Diet, inflammation and skeletal muscle mass in women

A thesis submitted to the University of East Anglia
in accordance with the requirements of the
Degree of Doctor of Philosophy

By

Eirini Kelaiditi, MSc

Norwich Medical School, University of East Anglia

August 2013

"I certify that the work contained in the thesis submitted by me for the Degree of Doctor of Philosophy is my own original work, except where due reference is made to other authors, and has not previously been submitted by me for a degree at this or any other university"

Signed

Eirini Kelaiditi

ACKNOWLEDGEMENTS

My deepest and sincere gratitude goes to my supervisors Dr Ailsa Welch and Prof Aedín Cassidy for giving me the opportunity to work on this project at the Department of Nutrition at UEA, and for their expert guidance, constant support, patience and constructive discussions throughout the PhD process. I am indebted to my primary supervisor, Ailsa Welch. Her enthusiasm for research and her dedication have been sources of inspiration for me. My sincere thanks go to Dr Amy Jennings, co-supervisor of this thesis, for her help and support whenever needed and for her useful comments.

I would like to thank Tim Spector from the Twins Unit, St Thomas' hospital (London, UK) and Alex Macgregor and Aedin Cassidy from UEA for facilitating access to the TwinsUK data.

I am grateful to my new supervisor, Dr Matteo Cesari for giving me the time and space to complete my thesis while working as a Postdoctoral Researcher in his group at the Institute of Aging (Gérontopôle), University Paul Sabatier III in Toulouse, France.

I am happy to thank my good friends Sara Hazim, Lawrence Barrera, Anna Wawer from UEA and Paula Sanchez for always being caring and supportive like a family. I also thank to Helen Flaherty, Fiona O'Neill, Henriette Finck and Lucy Bain from UEA for all our nice discussions and their support and friendship. Special thanks go to my lovely friends Marianthi Zarife and Dimitra Ioannou from back home (Greece) for always being supportive over the last years.

Most of all, I would like to thank my wonderful parents Athina and Fotios Kelaiditis, my beloved twin brother Christos Kelaiditis, and my lovely grandparents Anneta and Stergios Kiosses. Your priceless love, caring, constant support and understanding have been remarkable from the beginning until the end of this process. Even though you were physically far, your thoughts and motivation at every stage encouraged me to continue this PhD. You have always been there for the challenging and the nice moments. I could have never accomplished my dreams without your love, and this thesis is dedicated to all the five of you.

ABSTRACT

Evidence is growing that diet, lifestyle factors and chronic inflammation influence sarcopenia. Sarcopenia is the progressive decline of muscle mass, strength and function that occurs with healthy ageing. The ageing process is also associated with a gradual increasing production of pro-inflammatory cytokines, which may potentially enhance the development of sarcopenia. Dietary longitudinal studies have shown associations between protein intake and muscle mass in older people but results of supplementation studies in enhancing muscle mass and strength are equivocal. Additionally, short-term dietary interventions with essential amino acid supplementation have shown promising effects on muscle protein synthesis. Emerging evidence suggests that a number of nutrients may be associated with muscle mass and strength either due to their anti-inflammatory properties or their involvement in muscle biology. However, there are currently few population studies examining the relative importance of specific nutrients in association with muscle mass, muscle strength and muscle quality. Therefore, this thesis aimed to examine associations between the habitual dietary intake of a range of micronutrients, and diet quality (assessed by five predefined diet quality scores) and indexes of muscle mass, strength and muscle quality in female participants aged 18-79 years from the TwinsUK cohort. An additional aim was to examine associations between diet and biomarkers of inflammation and to investigate whether diet could also influence the relationship between muscle mass and inflammation. The results suggested a significant positive association between intakes of vitamins C and E, magnesium, potassium and a range of carotenoids and indexes of muscle mass with scale of associations ranging between 1.5-4.6%. However, no associations were observed for protein and essential amino acid intakes. Higher adherence to the Mediterranean Diet score (MDS), Healthy Diet Indicator (HDI), Diet Quality Index (DQI), Alternate Healthy Eating Index (AHEI), and DASH-style score was significantly associated with measurements of muscle mass, with associations ranging between 1-3% between quintiles. Furthermore, a number of nutrients and the HDI and AHEI scores were inversely associated with plasma levels of the inflammatory marker C-reactive protein (*CRP*). Interestingly, intakes of magnesium, potassium, vitamin C, carotene, β-carotene, glutamine, and the MDS, HDI and AHEI scores attenuated the association between indexes of muscle mass and CRP by 1-8%, inferring that these components mediate the relationship between muscle mass and inflammation. In conclusion, the findings of this thesis emphasise the importance of consumption of a variety of plant-based nutrients and of overall diet quality for the conservation of muscle mass, and shed new light on the influence of these dietary components on sarcopenia related inflammation.

LIST OF ABBREVIATIONS

Abbreviation	Description
AHEI	Alternate healthy eating index
aMED	Alternate Mediterranean diet score
AP-1	Activator Protein 1
BCAAs	Branched-chain amino acids
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CAF	Central abdominal fat
CDC/AHA	American Heart Association Consensus panel and the Centers for Disease Control and Prevention
CRP	C-reactive protein
CVD	Cardiovascular disease
CT scan	Computed tomography scans
C1QTNF9	C1q and tumor necrosis factor-related protein 9
DASH-style score	Based on Dietary Approaches to Stop Hypertension diet
DEXA scan	Dual energy X-ray absorptiometry scan
DRV	Daily reference value
DQI	Diet quality index
DZ twins	Dizygotic twins
EPIC study	European Prospective Investigation into Cancer and Nutrition
FFM	Fat free mass
FFM%	Percentage fat free mass
FFMI	Fat free mass index
FFQ	Food frequency questionnaire
GH	Growth hormone
GPPAQ	General Practice Physical Activity

