chapman, 2007). Aging is associated with decline in appetite and food intake which is probably physiological, but may contribute to the development of pathological anorexia and under-nutrition. Indeed, reduced appetite is a reliable predictor of future weight loss in the elderly; appetite scores obtained from the Simplified Nutritional Appetite Questionnaire (SNAQ) have been found to predict future weight loss in older people (Wilson et al., 2005).

Appetite loss may be caused by inflammation. Inflammation is the immune system's response to an acute infection or illness and is the result of the production of several pro-inflammatory cytokines including interleukin-1 (IL-1), IL-2, IL-6, IL-8, tumour necrosis factor-α (TNF-α) and interferon-γ (IFNγ) (Wong and Pinkney, 2004). These pro-inflammatory cytokines, when persistently elevated, can reduce appetite by actions on the hypothalamus and other neural centres, by altering gastric function and by modifying the regulation of appetite controlling hormones (Wong and Pinkney, 2004). Anti-inflammatory cytokines, such and IL-4 and IL-10 act to down-regulate pro-inflammatory cytokine production (Opal and DePalo, 2000). An imbalance between pro-inflammatory and anti-inflammatory cytokines is thus thought to lead to the cachexia of many chronic diseases (Plata-Salaman, 2001).

Ageing itself may be a low-level pro-inflammatory state (Sakuma and Yamaguchi, 2010). It might therefore be that the anorexia of ageing is due, at least in part, to increased inflammation. If so, it might be expected that there would be a positive connection between pro-inflammatory markers and reduced appetite even in apparently healthy individuals across the adult age range. Little is known about these possible connections.

This study explored the associations of appetite with markers of inflammation and body composition in healthy adults. It was hypothesised that there would be associations between increased inflammation and reduced appetite even in this group of healthy individuals, but probably not between markers of inflammation and adverse body composition changes, as these are likely to be later effects of under-nutrition.

7.2 Methods

7.2.1 Participants

Healthy subjects (ages 18 to 82 years) were recruited from the western suburbs of Adelaide into the Cytokine, Adiposity, Sarcopenia and Ageing Study (CASA). The recruitment method was similar to that described for other larger population studies conducted in the same catchment area, the North West Adelaide Health Study (Adams et al., 2003). Telephone numbers from the electronic White Pages were randomly selected, and willing subjects, aged 18 or over, with no exclusion criteria, were invited to participate. Subjects able to comply with the study protocol and who reported weight stability over the preceding three months were included in the study. Those with confirmed inflammatory diseases, pregnant and those who had been ill in the preceding three months or in the two weeks following blood sampling, were excluded. This study had ethics approval from the Central Northern Adelaide Health Service Ethics of Human Research Committee and all participants provided written informed consent.

7.2.2 Body composition measures

Body composition was assessed by measurement of height; weight; waist circumference; fat mass (FM) and fat free mass (FFM) by dual x-ray absorptiometry (DXA) (Lunar PRODIGY whole body scanner; GE Medical Systems, Madison, WI) scan; and bioelectrical impedance analysis (BIA) (Quantum II BIA Analyser, RJL system).

7.2.3 Appetite

Participants completed the SNAQ questionnaire, giving one of five responses to four questions regarding appetite, satiety, taste and meal frequency (Opal and DePalo, 2000). SNAQ gives a score out of 20, with higher scores indicating greater appetite. SNAQ has been found to predict weight loss over a six month period with 81.6 % sensitivity and 84.6 % specificity for people over 60 years of age (Wilson et al., 2005).

7.2.4 Exercise score

Exercise was assessed using Australian National Health Survey questions (Plata-Salaman, 2001). Scores for exercise intensity were 3.5 for walking, 5.0 for moderate activity and 7.5 for high intensity activity. Exercise intensity score was multiplied by minutes per fortnight for each exercise intensity to give total exercise level. This total level was classified as 'sedentary' (< 100), 'low level' (100 <1600), 'moderate level' (1600 – 3200 or > 3200 and less than 2 h of vigorous exercise) or 'high level' (> 3200)'.

7.2.5 Data collection

Fasting blood samples were collected and body composition measured by BIA in the morning, and body composition by DXA was measured either the afternoon of the same day or on another day but within two weeks. Plasma samples were stored at –80°C until analysis. Cytokine concentrations were measured using LINCOplex kits. Trace values < 0.08 pg/L for cytokines were recorded as zero values.

7.2.6 Statistical analysis

SNAQ scores were normally distributed. Other continuous study variables were non-normally distributed and are presented as medians (inter-quartile range). Categorical variables are presented as frequencies.

Relationships between the total SNAQ score and the study variables were assessed using Spearman rank correlation tests for non-parametric variables. Cytokines and anthropometric variables were included in a multiple regression analysis along with for age, gender and smoking status. Continuous data were log transformed prior to inclusion in this analysis. Statistical analysis was carried out using SPSS statistical program (17.0, SPSS, Chicago, USA) with statistical significance set at $P< 0.05$.

7.3 Results

180 subjects with complete results were included in the study. Median age was 52 years with a range of 18-82 years. SNAQ total scores ranged from 12-20 (out of 20), with a median score of 17. 15 participants (7.8%) had low SNAQ scores (defined as ≤ 14). Table 7.1 shows baseline subject characteristics.

Table 7.1: Baseline participant characteristics (n=180)

Continuous Variables	**Median (Inter-Quartile Range)**
Background Variables	
Age (years)	**52 (40-62)**
SNAQ appetite scores	**17.0 (16.0-18.0)**
Circulating Cytokine Concentrations	
IL-1β (pg/ml)	**0.50 (0.0-1.8)**
IL-2 (pg/ml)	**1.46 (0.0 - 8.0)**
IL-4 (pg/ml)	**0.0 (0.0 - 15.8)**
IL-6 (pg/ml)	**1.95 (0.25-5.9)**
IL-10 (pg/ml)	**3.9 (0.0-13.8)**
TNF-α (pg/ml)	**3.5 (1.9 - 5.4)**
HS-CRP (mg/L)	**1.2 (0.6 - 2.3)**
Anthropometric Measures	
BMI (kg/m^2)	**25.6 (23.0 - 28.7)**
Waist Circumference (cm)	**87.2 (76.3 - 96.7)**
Total Lean Mass DXA (kg)	**44.5 (38.1 - 56.8)**
Total Fat DXA (Kg)	**24.1 (17.1 - 30.2)**
Nutritional Biomarkers	
Haemoglobin (g/L)	**140.0 (129.0 - 150.0)**
Lymphocyte (g/L)	**1.8 (1.6-2.2)**
Albumin (g/L)	**39.0 (37.0 - 41.0)**
Categorical Variables	**n (%)**
Background Variables	
Gender	**106 (58.9 %) females; 74 (41.1%) males**
Smoking Status	**19 (10.6%) smokers**
Exercise Level	
Sedentary	**31 (17.2 %)**
Low Level	**75 (41.7%)**
Moderate Level	**39 (21.7%)**
High Level	**35 (19.4%)**

The results of the univariate regression analysis of the relationship between SNAQ appetite scores and continuous study variables are shown in Table 7.2. Both IL-6 and IL-10 concentrations were positively related to appetite. There were also strong significant associations between concentrations of a number of cytokines, including IL-6 with both IL-1β ($r = .353$, $P < 0.001$) and IL-10 ($r = .410$, $P<0.001$). By multivariate analysis (Table 7.3) non-smokers had higher appetite scores than smokers and men higher scores than women. IL-1β concentrations were negatively and IL-10 concentrations positively associated with appetite. None of the body composition variables showed any association with SNAQ score from either the univariate or multivariate analyses.

Table 7.2: Univariate Regression Analysis of relationships between total SNAQ appetite score and Continuous Study Variables (n=180)

Variable	R	*P*
Background Variables		
Age (years)	**0.016**	**0.836**
Exercise Score	**0.062**	**0.407**
Nutritional Biomarkers		
Haemaglobin (g/L)	**0.053**	**0.463**
Lymphocyte (g/L)	**0.040**	**0.585**
Albumin (g/L)	**0.038**	**0.601**
Cytokines		
IL-1β (pg/ml)	**0.033**	**0.637**
IL-2 (pg/mL)	**0.034**	**0.652**
IL-4 (pg/mL)	**0.041**	**0.584**
IL-6 (pg/mL)	**0.153**	**0.041**
IL-10 (pg/mL)	**0.210**	**0.005**
TNF-α (pg/mL)	**0.089**	**0.222**
HS-CRP (mg/mL)	**0.086**	**0.239**
Anthropometric Measures		
BMI (kg/m^2)	**0.039**	**0.599**
Waist Circumference (cm)	**0.058**	**0.425**
Total Lean Mass DXA (kg)	**0.064**	**0.374**
Total Fat DXA (kg)	**0.050**	**0.494**

Table 7.3: Multivariate Analysis of relationship between Study Variables and total SNAQ score (n=180)

Variable	βCoefficient	t	*P*
Background Variables			
Age (years)	**0.042**	**0.472**	**0.638**
Gender	**-0.367**	**-2.287**	**0.024***
Smoking Status	**-0.172**	**-2.176**	**0.031†**
Cytokines			
IL-1β	**-0.379**	**-2.739**	**0.007**
IL-2	**0.157**	**1.018**	**0.310**
IL-4	**0.057**	**0.535**	**0.593**
IL-6	**0.085**	**0.806**	**0.422**
IL-10	**0.248**	**2.598**	**0.010**
TNF-α	**0.035**	**0.392**	**0.696**
CRP	**-0.165**	**-1.868**	**0.064**
Anthropometric Measures			
BMI	**-0.227**	**-0.971**	**0.333**
Waist	**0.372**	**1.739**	**0.084**
Lean	**0.281**	**1.631**	**0.105**
Fat	**-0.058**	**-0.255**	**0.799**
Exercise Score	**0.117**	**1.471**	**0.143**

*SNAQ scores higher in men than women; †SNAQ scores higher in non-smokers than smokers.

7.4 Discussion

In this novel study of appetite in healthy people, appetite as measured by the SNAQ questionnaire was associated negatively with circulating serum levels of IL-1β and positively with IL-10 levels, but was not associated with any measure of body composition or nutritional biomarker – albumin, lymphocyte count and haemoglobin.

The negative association between IL-1βand appetite found in this study is consistent with previous reports in humans with inflammatory conditions such as cancer (Janik et al., 1997), renal failure (Cheung et al., 2010) eating disorders (Corcos et al., 2003) and depression (Andreasson et al., 2007). Our finding is also consistent with the known pro-inflammatory effects of IL-1βand the results of animal studies. In rodents, food intake is suppressed in a dose-dependent manner by IL-1β (Wong and Pinkney, 2004, Lukats et al., 2005). Additionally, IL-1βknock-out mice are of normal size and weight, but resistant to inflammation-induced weight loss (Wong and Pinkney, 2004). Of interest older mice lose more weight in response to IL-1 administration than young adult mice (Nelson et al., 1999).

The positive association between IL-10 and appetite is consistent with the anti-inflammatory actions of this cytokine. IL-10 is believed to suppress immune responses by inhibiting pro-inflammatory cytokine production (Opal and DePalo, 2000, Frossard and Eigenmann, 2008). For example, IL-10 has been found to be protective against weight loss induced by both pro-inflammatory cytokines (Ropelle et al., 2010) and bacteria-mimicked infection (Hollis et al., 2010) in rodent studies.

The finding that IL-6 was associated with appetite in the univariate analysis, but not associated in the multivariate analysis is probably because IL-6 concentrations are significantly associated with those of other cytokines, such as IL-1β and IL-10 which have more powerful effects on appetite. Consistent with the strong association observed between IL-6 and IL-10 concentrations ($r = 0.353$, $P < 0.001$), IL-6 has been found to up-regulate IL-10 during acute inflammation (Steensberg et al., 2003).

In the present study there was no association between appetite and circulating levels of either TNF-α or, CRP. TNF-α is a pro-inflammatory cytokine which has been associated with reduced appetite in patients with chronic diseases such as renal failure (Oner-Iyidogan et al., 2011) and levels of CRP, an inflammatory marker, have been associated with appetite decline in patients with chronic

disease (Kalantar-Zadeh et al., 2004, Del Fabbro et al., 2010). The lack of an association with appetite in the present study is perhaps because our subjects were healthy and TNFα and CRP effects on appetite occur later in the pathways of chronic and inflammatory diseases.

Low appetite leads to reduced food intake, which in turn, often results in weight loss (Wilson et al., 2005). Loss of appetite due to inflammation might therefore result in reduced lean tissue stores. We found, however, no such association in our study, a finding supported by a recent study of community elders in Malaysia, where appetite was also not associated with body composition (Mohamad et al., 2010).

Our results may provide some insight into the order in which changes leading to under-nutrition occur. It is not known if the muscle mass loss that often follows appetite reduction in older people leads to a pro-inflammatory state, or if inflammation leads to reduced appetite and food intake and subsequently to adverse body composition changes. Our findings support the latter sequence, at least in certain circumstances. In apparently healthy people there appears to be already present an association between inflammation and reduced appetite, without adverse effects on body composition, which we postulate would only occur with more prolonged and severe effects on food intake and nutrition.

This study was limited by a relatively small sample size. Nevertheless, subjects were randomly chosen from the community and thus reflect the situation in apparently healthy adults. A further limitation is that dietary background was also not assessed in this study and that SNAQ has not yet been validated against objective food intake (Mohamad et al., 2010), although it has been shown to predict future weight loss (Wilson et al., 2005). Dietary intake was not assessed in this study. Because it is possible that body composition and weight loss may reflect long term nutrition, whereas appetite and inflammation reflect short term nutrition (Kalantar-Zadeh et al., 2003), it would be interesting to follow these subjects to assess longer-term relationships between inflammation, appetite, body weight change and nutritional status and we are now planning such a follow-up study.

In summary, the major finding of the present study is that appetite in healthy people is associated with several inflammatory markers but not with any measures of body composition or nutritional bio-markers. Further follow-up is needed to explore the possibility that this may predict future weight loss and increased likelihood of developing under-nutrition.

Discussion, future directions and conclusion

Sarcopenia, the age-related loss of muscle mass, strength and function is not only common in older people but has many adverse health consequences effects (Cruz-Jentoft et al., 2010). These health consequences have been estimated to pose a significant economic burden on the healthcare system, and if not addressed appropriately is potentially unsustainable, especially with our ageing population (Janssen et al., 2004b).

Conclusively diagnosing sarcopenia in order to prevent the progression of the condition is, however, an ongoing challenge for doctors. Clinical symptoms are often silent and elusive. The point at which the muscle changes become pathological remains an issue of debate. The impact of functional decline and eventually significant disability is often late in the course of the condition and often beyond any remediable intervention. Early detection is likely important in clinical practice in order to forestall the debilitating and costly outcomes of the condition.

Internationally, experts are increasingly calling for clinicians to look for the presence of sarcopenia and intervene early. Nevertheless, research into sarcopenia in Australia remains limited and it is clear that more collaborative effort is required to progress research in the Australian context. The research presented in this PhD adds to the current literature and will contribute to clinical practice in relation to sarcopenia.

The European Working Group on Sarcopenia in Older People (EWGSOP) has identified three different methods to derive cut-off values for the low muscle mass characteristic of the condition, i.e. low skeletal muscle index [SMI] (Cruz-Jentoft et al., 2010):

a) SMI cut-off values of < 2 standard deviation (SD) of a young reference population;
b) SMI cut-off values< the lowest 20% of the older study population; and
c) the linear regression method where the cut-off values are < lowest 20% of residual of the linear regression models of ASM adjusting for fat mass and height in men and women.

It has previously been suggested that deriving the cut-off values from a younger reference group and the lowest 20% of the older study population yields similar SMI cut-off values (Baumgartner et al., 1998). More recent studies in different countries have found either a higher or lower relative SMI cut-off value when using a younger reference group. The research published and presented as Chapter 3 sought to clarify and determine which of these strategies was most relevant in the

Australian context. During the current research, the three methods of defining low muscle mass, in combination with low grip strength, were used to determine the prevalence of sarcopenia in community dwelling Australians. Unfortunately, low walk speed was not assessed during the North West Adelaide Health Study and, therefore, was one source of valuable information unavailable to confirm low muscle performance.

Chapter 3 confirmed that the resulting prevalence figures varied according to the method of assessment used, indicating the importance of reaching a consensus about the preferred method for assessing sarcopenia in Australia. Furthermore, based on the results of the research reported in Chapter 3, it was concluded that the best ways to identify SMI cut-off points in order to define low muscle mass, was either to use the gender specific lowest 20% of the SMI of the older population (aged 65 years and over) or the residual from applying the linear regression method since these two methods yielded similar prevalence rates for sarcopenia. Using dual absorptiometry x-ray (DXA) as a means to measure muscle mass, SMI cut-off values of $<7.36 kg/m^2$ for men and $<5.81 kg/m^2$ for women were identified using the gender specific lowest 20% of the SMI of the older population. Cut-offs of <-2.15 for men and <-1.42 for women were identified using linear regression method.

Using cut-offs determined from identifying the lowest 20% of the SMI in older people, *the overall prevalence of sarcopenia for men* was 6.2%, while prevalence based on linear regression was 6.4%. *For women*, the corresponding rates were 9.3% and 8.5% respectively. For men and women *aged 80 years and over,* the percentage of individuals displaying sarcopenia doubled, with prevalence rates ranging from 16.9% (men) and 16.1% (women) as identified from lowest 20% of the SMI, to 19.7% (men), and 22.6% (women) as identified from linear regression method. These prevalence figures confirm that sarcopenia is common in Australia, especially in people aged 80 years and older, the fastest growing demographic in Australia, essentially affecting one in five individuals.

Frail subjects from the residential aged care setting or who were homebound did not participate in the studies reported in Chapter 3. The prevalence of sarcopenia in these settings is likely to be higher than general population. Confirmation of this likely prevalence is, however, required.

The current best practice method to assess muscle mass is through DXA. However, for those who are frail and homebound, as well as those residing in residential aged care, attendance at a healthcare facility to undergo DXA can be challenging and distressing. Furthermore, there is also a

financial cost associated with the test, which not only includes transport to and from the health care facility but a fee for the DXA itself. It is therefore understandable that many clinicians will find it difficult to implement the call to identify sarcopenia in clinical practice unless a practical method to screen for low muscle mass is developed. Until that point is reached, assessment and treatment of sarcopenia remain insufficient, to the detriment of personal health and the national health care budget.

Chapter 4 described how biochemistry and anthropometric variables were examined as part of a prediction equation (PE) to assess for lean body mass (LBM). Prior to four international publications (Prado et al., 2009, Janssen et al., 2002, Janssen et al., 2004a) which all concluded that the preferred measurement for sarcopenia should be appendicular skeletal muscle mass (ASM), low LBM was commonly used in studies investigating for sarcopenia. The published study presented as Chapter 4 was undertaken before publication of the four articles and used low LBM as an assessment and diagnostic tool. It is also known that sarcopenia as defined by low LBM is associated with increased toxicity during chemotherapy (Prado et al., 2009). Therefore, estimation of LBM may allow for individualization of chemotherapy treatment, reducing the risk of toxic effects, resulting in better therapy outcomes for oncology patients (Prado et al., 2009).

Chapter 4 discussed how the addition of biochemistry variables to basic anthropometric measures [weight, body mass index (BMI), age and gender] marginally improved the performance of the PE to assess LBM. Although the marginal improvement was not sufficient to justify the increased costs from biochemistry tests in relation to nutrition management, in an oncological setting, where drug prescription should take account of LBM to reduce toxicity, the additional cost may be justified. Further research is required to support the interpretation of this data in practice, however. The study published in Chapter 4 identified the following anthropometric PE for LBM: 22.932326 + 0.684668 (weight) -1.137156 (BMI) - 0.009213 (age) + 9.940015 (if male) (Yu et al., 2013).

Whilst undertaking the research reported in Chapter 4, a technical difficulty arose because different DXA machines were used in the development and validation cohorts. The Lunar Prodigy whole-body scan (GE Medical Systems, Madison, WI) was used in the Cytokine, Adiposity, Sarcopenia and Ageing (CASA) study (i.e. development cohort). The Norland densitometer XR36 (Norland Medical Systems, Fort Atkinson, Wisconsin, USA) was used with the validation cohort. To

overcome this challenge, a new collaboration with Associate Professor Leigh Ward from the Department of Biochemistry at the University of Queensland was established. Associate Professor Ward developed a correction factor which allowed the conversion of data from the Norland to become the Lunar equivalent, thus allowing for the comparison of DXA results from the two cohorts (CASA and Validation Cohort) (Maple-Brown et al., 2012a, Yu et al., 2013).

A research methodology similar to that used in Chapter 4 was also used to develop and validate an anthropometric PE for ASM (PE_{ASM}) during the published study that is presented in Chapter 5. The following PE for ASM was derived: 10.047427 + 0.353307 (weight) - 0.621112 (BMI) - 0.022741 (age) + 5.096201 (if male). The study confirmed that this PE can predict ASM with acceptable accuracy (Visvanathan et al., 2012).

To further define the use of this PE_{ASM} in a clinical context, the diagnostic accuracy of this PE in screening for low muscle mass was examined (Chapter 6). The diagnostic accuracy of the PE_{ASM} in combination with low grip strength to screen for sarcopenia was also investigated. It was observed that the cut-offs for low muscle mass (i.e. low SMI) in Australia when the PE_{ASM} was applied were <8.28 Kg/m^2 in men and <5.97 Kg/m^2 in women. This study also confirmed that when compared to the DXA method for ASM assessment, with these figures as the cut-offs, the PE_{ASM} method exhibited high sensitivity and negative predictive values and, so, was best used as a 'rule out' screening test.

In other words, when applying this PE_{ASM} in clinical practice, negative results essentially suggest a very low probability that individuals have low muscle mass. The current study also demonstrated that using the PE_{ASM} to identify low muscle mass, when combined with low grip strength, was able to reduce the number of people eventually needing assessment by DXA to confirm the presence of sarcopenia, thus additionally providing cost-savings. The use of anthropometric PE_{ASM} means that it is now possible to identify at-risk groups, including in settings with difficult access to DXA, such as residential aged care; thus allowing for early institution of effective intervention to prevent further decline in physical function.

Understanding the pathophysiology of sarcopenia will assist in the development of prevention and treatment strategies. Research into the association between cytokines and appetite as assessed by the Simplified Nutritional Appetite Questionnaire (SNAQ) was discussed in Chapter 7. Impaired

SNAQ scores have been associated with future weight loss, which is associated with muscle mass loss and therefore contributes to the development of sarcopenia.

In Chapter 7, SNAQ scores were demonstrated to be negatively associated with the pro-inflammatory cytokine IL-1ß and positively associated with the anti-inflammatory cytokine IL-10 in a group of healthy subjects without weight loss. Therefore, inflammation is associated with reduced appetite, even in apparently healthy individuals. This suggests that inflammation may play a role in the process leading to the eventual phenotypic expression of sarcopenia manifesting firstly with appetite reduction. Effort of early effective intervention targeting inflammation may perhaps need to start even earlier in life, prior to weight loss, and may include screening for appetite loss.

8.1 Significance and contribution

This PhD research has generated new knowledge and contributed significantly to the research literature in the area of sarcopenia and ageing.

The major clinical contribution arising from this PhD is the development of a 'rule out' screening test for sarcopenia, incorporating a novel anthropometric PE_{ASM}. This practical and easy to implement screening tool allows for screening in settings where access to DXA is difficult. It also contributes to cost savings by reducing the need for DXA assessments in those patients identified as not at risk of having sarcopenia by the newly developed screening tool.

Also, as a direct result of this PhD research, there is now increased awareness in Australia that sarcopenia is common, especially in people aged 80 years and older. This research has been published in two local newsletters: the *Basil Hetzel Institute*, readers of which are predominantly donors to the research institute, researchers and some health professionals, and *In Central*, read by a wider range of health care professionals (Appendix 12). Sarcopenia will no longer remain a hidden public health issue in Australia as a result of this research.

The results of my investigations have demonstrated that the use of different methods to identify cut-offs for low muscle mass result in differing prevalence figures for sarcopenia. It is clear that there is a need for consensus as to the preferred method for successful prevention or remediation of the condition. My research suggests that it is preferable to use either the gender specific lowest 20% of the SMI of populations aged 65 years and over or the residual of the linear regression method to identify low muscle mass cut-offs given that both these methods resulted in similar prevalence figures for sarcopenia within the community.

A further contribution from the research is the development of an anthropometric PE for LBM that might have a purpose within the oncological setting, where it has been demonstrated that low LBM is associated with increased risk of chemotherapy toxicity.