	Questionnaire
GSH/GSSG	Ratio of reduced to oxidised glutathione
HCLP diet	High carbohydrate low protein diet
HDI	Healthy diet indicator
HEI	Healthy eating index
HPLC diet	High protein low carbohydrate diet
HRT	Hormone replacement therapy
hsCRP	High-sensitive CRP
IGF-1	Insulin-like growth factor 1
IL-1ß	Interleukin-1ß
IL-6	Interleukin 6
LDL	Low density lipoprotein
MDS	Mediterranean diet score
MZ twins	Monozygotic twins
n-3 PUFAS	n-3 polyunsaturated fatty acids
NDNS	National Diet and Nutrition Survey
NFCS	Nationwide Food Consumption Survey
NF-*k*B	Nuclear factor kappa-B
NSAID	Non-steroid anti-inflammatory drugs
NSP	Non-starch polysaccharides
PPBP	Pro-platelet basic protein
RDA	Recommended daily allowance
ROS	Reactive oxygen species
sTNF-aRII	Soluble tumor necrosis factor alpha receptor II
TNF- α	Tumor necrosis factor-αlpha
USDA	United States Department of Agriculture
1,25-(OH)(2)D(3)	1-alpha, 25-Dihydroxyvitamin D(3)

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 5

CHAPTER 9

LIST OF TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 7

CHAPTER 8

PUBLICATIONS AND PRESENTATIONS

Publications:

Kelaiditi E, Jennings A, Cassidy A, Macgregor A, Spector T, Welch A (2012) Plant-based nutrient intake is positively associated with fat free mass index. *Proceedings of the Nutrition Society*, 71 (OCE2), E191.

Kelaiditi E, Cassidy A, Macgregor A, Spector T, Welch A (2011) Diet quality is positively associated with indexes of muscle mass. *Proceedings of the Nutrition Society*, 70 (OCE4), E190.

Presentations:

Kelaiditi E, Jennings A, Cassidy A, MacGregor A, Spector T, Welch A (2012) *Plant based vitamin and mineral intake is positively associated with indexes of muscle mass.* Nutrition Society summer meeting 2012, University of Belfast. Proceedings of the Nutrition Society.

Kelaiditi E, Jennings A, Cassidy A, MacGregor A, Spector T, Welch A (2012) *Plant based vitamin and mineral intake is positively associated with indexes of muscle mass.* Postgraduate Research student conference 2012, University of East Anglia.

Kelaiditi E, Cassidy A, MacGregor A, Spector T, Welch A (2011) *Diet quality is positively associated with indexes of muscle mass.* Nutrition Society summer meeting 2011, University of Reading. Proceedings of the Nutrition Society.

Kelaiditi E, Cassidy A, MacGregor A, Spector T, Welch A (2011) *Diet quality is positively associated with indexes of muscle mass.* Postgraduate Research student conference 2011, University of East Anglia.

Kelaiditi E, Cassidy A, MacGregor A, Spector T, Welch A (2011) *Diet quality is positively associated with indexes of muscle mass.* UEA Postgraduate Research Showcase

TABLE OF CONTENTS

"None are so old as those who have outlived enthusiasm"

Henry David Thoreau

"*The three generations*" ©Eirini Kelaiditi

Chapter 1

General Introduction

1.0 Introduction

1.0.1 The ageing population

Life expectancy has been considerably improved over the past century and in recent years these increases in life expectancy have been attributed to a decline in later-life mortality (Oeppen and Vaupel 2002) (**Figure 1.1**). However, despite the population living longer, this greater number of life years has been associated with increases in chronic morbidity and mortality, including cardiovascular disease (Wald *et al.* 2011), diabetes (Fagot-Campagna A *et al.* 2005), and cancer (Vasto S *et al.* 2009), for which age is an independent risk factor (Fontana 2009).

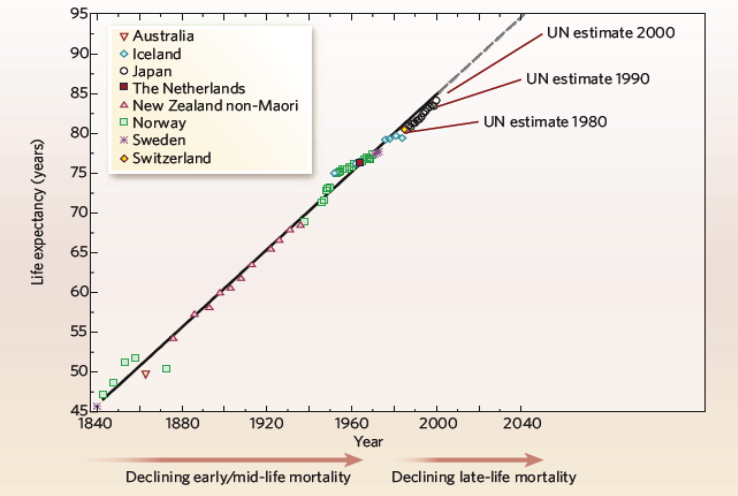


Figure 1.1: Life expectancy increase over the last 200 years[1].

There is a trajectory in ageing worldwide, which will to lead to an increase in age-related diseases with profound implications both socially and economically. Globally the number of people aged over 60 years was 11 % of the total population in 2011 and the UK is following a similar pattern.

[1] Adapted from Oeppen J & Vaupel JW (2002) *Science* 296, 1029-1031.

It is predicted that this rate is going to increase globally and by 2050 the number of people aged 60 and over will rise to 22% of the total population (approximately 2 billion). There will be concurrent increases for the number of people aged 80 years and over with numbers expected to double to 4% of the world population (approximately 400 million) (**Figure 1.2**) (Beard JR *et al.* 2011).

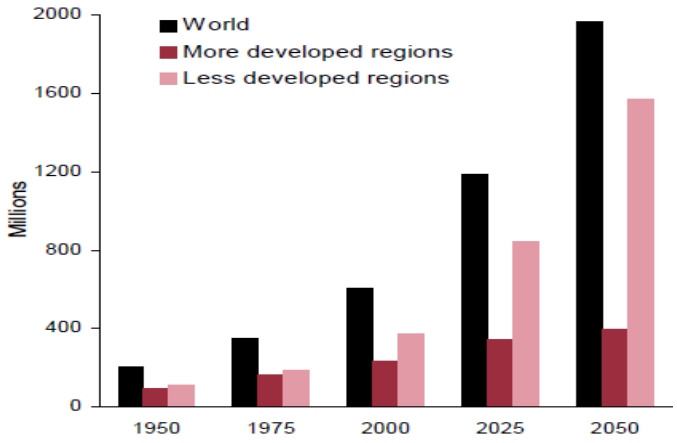


Figure 1.2: Population aged 60 years and over (world and development regions), 1950-2050[2].

Ageing is associated with significant declines in physical, cognitive and social function that contribute to loss of independence and quality of life, which can further lead to substantial economic costs (Gonyea 2005). It has been suggested that better living standards, improved lifestyle (by promoting a healthy diet, physical activity and non-smoking), better education, and greater access to quality healthcare services could effectively contribute to the improvement of quality of life in the elderly (Britton *et al.* 2008). Therefore, examining influences on the ageing process is important for social and economic reasons.

[2] Adapted from Beard JR et al (2011) Geneva: World Economic Forum.

1.0.2 Determinants of successful ageing

Given the global trends in population ageing, a multitude of epidemiological observations have focused on identifying risk factors that lead to functional decline (Stuck *et al.* 1999), mortality and morbidity (Strawbridge *et al.* 1996), and hence to diminished health and low quality of life. Suggested risk factors associated with poor health and quality of life include cognitive impairment, depression and comorbidities. Changes in body composition have been suggested as an issue in terms of health risk, as well as low levels of physical activity, smoking, poor self-perceived health, low frequency of social contacts and vision impairment (Stuck *et al.* 1999). However, nutrition has not always been included as a factor that might influence health and quality of life, although research has shown that nutrition interacts with genotype to influence health and ageing (Mathers 2002).

To date, few epidemiological studies have examined modifiable behavioural factors that positively predict healthy living in older cohorts (Khaw *et al.* 2008, Peel *et al.* 2005). In a systematic review determinants that were associated with healthy ageing included not smoking, being physically active, maintaining body mass index (BMI, kg/m^2) within normal ranges, and moderate intake of alcohol. Moreover, positive health behaviours and practices such as combined increased physical activity and not smoking were shown to enhance healthy ageing. In the same systematic review findings for the influence of diet on healthy ageing were inconclusive as dietary intake was measured in only three of the eight selected studies (Peel *et al.* 2005). Findings showed that a high quality diet, based on the

Mediterranean diet, was associated with maintenance of health status in a European study among men and women aged 70 to 75 years from the SENECA cohort (Haveman-Nies *et al.* 2003). Moreover, lower values for a Japanese diet score (based on questions concerning usual intake of ten common Japanese foods and ten Western foods, calculated as the ratio of intake of Japanese foods to total intake) were associated with healthy ageing among men aged 85 years after 12 years follow-up (Reed *et al.* 1998). However, the frequency of eating breakfast or snacking was not a predictor of healthy ageing in this systematic review.

Considering that ageing and overall health are influenced strongly by lifestyle, more recent, results from the Norfolk arm of the European Prospective Investigation into Cancer and Nutrition (EPIC) study have shown that combined health behaviors, including non-smoking, consumption of five or more servings of fruit and vegetable per day, consumption of less than 14 units alcohol per week, and physical activity predicted a four-fold difference in total mortality over an average 11 years of follow-up in men and women (Khaw *et al.* 2008). This equated to an estimated impact of 14 years in chronological age (**Figure 1.3**).

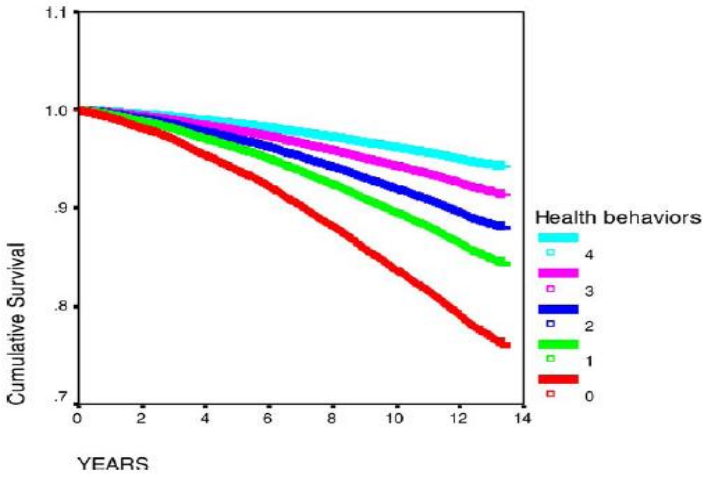


Figure 1.3: Survival curves according to the number of health behaviours in men and women aged 45-79 years from the EPIC-Norfolk study. Analysis was adjusted for age, sex, body mass index and social class and the participants were free from cardiovascular disease and cancer. Cumulative survival was about 75% for those scoring zero and increased to 95% for those scoring four[3].

Therefore, there is a major challenge for research to investigate how to improve the quality of life and how diet may promote healthy and successful ageing. Research in this direction will inform public health policies to implement evidence-based research findings for the development of prevention plans and interventions for lifestyle modification, including dietary changes with a focus on improving the well-being and quality of life in the elderly.

1.0.3 Healthy ageing

Healthy ageing is a complex and multifactorial process, characterised by progressive physiological and pathological deterioration over time, it can however be influenced by environmental changes, including nutrition. Indeed, there is evidence that greater adherence to healthy dietary patterns,

[3] Adapted from Khaw K-T et al. (2008) *PLoS Med* 5(1), e12.

such as the Mediterranean diet (high in consumption of fruits and vegetable, legumes, cereals, fish, moderate red wine and low in consumption of red and processed meats and dairy products) have been associated with lower risk of overall mortality (Trichopoulou *et al.* 2005) and age-related diseases, including cardiovascular disease, cancer, Parkinson's and Alzheimer's disease (Sofi *et al.* 2008). Ageing is also associated with progressive increased susceptibility to disease risk and mortality (Gil del Valle 2011), and there are many physiological alterations that occur with ageing and influence health status, including changes in body composition (Cruz-Jentoft *et al.* 2010).