8.2 Future directions

As a result of the research presented in this thesis, the following future research possibilities have been identified, and will be pursued by the research group of which I am a member over the next three years:

- The health, quality of life and economic consequences of sarcopenia in community dwelling South Australians are currently unknown. Both the North West Adelaide Health Study and Florey Adelaide Male Ageing Study will be used in future to determine some of the health consequences. The use of other Australian cohort studies of older people will also be considered. Completing such studies in Australia is important, as it will alert health funders and decision makers to the costs of sarcopenia to both individuals as well as society.
- There is a lack of research internationally in relation to the prevalence and consequences of sarcopenia in recipients of aged care services including residential aged care (i.e. frailer individuals). Future research will involve collaboration with local aged care service providers and their consumer groups to undertake this research.
- Building on the proposed collaboration, the performance of the various anthropometric prediction equations against DXA in residential aged care residents will be investigated. We will also further investigate the use of the best performing prediction equation as part of a screening method for sarcopenia in the residential aged care setting.
- Using other national cohorts, especially those with gait speed assessed, the proposed screening tool will be further validated. This will be of particular interest as a major limitation of the research reported in this PhD was because gait speed was not assessed within the North West Adelaide Health Study. Access will be sought to the Dynamic Analyses to Optimise Ageing (DYNOPTA) study in order to access studies with the necessary variables.
- Several other researchers internationally have proposed alternative screening methods. Using the research studies proposed earlier, our screening method will be compared to other reported screening methods to help clinicians determine the most appropriate screening method for use in various clinical settings: community or residential aged care.

In conclusion, the current study has confirmed that sarcopenia is common in older Australians but there remains a need to reach a consensus as to the best method to assess for low muscle mass. An anthropometric PE_{ASM} for low muscle mass that can be incorporated with assessment for low muscle performance to screen for sarcopenia has been proposed, along with cut-offs for low muscle mass that can be applied in the Australian context.

The proposed 'rule out' screening test for use in the primary or aged care setting will reduce the number of DXAs required to confirm the presence of sarcopenia, resulting in cost savings. This research will contribute to increased awareness, which in turn will increase the desire to look for this health condition. Through screening, at-risk individuals can be identified for the diagnosis to be confirmed and treatment to commence. Furthermore, preventative strategies, such as better nutrition and increased physical activity, can also be encouraged in those thought to be at-risk.

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Appendices

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Appendix 1

Information pack

HEALTHY MUSCLE & BODY COMPOSITION STUDY

Formal Title: CASA (Cytokines, Adiposity, Sarcopenia and Ageing) Study **Protocol Number:** 2006009

Invitation to participate

We invite you to participate in a research project which we believe is of potential importance.

However, before you decide whether or not you wish to participate, we need to be sure that you understand

why we are doing it, and

what it would involve if you agreed.

We are therefore providing you with the following information. Please read it carefully and be sure to ask any questions you have. The doctor conducting the research will be happy to discuss it with you and answer any questions that you may have. You are also free to discuss it with outsiders if you wish (ie family, friends and/or your local doctor).

Participation is voluntary

Participation in any research project is voluntary. If you do not wish to take part, you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage without providing a reason.

BACKGROUND TO THE STUDY

What is the research about?

Obesity and under-nutrition are both very common and have negative health outcomes. Obesity means having too much fat tissue, whilst being under-nourished usually causes a lack of muscle tissue. However, these syndromes may overlap; that is, someone who has excess fat may also lack muscle.

This study is investigating the relationship between special inflammatory proteins found in people's blood that act as chemical messengers between cells (***cytokines)***, fat tissue (***adiposity***), lack of muscle (***sarcopenia***) and ageing. Currently it is not known what happens to various cytokines as people age. Also, there is a lack of understanding how cytokine measurements are related to appetite, body fat and muscle. It is also not known if there is a relationship between cytokine measurements and various hormones (insulin, insulin-like growth factor, vitamin D) involved in appetite, lean and fat mass control.

Why is the research being done?

A lack of lean mass represents reduced nutritional health. There is a need to understand these relationships so that further research can be conducted to determine possible management strategies for these two important syndromes. Currently, total lean mass is assessed by performing a DEXA scan (osteoporosis scan). It would be beneficial to doctors if they could use a simple blood test to estimate someone's lean mass.

Who is sponsoring it, and are they paying the researcher or his/her department to do the research?

This study is funded by the following grants: (1) Vincent Fairfax Family Fellowship grant awarded by the Royal Australasian College of Physicians Research Foundation and (2) an Establishment Grant awarded by The University of Adelaide. The investigators are not receiving a salary from this grant.

How and why have I been chosen as a possible participant in the research?

Your phone number has been selected randomly from all telephone listings in the state, from the Electronic White Pages. We are looking for 200 healthy volunteers aged 18 years and over from the western suburbs of Adelaide.

PROCEDURES AND TREATMENT

Will I have to come back to the clinic more often or remain in the hospital longer than would normally be the case? What will I be asked to do at each visit?

You will only have to come to the hospital twice at the most for this study. For the initial visit (approximately one hour), you will need to fast (no food after 10pm, only sips of water after midnight) and we will obtain a blood sample from you (50mls- 6 dessert spoons). We would like you to refrain from alcohol, cigarettes and exercise the day prior to the study.

You also need to have not experienced any acute illness (new illness requiring a visit to your doctor or being hospitalised) in the one month before your first visit. We would also need to know of any illnesses in the two weeks after you have given us a blood sample. Your blood sample will have the following tests:

- FBE (Full Blood Examination) – to check for anaemia and infection;
- MBA20 (Multiple Blood Analysis) – to check kidney and liver function, fasting glucose (blood sugar) and cholesterol;
- CRP (C-Reactive Protein) – a special type of protein produced by the liver and used to check for inflammatory conditions such as rheumatoid arthritis
- Parathyroid hormone – a hormone involved in bone health;
- 25-OH (Hydroxy) Vitamin D – to determine a deficiency or excess amount of Vitamin D (important for bone health);
- and CK/CK-MM (Creatinine Kinase/Isoenzymes) - enzymes found mainly in the heart and skeletal muscle, the levels of which are used as a guide to how healthy these muscles are.

At the health examination, you will undergo a body composition study using bio-electrical impedance. This is a test that tells us how much muscle and fat you have. This is frequently performed at some health centres. You will lie on a bed and electrodes will be placed on your wrist and ankles. A small electrical current (not dangerous) will pass through your body and from the differences in charges, the machine will give us an estimation of your body fat and muscle content.

Additionally we will also measure your muscle strength by measuring the strength of your grip via a dynamometer. We will also ask you to stand up from a chair, walk, turn around and sit again. This will be timed. Furthermore, we will ask you to stand up from a chair, sit and stand again six times and we will time this. We will measure your waist, hip and thigh circumference with a tape measure, and take your blood pressure. We will ask you to complete a very simple questionnaire about your appetite. We will also record your medical and medication history.

During a second appointment, you will have a DEXA scan. This is a scan similar to that performed for the assessment of osteoporosis. The same scan used to assess the quality of your bones can also give us information about your muscle and fat tissue. The DEXA scan involves a X-ray of your whole body (estimating your total fat and muscle tissue) and this scan is similar to the scan commonly performed to assess osteoporosis risk. During a DEXA x-ray scan, you will be required to lie on a bed with a pillow under your head and a cushion under your knees. The bed will then be slid beneath the x-ray camera. The scan will take approximately 30 minutes and has a very low radiation dose. You will only be exposed to 0.14% of the background radiation that you are normally exposed to per year. Because of the small radiation exposure women of reproductive age will need to be sure that they are not pregnant before having this test.

We do not expect there to be any adverse events from the tests. There may only be some slight discomfort during the blood taking procedure.

What treatment will I get if I do take part? Will this be different from the treatment I would get otherwise? If so, how and in what ways?

You will not be receiving any treatment as part of this study. You will receive information about your blood tests and body composition measurements if you would like to know these results.

Are there any factors, which would exclude me from participating, like pre-existing illness, the possibility of becoming pregnant or other drugs being taken?

You must be at least 18 years of age. Women who have not experienced the menopause will need to provide their blood sample in the first 14 days of their menstrual cycle. You must not be pregnant. Your weight should have been stable over the previous 3 months and you should not have suffered an illness over the previous 1 month. Should you become ill 2 weeks after the blood test, please let the investigators know (they will also ring you to check).

People who are under-weight or over-weight (as determined by Body Mass Index) are excluded from this study. We prefer that you have no chronic diseases and are not on medications that cannot be safely stopped 24 hours prior to blood sampling. You should not have been vaccinated in the six weeks prior to providing us with a blood sample.

WHAT ARE THE DISCOMFORTS, RISKS AND SIDE EFFECTS?

Will there be any discomfort?

It is unlikely that you will experience discomfort from the bio-electrical impedance study. At the most, it is envisaged that you may only experience minor discomfort from the blood testing. It is unlikely that you will have any long lasting side effects from participating in this study. The risks associated with radiation from DEXA (a type of x-ray machine) measurements of body composition and bone density are low and are considered in the medically acceptable range. This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to a naturally occurring background of radiation and receives a dose of about 2 millisieverts (mSv) per year. The effective dose from this study is about 0.0001mSv. At this dose level, no harmful effects of radiation have been demonstrated, as any effect is too small to measure. The risk is believed to be minimal.

What would happen if I were to feel severe discomfort or pain?

Please contact Dr Renuka Visvanathan, via The Queen Elizabeth Hospital switchboard operator, on (tel) 8222 6000 if you have concerns.

What will happen to the information collected?

Even though we may publish the collective results, your individual results and information will not be revealed. Computerised data will be decoded. We will provide you copies of your results if you wish to receive them. Information will be stored for 15 years.

Blood samples

For funding and technical reasons, we have organised with one of the investigators from the USA (Professor Banks) to analyse some of the stored blood samples for this study. Approximately 10 mls of stored blood (a little more than one tablespoon) will be sent to the USA for analysis. These samples will be coded and your name will not be recorded on the bottles. A small amount will be stored for secondary transfer in case the first transfer of the sample to the United States is not successful. We will also be seeking separate consent to contact you (or your next of kin in the event of your death) at a later stage regarding possible further research studies on this stored blood.

What are my rights?

If you become injured during this study, and your injury is a direct result of the effects of study procedures, The Queen Elizabeth Hospital will provide reasonable medical treatment. Your participation in this study shall not affect any other right to compensation you may have under common law. If you have any queries, you can contact Dr Renuka Visvanathan (Chief Investigator) at The Queen Elizabeth Hospital on (tel) 8222 6000.

Is there any payment for participation?

There will be a reimbursement of $20 to each participant in the study, once they have completed both phases of the study (ie clinic examination and DEXA procedure).

What are the direct benefits?

It is unlikely that the results of this study will benefit you personally but it will help us understand the relationship between cytokines (proteins), fat tissue, muscle, hormones and appetite. We will also be able to assess if commonly performed blood tests can be used to assess lean mass.

What if I have a question about the study?

You can contact Dr Renuka Visvanathan at The Queen Elizabeth Hospital on (tel) 8222 6000 for further information. The Central Northern Adelaide Health Service Ethics of Human Research Committee (TQEH & LMHS) has approved this study. Should you wish to speak to a person not directly involved in the study in relation to:

- matters concerning policies,
- your rights as a participant;
- information about the conduct of the study;
- or should you wish to make a confidential complaint;

you may contact the Executive Officer of the Ethics Committee, on (tel) 8222 6841.

Health Body and Muscle Composition Study
PAST MEDICAL HISTORY

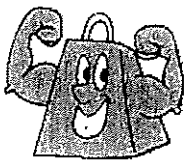
Please tick the box for any conditions the participant has now or in the past.

AREA	*ACCEPTABLE*	*EXCLUDED*
Heart – (Cardiovascular)	❒ **Atrial Fibrillation** ❒ **Vascular Replacements** ❒ **Pacemaker Insertion** ❒ **Asymptomatic abdominal aortic aneurysm**	❒ **Angina** ❒ **Heart Attack** ❒ **Angioplasty** ❒ **Heart By-pass surgery**
Lungs (Respiratory)	❒ **Mild asthma** (using puffers only for treatment) ❒ **Mild COPD** (using puffers only for treatment) ❒ **Obstructive Sleep Apnoea** ❒ **Resolved pneumothorx or pleural effusion – occurred > 2 years ago.** ❒ **Pulmonary Embolism – no longer on warfarin.**	❒ **Moderate to severe asthma** (Using oral steroids for treatment and/or has been hospitalised for this condition) ❒ **COAD** (Chronic Obstructive Airways Disease) – (Uses oral steroids for treatment and/or has been hospitalised for this condition) ❒ **Interstitial Lung Disease** – Inflammation and subsequent scarring of the lungs (Including pulmonary fibrosis) ❒ **Asbestos Lung Disease** ❒ **Bronchiectasis** (Abnormal stretching and enlarging of the respiratory passages due to blockage by excess mucus). ❒ **Current Emphysema** ❒ **Active TB** ❒ **Carcinoid disease**
Gastro-Intestinal Tract	❒ ***Healed*** **peptic ulcer disease** (Stomach ulcer / duodenal ulcer) ❒ **GORD** (Gastro-oesophgeal Reflux Disease) – (Reflux) ❒ **Colonic Polyps** (they are benign) ❒ **Irritable Bowel Syndrome** ❒ **Gallstones/ Resolved Cholecystitis**	❒ ***Active*** **Peptic Ulcer Disease** ❒ **Inflammatory bowel disease** (Includes Crohn's disease / ulcerative colitis) - ? Irritable bowel disease is not part of this category ❒ **Wilson's disease**
Kidneys	❒ **Renal Cysts**	❒ **Renal Failure** ❒ **Glomerulonephritis** ❒ **Renal Dialysis**
Liver	❒ **Fatty Liver**	❒ **Liver Disease** ❒ **Hepatitis**
Endocrine / Hormones	❒ **Hypothyroidism**	❒ **Diabetes** ❒ **Addison's Disease** ❒ **Pituitary Disease** ❒ **Hyperparathyroidism / Parathyroid adenoma**
Vascular Disease	❒ **Varicose veins**	❒ **Peripheral vascular disease** (Disease occurring in the blood vessels at the extremities, usually the hands and the feet) ❒ **Leg vessel by-pass** (Generally in veins which have difficulty returning blood to the heart- sometimes needed to fix varicose veins)

Please tick the box for any conditions the participant has now or in the past.

AREA	*ACCEPTABLE*	*EXCLUDED*
Brain and other Neurological Conditions	❒ **Benign positional vertigo** ❒ **Menniere's disease** ❒ **Seizures** ❒ **Resolved Meningitis** ❒ **Subdural Haematoma** ❒ **Resolved Sub-arachnoid Haemorrhage**	❒ **Mini stroke** ❒ **Stroke** ❒ **Carotid endarterectomy.** (A procedure where plaque (fatty build-up) is removed from the lining of the carotid artery- the main artery in the neck). ❒ **Motor Neurone Disease** ❒ **Multiple Sclerosis** (MS) ❒ **Huntington's disease** ❒ **Dementia** ❒ **Parkinson's disease**
Chronic Fatigue Conditions		❒ **Chronic Fatigue Syndrome** ❒ **Fibromyalgia**
Psychiatric / Psychological Conditions	❒ **Depression** ❒ **Schizophrenia** ❒ **Bipolar Disorder** **(not on medication n**	❒ **Drug Addiction**
Infections	❒ **Resolved Influenza** ❒ **Resolved Chicken pox** ❒ **Resolved Measles** ❒ **Resolved Mumps** ❒ **Ear Infection (not in the last 3 months)** ❒ **Resolved Epstein Barr Virus** (Glandular Fever is a common disease caused by this virus).	❒ **Severe Ongoing Infections** ❒ **Subacute bacterial endocarditis** (Bacterial infection of the heart) ❒ **Osteomylitis** (Infection of the bone) ❒ **HIV/ AIDS** ❒ **Tuberculosis** (TB)
Cancer	❒ **Skin Cancers**	❒ **All cancers except skin cancer**
Skin	❒ **Eczema** ❒ **Psoriasis**- Round or oval red plaques ❒ **Dermatitis** ❒ **Scabies** ❒ **Burns**	
Eyes	❒ **Glaucoma** ❒ **Cataracts** ❒ **Macula degeneration**	
Gallbladder	❒ **Gallstones**	
Genito- Urinary Conditions	❒ **Benign Prostate Hypertrophy** (Normal enlargement of the prostate gland-men only- that occurs with age)	

Questionnaire



Healthy Muscle and Body Composition (HMBC) Study

Please read the following instructions before answering the questions.

1. Please complete all the questions as per the instructions by placing a tick in the box ☑ that most closely corresponds to your answer.

2. Your answers will remain strictly confidential. Results of the study may be published in a medical journal, but no information that may lead to the identification of any individual will be released.

3. This questionnaire should take approximately 5 minutes to complete.

4. If you have any problems with this questionnaire, please contact:
   Sandy (Clinic Co-ordinator) on ☎ 8222 7866.

5. When you have completed the questionnaire, please bring it with you to your clinic appointment at The Queen Elizabeth Hospital.

Thank you

A. APPETITE

A1 My appetite is...

(tick one box only)

- ☐ 1 Very poor
- ☐ 2 Poor
- ☐ 3 Average
- ☐ 4 Good
- ☐ 5 Very Good

A2 When I eat, I feel full after...

(tick one box only)

- ☐ 1 Eating only a few mouthfuls
- ☐ 2 Eating about a third of a plate/meal
- ☐ 3 Eating over a half of a plate/meal
- ☐ 4 Eating most of the food
- ☐ 5 Hardly ever

A3 Food (generally) tastes...

(tick one box only)

- ☐ 1 Very poor
- ☐ 2 Poor
- ☐ 3 Average
- ☐ 4 Good
- ☐ 5 Very Good

A4 Normally I eat...

(tick one box only)

- ☐ 1 Less than 1 regular meal a day
- ☐ 2 1 meal a day
- ☐ 3 2 meals a day
- ☐ 4 3 meals a day
- ☐ 5 More than 3 meals a day (inc snacks)

B. ALCOHOL

B1 How often do you usually drink alcohol?

(tick one box only)

- ☐ 1 I don't drink alcohol **(Go to C1)** →
- ☐ 2 Less than once a week
- ☐ 3 Enter number of days per week

  _____

- ☐ 4 Refused **(Go to C1)** →

B2 A Standard Drink is equivalent to a schooner of full strength beer, a glass of wine or a nip of spirits. On a day when you drink alcohol, how many drinks do you usually have?

(tick one box only)

1 Enter number of drinks you usually consume on a day when you drink

_____

- ☐ 2 Refused

C. SMOKING

C1 Do you currently smoke?

(tick one box only)

- ☐ 1 Yes
- ☐ 2 No **(Go to C3)**
- ☐ 3 Occasionally

C2 How many cigarettes do you usually smoke a day?

- 1 Enter number of cigarettes _____ **(Go to C6) ↓**
- ☐ 2 Less than one **(Go to C6) ↓**
- ☐ 3 Only smoke cigars or pipes **(Go to C6) ↓**

C3 Have you ever smoked regularly (that is, at least once a day)?

(tick one box only)

- ☐ 1 Yes
- ☐ 2 No **(Go to D1) ↓**

C4 How many cigarettes did you usually smoke a day?

- 1 Enter number of cigarettes _____
- ☐ 2 Less than one
- ☐ 3 Only smoke cigars or pipes

C5 How old were you when you last gave up smoking?

- 1 Enter age _____
- ☐ 2 Can't remember

C6 At what age did you first start smoking daily?

- 1 Enter age _____
- ☐ 2 Can't remember

D. EXERCISE

The next questions are about exercise you may do for sport, recreation or fitness.

D1 In the last two weeks, did you do any *walking* for sport, recreation or fitness?

(tick one box only)

- ☐ 1 Yes
- ☐ 2 No **(Go to D4) →**

D2 How many times did you do any *walking* for exercise in the last two weeks?

- 1 Enter number of TIMES _____
- ☐ 99 Don't know

D3 What was the total amount of time you spent *walking* in the last two weeks?

- 1 Enter number of HOURS _____
- 2 Enter number of MINUTES _____

D4	In the last 2 weeks, *(apart from walking)* did you do any exercise which caused a moderate increase in your heart rate or breathing?	*(tick one box only)* ☐ 1 Yes ☐ 2 No **(Go to D7)** ↓
D5	How many times did you do any *moderate exercise* in the last two weeks?	1 Enter number of TIMES _____ ☐ 99 Don't know
D6	What was the total amount of time you spent doing *moderate exercise* in the last two weeks?	1 Enter number of HOURS _____ 2 Enter number of MINUTES _____
D7	In the last 2 weeks, did you do *any other exercise* which caused a large increase in your heart rate or breathing, that is, *vigorous exercise*?	*(tick one box only)* ☐ 1 Yes ☐ 2 No **(Go to D10)** ↓
D8	How many times did you do any *vigorous exercise* in the last two weeks?	1 Enter number of TIMES _____ ☐ 99 Don't know
D9	What was the total amount of time you spent doing *vigorous exercise* in the last two weeks?	1 Enter number of HOURS _____ 2 Enter number of MINUTES _____ ☐ 99 Don't know
D10	Do you participate in any regular professional sporting activities?	*(tick one box only)* ☐ 1 Yes ☐ 2 No
D11	Are you currently representing South Australia or Australia in sport?	*(tick one box only)* ☐ 1 Yes ☐ 2 No

Thank you very much for taking the time to complete this questionnaire.

Please make sure that you have answered all the questions.
Please bring this questionnaire with you to the clinic appointment.

If you have any problems or questions in completing this questionnaire, please telephone Sandy (Clinic Co-ordinator) on ☎ 8222 7866.

ID:

Healthy Muscle and Body Composition Study

MEDICATIONS RECORD

(This includes vitamins, oral contraceptive pill, implants, patches, respiratory puffers etc)

Name of medication	Reason for taking	Dose	Strength
Example only: *Dimetapp*	*Hay fever*	*Twice daily*	*40mg*

Government of South Australia
Central Northern Adelaide
Health Service

CONSENT FORM

HEALTHY MUSCLE & BODY COMPOSITION STUDY

Formal Title: CASA (Cytokines, Adiposity, Sarcopenia and Ageing) Study **Protocol Number:** 2006009

I, the undersigned .....

hereby consent to my involvement in the research project explained above.

- I have read the information sheet, and I understand the reasons for this study. The research worker has explained the ways in which it will affect me. My questions have been answered to my satisfaction. My consent is given voluntarily.
- I understand that the purpose of this research project is to improve the quality of medical care, but my involvement may not be of benefit to me.
- I understand that blood will be frozen and stored (as plasma) at The Queen Elizabeth Hospital and later sent to St Louis, USA for further analysis; and a small amount stored for secondary transfer in case the first transfer of the sample to the United States is not successful.
- The details of the research project have been explained to me, including:
  - the expected time it will take;
  - the nature of any procedures being performed, and the number of times they will be performed;
  - the nature of any medications I may be given;
  - any discomfort which I may experience.
- I have been given the opportunity to have a member of family or a friend present while the project was explained to me.
- My identity will be kept confidential, and nothing will be published which could possibly reveal my identity.
- My involvement in the study will not affect my relationship with my medical advisers. I understand that I am able to withdraw from the study at any stage without having to give a reason, and that by withdrawing it will not affect my treatment at this hospital in the future.

Patient signature: _____ **Date:** ___/___/___

Witness signature: _____ **Date:** ___/___/___

Witness name: _____

ID:

Healthy Muscle & Body Composition Study

STORAGE AND POSSIBLE FUTURE USE OF BLOOD SAMPLE AND NEXT OF KIN DETAILS

For funding and technical reasons, we have organised with one of the investigators (Prof Williams Banks from St Louis University in the United States of America) to analyse some of the stored blood samples for this study. Approximately 10mls of stored blood (a little more than one tablespoon) will be sent to Prof Banks for analysis. These samples will be coded and your name will not be recorded on the bottles.

We would also like to store a small amount of your blood sample (as plasma) in Adelaide, just in case the transfer of the sample to the United States is not successful (see General Consent Form).

We are seeking your consent here **to be able to contact you at a later stage** when we may speak to you about additional research studies and to then seek your permission using this stored blood. You can either refuse now to allow your samples to be used for future studies, or when we contact you at a later time. This would not jeopardise your participation in this study.

PART A *(Please choose ONE of the following options)*

OPTION 1

I, the undersigned _____

do NOT wish my stored blood sample to be used for future research studies.

Patient signature: _____ **Date:** / /

Witness signature: _____ **Date:** / /

Witness name: _____

OR

OPTION 2 *(See over page for additional option regarding contacting next of kin)*

I, the undersigned _____

consent to being contacted at a possible later stage regarding permission to use my stored blood sample for additional research studies.

Patient signature: _____ **Date:** / /

PART B

If you HAVE consented to option 2 (being contacted at a later stage regarding permission to use your stored blood sample for additional research studies) ...

... **we are seeking your consent here** to contact your NEXT OF KIN at a possible later stage, ONLY in the event that you have died, to seek their permission to conduct additional research studies using your stored blood.