Two parallel trends occur with physiological ageing, sarcopenia and chronic inflammation. Sarcopenia is a condition characterised by progressive loss of skeletal muscle mass, strength and muscle quality (muscle strength corrected for muscle mass) with physiological ageing, and has been associated with an increased risk of adverse outcomes, such as physical disability, poor quality of life and death (Baumgartner 2000, Cesari *et al.* 2009, Cruz-Jentoft *et al.* 2010). The second factor, chronic inflammation, has been clearly established in large epidemiological studies in older adults as having an influence on the ageing process and age-related diseases (Singh and Newman 2011). Notably, it is evident that there is a link between sarcopenia and age-related chronic inflammation, as it has been shown that increased levels of inflammation can be detrimental for muscle mass in both humans (Anker *et al.* 1999) and in animal models (Hoshino *et al.* 1991),

suggesting that age-related chronic inflammation is an important underlying mechanisms of sarcopenia (Chung *et al.* 2009).

Diet is one environmental factor that can potentially influence sarcopenia and inflammation. Although physical activity and smoking are known influences on sarcopenia, to date, diet has been less investigated at a population level (Houston *et al.* 2008, Robinson SM *et al.* 2008, Scott *et al.* 2010, Stookey JD *et al.* 2005). Given that sarcopenia and inflammation associated with the ageing process can lead to increased morbidity and low quality of life, it is emerging that targeting sarcopenia and inflammation through healthy dietary modifications, to potentially compress morbidity and delay the onset of disability, may promote successful and healthy ageing and quality of life.

1.1 Mechanisms associated with the ageing process

Ageing process is driven by a progressive, lifelong accumulation of a variety of molecular and cellular damage, which leads to age-related frailty, disability, disease and eventually death (Kirkwood 2005). This damage is essentially random, but as it is accumulated with age and it is regulated by complex maintenance and repair pathways, which may be modulated by metabolic factors (**Figure 1.4**) (Kirkwood 2008). These pathways are further enhanced by stress, inflammation, poor diet and adverse environment and can be delayed by better diet, lifestyle and a favourable environment.

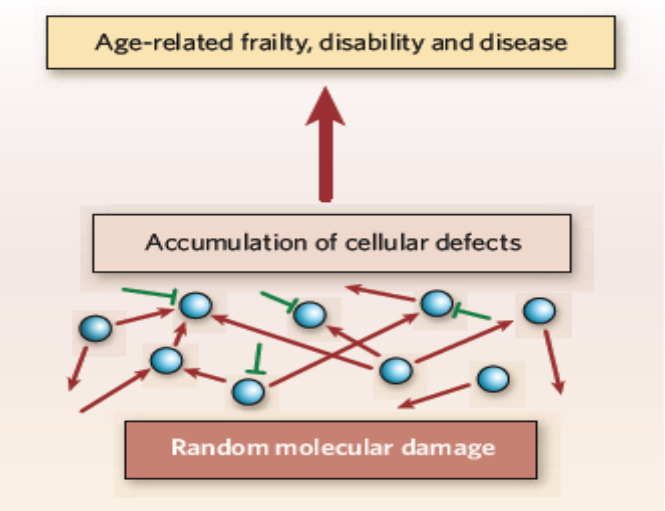


Figure 1.4: Schematic illustrating suggested mechanisms associated with the ageing process[4].

In addition, the ageing phenotype is linked to a reduced function at a cellular and molecular level, because of inter-cell differences in gene expression in older individuals due to altered epigenetic marks, which lead to low tissue function (Mathers and Ford 2009).

It is thought that major contributors that underpin cellular and molecular damage are oxidative stress and inflammation (Chung *et al.* 2009, Gil Del Valle 2010). There is already plenty of evidence that supports the theory of oxidative damage by reactive oxygen species (ROS) or by the dysfunction of mitochondria – the organelles within cells that generate energy - on the ageing process (Finkel and Holbrook 2000). Specifically, it is suggested that DNA damage accumulates with ageing possibly due to enhanced generation

[4] Adapted from Kirkwood (2008) *Nature* 451, 644-647.

in ROS and a decreased capacity of DNA repair with age (Burkle *et al.* 2002, Promislow 1994).

Interestingly, studies in long-lived genetic mouse models have shown that under optimal conditions with minimum stress, oxidative stress and damage have been shown to play little role in ageing, whereas, under chronic stress associated with diseases, such as Alzheimer disease, type II diabetes, and cardiovascular disease, oxidative stress and damage plays an important role in ageing, suggesting that ageing and age-related diseases share the common pathway of cumulative oxidative stress (Salmon *et al.* 2010, Selman and Withers 2011).

Accumulation of oxidative stress and damage as well as oxidative stress-induced redox imbalances can further activate a range of inflammatory mediators and increase systemic inflammation (Chung *et al.* 2009). Therefore, both the deregulation of the immune system and the redox imbalance with ageing implement the age-related inflammatory hypothesis (Chung *et al.* 2011). Notably, a number of epidemiological studies have examined the influence of inflammation in the process of ageing and age-related diseases in older adults, suggesting that markers of inflammation were elevated in older adults, and particularly interleukin-6 (IL-6) most robustly predicted various age-related diseases, disability, and mortality (Singh and Newman 2011).

Overall, the complexity of the ageing process is highlighted by the multiplicity of the mechanisms which contribute to its aetiology and should be considered in synergy in future research on ageing.

1.2 Sarcopenia

Since the ageing population is projected to increase, there is an emerging interest in examining environmental influences on sarcopenia. Sarcopenia is described by a progressive decline of muscle mass, strength and function as a consequence of healthy ageing, and accompanied by a number of adverse outcomes, including physical disability, decreased mobility, increased frailty and incidence of falls and increased risk of mortality (Cruz-Jentoft *et al.* 2010).

1.2.1 Importance of muscle mass and strength

Conservation of muscle mass is important for health. Skeletal muscle plays a major role in the body's movement and maintenance of stability and in protein metabolism, because it serves as a source of amino acids to maintain protein synthesis in essential tissues and vital organs when amino acid absorption from the gut is limited (Lang *et al.* 2010). Furthermore, in the fasting state, muscle mass can serve as a precursor for hepatic gluconeogenesis by supplying blood amino acids (Wolfe 2006). In addition, alterations in muscle metabolism can importantly affect chronic diseases, such as heart disease and cancer, which are often associated with extensive

loss of muscle mass and strength as well as metabolic function and this can be crucial in determining survival (Kadar *et al.* 2000).

Preservation of muscle strength and muscle quality is also important as low muscle strength and muscle quality have been related with increased physical disability, frailty, incidence of falls, and morbidity, and also with lower functional performance, and quality of life (Bassey 1997). Low muscle strength and muscle quality have also been shown to be predictive of mortality and disability (Clark 2008, Giampaoli *et al.* 1999, Newman *et al.* 2006, Rantanen *et al.* 1999, Rantanen *et al.* 2003).

1.2.2 Fat free mass and ageing

It is known that ageing is associated with changes in body composition characterised by increases in body weight and changes in the distribution of adipose tissue, and decreases in fat free mass, which start after young adulthood (Nooyens *et al.* 2009, Stenholm *et al.* 2008). Fat free mass is composed of non-fat lean soft tissues and bone mineral content and is an important metabolically active component of body composition (Nelson *et al.* 1992). Skeletal muscle mass represents the largest fraction of the fat free body mass (Heymsfield *et al.* 1990) and therefore, whole body fat free mass is commonly used in epidemiological studies (Jourdan *et al.* 2012).

Previous cross-sectional studies have shown that advanced age was associated with lower fat free mass (Janssen *et al.* 2000, Kyle *et al.* 2001). Longitudinal studies have also shown a similar trend with increased age

associated with lower fat free mass and higher fat mass over time of follow-up (Ding *et al.* 2007, Guo *et al.* 1999, Kyle *et al.* 2006). However, most of these studies were in older populations aged 60 years and over, where changes in body composition are expected (Ding *et al.* 2007, Kyle *et al.* 2001). Presently, only one cross-sectional study has evaluated age-related body composition differences in a cohort including younger adults (aged 18 to 88 years) (Janssen *et al.* 2000). The results showed that differences in muscle mass started at age thirty years, but differences in absolute muscle mass were more noticeable after fifty years of age in both men and women (an example of this effect is shown in **Figure 1.5** A and B). One retrospective study among a representative healthy Italian population of men and women also, included younger adults (aged 20 to 80 years) and it showed that fat free mass index was decreased only in the oldest group of women (70-80 years) (Coin *et al.* 2008).

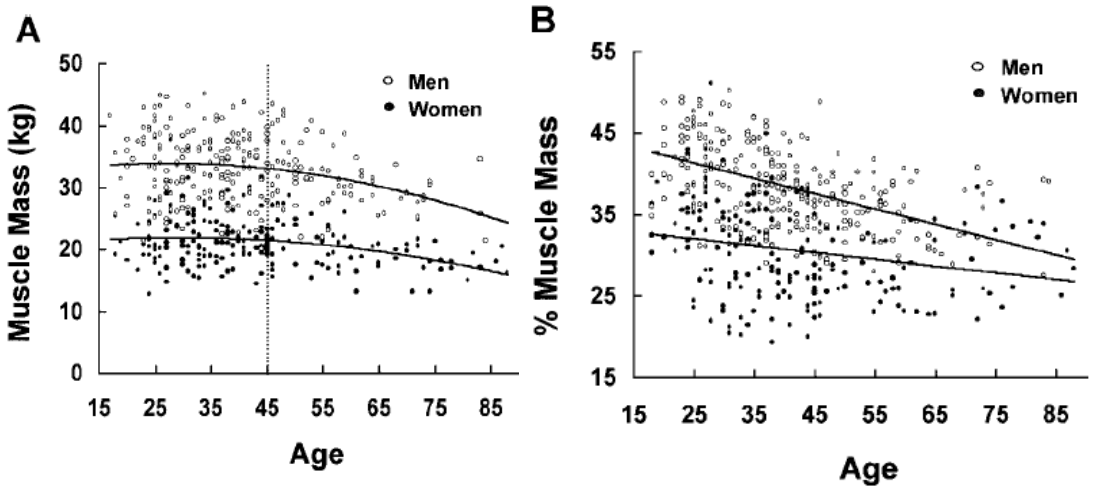


Figure 1.5 Adapted from Janssen *et al.* 2000 A) Curvilinear relationship between muscle mass (kilograms) and age in men and women aged 18-88 years, with changes in muscle mass occurring at ~ 45 years in both men and women. **B)** Linear relationship between relative muscle mass with age, which was greater in men ($p < 0.01$) than in women.

Overall, body composition differences are known to occur with age more likely as a result of an imbalance between energy intake and energy expenditure, which are associated with an increased sedentary lifestyle, and characterised by progressive increases in fat mass and decline in fat free mass, but other aspects of nutrition may also influence fat free mass (Kyle *et al.* 2001). Moreover, prevalence rates of sarcopenia may vary between different studies because of differences in methods used to assess fat free mass, but dual-energy x-ray absorptiometry scan (DEXA) has shown to be a reliable method for measuring fat free mass and this method was used for the purposes of this project (Cruz-Jentoft *et al.* 2010).

Therefore, it is important that studies examine body composition differences in cohorts including younger adults, as age-related decline of muscle mass starts to develop in the third decade and continuously throughout the rest of life (Cesari and Pahor 2008). This might be a useful consideration for future clinical interventions on sarcopenia, including participants of a wider age range and target the progress of sarcopenia earlier in adult life.