OPTION 3	
I, the undersigned	
consent to my next of kin (as detailed below) to be contacted at a possible later stage regarding permission to use my stored blood sample for additional research studies.	
Patient signature:	**Date:** / /

Witness signature:	**Date:** / /
Witness name:	

NEXT OF KIN		
Title:	Mr / Ms / Mrs / Miss	
Family Name:		
First Name:		
Address:		
	Suburb:	
	State:	P/Code:
Telephone Number:	Home:	Work:
	Mobile:	
Relationship to you:	Husband / Wife / Father / Mother / Son / Daughter / Brother / Sister / Uncle / Aunt / Cousin / Friend /	
	OR Other (specify):	

ID:

Healthy Muscle and Body Composition Study

CLINIC RESULTS

Would you like to receive a copy of your results? 1 Yes ☒ 2 No ☐

GENERAL PRACTITIONER CONTACT DETAILS

Please complete the details below if you would like us to send the results from your clinic appointment to your general practitioner.

GP's Name:

GP Practice Name

Address:

Suburb:

State: P/Code:

Telephone Number:

Appendix 2

Running sheet in clinical procedure

Surname

Healthy Muscle & Body Composition Study

RUNNING SHEET FOR CLINICAL PROCEDURES

PARTICIPANT INFORMATION

Appointment date	/ /	Study ID	
Appointment time	am	Gender	☐ 1 Male ☐ 2 Female
Age		Date of birth	/ /

REMINDER to update ID, gender and age over page - surname to be cut off sheet after appt

ADMINISTRATIVE INFORMATION

SECTION 1

☐ Questionnaire has been completed (check to see if all questions are answered)

SECTION 2

1 ☐ Explained procedure to participants (blood test, blood pressure, body measurements, functional tests, DEXA)

2 ☐ Participant has read and signed **clinic** consent form

3 ☐ Participant has read and signed **blood storage** consent form

4 ☐ Participant's medical history (from telephone interview) has been verified

4a	Has participant **fasted** since midnight today?	☐ 1 Yes	☐ 2 No
4b	Has participant refrained from using **alcohol** in the last 24 hours?	☐ 1 Yes	☐ 2 No
4c	Has participant refrained from **smoking** in the last 24 hours?	☐ 1 Yes	☐ 2 No
4d	Has participant not had **vigorous physical activity** in the last 24 hours?	☐ 1 Yes	☐ 2 No
4f	Did the participant take any medication in the last 24 hours? (does *not* include yesterday morning)	☐ 1 Yes	☐ 2 No

4g ☐ Medication sheet checked

SECTION 1 continued

Study ID | Gender Male / Female | Age

5a Weight (kg) | 5b Height (cm)

5c BMI *(Calculation of BMI = weight (kg) divided by height (m²)*

BMI Exclusion Criteria

If aged 18 to 70 years of age - BMI less than 21 kg/m² or greater than 28 kg/m²

If aged 70 years and over - BMI less than 23 kg/m² or greater than 35kg/m²

6 Dominant hand: ☐ 1 Right-handed ☐ 2 Left-handed

SECTION 2 continued

7 Hydration ☐ 1 Normal ☐ 2 Oedematous ☐ 3 Dehydrated

8 Blood taken from non-dominant arm ☐ 1 Yes ☐ 2 No

8a Exercise Level ☐ 1 No exercise ☐ 2 Some exercise ☐ 3 Moderate exercise ☐ 4 Daily exercise ☐ 5 Athletic

8b Body Frame ☐ 1 Small ☐ 2 Medium ☐ 3 Large

SECTION 3

9 Bio-electrical impedance study *Calculation from measurements*

9a Resistance | 9c Fat Free Mass

9b Reactance | 9d Fat Mass

☐ 0 Not completed | 9e Total Body Water

10 1st Blood pressure (mmHg) | 10a 1st Systolic | 10b 1st Diastolic

11 DEXA Appointment ☐ 1 Participant to attend same day as clinic

☐ 2 Participant was given information to make their own appointment

2nd Blood pressure (mmHg) | 10c 2nd Systolic | 10d 2nd Diastolic

12 Waist (cm) | 12a W1st | 12b W2nd | 12c W3rd

13 Hip (cm) | 13a H1st | 13b H2nd | 13c H3rd

14 Thigh (cm) | 14a T1st | 14b T2nd | 14c T3rd

15 Chair rise timed test (sec) 5x ☐ 0 No | Total time

16 6 metre walk test (sec) ☐ 0 No | Total time

17 Hand grip strength | 17a 1st | 17b 2nd | 17c 3rd

Appendix 3

Follow up letter and results

Government of South Australia
Central Northern Adelaide
Health Service

The Queen Elizabeth
Hospital
28 Woodville Road
Woodville South SA 5011
ABN 21 464 837 384
www.nwahs.sa.gov.au

Date:

To:

Dear

RE: Name DOB

I am writing to inform you that the lady/gentleman had kindly agreed to participate in a research study [Muscle and Body Composition (HMBC) study] at the Queen Elizabeth Hospital.

In this study, we are interested in examining the complex interaction between fat, muscle, age and various cytokines. In addition, we hope to find a simple blood test to estimate lean mass.

With this letter, I am attaching some of the test results for your information (and to be managed if required). It is expected that the participant will approach you to discuss some of the results if they are concerned.

Should you have any queries, please contact me through the TQEH switchboard on 08- 82226000.

Thank you

Yours sincerely,

Dr Solomon Yu

Government of South Australia
Central Northern Adelaide Health Service

The Queen Elizabeth Hospital
28 Woodville Road
Woodville South SA 5011
ABN 21 464 837 384
www.nwahs.sa.gov.au

Date:

To:

Dear

RE: HEALTHY MUSCLE AND BODY COMPOSITION (HMBC) STUDY

Thank you for having participated in this study. Below are some of the results from the investigations we conducted. We strongly encourage you to discuss these results (especially if they are not within the normal range) with your general practitioner. We would like to emphasise that even if the results are mildly abnormal, they are often remediable with the assistance from your GP. If you had initially agreed, we would have sent copies of most of your blood results to your GP. However, we provide here additional details of your body composition measure.

Your blood pressure was		mmHg	(normal <120/80 mmHg)
Your body mass index was		kg/m^2	

Blood Tests

Please not that your cholesterol/triglyceride and glucose test can be improved by adhering to a good balanced diet and exercise. Vitamin D/Calcium is important for bone health, if you are at risk of osteoporosis, speaking to your doctor about it, performing weight bearing exercise, some sunlight exposure and perhaps some calcium (obtainable from diary products) and vitamin D supplementation would be beneficial. For some advice on diet plans- go to www.mypyramid.gov.

Haemoglobin		g/L (112-155)	Are you anaemic?	Y/N
White Cell Count		$x10^9$ (4-11)	Is this acceptable?	Y/N
Platelets		$x10^9$ (150-400)	Is this acceptable?	Y/N
Total cholesterol		mmol/L (<5.5)	Is this acceptable?	Y/N
Total triglycerides		mmol/L (0.3-2.0)	Is this acceptable?	Y/N
Creatinine		mmol/L (50-120)		
eGFR		$mL/mri/1.73m^2$	Your kidney function is normal?	Y/N
Fasting Glucose		mmol/L (3.8-5.5)	Is this acceptable?	Y/N
Vitamin D level (Vitamin D is made in the skin from sunlight and there is also some input from the kidney)		mmol/L (60-160).	Is this normal?	

Government of South Australia
Central Northern Adelaide
Health Service

The Queen Elizabeth Hospital
28 Woodville Road
Woodville South SA 5011
ABN 21 464 837 384
www.nwahs.sa.gov.au

Parathyroid Hormone Level (An endocrine gland near the thyroid gland and is involved in your bone health)		pmol/L (0.8-5.5)	Is this normal? Sometimes parathyroid hormone levels are elevated if you are Vitamin D deficient.	Y/N
Ionised calcium level		mmol/L (1.10-1.25)	Is this normal?	Y/N
Bio-electrical impedance Study (Fat / Lean Mass Estimation)				
% Total fat mass =		% (target = %)		
% Total fat free mass =		% (target = %)		
DEXA - copy of results attached (Bone density and Fat / Lean Mass Estimation)				
Total Z score =				
Are you at risk of osteoporosis?	Y/N		If yes, please discuss results with your Doctor	
Total fat mass =	G			
Total fat free mass =	g			

Should you have any concerns, please contact me through the TQEH switchboard on 08- 82226000.

Thank you.

Yours truly,

Dr Solomon Yu

Appendix 4

Cohort Profile: The North West Adelaide Health Study (NWAHS)

Grant, J.F., Taylor, A.W., Ruffin, R.E., Wilson, D.H., Phillips, P.J., Adams, R.J.T., Price, K. & the North West Adelaide Health Study Team (2009). Cohort Profile: The North West Adelaide Health Study (NWAHS).
International Journal of Epidemiology, v. 38 (6), pp. 1479-1486

NOTE:
This publication is included in the print copy
of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1093/ije/dyn262

Appendix 5

Cohort Profile: The Florey Adelaide Male Ageing Study (FAMAS)

Martin, S., Haren, M., Taylor, A., Middleton, S., Wittert, G. & Members of the Florey Adelaide Male Ageing Study (FAMAS) (2007). Cohort Profile: The Florey Adelaide Male Ageing Study (FAMAS).
International Journal of Epidemiology, v. 36 (2), pp. 302-306

NOTE:
This publication is included in the print copy
of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1093/ije/dyl279

Appendix 6

Statement of authorship: Chapter 2 Sarcopenia in older people

Statement of Authorship

Title of Paper	Sarcopenia In Older People
Publication Status	○ Published, ☑ Accepted for Publication, ☒ Submitted for Publication, ○ Publication style
Publication Details	Solomon Yu, Kandiah Umapathysivam, Renuka Visvnathan. Sarcopenia in Older People. ~~Submitted for publication.~~ Int J Evid Based Healthc. 2014 Accepted for Publication 7/7/2014.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Solomon Yu		
Contribution to the Paper	Conceptualised the literature reveiw, reviewed articles,constructing summary table, interpreting information from multiple sources and acted as key corresponding author		
Signature		Date	2/6/14

Name of Co-Author	Kandiah Umapathysivam		
Contribution to the Paper	Provided supervision, overseeing the manuscript development and final approval and editing of the manuscript		
Signature		Date	20/5/2014

Name of Co-Author	Renuka Visvanathan		
Contribution to the Paper	Supervised development of literature review. Involved with project conception, Provided help with evaluation and editing of manuscript		
Signature		Date	20/5/14

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Appendix 7

Statement of authorship and published manuscript: Chapter 3, The impact of low muscle mass definition on the prevalence of sarcopenia in older Australians.

Statement of Authorship

Title of Paper	The impact of Low Muscle Mass definition on the Prevalence of Sarcopenia in older Australians
Publication Status	☒ Published, ○ Accepted for Publication, ☒ Submitted for Publication, ○ Publication style
Publication Details	Yu Solomon, Sarah Appleton, Robert Adams, Ian Chapman, Gary Wittert, Thavarajah Visvanathan, Renuka Visvanathan. The impact of Low Muscle Mass definition on the Prevalence of Sarcopenia in older Australians. ~~Submitted for Publication.~~ BioMed Research Internationl. 2014 ; Article ID 361790, 7 pages. http://dx.doi.org/10.1155/2014/361790

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Solomon Yu		
Contribution to the Paper	Involved in study conception, constructed study database, performed all the statistical analyses, interpreting results, prepare manuscript and acted as key corresponding author		
Signature		Date	2/6/14

Name of Co-Author	Sarah Appleton		
Contribution to the Paper	Assistance in data analysis and evaluated the final of version of the manuscript		
Signature		Date	5/5/2014

Name of Co-Author	Robert Adams		
Contribution to the Paper	Assisted in research supervision and contributed to manuscript. Provided access to database from NWAHS for validation. Evaluation final version of manuscript		
Signature		Date	5/5/2014

Name of Co-Author	Ian Chapman		
Contribution to the Paper	Assisted in research supervision and contributed to manuscript.		
Signature		Date	13/5/14

Statement of Authorship

Title of Paper	The impact of Low Muscle Mass definition on the Prevalence of Sarcopenia in older Australians
Publication Status	✓ Published, ○ Accepted for Publication, ⊗ Submitted for Publication, ○ Publication style
Publication Details	Yu Solomon, Sarah Appleton, Robert Adams, Ian Chapman, Gary Wittert, Thavarajah Visvanathan, Renuka Visvanathan. The impact of Low Muscle Mass definition on the Prevalence of Sarcopenia in older Australians. ~~Submitted for Publication~~. BioMed Research International. 2014 ; Article ID 361790, 7 pages. http://dx.doi.org/10.1155/2014/361790

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Gary Wittert		
Contribution to the Paper	Assisted in research supervision. Provided database from FAMAS for validation. Evaluated final version of manuscript.		
Signature		Date	13/05/14

Name of Co-Author	Thavarajah Visvanathan		
Contribution to the Paper	Assisted in research supervision and contributed to manuscript.		
Signature		Date	24/5/14

Name of Co-Author	Renuka Visvanathan		
Contribution to the Paper	Involved with project conception, development of overall research plan and study oversight		
Signature		Date	20/5/14

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

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Hindawi

Research Article

The Impact of Low Muscle Mass Definition on the Prevalence of Sarcopenia in Older Australians

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Background. Sarcopenia is the presence of low muscle mass and low muscle function. The aim of this study was to establish cutoffs for low muscle mass using three published methods and to compare the prevalence of sarcopenia in older Australians. *Methods.* Gender specific cutoffs levels were identified for low muscle mass using three different methods. Low grip strength was determined using established cutoffs of <30 kg for men and <20 kg for women to estimate the prevalence of sarcopenia. *Results.* Gender specific cutoffs levels for low muscle mass identified were (a) <6.89 kg/m^2 for men and <4.32 kg/m^2 for women, <2 standard deviation (SD) of a young reference population; (b) <7.36 kg/m^2 for men and <5.81 kg/m^2 for women from the lowest 20% percentile of the older group; and (c) < −2.15 for men and < −1.42 for women from the lowest 20% of the residuals of linear regressions of appendicular skeletal mass, adjusted for fat mass and height. Prevalence of sarcopenia in older (65 years and older) people by these three methods for men was 2.5%, 6.2%, and 6.4% and for women 0.3%, 9.3%, and 8.5%, respectively. *Conclusions.* Sarcopenia is common but consensus on the best method to confirm low muscle mass is required.

1. Introduction

Sarcopenia commonly affects older people and is characterized by loss of both muscle mass and strength [1, 2]. Sarcopenia is associated with disability, a loss of independence, and reduced quality of life [3]. In one American study, sarcopenia and its consequences were estimated to cost the US healthcare system US$18 billion [4]. Sarcopenia is therefore a costly issue to the healthcare system [4, 5].

The European Working Group on Sarcopenia in Older People (EWGSOP) has recently defined sarcopenia as a combination of both low muscle mass and low muscle function [1]. Grip strength is one method to assess muscle function [1]. Low grip strength cutoffs of <30 kg for men and <20 kg for women are recommended and derived from receiver operating characteristic (ROC) curves predicting walking speeds slower than 0.8 m/s [6]. Appendicular skeletal muscle mass (ASM) is commonly assessed using dual absorptiometry X-ray assessment (DXA). The EWGSOP identifies three different methods to define low muscle mass [1]. With the oldest method, gender specific cut-off values for low muscle mass are derived from a younger reference group (<2 standard deviation, age 18–40 years) and cut-off values of <7.26 kg/m^2 for men and <5.50 kg/m^2 for women were reported in the original paper [2]. With the second method, cut-off points for low muscle mass are derived from gender specific lowest 20% of a predictive population, thus circumventing the need for a younger reference group [7].

Cut-off points similar to those identified by the Newman and colleagues have been reported, <7.23 kg/m^2 for men and <5.67 kg/m^2 for women [7, 8]. The third method adjusts for fat mass and is derived from the gender specific lowest 20% of the distribution of residuals of the linear regression on appendicular lean mass adjusted for fat mass and height and cutoffs of < -2.29 kg for men and < -1.73 kg for women are reported [7].

To date, there have only been three studies in Australia investigating the prevalence of low muscle mass but only one has reported on the prevalence of sarcopenia (i.e., low muscle mass and low muscle strength) in the community [9–11]. Scott et al. reported a 5% prevalence of sarcopenia in those aged 50–79 years and using the lowest 20% distribution of the predictive population to identify the cut-off points for both low muscle mass and low grip strength [9]. In a second Australian study, cut-off points of <4.85 kg/m^2 derived from a young reference group were used to identify that 3.2% of older women residing in low level aged care have sarcopenia [10]. The third Australian study examined the prevalence of low ASM in older (≥70 years) men living in the community using the linear regression and the gender specific lowest 20% method and reported a prevalence rate ranging from 15% in those aged 70 to 74 years to 26% for those aged 80–84 years and increasing to 45% for those aged 85–89 years [11].

To date, no study in Australia has examined the prevalence of sarcopenia in both men and women and compared all three methods to identify low muscle mass. The aims of this study were to firstly establish gender specific cut-off points for low skeletal muscle mass using the three methods as identified by the EWGSOP and then report the prevalence of sarcopenia in older (aged 65 years and older) Australians living in the community.

2. Methods

2.1. Study Cohorts. Three cohorts were investigated in this study: The Cytokine, Adiposity, Sarcopenia and Ageing Study (CASA), the North West Adelaide Health Study (NWAHS), and the Florey Adelaide Male Ageing Study (FAMAS) [12–14]. The three cohorts were combined to derive two broad population groups: younger reference population (aged 18–40 years; CASA and FAMAS) and older group (aged ≥65; FAMAS and NWAHS) (see Figure 1). For the purpose of this study, only those participants with a complete set of information on weight, height, grip strength, and DXA were included in the analysis.

The methodology of recruitment was similar for all three cohorts and has been described in detail elsewhere [12–14]. Ethical approval was obtained from the Central Northern Adelaide Health Service Ethics of Human Research Committee. All participants in the three cohort studies provided written, informed consent. Briefly, all households in the northern and western region of Adelaide with a telephone number listed in the Electronic White Pages were eligible for selection into the study. Selected households were sent an approach letter and brochure informing them about the study. The person who was last to have a birthday and aged 18 years or older was invited to participate in a short telephone interview. Interviews were conducted using computer-assisted telephone interview (CATI) technology. Selected persons were deemed "nonreplaceable" and, if the selected person was not available, interviews were not conducted with alternative household members. Up to six telephone calls were made to each household before the selected individual was classified as noncontactable. Respondents to the telephone interview were asked a number of health-related and demographic questions. Following the recruitment interview, respondents were invited to make an appointment to attend clinic for biomedical examination and investigations.

NWAHS. 4060 adults were included in the baseline biomedical examination between December 1999 and July 2003. 3566 participants attended the followup (median 4 years) between May 2004 and February 2006. Of these, a total of 1553 participants aged 65 years and older (men = 724, women = 829) were included in the analysis [12].

FAMAS. 1195 community dwelling men aged between 35 and 80 years from the north west regions of Adelaide were recruited between August 2002 and April 2005. Of these, 295 men were aged 65 years and older [13].

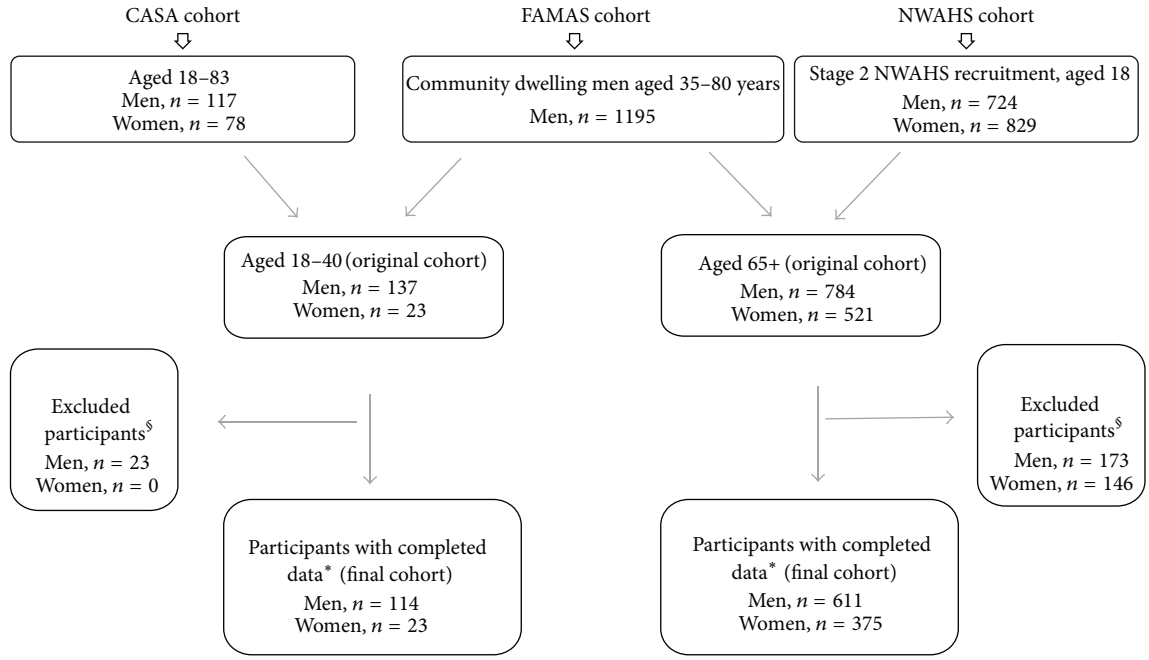
CASA. Healthy subjects aged 18 to 83 years (n = 195) were recruited from the western suburbs of Adelaide (2005–mid-2007). In this study, as the aim was to recruit a "healthier" population and so there were additional criteria. To participate in this study, subjects had to be 18 years and older, be able to comply with the study protocol, and be weight stable over the preceding three months. Those with a serious medical illness, inflammatory disease, an acute illness in the previous three months or in the two weeks following blood sampling, unable to stop medications for three days prior to blood sampling, in receipt of vaccinations, and pregnant were excluded from the study [14].

2.2. Measurements

Anthropometry. Height (m) was measured with shoes off to the nearest 0.1 cm. Weight (kg) was measured wearing light clothing to the nearest 0.1 kg. Body mass index (BMI, weight/height^2) was calculated. Three measurements of the waist and hip were taken and the mean for each was calculated [12].

Grip Strength. Grip strength (kg) was measured three times with each dominant hand using a grip dynamometer (Lafayette Instrument Company, IN, USA [CASA and NWAHS], Smedley, Chicago, IL [FAMAS]) while subjects were sitting with their arm supported by a horizontal surface. The mean of the three readings was used in this study [15].

Dual Energy X-Ray Absorptiometry (DXA). Appendicular skeletal muscle mass (ASM) in this study was defined as the sum of lean soft-tissue masses for arms and legs, assuming that all nonfat and nonbone tissue are skeletal muscle. *CASA*: ASM was determined using a Lunar PRODIGY whole-body scanner (GE Medical Systems, Madison, WI) in conjunction



*Completed data = those with completed measurements of DXA, grip strength, and anthropometric measurements (weight and height)

§Missing either one or more of the components of DXA, grip strength, and anthropometric measurements

Figure 1: Cohorts combined to develop the younger reference (aged 18–<40) and older study group (aged 65+).

with Encore 2002 software. *NWAHS* and *FAMAS*: A Lunar PRODIGY scanner (GE Medical Systems, Madison, WI) in conjunction with Encore 2002 software and a DPX+ (GE Medical Systems, Madison, WI) scanner in conjunction with LUNAR software version 4.7e were used. Cross-calibration analysis reported no significant differences between the 2 machines [16].

2.3. Statistical Analysis. SPSS 19 for Windows software (SPSS, Inc., Chicago, IL) was used for statistical analysis. Descriptive data was expressed as mean ± standard deviation (SD). Independent two-sample t-test was used to assess the mean difference in the characteristics variables between men and women. Low muscle mass was identified using the three different methods: (a) Baumgartner's method whereby cut-off values of ASM were <2 standard deviation (SD) of a young reference population, (b) the 20% gender specific method where cutoffs were derived for the lowest 20% of the older study population, and (c) the linear regression method where the lowest 20% of residual of the linear regression models of ASM adjusting for fat mass and height in men and women were applied to the older study population to derive cut points. As walk speed was not available within the NWAHS cohort, grip strength was used to determine muscle function and cutoffs of <30 kg for men and <20 kg for women were applied [6]. $P < 0.05$ was considered statistically significant.

3. Results

Figure 1 illustrates the flow diagram in establishing the two study populations from the three cohorts. For the young reference group, from the CASA and FAMAS cohort, there were a total of 137 men and 23 women aged 18–40 years. Of these, 23 men were excluded because of insufficient data. There were no statistically significant differences between the original and final cohorts in terms of age (35.7 ± 4.9 versus 35.5 ± 5.3 years, $P = 0.75$), weight (88.0 ± 16.3 versus 87.7 ± 15.9 kg, $P = 0.99$), height (1.8 ± 0.1 versus 1.8 ± 0.1 m, $P = 0.98$), BMI (27.9 ± 4.6 versus 27.8 ± 4.6 kg/m^2, $P = 0.98$), % fat (26.7 ± 8.5 versus 26.7 ± 8.5%, $P = 0.97$), ASM (28.6±4.3 versus 28.6±4.3 kg, $P = 0.83$), SMI (9.1±1.1 versus 9.1±1.1 kg/m^2, $P = 0.85$), and grip strength (52.2±10.8 versus 51.6 ± 11.1, $P = 0.68$). For the older group, from the FAMAS and NWAHS cohorts, there were 784 men and 521 women (Figure 1). 173 men and 146 women were excluded because of incomplete data. Consequently, the final cohort consisted of 611 men and 375 women. Women in the original cohort were significantly older than the women in the final cohort (74.0±6.3 versus 73.2±6.0 years, $P = 0.05$). No age difference was noted for men (73.0±6.0 versus 72.7±5.7 years, $P = 0.30$). There were no statistically significant differences between the original and final cohort in terms of weight (81.8±13.6 versus 81.8 ± 13.3 kg, $P = 0.96$), height (1.7 ± 0.1 versus 1.7 ± 0.1 years, $P = 0.85$), BMI (27.9 ± 4.3 versus 27.9 ± 4.2 kg/m^2,

Table 1: Characteristics of subjects from the younger reference group and older adults (aged ≥ 65) in the NWAHS and FAMAS included in the analysis.

Characteristics	Younger reference population 18 +< 40 years (FAMAS and CASA)			Older study population 65+ years (NWAHS and FAMAS)		
	Men (n = 117) mean (SD)	Women (n = 23) mean (SD)	P values	Men (n = 611) mean (SD)	Women (n = 375) mean (SD)	P values
Age (SD), years	35.5 (5.3)	31.2 (7.3)	0.01	72.7 (5.7)	73.2 (6.0)	0.21
Weight (SD), kg	87.7 (15.9)	69.3 (15.3)	<0.001	81.8 (13.3)	69.4 (12.4)	<0.001
Height (SD), m	1.8 (0.1)	1.7 (0.1)	<0.001	1.7 (0.1)	1.6 (0.1)	<0.001
BMI (SD), kg/m^2	27.8 (4.6)	25.5 (5.5)	0.03	27.9 (4.2)	27.8 (4.7)	0.79
% Fat	26.7 (8.5)	29.9 (11.6)	0.22	28.6 (6.9)	40.2 (6.9)	<0.001
ASM (SD), kg	28.6 (4.3)	18.4 (4.1)	<0.001	24.0 (3.2)	16.1 (2.4)	<0.001
SMI (SD), kg/m^2	9.1 (1.1)	6.7 (1.2)	<0.001	8.2 (0.9)	6.4 (0.8)	<0.001
Chronic conditions	%	%		%	%	
Cardiovascular Disease	1.7	0.0	0.54	24.1	17.8	0.019
Diabetes	1.7	0.0	0.54	24.4	19.1	0.050
Hypertension	27.6	4.5	0.02	77.3	69.7	0.007
Hypercholesterolemia	44.7	13.6	0.06	31.1	50.3	<0.001
Arthritis	0.9	0.0	0.66	33.7	61.5	<0.001
Number of prescribed medications						
0	92.2	54.5	<0.001	15.1	6.3	0.02
1–3	7.8	45.5		37.1	39.7	
4–6	0.0	0.0		25.8	32.9	
≥7	0.0	0.0		22.0	21.1	

SMI, skeletal muscle index; ASM, appendicular skeletal muscle mass; BMI, body mass index; SD, standard deviation; NS, not significant ($P > 0.05$); NA, not applicable; cardiovascular disease, ischemic heart disease, acute myocardial infarction, stroke, and angina; diabetes, self-reported, Dr diagnosed, FPG ≥ 7.0 mmol/L, or HbA1c ≥ 6.5%; hypertension, BP ≥ 140/90, or already on treatment; hypercholesterolaemia, serum total cholesterol ≥5.5 mmol/L; arthritis, self-reported osteo- or rheumatoid.

$P = 0.88$), % fat (28.6 ± 6.9 versus 28.6 ± 6.9%, $P = 0.95$), ASM (23.9 ± 3.3 versus 24.0 ± 3.2 kg, $P = 0.92$), SMI (8.2 ± 0.9 versus 8.2 ± 0.9 kg/m^2, $P = 0.94$), and grip strength (37.2 ± 8.9 versus 37.6 ± 8.9 kg, $P = 0.37$).

Table 1 shows the characteristics of participants in the final cohort aged 18–40 and aged 65 years and older. Comparing men to women in the younger reference group, men were significantly older (35.5±5.3 versus 31.2±7.3 years, $P = 0.01$), heavier (87.7 ± 15.9 versus 69.3 ± 15.3 kg, $P < 0.001$), and taller (1.8 ± 0.1 versus 1.7 ± 0.1 m, $P < 0.001$) and had higher BMI (27.8 ± 4.6 versus 25.5 ± 5.5 kg/m^2, $P = 0.03$) and SMI (9.1 ± 1.1 versus 6.7 ± 1.2 kg/m^2, $P < 0.001$) than women. Similar to the younger population group, older men were significantly heavier (81.8 ± 13.3 versus 69.4 ± 12.4 kg, $P < 0.001$), and taller (1.7 ± 0.1 versus 1.6 ± 0.1 m, $P < 0.001$) and with higher values for ASM (24.0±3.2 versus 16.1±2.4 kg, $P < 0.001$) and SMI (8.2 ± 0.9 versus 6.4 ± 0.8 kg/m^2, $P < 0.001$) than women. Interestingly, there was no difference in the BMI (27.9±4.2 versus 27.8±4.7 kg/m^2, $P = 0.79$) between the older men and women. The spread of various chronic conditions was shown in Table 1 with higher prevalence of chronic conditions amongst the older population compared to the younger population.

In men, low grip strength (Table 2) was noted in approximately 14% of men aged between 65 and less than 80 years and almost half of men aged 80 years and older. A higher proportion of women (i.e., 33.5%) between 65 years and less than 80 years had low grip strength compared to men. Similarly, 63% of women aged 80 years and older had low grip strength and this was higher in proportion within the same age group of men.

The cut-off points (Table 2) for low muscle mass identified were as follows:

(a) <6.89 kg/m^2 for men and <4.32 kg/m^2 for women by Baumgartner's method;

(b) <7.36 kg/m^2 for men and <5.81 kg/m^2 for women by the 20% gender specific method;

Table 2: The prevalence of low muscle mass and low grip strength in the North West Adelaide Health Study (NWAHS) and Florey Adelaide Male Ageing Study (FAMAS) based upon dual absorptiometry X-ray assessments of appendicular skeletal muscle mass.

	Low grip strength (*n*%)	Low SMI (*n*%)	Low SMI (*n*%)	Low SMI (*n*%)
	EWGSOP Criteria [6]	<2 SD below mean of younger reference group (FAMAS and NWAHS) (Table 1)	Gender specific lowest 20% of study group (FAMAS and NWAHS)	Residuals of linear regression on appendicular lean mass adjusted for fat and height (FAMAS and NWAHS)
		NWAHS + FAMAS men		
Cut-offs	<30 Kg	<6.89 Kg/m^2	<7.36 Kg/m^2	<−2.15 Kg
65 −< 80 (n = 540)	78 (14.4)	38 (7.0)	92 (17.0)	101 (18.7)
80+ (n = 71)	32 (45.1)	9 (12.7)	29 (40.8)	21 (29.6)
Total 65+ (n = 611)	110 (18.0)	44 (7.2)	121 (19.8)	122 (20)
		NWAHS female		
Cutoffs	<20.0 Kg	<4.32 Kg/m^2	<5.81 Kg/m^2	<−1.42 Kg
65 −<80 (n = 313)	105 (33.5)	0 (0)	56 (17.9)	63 (20.1)
80+ (n = 62)	39 (62.9)	1 (1.6)	18 (29)	12 (19.4)
Total 65+ (n = 375)	144 (38.4)	1 (1.6)	74 (19.7)	75 (20)

(c) <−2.15 for men and <−1.42 for women using the linear regression method. The linear regression model was ASM (kg) = −18.24 + 23.09 × height (m) + 0.11 × total fat mass for men and ASM (kg) = −15.84 + 18.18 × height (m) + 0.11 × total fat mass for women.

The prevalence of low muscle mass ranged between 7 and 18% for men aged between 65 and 80 years but increased to between 12 and 29.6% for men aged 80 years and older (Table 2). However, for women, there was no increase in the reported prevalence with increasing age with the prevalence of low muscle mass ranging from 0 to 20.1% in those aged between 65 and <80 years and remaining between 1.6–19.4% in those aged 80 years and older. The prevalence reported by the 20% gender specific method and linear regression method was similar and much higher than the prevalence reported by Baumgartner's method.

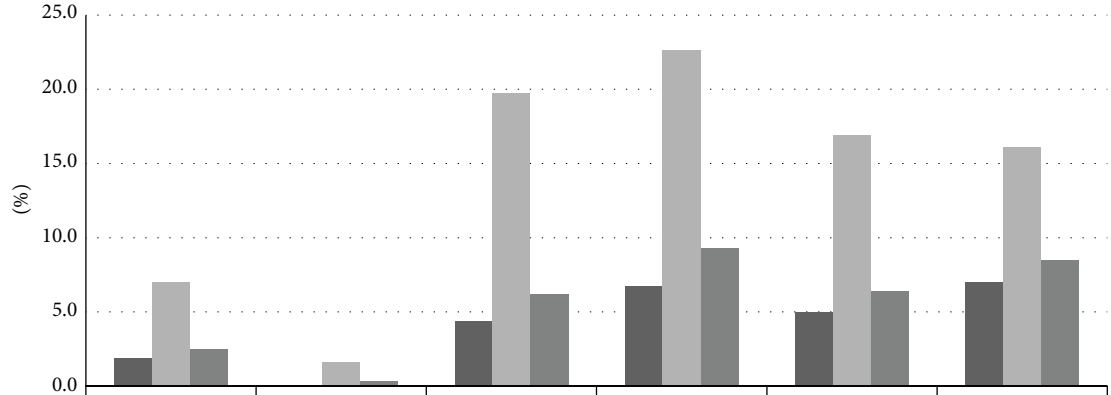
Figure 2 shows that the prevalence of sarcopenia was higher in men (7–19.7%) and women (1.6–22.6%) aged 80 years and older compared to men (1.9–5.0%) and women (2.5–7.0%) aged between 65 and <80 years. The prevalence of sarcopenia in people aged 65 years and older in this study was between 2.5% and 6.4% for men and between 0.3% and 9.3% for women. The overall prevalence of sarcopenia as estimated by Baumgartner's method, the lowest 20% method, and linear regression method was 1.6%, 7.4%, and 7.2%, respectively.

4. Discussion

The key finding from this study is that in combination with grip strength, different methods of determining low muscle mass result in different sarcopenia prevalence. The cut-off points for low muscle mass derived by the gender specific lowest 20% method and the linear regression method yielded similar prevalence rates for low muscle mass and sarcopenia. Also, the cutoffs generated by these two methods, in this study, were similar to those reported by EWGSOP [1]. However, the cutoffs derived by the Baumgartner method (<6.89 kg/m^2 for men and <4.32 kg/m^2 for women), in this study, were much lower than that previously reported (<7.26 kg/m^2 for men and <5.50 kg/m^2 for women) [2]. Our findings of a lower cutoff than that previously reported was similarly noted in an Australian study of women (<4.85 kg/m^2) [10]. Researchers from Korea have recently reported similar SMI cut-off values (6.58 kg/m^2 for men and 4.59 kg/m^2 for women) [17]. The mean ASM for the younger reference population in this study was lower than that reported in the Baumgartner (28.6 kg versus 30.6 kg for men and 18.4 kg versus 20.9 kg) study and this is potentially contributing to the difference in the reported cut-off values [2]. Importantly, the sample size making up the younger reference population in our study was small and so there is a need to derive cutoffs from a larger cohort of younger people before firm conclusions can be reached.

Using the lowest 20% method and linear regression method to define low muscle mass, the prevalence of sarcopenia reported in this study was approximately 6.2% for men and 9% of women aged 65 years. To the best of our knowledge, there has only been one other Australian study which used the lowest 20% method to define low muscle mass [9]. In that study, the overall sarcopenia prevalence rate was 5% [9]. We observed a higher overall prevalence rate at 7.6% and this is likely due to older age group in our study population compared with the population in the other Australian study (72.7 ± 5.7 versus 61.7 ± 7.1 years in men and 73.2 ± 6.0 versus 61.0 ± 6.8 years in women) [9].

Consistent with other studies, the prevalence of low muscle mass increased with age in men and was higher in



	SMI reference of younger group (FAMAS and CASA) + low grip strength		Gender specific lowest 20% (FAMAS and NWAHS) + low grip strength		Residuals of linear regression (FAMAS and NWAHS) + low grip strength	
	Men	Women	Men	Women	Men	Women
60–<80	1.9%	0.0%	4.4%	6.7%	5.0%	7.0%
80+	7.0%	1.6%	19.7%	22.6%	16.9%	16.1%
Total 65+	2.5%	0.3%	6.2%	9.3%	6.4%	8.5%

SMI: skeletal muscle index

Figure 2: Comparison of prevalence rate of sarcopenia as defined by EWGSOP, by using different methods of SMI cut points derivation with a low grip strength (<30 kg for men and <20 kg for women).

those aged 80 years and older compared to those between 65 and <80 years using all three methods [18]. However, in women, this relationship was not seen with the linear regression method, which also accounts for fat mass. Fat mass reduces with increasing age in women but not in men [19]. In this study, the prevalence of low grip strength increased with age in both men and women. A greater proportion of women however met the criteria of low grip strength compared to men in older age. It is well known that a decline in sex hormones with increasing age (andropause and menopause) contributes to decline in strength [20].

Both the FAMAS and the NWAHS cohorts did not include subjects from residential care facilities where the prevalence of sarcopenia is likely higher. The requirement for subjects to attend a hospital based clinic also made it very likely that frail individuals may have been less likely to participate. Therefore, the reported prevalence in this study is likely to be an underestimate of the true prevalence of sarcopenia in the community. Subjects enrolled in these studies were predominantly Caucasian and so the findings from this study are not generalizable to the wider multicultural Australian population. Ethnic specific cutoffs need to be determined and future research including different ethnic population groups is important.

5. Conclusion

To conclude, the prevalence of sarcopenia varies depending on the method used to estimate the cut-off values for low muscle mass. Therefore, a consensus is required to identify the preferred method to define Sarcopenia. This will allow for pooling of research data. However, sarcopenia is common in the community. Given that sarcopenia is linked to morbidity and costs [4], early recognition and intervention through exercise and nutritional programs may contribute to healthy ageing outcomes and so a reduction in health costs [21].

Conflict of Interests

Solomon Yu, Sarah Appleton, Thavarajah Visvanathan, Ian Chapman, Robert Adams, and Gary Wittert have no conflict of interests to declare. Renuka Visvanathan is a member of the Nestle Nutrition Australia Malnutrition in the in the Elderly Board and receives an honorarium for this activity. She has previously participated in the MNA Initiative and the PROT-AGE group which received educational grants from Nestle Inc.

Authors' Contribution

Solomon Yu, Renuka Visvanathan, and Thavarajah Visvanathan were involved with project conception, development of overall research plan, and study oversight. Solomon Yu was responsible for data collection with the CASA population and was also primarily responsible for data analysis with assistance from Sarah Appleton. Solomon Yu led the preparation of this paper with significant contribution from all coauthors.

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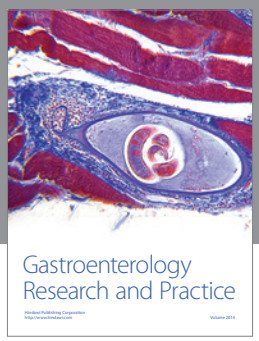

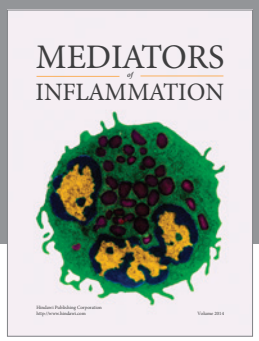

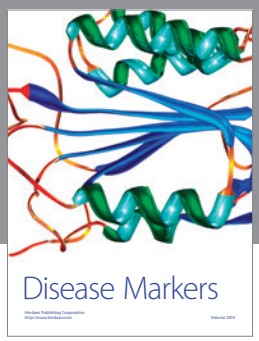

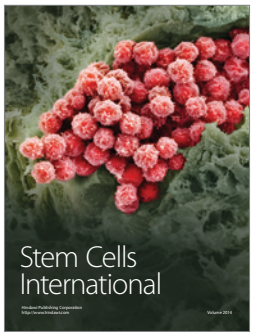

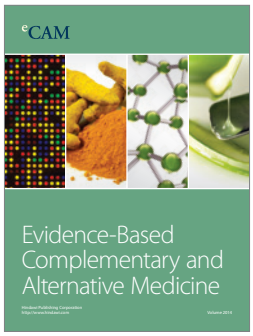

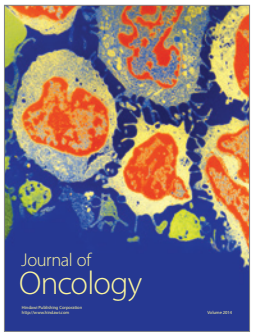

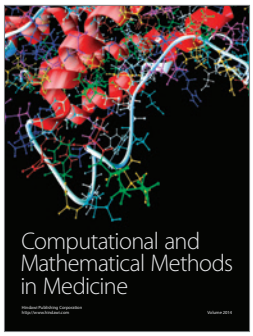

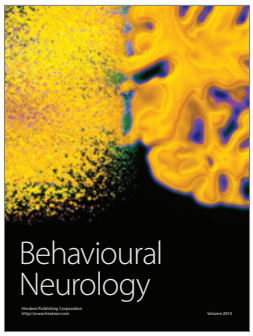

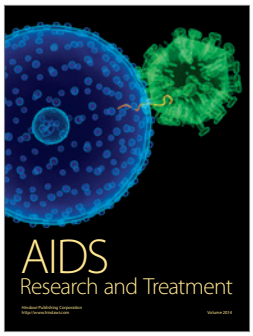

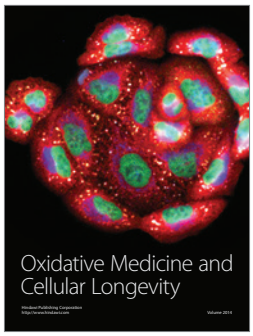

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Appendix 8

Statement of authorship and published manuscript: Chapter 4, Lean body mass, the development and validation of prediction equations in healdthy adults

Statement of Authorship

Title of Paper	Lean Body Mass: the development and validation of prediction equation in healthy adults
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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Solomon Yu		
Contribution to the Paper	Conducted data collection, constructed study database, performed all the statistical analyses, prepare manuscript and acted as key corresponding author		
Signature		Date	2/6/14

Name of Co-Author	Thavarajah Visvanathan		
Contribution to the Paper	Assisted in research supervision and final evalution of the manuscript.		
Signature		Date	24/5/14

Name of Co-Author	John Field		
Contribution to the Paper	Advised on statistical analysis. Assisted with the derivation of the lean body mass prediction equations. Contributed to the final version of the manuscript.		
Signature		Date	22 Mar 2014

Name of Co-Author	Ward C Leigh		
Contribution to the Paper	Assisted in calibrating the dataset on the difference between the dual energy absorptiometry. Evaluated and contributed to manuscript preparation		
Signature		Date	1/04/2014

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Ian Chapman
Contribution to the Paper	Assisted in research supervision and contributed to manuscript.
Signature	Date: 13/5/14

Name of Co-Author	Robert Adams
Contribution to the Paper	Assisted in research supervision. Provided database from NWAHS for validation. Evaluation final version of manuscript
Signature	Date: 5/5/14.

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Contribution to the Paper	Assisted in research supervision. Provided database from FAMAS for validation. Evaluated final version of manuscript.
Signature	Date: 12/05/14

Name of Co-Author	Renuka Visvanathan
Contribution to the Paper	Assisted in conceptualising and study design, supervised development of the project and help with data interpretation, prepare the manuscript.
Signature	Date: 20/5/14

Appendix 8

BMC Pharmacology & Toxicology

RESEARCH ARTICLE

Lean body mass: the development and validation of prediction equations in healthy adults

Solomon Yu[1,2,3,6*], Thavarajah Visvanathan[4], John Field[3], Leigh C Ward[5], Ian Chapman[3], Robert Adams[6], Gary Wittert[3] and Renuka Visvanathan[1,2,3,6]

Abstract

Background: There is a loss of lean body mass (LBM) with increasing age. A low LBM has been associated with increased adverse effects from prescribed medications such as chemotherapy. Accurate assessment of LBM may allow for more accurate drug prescribing. The aims of this study were to develop new prediction equations (PEs) for LBM with anthropometric and biochemical variables from a development cohort and then validate the best performing PEs in validation cohorts.

Methods: PEs were developed in a cohort of 188 healthy subjects and then validated in a convenience cohort of 52 healthy subjects. The best performing anthropometric PE was then compared to published anthropometric PEs in an older (age ≥ 50 years) cohort of 2287 people. Best subset regression analysis was used to derive PEs. Correlation, Bland-Altman and Sheiner & Beal methods were used to validate and compare the PEs against dual X-ray absorptiometry (DXA)-derived LBM.

Results: The PE which included biochemistry variables performed only marginally better than the anthropometric PE. The anthropometric PE on average over-estimated LBM by 0.74 kg in the combined cohort. Across gender (male vs. female), body mass index (< 22, 22- < 27, 27- < 30 and ≥30 kg/m^2) and age groups (50–64, 65–79 and ≥80 years), the maximum mean over-estimation of the anthropometric PE was 1.36 kg.

Conclusions: A new anthropometric PE has been developed that offers an alternative for clinicians when access to DXA is limited. Further research is required to determine the clinical utility and if it will improve the safety of medication use.

Keywords: Lean body mass, Weight, Older people, Drugs

Background

With increasing age, there is a decline in lean body mass (LBM) and very often an increase in adiposity [1]. The decline in LBM may also be accompanied by a reduction in physical function and when a pathological threshold is reached, the person is said to have sarcopenia [2]. In recent times, sarcopenia has been recognized as an independent predictor of drug related adverse outcomes in the oncology setting where muscle wasting can be common [3,4]. Drug-related adverse effects are defined as medical events related to the use of medication which may result in disability, hospital admissions or death [5]. In patients with cancer, the use of LBM might be superior to body surface area (BSA) [6]. For example, in a prospective study of colon cancer patients treated with 5-fluorouracil (5-FU), the incidence of dose limiting toxicity was examined with respect to conventional dosing of 5-FU/m^2 of BSA versus 5-FU/kg of LBM. LBM was a better predictor of toxicity ($p = 0.011$) but not BSA [6]. Similar findings have been reported in other studies [7,8]. In anaesthesia, propofol pharmacokinetic parameters scaled linearly to LBM is also said to provide for improved dosing in adults [9]. Therefore, accurate measurement of LBM may have clinical application in improving drug prescribing safety and efficacy, especially in older people where loss of lean mass is common.

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A major impediment to the routine clinical use of LBM is the reliance on relatively inaccessible or expensive methods of body composition measurements. Computed tomography (CT), magnetic resonance imaging and dual absorptiometry x-ray (DXA) are used to assess LBM but these methods may be difficult to access in clinical practice (e.g. frail or rural patients) [10]. Although the bioelectrical impedance analysis (BIA) method is portable, it still requires the purchase of special equipment and it's accuracy is also dependent on many other factors such as state of hydration, food intake and exercise [11].

Total body weight consists of fat mass and fat free mass. Fat free mass (FFM) consists of bone, muscle, vital organs and extracellular fluid. LBM differs from FFM in that lipid in cellular membranes are included in LBM but this accounts for only a small fraction of total body weight (up to 3% in men and 5% in women) [12]. In the literature, bone mass has at times been included in LBM and at other times not included [4,13].

Anthropometric-based prediction equations (PEs) have been examined as an alternative in measuring LBM in settings where access to these accurate methods is limited. In a very recent study of older (≥70 years) Australian men, FFM as estimated by three PEs were compared to FFM as estimated by DXA (FFM_{DXA}) [14]. The three PEs were the Heitmann, Janmahasatian and Deurenberg equations as shown below:

Heitmann equation [15]*:*

$$\text{Body fat (kg)}_{\text{male}} = (0.988 \times \text{BMI}) + (0.242 \times \text{weight}) + (0.094 \times \text{age}) - 30.180$$

$$\text{Body fat (kg)}_{\text{female}} = (0.988 \times \text{BMI}) + (0.344 \times \text{weight}) + (0.094 \times \text{age}) - 30.180.$$

Janmahasatian equation [12] *:*

$$\text{FFM (kg)}_{\text{female}} = (9270 \times \text{weight}) / (8780 + (244 \times \text{BMI})$$

$$\text{FFM (kg)}_{\text{male}} = (9270 \times \text{weight}) / (6680 + (216 \times \text{BMI})$$

Deurenberg equation [16]*:*

$$\text{Body fat}(\%) = (1.2 \times \text{BMI}) + (0.23 \times \text{Age}) - (10.8 \times \text{Sex}) - 5.4$$

Male = 1, Female = 0

For two of the PEs (Heitmann and Deurenberg equations), FFM was calculated by subtracting fat mass from total body mass. In defining the FFM and LBM, the authors in that study proposed that FFM and LBM could be used interchangeably. Mitchell et al. reported that FFM as estimated by Deurenberg equation had the smallest mean difference and overestimated FFM_{DXA} for overweight men but underestimated FFM_{DXA} for all other body mass index (BMI) subgroups [14]. The Heitmann and Janmahasatian equations, on the other hand, overestimated FFM_{DXA} across various BMI categories [14].