1.2.3 Muscle strength and ageing

Muscle strength is another important component of sarcopenia and ageing has been associated with lower muscle strength in a number of epidemiological studies (an example of this effect is shown in **Figure 1.6**) (Frontera *et al.* 1991, Hughes *et al.* 2002, Lynch *et al.* 1999, Rantanen *et al.* 1998). The most common measurements used in epidemiological studies assessing muscle strength are grip, hip and knee strength. Grip strength is an

important measurement for overall health, as it has been associated with an increased risk of disability, morbidity and mortality in both men and women, with declined quality of life in older individuals, and has been suggested as a useful marker of muscle function and sarcopenia (Gale *et al.* 2007, Laukkanen *et al.* 1995, Rantanen 2003, Rantanen *et al.* 1999, Rantanen *et al.* 2003, Sayer *et al.* 2006, Syddall *et al.* 2003).

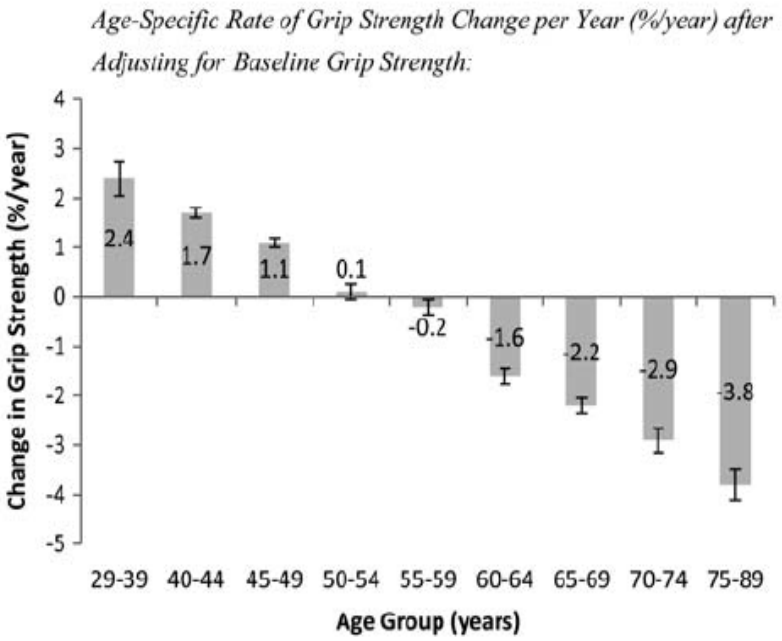


Figure 1.6 Adapted from Forrest *et al.* 2012 Changes in hand grip strength during 4.5 years of follow-up in Afro-Caribbean men aged 29 to 89 years, with considerable decreases starting after fifty years of age.

1.2.4 Relationship between muscle mass and strength

Epidemiological studies have shown that after the age of 50 years muscle mass declines of 1-2 % per year on average, and muscle strength declines at a rate of 1.5 % per year, increasing to 3 % per year after the age of 60 years (Evans 1997, Hughes *et al.* 2002, Morley *et al.* 2001, Roubenoff and

Hughes 2000). The relationship between muscle mass and strength is well established (Baumgartner *et al.* 1998, Janssen *et al.* 2000, Rolland *et al.* 2008, Rosenberg 1997) however it may not necessarily be linear. It has been reported that older adults with greater muscle mass did not exhibit greater muscle strength (Park *et al.* 2006). Similarly, there is evidence that increases in muscle strength with resistance training in women were not followed by increases in muscle mass (Raue *et al.* 2009). Interestingly, recent data from the National Health and Nutrition Examination Survey (NHANES, 1999-2002) showed that in US men and women (aged >50 years) muscle mass and muscle strength were positively correlated (r = 0.365, P < 0.001) after adjusting for age and gender (Chen *et al.* 2013). In the same study, muscle mass explained 13.3 % of variance in muscle strength independent of the effect of age and gender (Chen *et al.* 2013). Comorbidity (e.g. obesity) may also modify the relationship between muscle mass and muscle strength (Chen *et al.* 2013) and therefore it is important that studies on sarcopenia examine both dimensions (Newman *et al.* 2003).

1.2.5 Muscle quality and ageing

Muscle quality which is defined as the ratio of muscle strength per unit of muscle mass has been used as an approach to measure the quality of muscles. Lower muscle quality has been associated with greater age (Lynch *et al.* 1999) and functional incapacities (Goodpaster *et al.* 2006). Although the preservation of muscle mass may be considerably important for the prevention of muscle strength loss during ageing, muscle quality may also be important in determining the loss of muscle strength with ageing (Barbat-

Artigas *et al.* 2012). To date, muscle quality has not been widely studied in relation to diet as such.

Therefore, it is important to examine body composition changes/differences with age, because it is believed that these changes will have significant implications for health status as they are associated with the development of chronic disease, and moreover are risk factors for physical disability, function, morbidity and early mortality, and consequently impaired quality of life (Atlantis *et al.* 2008, Baumgartner 2000).

1.2.6 Prevalence and epidemiology of sarcopenia

The increase in the average age of the population will ultimately increase the prevalence of sarcopenia, which appears to vary between different cohorts from 5-13 % on average in people aged 60-70 years and 11-50 % in people aged more than 80 years. This suggests that the prevalence of sarcopenia is considerably higher in the elderly (von Haehling *et al.* 2010). The estimates of the prevalence of sarcopenia are not well established and vary widely, due to differences among cohorts relating to the study population, the definition, and the different methods employed to assess muscle mass, including dual-energy x-ray absorptiometry scans (DEXA), magnetic resonance imaging, computed tomography (CT scan), and bioelectrical impedance analysis (BIA) (Abellan Van Kan 2009, Morley 2008). Since the prevalence rates are higher in the elderly, it might be important to prevent sarcopenia earlier in adult life.

Notably, the decline of muscle strength is higher in sedentary people and males with rates almost two times higher in men compared to women (Gallagher *et al.* 1997). Moreover, although men have on average greater amounts of muscle mass than women, they also have lower survival compared to women, implying that sarcopenia may potentially become a greater public health concern among female than male populations (Abellan Van Kan 2009, Roubenoff and Hughes 2000).