The addition of biochemistry variables might improve the performance of prediction equations but few studies have examined this. Creatine Kinase (CK) is found predominantly in skeletal muscle and serum levels were associated with the lean muscle mass [17]. There has only been one study evaluating the relationship between LBM and plasma creatine kinase activity (CK) and a weak and partial correlation ($r < 0.262$) between log CK and LBM was reported [18]. Serum albumin has also been reported to reflect protein reserve and lower albumin levels have been shown to be associated with loss of lean mass [19].

Therefore, the aims of this study were to develop and validate PEs for LBM with anthropometric and biochemistry variables against DXA.

Methods

The Central Northern Adelaide Health Service Ethics of Human Research Committee approved this study. All participants provided written informed consent.

Study cohorts

Four study cohorts were investigated in this study: a) the Cytokine, Adiposity, Sarcopenia and Ageing (CASA) cohort; b) the validation cohort (VC); c) the North West Adelaide Health Study (NWAHS) cohort and d) the Florey Adelaide Male Ageing Study (FAMAS) cohort. CASA was used to derive the PEs for LBM which included anthropometric and biochemistry variables. The selected LBM PEs were then validated in a second independent cohort, the VC (n = 52). As sarcopenia is more prevalent in older populations, validation of the best performing PE and other published FFM PEs (Heitmann, Janmahasatian and Deurenberg equations) were then undertaken in the larger population representative NWAHS and FAMAS cohorts (n = 2287, age ≥ 50 years).

CASA

195 population representative healthy subjects (age 18 to 83 years) were recruited from the western suburbs of Adelaide [20]. The inclusion criteria were: being aged 18 and above, able to comply with study protocol and weight stable over the last 3 months. We excluded those with a serious medical illness, an acute illness in the pass 3 months or in the 2 weeks following blood sampling, an inability to stop medications for 3 days prior to blood sampling, being in receipt of vaccinations and pregnancy. In undertaking the analysis, data from 7 subjects were excluded due to haemolysed or insufficient blood samples.

VC

This was a convenience sample of 52 healthy subjects (age 22 – 83 years) recruited through advertisement for another study [21]. Subjects with known medical illness including gastrointestinal disease or symptoms, significant respiratory, renal or cardiac disease and who were pregnant were excluded from this study.

NWAHS

This is a longitudinal study of community dwelling adults aged eighteen years and older. The population which is a representative biomedical cohort of predominantly of mixed European descent has been described in detail previously [22]. DXA scans were offered to NWAHS participants who were aged ≥ 50 years at follow up (median time = 4 years). Participants with complete anthropometric and DXA measurements at follow up (2004–06) aged ≥50 were included in this analysis (n = 1575).

FAMAS

This male only cohort has also been described in detail elsewhere [23]. The recruitment process was very similar to that used for the NWAHS and so the men in FAMAS were comparable with men in the same age groups from the NWAHS study and of mixed European descent [24]. DXA measurements at baseline (2002–2005) were obtained on 700 participants aged 50 years and over.

Measurements

Anthropometry

Height (m) was measured without shoes using a wall-mounted SECA stadiometer to the nearest 0.1 cm. Weight (kg) was measured wearing light clothing to the nearest 0.1 kg (A&D FV platform scales 0.5 – 150 kg). Body mass index (BMI, weight/height^2) was calculated. The healthy BMI for older people is said to be between 22–27 kg/m^2 [25]. Caucasians with BMI > 30 kg/m^2 were classified as obese [26].

Dual Energy X-ray Absorptiometry (DXA)

DXA analysis in all cohorts measured 3 compartments of the total body composition; fat mass, LBM and bone mineral content. For the purpose of this study, LBM refers to soft tissues and muscle mass, but excludes fat and bone mass. CASA: A Lunar PRODIGY whole-body scanner (GE Medical Systems, Madison, WI), in conjunction with Encore 2002 software, was used to estimate LBM. The majority of subjects underwent DXA within 2 hours of attending the morning clinic when blood sampling occurred. VC: A Norland densitometer XR36 (Norland Medical Systems, Fort Atkinson, Wisconsin, USA), in conjunction with Illuminatus 4.2.4a software, was used to estimate LBM. The DXA was performed on a separate study day but within 2 weeks of blood sampling and given that the subjects were healthy, it is unlikely that there would have been significant change in body composition within that time frame. To account for differences between machines, LBM data from the VC had a correction factor applied to convert the data to Lunar equivalent [27]. NWAHS and FAMAS: The fan-beam Lunar PRODIGY (GE Medical Systems, Madison, WI) in conjunction with Encore 2002 software and a pencil-beam DPX + (GE Medical Systems, Madison, WI) in conjunction with LUNAR software version 4.7e were used. Cross-calibration analysis had been undertaken and no differences between these 2 densitometers were reported [28].

Blood analyses

For both the CASA and VC cohorts, a venous sample was obtained from each participant after an overnight fast. Both cohorts were asked to refrain from smoking, consuming alcohol or vigorous exercise in the 24 hours before the clinic appointment. Last regular medications were taken the day before and the morning dose was held until after venous sampling. For CASA, the blood was placed in ethylenediaminetetraacetic acid (EDTA) tubes and transported immediately to the Institute for Medical and Veterinary Sciences Laboratories (IMVS) in South Australia for analysis. The blood was centrifuged at 5000 rpm for 7 minutes and analyzed immediately at 37°C. For the VC, samples that had been centrifuged and stored at −70°C were transferred to be processed by the IMVS using the same methodology. The measured coefficients of variation (CV) were: alanine transferase (ALT, 1.98%), aspartate transaminase (AST, 2.8%),albumin (2.8%), creatinine (3%), lactate dehydrogenase (LDH, 2.2%), creatinine kinase (CK, 2.2%) and high sensitivity C-reactive protein (hsCRP, 1.4%). A Beckman Coulter AU 2700 was used to perform the blood analysis and the methods, reagents and calibration were as per manufacturer instructions.

Statistical analysis

Demographic characteristics in both groups were expressed as mean ± standard deviation (SD). Independent samples *t* test was used to compare means between the two cohorts. Differences between methods of LBM measurements in the same cohort were examined by paired *t* test. PEs for LBM were developed from CASA where the independent variable was DXA derived LBM. The initial 10 independent variables were gender, age, weight, height, body mass index, albumin, AST, LDH, CK and hsCRP. The best PEs (as assessed by adjusted R^2: the proportion of the variance of the dependent variable accounted for by the independent variables, and adjusted for the number of independent variables) were developed considering up to 6 equations with *n* predictors.

For each *n*, the PE for validation was selected by considering the adjusted R^2 value and likely clinical utility. In the VC, LBM was calculated from the developed prediction equations (LBM_{PE}) and compared with DXA derived LBM (LBM_{DXA}).

The anthropometric PE was also compared to other known PEs [12,15,16] in the NWAHS and FAMAS cohorts.

To assess the accuracy and predictive performance of the prediction equations against LBM_{DXA}, a regression analysis as proposed by Lin [29] was undertaken and the concordance correlation coefficient (ρ_c) was derived. ρ_c measures how much the data deviates from the line of identity representing congruence between the methods. It is a product of Pearson correlation (ρ) and bias correction factor (C_b): $\rho_c = \rho\ C_b$ [30].

In addition, to assess the level of agreement between the two methods, Bland-Altman analysis was performed to obtain the 95% limits of agreement [31]. Furthermore, the goodness of fit with root mean square error (RMSE) and bias (mean error [ME]) was also determined. RMSE and ME were calculated according to the method of Sheiner and Beal [32]. When the 95% confidence interval of the ME includes 0 (i.e. no error), it indicates that the model is not biased. In this study, mean difference was taken to be the same as ME. This gives an estimation of R^2 and the standard error of the estimate [SEE]. SPSS 11.5 for Windows software (SPSS, Inc., Chicago, IL) and the R statistical language (R Foundation for Statistical Computing, Vienna, Austria) were used for the analyses. $P < 0.05$ was considered statistically significant.

Results

The CASA and VC cohorts were similar in age (CASA mean [SD] 49.2 [17.0] vs. VC 50.6 [15.7] years), but younger than the NWAHS (64.7 [9.84] years) and FAMAS (62.3 [8.2] years) cohorts. The BMI (23.7 [2.3] vs. 26.7 [5.2] kg/m^2) and CK (93.3 [54.7] vs. 114.3 [66.0] U/L), were significantly lower in the VC compared to the CASA. LDH (194.4 [37.8] vs. 175.0 [37.4] U/L) and albumin (40.4 [2.5] vs. 39.1 [3.1] g/L) were significantly higher in the VC compared to the CASA. No significant differences between the two cohorts were noted for hsCRP or LBM. The BMI of subjects in the NWAHS and FAMAS studies were higher at 28.2 [4.8] and 28.6 [4.6] kg/m^2 respectively.

Based on adjusted R^2 and potential clinical utility, the following PEs were selected for further validation in the VC:

$$LBM_{PE1} = 22.93 + 0.68(weight) - 1.14(BMI) - 0.01(age) + 9.94(if\ male)\ SEE = 3.61, R^2 = 90.7$$
$$LBM_{PE2} = 22.06 + 0.67(weight) - 1.11(BMI) + 9.76(if\ male) + 0.01(CK)\ SEE = 3.56, R^2 = 91.0$$
$$LBM_{PE3} = 21.19 + 0.67(weight) - 1.04(BMI) + 9.51(if\ male) - 0.56(CRP) + 0.01(CK)\ SEE = 3.47, R^2 = 91.4$$
$$LBM_{PE4} = 23.17 + 0.64(weight) - 0.91(BMI) + 9.45(if\ male) + 0.02(CK) - 0.58(CRP) - 0.02(LDH)\ SEE = 3.38, R^2 = 91.9$$

Table 1 compares $LBM_{PE1\text{-}4}$ to LBM_{DXA} in the VC. LBM predicted by all PEs was highly correlated with LBM_{DXA}. Concordance correlations, a measure of the degree to which the data lie on the line of identity, were all around 0.9 and similar to the Pearsons correlation coefficient. All PEs over-estimated LBM_{DXA}, ranging from 1.9% for $PE_1$to 4.1% for PE_4. The limits of agreement were similar for all PEs, approximately ± 15%. With increasing number of variables, there were reducing RMSE and mean error indicating improving precision and reducing bias. Because of the costs involved with blood investigations and the marginal benefits, only the anthropometric $PE_1$was selected for further comparison in the combined NWAHS and FAMAS cohorts (Tables 2, 3 and 4). Furthermore, biochemistry was not readily available from those cohorts.

Table 2 compares the performance of various PEs including PE_1 against LBM_{DXA} in the total combined NWAHS and FAMAS cohorts as well as in the two gender groups, men and women. All PEs over-estimated the LBM_{DXA} in the total group. PE_1 demonstrated a lower mean error and RMSE score than the Heitmann and Janmahasatian equations in the total population, men and women cohorts. The Deurenberg equation performed the best in the total population with the lowest mean error and RMSE. However, when reviewed within gender groups, PE_1 performed better than the Deurenberg equation in women where both equations over-estimated LBM. In men, the Deurenberg equation under-estimated LBM whilst all other equations over-estimated LBM.

Table 3 compares the performance of the various PEs across age groups (60–64, 65–79, ≥80). PE_1 consistently over-estimated LBM_{DXA} across the age groups but performed better (lowest ME, RMSE values and higher concordance correlation coefficient) than the Janmahasatian and Heitmann equations. The Deurenberg equation did not perform as well as PE_1 in the 50- < 65 years age group and the ≥ 80 years age group and over-estimated LBM in the 50- < 65 years age group but under-estimated LBM in the other two age groups.

Table 4 compares the performance of the various PEs across various BMI groups. Once again, PE_1 has the smallest ME and RMSE compared with the Janmahasatian and Heitmann equations across all the BMI groups analyzed but all of these consistently over-estimated LBM_{DXA} across the various BMI groups. PE_1, in comparison with the Deurenberg equation has a lower ME and RMSE in the obese BMI (>30 kg/m^2) and underweight BMI

Table 1 Validation of PE LBM in healthy adults from the Cytokine, Adiposity, Sarcopenia and Ageing (CASA) study cohort (n = 195) against DXA derived LBM in the validation cohort (n = 52)

	Mean (SD), kg	Mean error (95%CI), kg	P-value for mean error	R	ρ_c (95% CI) [C_b]	95% limits of agreement	RMSE (95% CI), kg
Total (n = 52)							
LBM_{DXA}	46.2 (9.49)						
LBM_{PE1}	48.1 (8.93)	1.88 (0.79, 2.97)	0.001	0.911*	0.891 (0.820, 0.935) [0.977]	−9.72, 5.96 (−20.7 to 12.6%)	4.32 (2.84, 5.80)
LBM_{PE2}	47.9 (8.95)	1.69 (0.62, 2.75)	0.003	0.915*	0.899 (0.832, 0.940) [0.982]	−9.20, 5.83 (−19.9 to 12.6%)	4.15 (2.70, 5.60)
LBM_{PE3}	47.7 (9.13)	1.50 (0.44 ,2.57)	0.006	0.917*	0.904 (0.840, 0.943) [0.986]	−8.99, 5.98 (−19.5 to 13.0%)	4.07 (2.63, 5.51)
LBM_{PE4}	47.1 (8.96)	0.86 (−0.22, 1.94)	0.114	0.914*	0.908 (0.846, 0.946) [0.994]	−8.44, 6.72 (−18.3 to 14.6%)	3.93 (2.51, 5.35)

*P-value <0.001, R = correlation, SD = Standard Deviation.
RMSE = root mean squared prediction error, CI = confidence interval, R = Pearson Correlation Coefficient, C_b = Bias Correction Factor, ρ_c = Concordance Correlation Coefficient.

(< 22 kg/m^2) groups. Interestingly, the Deurenberg equation has less bias and better precision than PE_1 in predicting LBM_{DXA} in the 22-27 kg/m^2 BMI group. The Deurenberg equation overestimated LBM_{DXA} except in the underweight and obese categories.

Discussion

In this study, prediction equations for LBM were developed and validated. It was hypothesized that the addition of biochemistry variables would result in an improvement in the performance of the PEs and this was seen. However, the improvement was marginal and insufficient to justify the additional costs.

A significant finding from this study wasthe development of a new anthropometric PE (PE_1) for LBM: *LBM = 22.932326 + 0.684668 (weight) -1.137156 (BMI) -0.009213 (age) + 9.940015 (if male)*. The close approximation to LBM_{DXA} generated by this equation was reflected by its small bias (ME = 0.74 kg) and precision (RMSE = 3.73 kg). It overestimated LBM_{DXA} across gender, age and BMI groups. This PE may be useful in care settings where access to DXA may be limited, providing clinicians a

Table 2 Performance of the CASA (LBM_{PE1}) and previously published FFM prediction equations in the NWAHS and FAMAS cohorts (age 50 years and over) in the combined cohort and by gender

	Mean (SD), kg	Mean error (95%CI), kg	P-value for mean error	R	ρ_c (95% CI) [C_b]	95% limits of agreement	RMSE (95% CI), kg
Total (n = 2287)							
LBM_{DXA}	50.62 (10.8)						
Heitmann equation	54.30 (10.7)	3.68 (3.53, 3.83)	<0.001	0.940*	0.888 (0.880, 0.896) [0.945]	−3.77, 11.1	5.24 (4.97, 5.51)
Janmahasatian equation	54.23 (11.0)	3.61 (3.46, 3.76)	<0.001	0.943*	0.884 (0.884, 0.899) [0.946]	−3.78, 11.0	5.17 (4.90, 5.44)
Deurenberg equation	50.64 (10.1)	0.02 (−0.14, 0.19)	0.777	0.931*	0.928 (0.923, 0.934) [0.998]	−7.89, 7.93	3.95 (3.70, 4.20)
LBM_{PE1}	51.36 (10.6)	0.74 (0.59, 0.89)	<0.001	0.942*	0.939 (0.934, 0.944) [0.998]	−6.58, 8.06	3.73 (2.48, 4.98)
Men (n = 1436)							
LBM_{DXA}	57.09 (7.50)						
Heitmann equation	60.56 (7.80)	3.46 (3.25. 3.67)	<0.001	0.863*	0.782 (0.764, 0.800) [0.906]	−11.5, 4.57	5.30 (4.93, 5.67)
Janmahasatian equation	61.18 (6.80)	4.09 (3.89, 4.29)	<0.001	0.852*	0.728 (0.707, 0.747) [0.853]	−12.0, 3.82	5.69 (5.32, 6.06)
Deurenberg equation	56.76 (6.80)	- 0.34 (−0.55, -0.12)	0.002	0.838*	0.834 (0.818, 0.848) [0.995]	−7.92, 8.60	4.14 (3.85, 4.43)
LBM_{PE1}	58.22 (6.11)	1.12 (0.92, 1.33)	<0.001	0.851*	0.822 (0.806, 0.837) [0.851]	−6.78, 9.02	4.11 (3.80, 4.42)
Women (n = 851)							
LBM_{DXA}	39.70 (5.30)						
Heitmann equation	43.74 (5.55)	4.04 (3.83, 4.26)	<0.001	0.833*	0.651 (0.620, 0.680) [0.782]	−10.3, 2.26	5.12 (4.75, 5.49)
Janmahasatian equation	42.50 (5.39)	2.81 (2.60, 3.01)	<0.001	0.837*	0.722 (0.693, 0.749) [0.872]	−8.91, 3.29	4.14 (3.83, 4.45)
Deurenberg equation	40.32 (4.90)	0.63 (0.39, 0.87)	<0.001	0.759*	0.751 (0.721, 0.779) [0.990]	−7.75, 6.49	3.61 (3.29, 3.93)
LBM_{PE1}	39.78 (5.11)	0.08 (−0.12, 0.28)	0.433	0.835*	0.835 (0.813, 0.854) [0.999]	−5.91, 6.07	2.99 (2.74, 3.24)

Mean Error = DXA-PE; LBM, Lean Body Mass; DXA, Dual X-ray absorptiometry; RMSE, Root Mean Square Error; CI, Confidence interval; SD, Standard Deviation; R, Pearson Correlation; C_b = Bias Correction Factor; ρ_c = Concordance Correlation Coefficient; *p-value <0.001.

Table 3 Performance of the CASA (LBM_{PE1}) and previously published FFM prediction equations in the NWAHS and FAMAS cohorts (age 50 years and over) across various age groupings

	Mean (SD), kg	Mean error (95%CI), kg	P-value for mean error	R	ρ_c (95% CI) [C_b]	95% limits of agreement	RMSE (95% CI), kg
Age 50–64, years (n = 1265)							
LBM_{DXA}	52.27 (11.2)						
Heitmann equation	56.47 (10.8)	4.20 (3.99, 4.40)	<0.001	0.944*	0.879 (0.868, 0.890) [0.932]	−11.6, 3.23	5.60 (5.26, 5.95)
Janmahasatian equation	55.62 (11.2)	3.35 (3.15, 3.55)	<0.001	0.948*	0.907 (0.897, 0.915) [0.956]	−10.6, 3.85	4.92 (4.61, 5.23)
Deurenberg equation	53.15 (10.0)	0.87 (0.66, 1.09)	<0.001	0.938*	0.929 (0.921, 0.936) [0.990]	−8.72, 6.98	4.02 (3.73, 4.31)
LBM_{PE1}	52.77 (10.7)	0.50 (0.30, 0.70)	<0.001	0.948*	0.946 (0.939, 0.951) [0.998]	−6.68, 7.68	3.62 (3.36, 3.88)
Age 65–79, years (n = 882)							
LBM_{DXA}	49.09 (9.91)						
Heitmann equation	52.23 (10.0)	3.14 (2.90, 3.38)	<0.001	0.933*	0.887 (0.873, 0.899) [0.951]	−10.5, 4.18	4.82 (4.35, 5.29)
Janmahasatian equation	53.03 (10.5)	3.93 (3.69, 4.18)	<0.001	0.933*	0.862 (0.846, 0.876) [0.925]	−11.5, 3.66	5.46 (4.97, 5.95)
Deurenberg equation	48.19 (9.14)	−0.90 (−1.15, -0.65)	<0.001	0.924*	0.916 (0.905, 0.926) [0.993]	−6.70, 8.50	3.90 (3.45, 4.35)
LBM_{PE1}	50.20 (10.2)	0.98 (0.73, 1.22)	<0.001	0.929*	0.925 (0.915, 0.934) [0.995]	−6.57, 8.53	3.90 (3.48, 4.32)
Age ≥80, years (n = 140)							
LBM_{DXA}	44.48 (8.64)						
Heitmann equation	46.71 (9.20)	2.23 (1.60, 2.85)	<0.001	0.929*	0.902 (0.868, 0.928) [0.969]	−9.05, 4.59	4.06 (3.20, 4.92)
Janmahasatian equation	48.46 (10.1)	3.97 (3.29, 4.66)	<0.001	0.936*	0.850 (0.806, 0.883) [0.906]	−11.4, 3.46	5.43 (4.31, 6.55)
Deurenberg equation	42.46 (8.41)	−2.03 (−2.58, -1.48)	<0.001	0.937*	0.911 (0.880, 0.934) [0.971]	−3.97, 8.03	3.61 (2.85, 4.37)
LBM_{PE1}	45.84 (9.81)	1.36 (0.80, 1.93)	<0.001	0.941*	0.923 (0.897, 0.943) [0.981]	−5.39, 8.11	3.63 (2.90, 4.36)

Mean Error = DXA-PE; LBM, Lean Body Mass; DXA, Dual X-ray absorptiometry; RMSE, Root Mean Square Error; CI, Confidence interval; SD, Standard Deviation; R, Pearson Correlation; C_b = Bias Correction Factor; ρ_c = Concordance Correlation Coefficient; *p-value <0.001.

practical alternative to assess LBM. Furthermore, it also provides a bedside option in hospitals for ill and frail patients where transport for DXA assessment may be difficult. Whilst BIA may be simple technique to be used at the beside, BIA may be affected by clinical factors such as ascites, hydration status, food intake and exercise and cannot be used in older people with pacemakers [11]. Skin fold measurements may be a cheaper option but the accuracy is operator dependent and the loss of subcutaneous tissue in older people may also affect accuracy [33].

Interestingly, the Deurenberg equation appeared to have less bias with a ME of 0.02 kg but similar precision with a RMSE of 3.95 when compared to the newly developed PE. However, across gender, age and BMI groups, it at times over-estimated and at other times under-estimated the LBM_{DXA} [14]. The newly developed PE_1 appeared to have better precision (smaller RMSE) and less bias (lower ME) than the Deurenberg equation only in women and in obese older individuals. In clinical settings where the dose normalization to LBM is required, an overestimation of LBM could potentially lead to higher incidence of dose limiting toxicity. Sarcopenia was an important predictor of toxicity in women with metastatic cancer and colon cancer receiving chemotherapy [4,6]. It was suggested that chemotherapy dose normalization to LBM may reduce the excess toxicity in women. PE_1 in our study potentially offers a more accurate estimation of LBM over Deurenberg equation in women and obese individuals and may have clinical utility in this two patient population groups.

This study had several limitations. Only 6% of the study population was under-weight with a BMI < 22 kg/m^2 and therefore, it remains important to validate this newly developed PE in an under-weight population where sarcopenia is likely to be common. Furthermore, only Caucasians were studied and therefore generalizing these results to other ethnic communities is not possible and ethnic specific PEs will need to be developed. Different DXA machines were used in the CASA and VC cohort studies. This may have affected the results as even in the same person, reported measurements of the same tissue mass can be different with different DXA machines [34]. The researchers adjusted for the difference between the machines in the validation aspects of this study but clearly, it would have been preferable to use the same DXA machine in both cohorts. The use of other anthropometry measurements such as calf or arm circumference may improve the performance of prediction equations and needs to be explored in future studies.

Table 4 Performance of the CASA (LBM_{PE1}) and previously published FFM prediction equations in the NWAHS and FAMAS cohorts (age 50 years and over) across various body mass index groupings

	Mean (SD), kg	Mean error (95%CI), kg	P-value for mean error	R	ρ_c (95% CI) [C_b]	95% limits of agreement	RMSE (95% CI), kg
BMI < 22 kg/m^2 (n = 135)							
LBM_{DXA}	42.45 (8.85)						
Heitmann equation	44.85 (7.65)	2.40 (1.85, 2.96)	<0.001	0.932*	0.885 (0.847, 0.914) [0.949]	−4.12, 8.92	4.04 (3.21, 4.87)
Janmahasatian equation	43.72 (9.26)	1.27 (0.77, 1.77)	<0.001	0.946*	0.937 (0.914, 0.955) [0.989]	−4.65, 7.19	3.21 (2.55, 3.87)
Deurenberg equation	41.26 (8.04)	−1.18 (−1.77, -0.60)	<0.001	0.921*	0.909 (0.876, 0.933) [0.986]	−8.04, 5.68	3.62 (2.86, 4.36)
LBM_{PE1}	43.52 (9.04)	1.08 (0.57, 1.59)	<0.001	0.944*	0.937 (0.913, 0.955) [0.993]	−4.92, 7.08	3.18 (2.53, 3.83)
BMI 22- < 27 kg/m^2 (n = 847)							
LBM_{DXA}	47.45 (9.18)						
Heitmann equation	50.67 (8.67)	3.22 (2.99, 3.44)	<0.001	0.933*	0.874 (0.860, 0.888) [0.938]	−3.42, 9.86	4.62 (4.26,4.98)
Janmahasatian equation	50.81 (9.71)	3.36 (3.13, 3.59)	<0.001	0.937*	0.880 (0.866, 0.893) [0.939]	−3.41, 10.1	4.77 (4.39, 5.15)
Deurenberg equation	47.91 (8.68)	0.45 (0.22, 0.68)	0.001	0.928*	0.925 (0.915, 0.934) [0.997]	−6.42, 7.32	3.46 (3.16, 3.76)
LBM_{PE1}	48.64 (9.45)	1.19 (0.96, 1.41)	<0.001	0.938*	0.930 (0.920, 0.938) [0.992]	−5.41, 7.79	3.51 (3.20, 3.82)
BMI 27- < 30 kg/m^2 (n = 596)							
LBM_{DXA}	52.00 (9.83)						
Heitmann equation	55.65 (9.48)	3.65 (3.36, 3.95)	<0.001	0.929*	0.867 (0.847, 0.883) [0.933]	−3.65, 10.9	5.16 (4.69, 5.63)
Janmahasatian equation	56.11 (9.75)	4.12 (3.83, 4.41)	<0.001	0.932*	0.857 (0.837, 0.874) [0.919]	−3.08, 11.3	5.47 (4.97, 5.97)
Deurenberg equation	52.58 (9.23)	0.59 (0.30, 0.88)	<0.001	0.928*	0.925 (0.912, 0.935) [0.996]	−6.72, 7.90	3.70 (3.35, 4.05)
LBM_{PE1}	52.80 (9.69)	0.81 (0.52, 1.09)	<0.001	0.933*	0.929 (0.918, 0.939) [0.997]	−6.37, 7.99	3.67 (3.31, 4.03)
BMI ≥30 kg/m^2 (n = 709)							
LBM_{DXA}	54.80 (11.7)						
Heitmann equation	59.30 (11.7)	4.50 (4.19, 4.80)	<0.001	0.937*	0.867 (0.847, 0.883) [0.933]	−3.80, 12.8	6.12 (5.53, 6.71)
Janmahasatian equation	58.93 (11.0)	4.13 (3.83, 4.43)	<0.001	0.937*	0.857 (0.837, 0.974) [0.919]	−4.02, 12.3	5.80 (5.22, 6.38)
Deurenberg equation	54.07 (10.6)	−0.74 (−1.08, -0.39)	<0.001	0.917*	0.925 (0.912, 0.935) [0.996]	−10.0, 8.55	4.70 (4.14, 5.26)
LBM_{PE1}	54.88 (11.3)	0.08 (−0.23, 0.38)	0.628	0.936*	0.929 (0.918, 0.939) [0.997]	−8.15, 8.31	4.11 (3.61, 4.61)

Mean Error = DXA-PE; LBM, Lean Body Mass; DXA, Dual X-ray absorptiometry; RMSE, Root Mean Square Error; CI, Confidence interval; SD, Standard Deviation; R, Pearson Correlation; C_b = Bias Correction Factor; ρ_c = Concordance Correlation Coefficient.
*p-value <0.001.

Conclusions

This study describes the development of a new prediction equation for LBM as estimated by DXA. This new PE consistently over-estimates across gender, age and BMI groups. There remains a need to confirm these findings in older and leaner cohorts, cohorts with diseases (e.g. renal failure), as well as other cohorts with varying ethnicity. The anthropometric PE is an alternative when access to DXA is difficult and this might occur with home bound frail older people as well as people residing in rural areas. The availability of simple and accuratemethods to estimate LBM might be the necessary catalyst required to support better prescribing to limit toxicity in the oncology setting.

Competing interests

TV, JF, JCW, IC, RA and GW have no conflicts of interest; SY received scholarship from University of Adelaide, Faculty of Health Sciences, Divisional Scholarship to support his PhD studies; RV received research grants from: i) University of Adelaide, Faculty of Health Sciences, Establishment Grant; ii) University of Adelaide, Faculty of Health Sciences, Early Career Grant; iii) Vincent Fairfax Family Foundation Research Fellowship through the Royal Australasian College of Physicians; iv) Bernie Lewis Foundation Grant through the Hospital Research Foundation; RVis a member of the Nestle Nutrition Australia Malnutrition In The Elderly Board and receives a honorarium for this activity. She has previously also been a member of Mini Nutritional Assessment (MNA) International Group to refine the MNA and more recently

attended the International Consensus Meeting On Protein Intake In The Elderly and both meetings were undertaken independently but supported by an educational grant from Nestle Pty Ltd. She has no other relationships or activities that could appear to have influenced the submitted work. LCW has provided consultancy services to ImpediMed Ltd. The authors declare that they have no competing interests.

Authors' contributions

RV, TV, RA and SY contributed to the initial study design. SY contributed to the data collection, data entry and statistical analysis. JF undertook the statistical analysis required to develop the PEs. LW performed the LBM data correction to adjust differences between dual x-ray absorptiometry machines in this study. All authors contributed to the interpretation of the analysis and preparation of this manuscript. All authors read and approved the final manuscript.

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Appendix 9

Statement of authorship and published manuscript: Chapter 5, Appendicular skeletal muscle mass: development and validation of anthropometric prediction equations

Statement of Authorship

Title of Paper	Appendicular Skeletal Muscle Mass: Development And Validation of Anthropometric Prediction Equations
Publication Status	◉ Published, ○ Accepted for Publication, ○ Submitted for Publication, ○ Publication style
Publication Details	Visvanathan R, Yu S, Field J, Chapman I, Adams R, Wittert G and Visvanathan T. Appendicular Skeletal Muscle Mass: Development and validation of prediction equations. The Journal of Frailty & Aging, 2012; 1(4):147-151.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Solomon Yu		
Contribution to the Paper	Involved in study design, conducted data collection, constructed study database, performed all the statistical analyses, involved in data interpretation and prepare the manuscript.		
Signature		Date	2/6/14

Name of Co-Author	Renuka Visvanathan		
Contribution to the Paper	Assisted in conceptualising and study design, supervised development of the project and help with data interpretation, prepare the manuscript.		
Signature		Date	20/5/14

Name of Co-Author	John Field		
Contribution to the Paper	Advised on statistical analysis. Assisted with the derivation of the lean body mass prediction equations. Contributed to the final version of the manuscript.		
Signature		Date	22 Mar 2014

Name of Co-Author	Ian Chapman		
Contribution to the Paper	Assisted in research supervision and contributed to manuscript.		
Signature		Date	13/5/14

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Robert Adams		
Contribution to the Paper	Assisted in research supervision. Provided database from NWAHS for validation. Evaluation final version of manuscript		
Signature		Date	5/5/2014

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Contribution to the Paper	Assisted in research supervision and final approval of the manuscript.		
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Contribution to the Paper			
Signature		Date	

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Appendix 9

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APPENDICULAR SKELETAL MUSCLE MASS: DEVELOPMENT AND VALIDATION OF ANTHROPOMETRIC PREDICTION EQUATIONS

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Abstract: *Objectives:* Sarcopenia is the loss of muscle mass and function seen with increasing age. Central to making the diagnosis of sarcopenia is the assessment of appendicular skeletal muscle mass (ASM). The objective of this study was to develop and validate novel anthropometric prediction equations (PEs) for ASM that would be useful in primary or aged care. *Design:* PEs were developed using best subset regression analysis. Three best performing PEs (PE1, PE2, PE3) were selected and validated using the Bland-Altman and Sheiner & Beal methods. *Setting:* Community dwelling adults in South Australia. *Participants:* 188 healthy subjects were involved in the development study. 2275 older(age ≥ 50years) subjects were involved in the validation study. *Measurements:* ASM was assessed using dual x-ray abosrptiometry (DEXA). Weight and height was measured and body mass index (BMI) estimated. *Results:* A strong correlation between PE derived ASM and the DEXA derived ASM was seen for the three selected PEs. PE3: ASM= 10.047427 + 0.353307(weight) - 0.621112(BMI) - 0.022741(age) + 5.096201(if male) performed the best. PE3 over-estimated (P<0.001) ASM by 0.36 kg (95% CI 0.28-0.44 Kg) and the adjusted R^2 was 0.869. The 95% limit of agreement was between -3.5 and 4.35 kg and the standard error of the estimate was 1.95. The root mean square error was 1.91(95% CI 1.80-2.01). PE3 also performed the best across the various age (50-65, 65-<80, 80+ years) and weight (BMI <18.5, 18.5-24.9, 25-29.9, ≥30 kg/m^2) groups. *Conclusions:* A new anthropometric PE for ASM has been developed for use in primary or aged care but is specific to Caucasian population groups.

Key words: Appendicular skeletal muscle mass, prediction equations, sarcopenia.

Introduction

A physiological decline in muscle mass averaging about 3 kilograms per decade is seen from the 4th decade of life (1). This decline may be accompanied by a gradual reduction in physical function and can become pathological when sufficiently severe resulting in a loss of autonomy, a condition referred to as sarcopenia (2, 3).

Sarcopaenia is a Greek word which literally means loss of tissue (sarx [flesh] + paenia [loss]), but is now generally taken to mean loss of lean tissue, and particularly skeletal muscle (2). More recently, the European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as not only the presence of low muscle mass but also included low muscle function (4). Similar to what is seen with the diagnosis of osteoporosis, the EWGSOP has defined that skeletal muscle index (SMI= appendicular skeletal muscle mass [ASM]/[height]^2) cut-offs < two standard deviation (SD) below young male and female reference groups (18- <40 years) are required in addition to loss of physical function to define the presence of sarcopenia. In the late 90s, SMI cut-offs of <7.26kg/m^2 for men and <5.5kg/m^2 for women were developed to identify sarcopenia and in that landmark study, >40% of men and women over the age of 80 years were identified as sarcopenic (5, 6).

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Clearly, it has become important that clinicians are able to easily estimate ASM in clinical practice to identify those at risk of sarcopenia (4). Computed tomography, magnetic resonance imaging and dual absorptiometry x-ray (DXA) are currently the recommended methods to assess ASM in research but may be difficult to access in some clinical settings (e.g. rural regions) as well as burdensome for some patient population groups such as the frail elderly who may be reluctant to attend tertiary centers for ASM assessment (7). Although the bio-electrical impedance analysis method is portable, it still requires the purchase of equipment that is not routinely used in clinical practice. Therefore, anthropometric prediction equations may have a role to play in primary or aged care settings. The aims of this study were to develop anthropometric PEs for ASM using DXA as the reference method and validate these newly developed PEs in South Australian population cohorts.

Methods

Study Cohorts

Three cohorts were investigated in this study: a) the Cytokine, Adiposity, Sarcopenia and Ageing Study (CASA) cohort; b) the North West Adelaide Health Study (NWAHS) cohort; and c) the Florey Adelaide Male Ageing Study (FAMAS) cohort.

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CASA: 195 population representative healthy subjects (age 18 to 83 years) were recruited from the western suburbs of Adelaide. The method used is similar to that used for the NWAHS (8). In undertaking the analysis, data from 7 subjects were excluded due to samples being haemolysed or insufficient. Subjects were selected randomly from the Electronic White Pages. Selected households were sent a letter and brochure about the study. The person in the household aged 18 years or over and who most recently had a birthday was eligible to participate in a brief telephone interview. A minimum of six telephone calls was made to each household before an individual was deemed non contactable. Subjects who were able to comply with the study protocol and who reported weight stability over the preceding 3 months were included in the study. Those with known inflammatory diseases, those who were pregnant and those who had been ill in the preceding 3 months or in the 2 weeks following blood sampling, were excluded.

NWAHS: This study cohort has previously been described in detail (9). Briefly, NWAHS is a representative biomedical cohort study of subjects of predominantly mixed European descent, aged at least eighteen years. Subjects living in residential care and those who could not attend the clinics or converse in English were excluded. There was under-representation in the younger age groups but over-representation in the older age groups. From December 1999 to July 2003, 4060 adults underwent baseline biomedical examination (69.4% of those completing the initial interview). At follow-up (May 2004 to Feb 2006, median time = 4.0 years), survey data was obtained on 88% (n=3574) and clinic data on 79% (n=3206) using the same methodology. Of the baseline sample, 100 subjects were deceased, 226 were unable to be contacted, and 160 refused further participation in the study. At follow-up, DXA scans were offered to NWAHS participants who were aged 50 years and over as part of the clinic assessment. DXA measurements were obtained on 1575 participants.

FAMAS: This male only study cohort has been described in detail elsewhere (10). Briefly, 1195 men age between 35 and 80 years from the North West regions of Adelaide were recruited between August 2002 and April 2005 to this longitudinal study. The recruitment process was very similar to that described for the NWAHS study and so, it was not surprising that the men in FAMAS were comparable with men in the same age groups from the NWAHS study and of mixed European descent (8). DXA measurements were obtained on 700 participants aged 50 years and over.

Measurements

Anthropometry: Height (m) was measured with shoes off using a wall-mounted stadiometer to the nearest 0.1cm. Weight (kg) was measured wearing light clothing to the nearest 0.1kg. Body mass index (BMI-weight/height^2) was calculated.

Dual Energy X-ray Absorptiometry (DXA): The DXA in all cohorts measured 3 compartments of the total body composition; fat mass, LBM and bone mineral content. In this study, the ASM refers to sum of lean soft-tissue masses for arms and legs. CASA: A Lunar PRODIGY whole-body scanner (GE Medical Systems, Madison, WI), in conjunction with Encore 2002 software, was used to estimate ASM. NWAHS and FAMAS: For both of these cohort studies, the same fan-beam Lunar PRODIGY (GE Medical Systems, Madison, WI) in conjunction with Encore 2002 software (as per the DC) and a pencil-beam DPX+ (GE Medical Systems, Madison, WI) in conjunction with LUNAR software version 4.7e were used. Cross-calibration analysis was undertaken and reported no differences between the 2 densitometers (11).

Ethics

The Central Northern Adelaide Health Service Ethics of Human Research Committee approved this study. All participants provided written informed consent.

Statistical Analysis

Demographic characteristics in both groups are expressed as mean ± standard deviation (SD). Differences between methods of ASM measurements in the same cohort were examined by paired t test. PEs for ASM were developed from CASA cohort where the independent variable included gender, age, weight, height and body mass index. The best anthropometric PE (as assessed by adjusted R^2: the proportion of the variance of the dependent variable accounted for by the independent variables, and adjusted for the number of independent variables) involving n = 1, … , four predictors was developed by considering all such equations with n predictors. For each n, the PE for validation was selected by considering the adjusted R^2 value and clinical utility in primary care. The developed PEs were then cross-validated in two combined populations (FAMAS and NWAHS cohorts). ASM was calculated from the developed prediction equations (ASMPE) and compared with DXA derived ASM (ASMDXA). To assess the accuracy and predictive performance of the prediction equations, the method of Bland-Altman was used to estimate the level of agreement, whereby the difference between the two measurements was plotted against the average of the two measurements (12). Precision (root mean square error [RMSE]) and bias (mean error [ME]) were calculated according to the method of Sheiner and Beal (13). When the 95% confidence interval of the ME included 0 (i.e. no error), this indicated that the model was not biased. Linear regression analysis was performed using ASMPE to predict ASMDXA. This gives an estimation of R2 and the standard error of the estimate [SEE]. In this study, mean difference was used interchangeably with ME. SPSS 11.5 for Windows software (SPSS, Inc., Chicago, IL) and R statistical language (R Foundation for Statistical Computing, Vienna, Austria) were used for the analyses. $P<0.05$ was considered statistically significant.

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Results

The mean age for CASA subjects was lower (50.6 ± 15.7 years) than for subjects from the NWAHS (64.7 ± 9.84) and FAMAS (62.3 ± 8.2) studies. Similarly, the subjects in the CASA (26.7 ± 5.2 kg/m^2) had lower BMI than the subjects in the NWAHS (28.2 ±4.8) and FAMAS (28.6 ± 4.6) studies. Given that the FAMAS (25.8 ± 3.75) study was a study of men only, then as expected that cohort had higher mean ASM values compared to the NWAHS (20.7 ± 5.25 Kg) and CASA (21.4 ± 2.14).

The three selected PEs are presented here:

PE1: ASM= 9.11472 + 0.36992(weight) -0.67551(BMI) + 5.00840 (if male)

[Standard Error of Estimate (SEE) 1.89; Adjusted R^2 (%) 90.4]

PE2: ASM= -27.879919 + 0.129727(weight) + 22.122674 (height) + 4.980820 (if male)

[SEE 1.93; Adjusted R^2 (%) 90.1]

PE3: ASM= 10.047427 + 0.353307(weight) - 0.621112(BMI) - 0.022741(age) + 5.096201 (if male)

[SEE 1.87; Adjusted R^2 (%) 90.6]

RMSE, ME and SEE measure the degree of error (precision) of the PEs against the reference method (ASMDXA). Lower values of RMSE, ME and SEE reflects a lower error rate and therefore a higher precision of the PE in predicting the ASMDXA. Table 1 compares ASMPE1-3 to ASMDXA. PE3 has the lowest SEE, ME and RMSE and therefore appears to be the most precise of the three PEs. For all equations, there was a significant over-estimation (i.e. mean error >0) of ASM when ASMPE1-3 were compared to ASMDXA (Table 1-3). When the performance of the PEs was compared against various older age cohorts [50-<65 vs. 65- <80 vs. ≥80] (Table 2), PE3 continued to perform the best across the age cohorts with the lowest SEE, ME and RMSE. The PEs were also compared across various BMI groupings [<18.5, 18.5-24.9, 25-29.9, ≥30 kg/m^2] (Table 3). Once again, PE3 performed slightly better (lower SEE, ME and RMSE) than PE1&2 across all BMI groupings except for the BMI category <18.5kg/m^2 where the sample size was small (n=7).

Discussion

This study reports on the development of three anthropometric prediction equations (PEs) for appendicular skeletal muscle mass (ASM) with DXA as the reference method and including common variables measured in clinical practice such as age, gender, weight, height and body mass index. The PEs were validated in older (50+ years), population representative combined cohorts of 2275 men and women in total. The main conclusion was that the following prediction equation performed the best when compared across various older age and BMI groups: PE3: ASM= 10.047427 + 0.353307(weight) - 0.621112(BMI) - 0.022741(age) + 5.096201(if male). This PE will be useful in primary care and aged care settings where access to alternate methods such as DXA and BIA is limited for example in rural regions and where patients may be too frail to attend hospital centers.

The findings from this study are consistent with those recently reported by a Chinese research group but different in that this PE has been developed for use in Caucasian populations and validated in a large cohort including older (45% ≥ 65 years) people where sarcopenia is more prevalent (14). Wen et. al, in their paper studied 729 individuals (age 18-69 years, mean age men 39 and women 41) and these subjects were randomized to either a development cohort and a validation cohort (14). Additionally, our research group found that PE3 with BMI included in addition to weight, age and gender performed better than the PEs with weight, height, age and gender only as variables. Similar to a previous study, the Chinese research group has proposed that equations with limb lengths and circumferences as additional variables may perform better and this requires further exploration in Caucasian and older population groups (15). It has been reported that squaring appendicular lean tissue circumferences creates a lean tissue area estimate and that by adding the product of the summed estimate of appendicular lean tissue areas and height, the total muscle mass may be estimated (15). To the best of our knowledge, Baungmartner et. al. first developed a PE for ASM in the late 1990s and this PE was used to determine the prevalence of sarcopenia in New Mexico (6). Baumgartner and

Table 1

Validation of the 3 prediction equations in the North West Adelaide Health Study and Florey Adelaide Male Ageing Study older (age > 50+ years) cohorts (N=2275)

	Mean Kg (SD)	Mean Error Kg (95%CI)	P-value for mean error	Adj R^2	SEE	95% Limits of Agreement	RMSE (95% CI)
ASMDXA	22.2 (5.39)						
ASMPE1	22.9 (5.49)	0.62 (0.54, 0.71)	<0.001	0.862	2.00	-3.50, 4.74	2.15 (2.04, 2.26)
ASMPE2	22.9 (5.41)	0.67 (0.58, 0.75)	<0.001	0.859	2.02	-3.45, 4.79	2.03 (1.92, 2.14)
ASMPE3	22.6 (5.44)	0.36 (0.28, 0.44)	<0.001	0.869	1.95	-3.63, 4.35	1.91 (1.80, 2.01)

SD- Standard Deviation, CI- Confidence Interval, SEE- Standard Error of the Estimate, RMSE- Root Mean Square Error

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Table 2
Comparison of the 3 prediction equations in the North West Adelaide Health Study and Florey Adelaide Male Ageing Study across different older (50+ years) age groups

	Mean Kg (SD)	Mean Error Kg(95%CI)	P-value for mean error	Adj R^2	SEE	95% Limits of Agreement	RMSE (95% CI)
Age 50-<65, years (n=1259)							
ASMDXA(VC)	23.3 (5.61)						
ASMPE1	23.6 (5.53)	0.33 (0.22, 0.44)	<0.001	0.877	1.97	-3.65, 4.31	2.02 (1.87, 2.17)
ASMPE2	23.6 (5.38)	0.37 (0.26, 0.48)	<0.001	0.874	1.99	-3.61. 4.36	2.03 (1.88, 2.18)
ASMPE3	23.4 (5.45)	0.22 (0.11, 0.32)	<0.001	0.879	1.95	-3.70, 4.14	1.97 (1.82, 2.12)
Age 65-<80, years (n=877)							
ASMDXA(VC)	21.3 (4.82)						
ASMPE1	22.3 (5.29)	0.95 (0.81, 1.09)	<0.001	0.841	1.92	-3.27, 5.15	2.31 (2.12, 2.50)
ASMPE2	22.3 (5.26)	1.01 (0.87, 1.15)	<0.001	0.842	1.92	-3.17, 5.19	2.32 (2.13, 2.50)
ASMPE3	21.9 (5.21)	0.54 (0.41, 0.68)	<0.001	0.846	1.89	-3.55, 4.63	2.11 (1.93, 2.29)
Age ≥80 years (n=139)							
ASMDXA(VC)	18.9 (4.22)						
ASMPE1	20.1 (5.11)	1.19 (0.87, 1.52)	<0.001	0.863	1.56	-2.72, 5.10	2.28 (1.83, 2.73)
ASMPE2	20.1 (5.27)	1.19 (0.84, 1.53)	<0.001	0.864	1.56	-2.92, 5.30	2.37 (1.92, 2.82)
ASMPE3	19.5 (5.04)	0.54 (0.23, 0.86)	0.001	0.868	1.53	-3.24, 4.32	1.96 (1.58, 2.34)

SD- Standard Deviation, CI- Confidence Interval, SEE- Standard Error of the Estimate, RMSE- Root Mean Square Error

Table 3
Comparison of the 3 prediction equations in the North West Adelaide Health Study and Florey Adelaide Male Ageing Study across different body mass index (BMI) groupings

	Mean Kg(SD)	Mean Error Kg(95%CI)	P-value for mean error	Adjusted R^2	SEE	95% Limits of Agreement	RMSE (95% CI)
BMI <18.5 kg/m² (n=7)							
ASMDXA(VC)	15.4 (4.41)						
ASMPE1	16.5 (4.00)	1.15 (-0.20, 2.49)	0.082	0.871	1.58	-1.76, 4.06	1.76 (0.72, 3.48)
ASMPE2	16.5 (4.66)	1.07 (-0.24, 2.38)	0.092	0.889	1.47	-1.77, 3.91	1.69 (0.12, 3.26)
ASMPE3	16.2 (4.05)	0.79 (-0.63, 2.21)	0.223	0.854	1.68	-2.28, 3.86	1.63 (0.29, 2.97)
BMI 18.5-24.9 kg/m² (n=543)							
ASMDXA(VC)	19.7 (4.54)						
ASMPE1	20.5 (4.86)	0.87(0.71, 1.03)	<0.001	0.850	1.76	-2.90, 4.64	2.07 (1.86, 2.28)
ASMPE2	20.6 (5.17)	0.92 (0.75, 1.09)	<0.001	0.849	1.77	-3.12, 4.96	2.22 (2.05, 2.47)
ASMPE3	20.2 (4.81)	0.54 (0.38, 0.69)	<0.001	0.858	1.71	-3.09, 4.17	1.89 (1.66, 2.12)
BMI 25-29.9 kg/m² (n=1008)							
ASMDXA(VC)	22.4 (5.02)						
ASMPE1	23.0 (5.03)	0.60 (0.48, 0.73)	<0.001	0.847	1.96	-3.41, 4.61	2.09 (1.92, 2.26)
ASMPE2	23.1 (5.02)	0.63 (0.51, 0.75)	<0.001	0.845	1.98	-3.40, 4.66	2.11 (1.94, 2.28)
ASMPE3	22.7(4.98)	0.33 (0.21, 0.45)	<0.001	0.854	1.92	-3.56, 4.22	1.97 (1.89, 2.05)
BMI ≥30 kg/m² (n=717)							
ASMDXA(VC)	24.0 (5.70)						
ASMPE1	24.4 (5.93)	0.46 (0.29, 0.62)	<0.001	0.858	2.15	-4.02, 4.94	2.29 (2.07, 2.51)
ASMPE2	24.5 (5.48)	0.52 (0.68, 0.36)	<0.001	0.859	2.14	-3.77, 4.81	2.20 (1.98, 2.42)
ASMPE3	24.2 (5.84)	0.28 (0.12, 0.44)	0.001	0.862	2.12	-4.09, 4.65	2.20 (1.98, 2.42)

SD- Standard Deviation, CI- Confidence Interval, SEE- Standard Error of the Estimate, RMSE- Root Mean Square Error

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colleagues included hip circumference and grip strength as variables (6, 14). Unfortunately, the inclusion of grip strength is likely to limit the use of the PE in primary or aged care as dynanometers are not routinely available in these clinical settings. However, greater accuracy of estimation may be of benefit in research practice and low grip strength is a criteria than can also be used in conjunction with low ASM to confirm the diagnosis of sarcopenia (4).