1.2.7 Aetiology and mechanisms of sarcopenia

The primary cause of sarcopenia is as yet unclear but multiple factors and mechanisms are involved in its aetiology. Age-related loss of muscle mass and strength can be attributed to an interaction of age-related environmental/lifestyle factors and genetic factors (Jones *et al.* 2009, Weber *et al.* 2010). These factors include hormonal changes, such as decreased levels of growth hormone – GH with age, insulin-like growth factor 1 – IGF-1, and testosterone, hormones that help to the growth and development of muscles (Baumgartner *et al.* 1999), insulin resistance (Park *et al.* 2009), chronic diseases (McDermott *et al.* 2004), increased catabolic activity and circulating levels of pro-inflammatory cytokines (such as interleukin 6 - IL-6 and tumor necrosis factor a - TNF-a) (Morley and Baumgartner 2004). Also, other factors including physical inactivity (Hughes *et al.* 2002, Peterson *et al.* 2010, Peterson *et al.* 2011), and altered nutritional status (such as decreased protein intake and malnutrition of ageing) (Chapman 2007, Katsanos *et al.* 2006, Volpi *et al.* 2003). Suggested mechanisms involve changes in muscle protein turnover, synthesis, loss of alpha-motor

neurons, and apoptosis (Baumgartner and Waters 2006). In addition, genetic heritability may partly explain the aetiology of sarcopenia as the heritability of muscle mass and strength may be up to 50-60%, suggesting that genetic factors control significantly the development of sarcopenia (Carey *et al.* 2007).

However, evidence is scarce on whether components of sarcopenia, including muscle mass, strength and muscle quality differ in relation to these risk factors. Each factor of the aetiology of sarcopenia potentially influences differently losses of muscle mass, strength, and therefore muscle quality. It is argued that improvements in muscle mass are more relevant for outcomes such as improvement of protein stores, but a contrary explanation is that improvements in muscle strength and function are more important for outcomes such as disability and mobility (Rolland *et al.* 2008). Findings from observational, intervention and experimental studies support the argument that different risk factors affect differently muscle mass and strength (Baumgartner *et al.* 1999, Goodpaster *et al.* 2006, Malbut *et al.* 2002, Morley and Baumgartner 2004). Notably, changes in lifestyle factors such as increased physical activity have shown to be beneficial for both muscle mass and strength (Roubenoff 2007). In contrast, administration of GH supplementation has shown increases in muscle mass, but not significant changes in muscle strength (Papadakis *et al.* 1996, Thompson *et al.* 1998). Moreover, it is seems that certain underlying mechanisms influence differently sarcopenia when age, gender or associated comorbidities are considered, highlighting the multiplicity of factors that

contribute to the development, progression and aetiology of sarcopenia (Doherty 2003).

Additionally, oxidative stress plays a major role in the pathogenesis of muscle mass and strength with age, caused by elevated damage of DNA, proteins and lipids of the ageing muscle (Jackson 2009). Chronic low-grade inflammation is also considered detrimental to skeletal muscle mass and strength in both humans and animal models (Howard *et al.* 2007, Siu *et al.* 2008). The underlying mechanisms involve enhancement of the redox-sensitive transcription factors such as Nuclear Factor kappa-light-chain enhancer of activated B cells (NF-kB), and Activator Protein 1 (AP-1) from reactive oxygen species (ROS). These two signaling pathways are involved in the regulation of pro-inflammatory cytokines, such as IL-6 and TNF-α, up-regulation of acute phase proteins, such as C-reactive protein, and inflammatory mediators, such as interleukin-10 (IL-10) (Janssen-Heininger *et al.* 2000).

Indeed, a number of epidemiological studies have shown an inverse relationship between muscle mass, strength and function and inflammatory cytokines (Barbieri *et al.* 2003, Payette *et al.* 2003, Schaap *et al.* 2009, Visser *et al.* 2002). These findings were also supported by animal studies showing that administration of IL-6 and TNF-a in rats were associated with changes in muscle metabolism, including increased skeletal muscle protein breakdown, decreased protein synthesis rate, decreased amino acid

concentration of skeletal muscle, and muscle wasting (Goodman 1991, Hoshino *et al.* 1991).

1.3 Diet and sarcopenia

The rate of age-related changes in muscle mass, strength and muscle quality varies across different populations. This implies that other factors such as diet, lifestyle, and genetics could influence muscle mass, strength, and function not only in the elderly, but throughout adult life (Robinson S *et al.* 2012). However, the influence of nutrition and better quality of diet on sarcopenia is not well studied (Robinson SM *et al.* 2008, Scott *et al.* 2010).

In recent years, it has been suggested that research should focus on examining associations between the "whole" diet, alongside individual foods and nutrients, and health status, as we consume a variety of foods and not simply nutrients, and therefore benefits in health status are more likely to be determined by the total diet (Jacobs *et al.* 2009). Diet scores examining dietary patterns of a population have being used for more than a decade as an approach to study the relationship between diet and chronic disease risk, because they examine nutrient and food synergies and interactions instead of examining a single nutrient's effect in relation to disease risk (Hu 2002). Despite this, the role of the "whole" diet, in terms of healthy eating patterns, on sarcopenia has not been researched yet.

A number of nutrients may be associated with muscle mass and strength either because they have been shown to be beneficial in supplementation studies, such as protein (Campbell *et al.* 2001, Castaneda *et al.* 1995), amino

acids (Borsheim *et al.* 2008, Scognamiglio *et al.* 2004, Solerte *et al.* 2008), and vitamin D (Dawson-Hughes 2008), or because they may play a role in muscle metabolism, such as magnesium and potassium, or due to their potential against oxidative stress and their anti-inflammatory properties, such as vitamins C and E, selenium, and carotenoids (Frassetto *et al.* 1998, Song *et al.* 2007, Song *et al.* 2005). However, little evidence exists on the influence of habitual dietary intake (either for nutrients or dietary patterns) on muscle mass, muscle strength and muscle quality (Robinson SM *et al.* 2008, Scott *et al.* 2010) and this will be discussed in the following sections.