A major strength of this study was the fact that the PEs were initially developed in a population representative and healthy cohort and subsequently validated in large population representative cohorts of older people. The study methodologies for the three cohort studies were similar and the DXA machines used were comparable. However, sarcopenia is most prevalent in under-weight and older people. Only small numbers of people aged 80 years or over (n=139) or people with BMIs less than 22kg/m^2 (n=132) had DXA assessments in these epidemiological cohorts and this provides some support to the notion that alternate methods of body composition assessments are required for frail and older populations groups as they may not wish to travel to hospitals for DXA assessment. It will be very important for PE3 to be further validated in the underweight and very old, especially those who are home or institution bound, the population group this PE is targeted towards. Ethnic specific PEs will need to be developed to assess ASM in different ethnic groups.

Conclusion

To summarize, this paper reports on a novel, anthropometric PE to assess ASM, which has application in the primary care and aged care settings. Combined with a physical function measure such as walk speed, this PE will contribute to the diagnosis of Sarcopenia allowing for early identification and management of at-risk individuals in these care settings (4). The next step is to validate this PE in a larger group of older (mean age $\geq$ 80 years) and underweight (BMI<22kg/m^2) people and explore the benefits of additional variables such as limb lengths or circumferences.

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Conflict of interest: There are no specific conflicts of interests to declare relevant to this study. R Visvanathan is a member of the Nestle Nutrition Australia Malnutrition In The Elderly Board and receives a honorarium for this activity.

Author Contributions: RV and TV were involved with project conception, development of overall research plan, study oversight and were involved in manuscript preparation. SY conducted the research, managed the database, analyzed the data and contributed significantly to manuscript preparation. JF undertook statistical analysis with regards to the development of the prediction equations and contributed to manuscript development. IC, GW, RA contributed to manuscript preparation. RA and GW facilitated access to the NWAHS and FAMAS study data.

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Appendix 10

Statement of authorship: Chapter 6, An anthropometric prediction equation for appendicular skeletal muscle mass in combination with a measure of muscle performance to screen for sarcopenia in primary and aged care

Statement of Authorship

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Solomon Yu		
Contribution to the Paper	Involved in study conception, constructed study database, performed all the statistical analyses, interpreting results, prepare manuscript and acted as key corresponding author		
Signature		Date	2/6/14

Name of Co-Author	Sarah Appleton		
Contribution to the Paper	Assistance in data analysis and evaluated the final of version of the manuscript		
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Name of Co-Author	Ian Chapman		
Contribution to the Paper	Assisted in research supervision and contributed to manuscript.		
Signature		Date	13/5/14

Name of Co-Author	Robert Adams		
Contribution to the Paper	Assisted in research supervision and contributed to manuscript. Assisted in research supervision and contributed to manuscript. Provided access to database from NWAHS for validation. Evaluation final version of manuscript		
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Statement of Authorship

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

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Appendix 11

Statement of authorship and published manuscript: Chapter 7, Inflammatory cytokines and appetite in health people

Statement of Authorship

Title of Paper	Inflammatory Cytokines and Appetite in Health People
Publication Status	⦿ Published, ○ Accepted for Publication, ○ Submitted for Publication, ○ Publication style
Publication Details	Dent E, Yu S, Visvanathan R, Piantadosi C, Adams R, Lange K, Chapman I. Inflammatory Cytokines and Appetite in Healthy People. Journal of Aging Research and Clinical Practice, 2012; 1(1):40-43.

Author Contributions

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Contribution to the Paper	Assisted in study design, collected all data, established the research database and evaluated the draft of the manuscript.		
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Contribution to the Paper	Performed all statistical analysis for the manuscript, interpreted data, lead in manuscript preparation and was corresponding author.		
Signature		Date	8/4/14

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Contribution to the Paper	Assisted in study designed, supervised the development of the porject, assited in data interpretation. Helped with manuscript editing and evaluation		
Signature		Date	20/5/14

Name of Co-Author	Cynthia Piantadosi		
Contribution to the Paper	Assisted with research supervising		
Signature		Date	7/5/14

Statement of Authorship

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Contribution to the Paper	Assisted in study designed, supervised the development of the porject, assited in data interpretation. Helped with manuscript editing and evaluation		
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INFLAMMATORY CYTOKINES AND APPETITE IN HEALTHY PEOPLE

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Abstract: *Background and Objectives:* Inflammation has been associated with reduced appetite and body composition changes in populations with established diseases. However, it is not known if an association exists between appetite, body composition and inflammation in healthy people. *Design:* To explore associations of appetite with markers of inflammation and body composition, data from the Cytokines, Adiposity, Sarcopenia and Ageing (CASA) study was analysed. *Setting:* Western suburbs, Adelaide, Australia. Participants: 180, population representative, healthy participants, aged 18 – 82 years, were studied. *Measurements:* Body composition was measured by both Dual X-ray absorbiometry (DXA) and bioelectrical impedance analysis (BIA). Appetite was assessed by the Simplified Nutritional Appetite Questionnaire (SNAQ). Circulating cytokine concentrations were measured. *Results:* Multiple regression analysis showed appetite scores were increased in non-smokers (P = 0.031) and men (P = 0.024), negatively associated with serum levels of the pro-inflammatory IL-1β (β coefficient = - 0.379, P = 0.007), and positively associated with serum levels of the anti-inflammatory cytokine IL-10 (β coefficient = 0.25, P = 0.010). There was no association between appetite and body composition. *Conclusions*: Appetite loss may reflect background inflammation even in apparently healthy people, and probably occurs before consequent changes in body composition. Further explorations of longer term appetite changes with respect to inflammation and body composition changes are needed.

Key words: Appetite, body composition, cytokine, inflammation.

Introduction

Under-nutrition is common among older people, even in developed countries (1-4) and is associated with serious consequences, including more frequent and prolonged hospital admissions (5), increased infection risk (6), functional decline (7) and reduced life expectancy (3). It is important to identify factors that might predict those older people more likely to lose weight and become under-nourished, so prevention and early treatment measures can be implemented.

Multiple methods have been used to define and diagnose under-nutrition in older people, but features commonly seen in this condition are weight loss (particularly muscle loss), reduced body weight, reduced appetite and sometimes cachexia (8). Aging is associated with decline in appetite and food intake which is probably physiological, but may contribute to the development of pathological anorexia and under-nutrition. Indeed, reduced appetite is a reliable predictor of future weight loss in the elderly; appetite scores obtained from the Simplified Nutritional Appetite Questionnaire (SNAQ) have been found to predict future weight loss in older people (9).

Appetite loss may be caused by inflammation. Inflammation is the immune system's response to an acute infection or illness and is the result of the production of several pro-inflammatory cytokines including interleukin-1 (IL-1), IL-2, IL-6, IL-8, tumour necrosis factor-α (TNF-α) and interferon-γ (IFNγ) (10). These pro-inflammatory cytokines, when persistently elevated, can reduce appetite by actions on the hypothalamus and other neural centres, by altering gastric function and by modifying the regulation of appetite controlling hormones (10). Anti-inflammatory cytokines, such and IL-4 and IL-10 act to down-regulate pro-inflammatory cytokine production (11). An imbalance between pro-inflammatory and anti-inflammatory cytokines is thus thought to lead to the cachexia of many chronic diseases (12).

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Ageing itself may be a low-level pro-inflammatory state (13). It might therefore be that the anorexia of ageing is due, at least in part, to increased inflammation. If so, it might be expected that there would be a positive connection between pro-inflammatory markers and reduced appetite even in apparently healthy individuals across the adult age range. Little is known about these possible connections.

This study explored the associations of appetite with markers of inflammation and body composition in healthy adults. It was hypothesised that there would be associations between increased inflammation and reduced appetite even in this group of healthy individuals, but probably not between markers of inflammation and adverse body composition changes, as these are likely to be later effects of under-nutrition.

Methods

Participants

Healthy subjects (ages 18 to 82 years) were recruited from the western suburbs of Adelaide into the Cytokine, Adiposity, Sarcopenia and Ageing Study (CASA). The recruitment methodology is similar to that described for other larger population studies conducted in the same catchment area, the North West Adelaide Health Study (14). Telephone numbers from the Electronic White Pages were randomly selected, and willing subjects, aged 18 or over, with no exclusion criteria, were invited to participate. Subjects able to comply with the study protocol and who reported weight stability over the preceding 3 months were included in the study. Those with confirmed inflammatory diseases, pregnant and those who had been ill in the preceding 3 months or in the 2 weeks following blood sampling, were excluded. This study had ethics approval from the Central Northern Adelaide Health Service Ethics of Human Research Committee and all participants provided written informed consent.

Body Composition Measures

Body composition was assessed by measurement of height; weight; waist circumference; Fat Mass (FM) and Fat Free Mass (FFM) by Dual X-Ray absorbiometry (DXA) (Lunar PRODIGY whole body scanner; GE Medical Systems, Madison, WI) scan; and Biolectrical Impedence Analysis (BIA) (Quantum II BIA Analyser, RJL system).

Appetite

Participants completed the SNAQ questionnaire, giving one of five responses to four questions regarding appetite, satiety, taste and meal frequency (11). SNAQ gives a score out of 20, with higher scores indicating greater appetite. SNAQ has been found to predict weight loss over a six month period with 81.6 % sensitivity and 84.6 % specificity for people over 60 years of age (9).

Exercise Score

Exercise was assessed using Australian National Health Survey questions (12). Scores for exercise intensity were 3.5 for walking, 5.0 for moderate activity and 7.5 for high intensity activity. Exercise intensity score was multiplied by minutes per fortnight for each exercise intensity to give total exercise level. This total level was classified as "sedentary" (< 100), "low level" (100 <1600), "moderate level" (1600 – 3200 or > 3200 and less than 2 h of vigorous exercise) or "high level" (> 3200)".

Data Collection

Fasting blood samples were collected and body composition measured by BIA in the morning, and body composition by DXA was measured either the afternoon of the same day or on another day but within 2 weeks. Plasma samples were stored at –80°C until analysis. Cytokine concentrations were measured using LINCOplex kits. Trace values < 0.08 pg/L for cytokines were recorded as zero values.

Statistical Analysis

SNAQ scores were normally distributed. Other continuous study variables were non-normally distributed and are presented as medians (inter-quartile range). Categorical variables are presented as frequencies.

Relationships between the total SNAQ score and the study variables were assessed using Spearman rank correlation tests for non-parametric variables. Cytokines and anthropometric variables were included in a multiple regression analysis along with for age, gender and smoking status. Continuous data were log transformed prior to inclusion in this analysis. Statistical analysis was carried out using SPSS statistical program (17.0, SPSS, Chicago, USA) with statistical significance set at $P < 0.05$.

Results

180 subjects with complete results were included in the study. Median age was 52 years with a range of 18-82 years. SNAQ total scores ranged from 12-20 (out of 20), with a median score of 17. 15 participants (7.8%) had low SNAQ scores (defined as ≤ 14). Table 1 shows baseline subject characteristics.

The results of the univariate regression analysis of the relationship between SNAQ appetite scores and continuous study variables are shown in Table 2. Both IL-6 and IL-10 concentrations were positively related to appetite. There were also strong significant associations between concentrations of a number of cytokines,

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including IL-6 with both IL-1β (r = .353, P <0.001) and IL-10 (r = .410, P<0.001). By multivariate analysis (Table 3) non-smokers had higher appetite scores than smokers and men higher scores than women. IL-1β concentrations were negatively and IL-10 concentrations positively associated with appetite. None of the body composition variables showed any association with SNAQ score from either the univariate or multivariate analyses.

Table 1
Baseline Participant Characteristics (n=180)

Continuous Variables	Median (Inter-Quartile Range)
Background Variables	
Age (years)	52 (40-62)
SNAQ appetite scores	17.0 (16.0-18.0)
Circulating Cytokine Concentrations	
IL-1β (pg/ml)	0.50 (0.0-1.8)
IL-2 (pg/ml)	1.46 (0.0 - 8.0)
IL-4 (pg/ml)	0.0 (0.0 - 15.8)
IL-6 (pg/ml)	1.95 (0.25-5.9)
IL-10 (pg/ml)	3.9 (0.0-13.8)
TNF-α (pg/ml)	3.5 (1.9 - 5.4)
HS-CRP (mg/L)	1.2 (0.6 - 2.3)
Anthropometric Measures	
BMI (kg/m²)	25.6 (23.0 - 28.7)
Waist Circumference (cm)	87.2 (76.3 - 96.7)
Total Lean Mass DXA (kg)	44.5 (38.1 - 56.8)
Total Fat DXA (Kg)	24.1 (17.1 - 30.2)
Nutritional Biomarkers	
Haemoglobin (g/L)	140.0 (129.0 - 150.0)
Lymphocyte (g/L)	1.8 (1.6-2.2)
Albumin (g/L)	39.0 (37.0 - 41.0)
Categorical Variables	**n (%)**
Background Variables	
Gender	106 (58.9 %) females; 74 (41.1%) males
Smoking Status	19 (10.6%) smokers
Exercise Level	
Sedentary	31 (17.2 %)
Low Level	75 (41.7%)
Moderate Level	39(21.7%)
High Level	35(19.4%)

Table 2
Univariate Regression Analysis of relationships between total SNAQ appetite score and Continuous Study Variables (n=180)

Variable	R	P
Background Variables		
Age (years)	0.016	0.836
Exercise Score	0.062	0.407
Nutritional Biomarkers		
Haemaglobin (g/L)	0.053	0.463
Lymphocyte (g/L)	0.040	0.585
Albumin (g/L)	0.038	0.601
Cytokines		
IL-1β (pg/ml)	0.033	0.637
IL-2 (pg/mL)	0.034	0.652
IL-4 (pg/mL)	0.041	0.584
IL-6 (pg/mL)	0.153	0.041
IL-10 (pg/mL)	0.210	0.005
TNF-α (pg/mL)	0.089	0.222
HS-CRP (mg/mL)	0.086	0.239
Anthropometric Measures		
BMI (kg/m²)	0.039	0.599
Waist Circumference (cm)	0.058	0.425
Total Lean Mass DXA (kg)	0.064	0.374
Total Fat DXA (kg)	0.050	0.494

Table 3
Multivariate Analysis of relationship between Study Variables and total SNAQ score (n=180)

Variable	β Coefficient	t	P
Background Variables			
Age (years)	0.042	0.472	0.638
Gender	-0.367	-2.287	0.024★
Smoking Status	-0.172	-2.176	0.031†
Cytokines			
IL-1β	-0.379	-2.739	0.007
IL-2	0.157	1.018	0.310
IL-4	0.057	0.535	0.593
IL-6	0.085	0.806	0.422
IL-10	0.248	2.598	0.010
TNF-α	0.035	0.392	0.696
CRP	-0.165	-1.868	0.064
Anthropometric Measures			
BMI	-0.227	-0.971	0.333
Waist	0.372	1.739	0.084
Lean	0.281	1.631	0.105
Fat	-0.058	-0.255	0.799
Exercise Score	0.117	1.471	0.143

★SNAQ scores higher in men than women; †SNAQ scores higher in non-smokers than smokers.

Discussion

In this novel study of appetite in healthy people, appetite as measured by the SNAQ questionnaire was associated negatively with circulating serum levels of IL-1β and positively with IL-10 levels, but was not associated with any measure of body composition or nutritional biomarker – albumin, lymphocyte count and haemoglobin.

The negative association between IL-1β and appetite found in this study is consistent with previous reports in humans with inflammatory conditions such as cancer (15), renal failure (16) eating disorders (17) and depression (18). Our finding is also consistent with the known pro-inflammatory effects of IL-1β and the results of animal studies. In rodents, food intake is suppressed in a dose-dependent manner by IL-1β (10, 19). Additionally, IL-1β knock-out mice are of normal size and weight, but resistant to inflammation-induced weight loss (10). Of interest older mice lose more weight in response to IL-1 administration than young adult mice (20).

The positive association between IL-10 and appetite is consistent with the anti-inflammatory actions of this cytokine. IL-10 is believed to suppress immune responses by inhibiting pro-inflammatory cytokine production (11, 21). For example, IL-10 has been found to be protective against weight loss induced by both pro-inflammatory cytokines (22) and bacteria-mimicked infection (23) in rodent studies.

The finding that IL-6 was associated with appetite in the univariate analysis, but not associated in the multivariate analysis is probably because IL-6 concentrations are significantly associated with those of other cytokines, such as IL-1β and IL-10 which have more powerful effects on appetite. Consistent with the strong association observed between IL-6 and IL-10 concentrations (r = 0.353, P <0.001), IL-6 has been found to up-regulate IL-10 during acute inflammation (24).

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In the present study there was no association between appetite and circulating levels of either TNF-α or, CRP. TNF-α is a pro-inflammatory cytokine which has been associated with reduced appetite in patients with chronic diseases such as renal failure (25) and levels of CRP, an inflammatory marker, have been associated with appetite decline in patients with chronic disease (26, 27). The lack of an association with appetite in the present study is perhaps because our subjects were healthy and TNFα and CRP effects on appetite occur later in the pathways of chronic and inflammatory diseases.

Low appetite leads to reduced food intake, which in turn, often results in weight loss (9). Loss of appetite due to inflammation might therefore result in reduced lean tissue stores. We found, however, no such association in our study, a finding supported by a recent study of community elders in Malaysia, where appetite was also not associated with body composition (28).

Our results may provide some insight into the order in which changes leading to under-nutrition occur. It is not known if the muscle mass loss that often follows appetite reduction in older people leads to a pro-inflammatory state, or if inflammation leads to reduced appetite and food intake and subsequently to adverse body composition changes. Our findings support the latter sequence, at least in certain circumstances. In apparently healthy people there appears to be already present an association between inflammation and reduced appetite, without adverse effects on body composition, which we postulate would only occur with more prolonged and severe effects on food intake and nutrition.

This study was limited by a relatively small sample size. Nevertheless, subjects were randomly chosen from the community and thus reflect the situation in apparently healthy adults. A further limitation is that dietary background was also not assessed in this study and that SNAQ has not yet been validated against objective food intake (28), although it has been shown to predict future weight loss (9). Dietary intake was not assessed in this study. Because it is possible that body composition and weight loss may reflect long term nutrition, whereas appetite and inflammation reflect short term nutrition (29), it would be interesting to follow these subjects to assess longer-term relationships between inflammation, appetite, body weight change and nutritional status and we are now planning such a follow-up study.

In summary, the major finding of the present study is that appetite in healthy people is associated with several inflammatory markers but not with any measures of body composition or nutritional bio-markers. Further follow-up is needed to explore the possibility that this may predict future weight loss and increased likelihood of developing under-nutrition.

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Appendix 12

Newsletter 1: *Research Impact*, The hospital research foundation and Newsletter 2: in *Central,* SA health, Government of South Australia.

Body Building; Important for Young and Old

Researchers at The Queen Elizabeth Hospital (TQEH) are undertaking a research project aimed at helping people preserve their weight and strength as they age to improve quality of life and help maintain independence.

Sarcopenia is a common condition in older people (65+) but many sufferers are not aware of it. It refers to the gradual loss of muscle mass and strength which has a number of negative side effects.

It is a condition that eighty-one year old Dorothy (pictured above) has been battling over the last two years.

"It takes me so much longer to do things now which really frustrates me – until I hit 80 I felt like I never had a thing wrong with me! I had a lot of strength," explained Dorothy.

"I am determined to keep my own home; I still clean and polish the furniture, but it's difficult. I get tight in the chest and find it hard to breathe. I love gardening but I can't do too much at a time anymore."

"You shouldn't give up in life, but it's frustrating..."

"You shouldn't give up in life, but it's frustrating."

While Dorothy has maintained her independence through admirable determination and a positive attitude, there is a clear need to prevent the negative effects of Sarcopenia.

Dr Solomon Yu (pictured right) from the Aged and Extended Care Services department at TQEH has identified that Sarcopenia is particularly common in the north-west population of Adelaide – up to 21% of people (about 1 in 5) who are aged 80+ living at home are suffering from Sarcopenia.

With this in mind, Dr Yu has been developing a predictive equation that will assist doctors in diagnosing Sarcopenia in the early stages, before people start experiencing the negative ramifications such as poor balance, trouble walking and climbing stairs, getting up from a chair, serious falls which can shake confidence and cause fractures which require risky surgery.

"At the moment we can identify Sarcopenia with a DXA scan. But there are limitations with this machine in terms of accessibility and affordability," explained Dr Yu.

"If you live in a nursing home, are housebound or in a rural area without access to the machine it makes it hard to measure and identify Sarcopenia."

"What we need to be able to do is screen people, using a tool like the one I have started to develop, to identify those people who need to go on to have a DXA assessment, rather than everyone going straight to a DXA, or having their problem missed all together."

"The preliminary equation I have developed uses measurements of simple elements such as weight and height, from which we can derive muscle mass."

"If we can identify the condition early by using a simple formula, just as we start to notice signs of Sarcopenia, we can implement preventative treatment; for example more exercise or an increase in protein in the diet."

The predictive equation that Dr Yu has developed is a promising step forward, however it requires further defining to ensure it is as accurate as possible.

Earlier this year Dorothy suffered a bad fall at home, impaling her head on a piece of furniture. A contributing factor to Dorothy's fall was impaired balance, a common result of Sarcopenia.

"It's these kinds of incidences we want to prevent through early intervention with people like Dorothy."

Dorothy believes research like Dr Yu's is extremely important.

"I would tell anyone considering supporting this work to think about it very seriously," said Dorothy.

"A younger person may not realise what can happen when you get older – it's not until you get to the age where you require assistance that you realise how important research like Dr Yu's is."

Dr Yu says that Sarcopenia is a significant, widespread problem that must be addressed.

"Body building is important in young age and older age! This research is helping put the spot-light on the importance of maintaining a healthy weight and strength as people age," said Dr Yu.

What can you do to maintain your strength & weight as you age?

1) Engage in regular physical activity – 3-5 times per week, resistive exercise (ie lifting weights) and endurance exercise (ie cycling, swimming or brisk walking), minimum of 30 minutes each session.

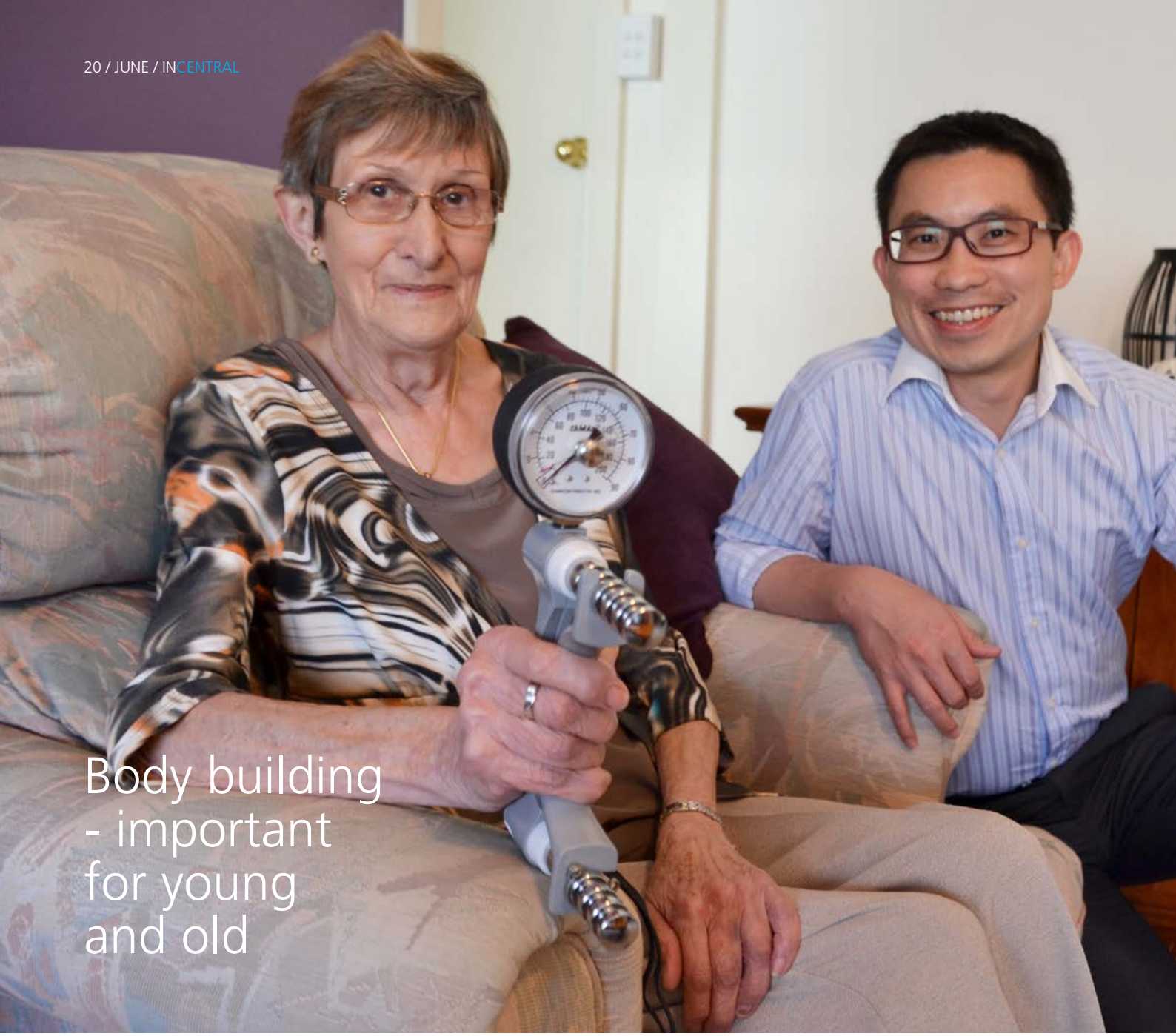
2) Eat a balanced diet with focus on a protein supplement such as Ensure or Sustagen.

3) Keep a diary of your weight to help you maintain a stable weight.

4) Modify your lifestyle to include incidental exercise, eg alighting 1 bus stop away from your intended destination.

5) Have regular check-ups with your GP to stay on top of your health.

Dorothy has been suffering with Sarcopenia for two years. Researchers at TQEH are working towards identifying loss of muscle earlier in older people so they can live stronger and maintain their independence.



Body building - important for young and old

Researchers at The Queen Elizabeth Hospital (TQEH) are undertaking a research project aimed at helping people preserve their weight and strength as they age to improve quality of life and help maintain independence.

Sarcopenia is a common condition in older people (65+) but many sufferers are not aware of it. It refers to the gradual loss of muscle mass and strength which has a number of negative side effects, including poor balance.

It is a condition that 81-year-old Dorothy has been battling over the last two years.

"It takes me so much longer to do things now which really frustrates me – until I hit 80 I felt like I never had a thing wrong with me! I had a lot of strength," explained Dorothy.

Dr Solomon Yu from the Aged and Extended Care Services department at TQEH has identified that Sarcopenia is particularly common in the north-west population of Adelaide.

He has been developing a predictive equation to assist doctors in diagnosing Sarcopenia in the early stages.

"If we can identify the condition early by using a simple formula, just as we start to notice signs of Sarcopenia, we can implement preventative treatment; for example more exercise or an increase in protein in the diet," he said.

Dorothy has been suffering with Sarcopenia for two years. Researchers at TQEH are working towards identifying loss of muscle earlier in older people so they can live stronger and maintain their independence.

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Appendix 13

Oral abstracts/Platform presentations

The Australian and New Zealand Society for Geriatric Medicine Annual Scientific Meeting, 17-19 June 2013, Adelaide Convention Centre, Adelaide, SA, Australia.

Yu S, Appleton S, Adams R, Chapman I, Wittert G, Visvanathan T, Visvanathan R. Prevalence of Sarcopenia in Community Dwelling Older Australian.(2013).*Australasian Journal on Ageing.*32 (Sppl 1):6-35.

The Australian and New Zealand Society for Geriatric Medicine Annual Scientific Meeting 2012.Dementia: Managing Not to Forget. 2-4 May 2012. Hilton Hotel, Sydney, Australia.

Yu S, Visvanathan T, Field J, Chapman I, Adams R, Wittert G, Visvanathan R. (2012). A prediction equation to aid diagnosis of sarcopenia in primary care.*Australasian Journal on Ageing.*31 (Sppl 1):16-33.

The Australian & New Zealand Society for Geriatric Medicine Annual Scientific Meeting, 5-7 May 2010, Hyatt Regency Coolum, Queensland, Australia.

Yu S, Adams RJ, Wilson DH, Chapman I, Phillips P and Visvanathan R. (2010). Development and validation of prediction equation for fat free mass using variables consisting of blood and weight measurements. *Australasian Journal on Ageing.*29 (Sppl 1):17.

Conjoint Scientific Meeting of the Australian & New Zealand Society for Geriatric Medicine, Internal Medicine Society of Australia & New Zealand in association with International Academy of Nutrition & Aging, 5–8 September 2007, Adelaide, Australia.

Yu S and Visvanathan, R. (2007). Estimation of fat free mass in routine clinical practice. *Internal Medicine Journal.*37 (Suppl.3):A64.

Sarcopenia In Community Dwelling Older Australians

Yu, S[1,2], *Appleton, S*[1], *Adams, R*[2], *Chapman, I*[2], *Wittert, G*[2], *Visvanathan, T*[4], *Visvanathan, R*[1,3]

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Aims: Sarcopenia is defined as the presence of a low skeletal muscle index (SMI) at least two standard deviations below the mean SMI in healthy young individuals aged between 18 to 40 years) and the presence of low physical function such as low grip strength. The aim of this study was to identify gender specific cut-offs for low skeletal muscle index (SMI) and then determine the prevalence of sarcopenia in community dwelling older Australians.

Methods: Three South Australian community cohorts were investigated. Gender specific cut-offs for low SMI were identified. Low SMI in conjunction with low grip strength (<30.3kg for men and <19.3Kg for women) confirmed the diagnosis of sarcopenia.

Results: The cut-off for low SMI was <6.88Kg/$m^2$for men and <4.41 Kg/m^2 for women. These were lower than those previously identified. Depending on the cut-offs used for SMI, between 7-15.5% of men and 1.6-12.9% of women aged 80 years or more were identified as having sarcopenia. In contrast, between 1.9-4.3% of men and 0-3.2% of women in the 65 to 79 year age group were classified as sarcopenic.

Conclusion: Sarcopenia is common in community dwelling older Australians aged 80 years and older with more men than women affected. The lower cut-offs identified in this study compared to those identified elsewhere decades ago raises concern about the impact of increasingly sedentary lifestyles and this warrants further investigation.

A Prediction Equation To Aid The Diagnosis of Sarcopenia In Primary Care

Yu S[1,2,3], Visvanathan T[4], Field J[2], Chapman I[2], Adams R[2,3], Wittert G[2,3] and Visvanathan R[1,2]

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Aim: Sarcopenia is common in older people and results in morbidity. The aim of this study was to develop and validate a prediction equation (PE) for appendicular skeletal muscle mass (ASMM) that would be useful in primary care settings.

Methods: ASMM was estimated using dual energy X-ray absorptiometry (DXA). Anthropometric measurements such as weight and height were made. Several PEs were derived by best subset regression analysis in a development cohort (DC) of 195 healthy subjects and then validated using the Bland-Altman and method in a validation cohort (VC) consisting of older (age 50+) subjects from 2 South Australian longitudinal studies- the North West Adelaide Health Study and the Florey Adelaide Male Ageing Study.

Results: The best performing PE demonstrated a strong correlation between the $ASMM_{PE}$ and the observed $ASMM_{DEXA}$ in the VC ($r=0.932$, p-value<0.001). The predictive performance was good (RMSE 1.91kg, CI: 1.8, 2.01). The mean bias was -0.36kg and the 95% limits of agreement were between -4.35 and 3.63. The PE performed equally well across various age (80+, 65-<80, <65) and weight (BMI <22, 22-27, >27 kg/m^2) groups.

Conclusion: This novel Australian PE has immediate practical application in primary care to aid the diagnosis and management of sarcopenia.

Development and Validation Of Prediction Equation For Fat Free Mass Using Variables Consisting Of Blood And Weight Measurements

Yu S[1,2], Field J[2], Chapman I[2], Wilson D[2], Adams R[2], Phillips P[3] ,and Visvanathan R[1,2]

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3 The Osteoporosis Centre, Department of Endocrine and Diabetes Service, The Queen Elizabeth Hospital, Central Northern Adelaide Health Service, South Australia

Aim: To develop and validate novel prediction equations (PEs) for fat free mass (FFM) from routine blood investigations.

Methods: FFM was estimated using dual energy X-ray absorptiometry (DEXA). Weight and blood investigations (creatinine kinase [CK], albumin, C-reactive protein [CRP], lactate dehydrogenase [LDH] and alanine aminotransferase [AST]) were performed following an overnight fast. Several PEs were derived by best subsets regression analysis in a development cohort (DC) of 195 healthy subjects (age 18-82 years; 40% men) and the PEs were validated using the Bland-Altman method in a validation cohort (VC) of 52 healthy subjects (age 22-82 years, 50% men).

Results: When 7 predictive variables were included, the PE accounted for 89.8% of the variance (adjusted R^2= 0.898, SEE=3.78). The ability to estimate variance decreased as variables were deleted and when the PE included only weight and gender as variables, the PE accounted for 85.9% of the variance (adjusted R^2=0.859, SEE=4.45). There was a strong correlation between the selected FFM_{PE} and the observed FFM_{DEXA} in the VC (r=ranging from 0.898-0.904, p-value<0.001). In the VC, the following equation- FFM = 8.8063 + 0.3813weight + 0.0332AST + 0.0227CK – 0.6278CRP + 0.2955Albumin – 0.044LDH + 11.2887 (if male) demonstrated a strong correlation (FFM_{PE} vs.FFM_{DEXA} ;r=0.904 ;P<0.001). The mean bias was -0.92Kg and the 95% limits of agreement were between 8.08 and -9.92Kg.

Conclusion: This novel study has identified several PEs for FFM that has immediate practical application. Economic considerations will influence the PE used.

ESTIMATION OF FAT FREE MASS IN ROUTINE CLINICAL PRACTICE

S Yu[1], R Visvanathan[1,2]

[1]*Aged and Extended Care Service, The Queen Elizabeth Hospital, Adelaide, South Australia,*
[2]*The Department of Medicine, University of Adelaide, Adelaide, South Australia*

Aim: With increasing age, there is loss of lean mass and this is termed sarcopenia. Sarcopenia is associated with morbidity and mortality but is most often not assessed in clinical practice. There is currently no simple way to estimate lean mass in the doctor's rooms as anthropometric measurements require some training and may not be accurate. The aim of this study was to determine what routine clinical blood measurements correlate with fat free mass (FFM) as estimated by dual energy x-ray absorptiometry (DEXA).

Method: A population representative sample of healthy subjects from the Western Suburbs of Adelaide was recruited. All subjects underwent DEXA evaluation and had venous blood drawn.

Results: Participants were aged between 18 and 82 years. 31.1% of subjects were male. There was no correlation between FFM and thigh circumference indicating that this was a less reliable measure of FFM. FFM was significantly correlated with grip strength and the timed 'get up and go test'. FFM was also found to be significantly correlated with the following commonly performed blood investigations: Creatine Kinase (CK), CK-MM, Aspartate Aminotransferase, Alanine Aminotransferase, Gamma-Glutamyl Transferase, albumin, urea and creatinine.

Conclusion: In a group of healthy people, many routinely performed blood tests are significantly correlated with FFM as estimated by DEXA. It remains to be seen if the same applies to other population groups with differing illnesses (eg. renal disease) and if these blood measurements can be used to monitor changes in FFM in clinical practice.

Appendix 14
Poster presentations

The Australian and New Zealand Society for Geriatric Medicine Annual Scientific Meeting, 28–30 May 2014, Grand Hyatt Melbourne, Melbourne, VIC, Australia.

Yu S, Appleton S, Adams R, Chapman I, Wittert G, Visvanathan T, Visvanathan R.(2014).The impact of low muscle mass definition on the prevalence of sarcopenia in older Australians.*Australasian Journal on Ageing*.33(Sppl 1):69.

9th Congress of the European Union of Geriatric Medicine Society (EUGMS), 2-4 October 2013, Venice Lido, Italy.

Yu S, Appleton S, Adams R, Chapman I, Wittert G, Visvanathan T, Visvanathan R. Sarcopenia in Community Dwelling Older Australian.

The impact of Low Muscle Mass definition on the Prevalence of Sarcopenia in older Australians

Solomon Yu[1,2,3], Sarah Appleton[3], Robert Adams[3], Ian Chapman[3], Gary Wittert[3], Thavarajah Visvanathan[4], Renuka Visvanathan[1,2,3]

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[4]Department of Anaesthesia, The Queen Elizabeth Hospital, Central Adelaide Local Health Network, South Australia, Australia.

BACKGROUND

Sarcopenia commonly affects older people and is characterized by loss of both muscle mass and strength[1]. It is associated with disability, a loss of independence, reduced quality of life and costly[1,2]. European Working Group on Sarcopenia in Older People (EWGSOP) has recently defined sarcopenia as a combination of both low muscle mass and low muscle function. EWGSOP proposed three different method to define low muscle mass[1]:

- •gender specific cut-off values as derived from a younger reference group (< 2 standard deviation, age 18-40 years)
- •gender-specific lowest 20% of appendicular skeletal muscle mass of a predictive population
- •gender-specific lowest 20% of the distribution of residuals of the linear regression on appendicular lean mass adjusted for fat mass and height

The aims of this study were to firstly establish gender specific cut-off points for low skeletal muscle mass using the three methods as identified by the EWGSOP and then report the prevalence of sarcopenia in older (aged 65 years and older) Australians living in the community.

METHOD

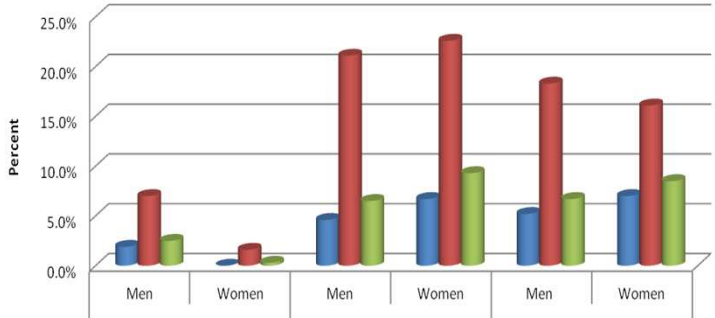
Three cohorts were investigated in this study: The Cytokine, Adiposity, Sarcopenia and Ageing Study (CASA), the North West Adelaide Health Study (NWAHS), and the Florey Adelaide Male Ageing Study (FAMAS). The three cohorts were combined to derive two broad population groups: younger reference population (aged 18-40 years; CASA and FAMAS; men, n=114 and women, n=23) and older group (aged ≥65; FAMAS and NWAHS; men, n=611 and women, n=375). Gender specific cut-offs levels were identified for low muscle mass using three different methods. Low grip strength was determined using established cut-offs of <30.3kg for men and <19.3kg for women to estimate the prevalence of sarcopenia.

RESULTS

The prevalence of low muscle mass and low grip strength in the North West Adelaide Health Study (NWAHS) and Florey Adelaide Male Ageing Study (FAMAS) based upon dual absorptiometry x-ray assessments of appendicular skeletal muscle mass

	Low grip strength (n%) Laurateni's Criteria	**Low SMI (n%)** <2 SD below mean of younger reference group (FAMAS and NWAHS) (Table 1)	**Low SMI (n%)** Gender Specific lowest 20% of study group (FAMAS and NWAHS)	**Low SMI (n%)** Residuals of linear regression on appendicular lean mass adjusted for fat and height(FAMAS and NWAHS)
NWAHS+FAMAS Men				
cut-offs	**<30.3 Kg**	**<6.89 Kg/m²**	**<7.36 Kg/m²**	**< -2.15 Kg**
65-<80 (n=540)	81 (15.0)	38 (7.0)	92 (17.0)	101 (18.7)
80+ (n=71)	33 (46.5)	9 (12.7)	29 (40.8)	21 (29.6)
Total 65+ (n=611)	114 (18.7)	44 (7.2)	121 (19.8)	122 (20)
NWAHS Female				
cut-offs	**<19.3 Kg**	**<4.32Kg/m²**	**< 5.81 Kg/m²**	**<-1.42 Kg**
65-<80(n=313)	105 (33.5)	0 (0)	56 (17.9)	63 (20.1)
80+ (n=62)	39(62.9)	1 (1.6)	18 (29)	12 (19.4)
Total 65+ (n=375)	144 (42.5)	1 (1.6)	74 (19.7)	75 (20)

Comparison of prevalence rate of sarcopenia as defined by EWSOP, by using different methods of SMI cut-points derivation with a low grip strength (<30.3kg for men and <19.3kg for women).



CONCLUSION

The key finding from this study is that in combination with grip strength, different methods of determining low muscle mass results in different sarcopenia prevalence. The cut-off points for low muscle mass derived by the gender specific lowest 20% method of a predictive population and the linear regression method yielded similar prevalence rates for low muscle mass and sarcopenia. Therefore, a consensus is required to identify the preferred method to define Sarcopenia. This will allow for pooling of research data. However, sarcopenia is common in the community. Given that sarcopenia is linked to morbidity and costs, early recognition and intervention through exercise and nutritional programs may contribute to healthy ageing outcomes and so, a reduction in health costs[3].

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Sarcopenia in Community Dwelling Older Australian

Yu S[1,2,3], Appleton S[2], Adams RJ[2], Chapman I[2], Wittert G[2], Visvanathan T[4] and Visvanathan R[1,2,3]

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BACKGROUND

•Sarcopenia commonly affects older people and is characterized by loss of both muscle mass and strength[1].

•The European Working Group on Sarcopenia in Older People (EWGSOP) has recommended that sarcopenia be defined as a combination of both low muscle mass and low muscle function. More specifically sarcopenia is defined as the presence of low skeletal muscle index (SMI) at least two standard deviations below the mean SMI in healthy young individuals aged between 18-40 years and the presence of low physical function such as low grip strength[1].

•To date, the prevalence of sarcopenia in Australia has not been investigated.

• The aims of this study were to:

1. Establish gender specific SMI cut-offs.
2. Determine the prevalence of sarcopenia in community dwelling older (aged 65 years and older) Australians.

METHOD

• Three South Australian community cohorts were investigated: The Cytokine, Adiposity, Sarcopenia and Ageing Study (CASA), Florey Adelaide Male Ageing Study (FAMAS) and North West Adelaide Health Study (NWAHS).

• Gender specific cut-offs (<2SD for healthy young 18-<40 years) for SMI were determined from the CASA and FAMAS study combined.

• Low SMI in conjunction with low grip strength confirmed the diagnosis of sarcopenia.

•Descriptive data was expressed as mean ± standard deviation (SD).

•Independent samples-t-test was used to assess the mean difference in the characteristics variables between the genders and groups (men in FAMAS and CASA). P<0.05 was considered statistically significant.

RESULTS

Table 1: Characteristics of subjects aged 18-<40 years from the Florey Adelaide Male Ageing Study (FAMAS) and The Cytokine, Adiposity, Sarcopenia and Ageing Study (CASA).

	Men Mean (SD)	**Women** Mean (SD)	**Total** Mean (SD)
FAMAS	**N=93**	**N=0**	**N=93**
Age (SD), years	37.4 (1.4)		37.4 (1.4)
Weight (SD), kg,	87.9 (15.2)		87.9 (15.2)
Height (SD),m	1.8 (0.1)		1.8 (0.1)
BMI (SD), kg/m^2	28.1 (4.3)		28.1 (4.3)
ASM (SD), kg	28.6 (4.2)		28.6 (4.2)
SMI (SD), kg/m^2	9.1 (1.1)		9.1 (1.1)
CASA	**N=24**	**N=23**	**N=47**
Age (SD), years	28.2 (8.0)	32.2 (7.3)	29.7 (7.8)
Weight (SD), kg,	87.0 (18.5)	69.3 (15.3)	78.3 (19.0)
Height (SD),m	1.8 (0.1)	1.7 (0.1)	1.7 (0.1)
BMI (SD), kg/m^2	26.8 (5.5)	25.5 (5.5)	26.2 (5.5)
ASM (SD), kg	28.5 (4.7)	18.4 (4.1)	23.5 (6.7)
SMI (SD), kg/m^2	8.8 (1.1)	6.7 (1.2)	7.8 (1.5)
Total (FAMAS + CASA)	**N=177**	**N=23**	**N=140**
Age (SD), years	35.5 (5.3)	31.2 (7.3)	34.8(5.9)
Weight (SD), kg,	87.7 (15.9)	69.3 (15.3)	84.7 (17.2)
Height (SD),m	1.8 (0.1)	1.7 (0.1)	1.8 (0.1)
BMI (SD), kg/m^2	27.8 (4.6)	25.5 (5.5)	27.4 (4.8)
ASM (SD), kg	28.6 (4.3)	18.4 (4.1)	26.9 (5.7)
SMI (SD), kg/m^2	9.1(1.1)	6.7 (1.2)	8.7 (1.4)
SMI Cut-off(DEXA)	6.86	4.31	

Table 2: The prevalence of sarcopenia in the North West Adelaide Health Study (NWAHS) and Florey Adelaide Male Ageing Study (FAMAS) based upon dual absorptiometry x-ray assessments of appendicular skeletal muscle mass

	Low grip strength n(%) Lauraten's Criteria[6]	**Pre-sarcopenia (n%)** Low SMI using DXA [Pre-Sarcopenia] <2 SD below mean of reference group (Table 1)	**Pre-sarcopenia (n%)** Low SMI using DXA [Pre-Sarcopenia] <2 SD below mean of reference group-Rosetta Study[2]	**Sarcopenian(%)** <2 SD below mean of reference group (Table 1) Low SMI DXA+ Low Grip strength	**Sarcopenia (n%)** <2 SD below mean of reference group-Rosetta Study[2] Low SMI DXA+ Low Grip strength
FAMAS Men					
cut-off	**<30.3 Kg**	**<6.86Kg/m^2**	**<7.26Kg/m^2**		
65 + (n=256)	6 (2.3)	22 (8.6)	41 (16.0)	1 (0.4)	1 (0.4)
65-<80 (n=252)	6 (2.4)	22 (8.7)	41 (16.3)	1 (0.4)	1 (0.4)
80+ (n=4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NWAHS Men					
cut-off	**<30.3 Kg**	**<6.86Kg/m^2**	**<7.26 Kg/m^2**		
65+ (n=355)	108 (30.4)	22 (6.2)	63 (18.0)	12 (3.4)	33 (9.3)
65-<80 (n=288)	75 (26.0)	13 (4.5)	39 (13.5)	7 (2.4)	22 (7.6)
80+ (n=67)	33 (49.3)	9 (13.4)	25 (37.3)	5 (7.5)	11 (16.4)
NWAHS+FAMAS Men					
cut-off	**<30.3 Kg**	**<6.86 Kg/m^2**	**<7.26 Kg/m^2**		
65+ (n=611)	114 (18.7)	44 (7.2)	105 (17.2)	15 (2.5)	34 (5.6)
65-<80 (n=540)	81 (15.0)	35 (6.5)	80 (14.8)	10 (1.9)	23 (4.3)
80+ (n=71)	33 (46.5)	9 (12.7)	25 (35.2)	5 (7.0)	11 (15.5)
NWAHS Female					
cut-off	**<19.3 Kg**	**<4.31Kg/m^2**	**<5.50 Kg/m^2**		
65+ (n=375)	144 (42.5)	1 (0.3)	24 (9.1)	1 (0.3)	18 (4.8)
65-<80(n=313)	105 (33.5)	0 (0)	25 (8.0)	0 (0)	10 (3.2)
80+ (n=62)	39(62.9)	1 (1.6)	9 (14.5)	1 (1.6)	8 (12.9)

CONCLUSION

• Sarcopenia was more common in men and the prevalence was highest in those 80 years and older, the fastest growing age group in Australia.

• This study provides preliminary cut-offs for the Australian population to support the diagnosis of sarcopenia in clinical practice but it would be prudent to reach a consensus cut-offs in a larger cohort.

• The cut-offs identified in this study are very much lower than those previously identified almost 20 years ago and this may indicate reduced muscle mass reserve in younger age in our community as a result of increasingly sedantary lifestyle.

• This may require urgent consideration and intervention.

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