1.3.1 Protein and amino acids

The importance of protein and amino acids, particularly the branched-chain amino acids in skeletal muscle protein synthesis and anabolic processes are evident (Fujita and Volpi 2006, Koopman and van Loon 2009, Volpi *et al.* 2003). Moreover, protein synthesis can be increased by higher essential amino acid intake or protein availability in both younger and older individuals (regardless of the fact that muscle mass decreases with age) (Paddon-Jones *et al.* 2004, Symons *et al.* 2007, Volpi *et al.* 2003). A disruption in the regulation of protein turnover in skeletal muscle can cause further imbalances in muscle protein synthesis. Hence, the role of protein intake and amino acids in the development of sarcopenia is of particular interest.

Observational studies have focused on associations between total protein intake and muscle mass mainly in populations aged more than 50 years. One

previous prospective study among women aged 75 ± 3 years found that those in the highest tertile of protein intake (> 87 g/d) had 5.3% and 6.6% higher whole body and appendicular lean mass, respectively compared with those in the lowest tertile (< 66g/d) after five years follow-up (Meng *et al.* 2009). A second prospective study among participants aged 70-79 years from the Health, Aging and Body Composition study (Health ABC) showed that those in the highest quintile of energy-adjusted protein intake (18.2% protein as a percentage of energy) lost ≈ 40% less lean mass and appendicular lean mass over three years follow-up, than did those in the lowest quintile (11.2% protein as a percentage of energy) (Houston *et al.* 2008). Similarly, an analysis of the China Health and nutrition Survey among older adults aged 50-70 years showed that those with higher protein intake (> 12.1% of energy) lost less midarm muscle area compared with those who had lower protein intake (< 10.4% of energy) over four years follow-up, although midarm muscle area is considered as an imprecise measure of lean mass (Stookey *et al.* 2005). Another previous prospective study found that energy-adjusted protein intake was a positive predictor of muscle mass over 2.6 years follow-up in participants aged 50 to 79 years, however no association was observed for muscle strength in this study (Scott *et al.* 2010). Only one previous cross-sectional study found that energy-adjusted protein intake was positively associated with grip strength in women aged 59 to 73 years, however in this study in the multivariate models limited adjustment for confounding factors was considered, as only age, height and gender were included in the regression models but not other

lifestyle factors that potentially influence the relationship (Robinson *et al.* 2008).

Two short-term intervention studies have examined the effects of protein intake on muscle mass and strength. One small study in 12 elderly women reported that after nine weeks those who consumed the higher protein diet 0.92 g/kg body weight/day had maintained muscle mass and function compared with those with an intake of 0.45 g/kg body weight/day (Castaneda *et al.* 1995). The other small study was a 14-week controlled dietary intake trial (where all feeds were supplied to the volunteers) providing the RDA for protein intake of 0.8 g protein/kg body weight/d) among 10 men and women aged 55 to 77 years. The intervention had no effect on whole body composition (% body fat, fat free mass, protein and mineral mass) although mid-thigh muscle area was decreased at the end of the 14 week intervention (Campbell *et al.* 2001). Nevertheless, supplementation with protein (after or separate from physical activity) in older subjects who frequently consumed adequate amounts of protein (≥ 0.8 g/kg body weight/day) did not further benefit the improvements in muscle mass and strength due to exercise (Campbell 2007).

However, supplementation studies that used essential amino acids were beneficial for muscle mass and muscle strength (Borsheim *et al.* 2008, Scognamiglio *et al.* 2004, Solerte *et al.* 2008). Indeed, amino acid supplementation (essential and non essential mix consisting of: 3.8 g/d leucine, 2 g/d lysine, 1.9 g/d isoleusine, 1.9 g/d valine, 1.1 g/d threonine, 0.4

g/d cysteine, 0.4 g/d histidine, 0.3 g/d phenylalanine, 0.2 g/d Methionine, 0.1 g/d tyrosine, 0.1 g/d tryptophan) at 12.2 g/d, three times per day for three months improved walking function by 48 m, and isometric hand grip strength by 2.6 kg in 44 sedentary and frail individuals aged > 65 years (Scognamiglio *et al.* 2004). Another small intervention study (*n*=12, males and females) showed that supplementation with two daily doses of 11 g/day of essential amino acids (including: 0.36 g/d histidine, 0.94 g/d isoleucine, 3.95 g/d leucine, 1.88 g/d lysine, 0.39 g/d methionine, 0.51 g/d phenylalanine, 1.05 g/d threonine, 0.82 g/d valine) plus 1.10 g/d arginine, in the form of capsules, for 16 weeks, improved lean body mass by 0.60 kg, and muscle strength by 22.2 % on average, in glucose intolerant subjects with mean (±SD) age 67 (±5.6) years (Borsheim *et al.* 2008). However, these two studies were poorly controlled and were not randomised as pre-intervention/baseline data were used as a "control" group, limiting the robustness of the study. In a third intervention study, among 41 subjects with sarcopenia aged 66-88 years, after supplementation with 8 g of essential amino acids twice per day for 18 months, significant increases in whole body lean mass were observed, although the magnitude of the effect was not reported (Solerte *et al.* 2008). These data suggest an association between amino acids and muscle mass and strength and provide evidence for further long-term randomised controlled trials, with large sample sizes, a wider age range and appropriately powered for the end points of interest.

These data suggest an association between amino acids and muscle mass and strength and provide evidence for further long-term randomised controlled trials, including bigger sample size and a wider age range.

1.3.2 Minerals (magnesium, potassium and selenium)

Magnesium is essential for muscle metabolism (Maguire and Cowan 2002). Poor magnesium intake may trigger the production and release of pro-inflammatory cytokines, such as interleukin-1ß (IL-1ß) and TNF-α, and contribute in the activation of low grade chronic inflammation (Kramer *et al.* 2003, Lukaski 2004). There is only one cross-sectional study in the literature reporting that energy-adjusted magnesium intake was a positive predictor of muscle mass over 2.6 years follow-up in participants aged 50 to 79 years (Scott *et al.* 2010). One study has shown a strong correlation between circulating magnesium and muscle performance and significant associations with hand grip strength (Dominguez *et al.* 2006). These findings are supported by studies applying supplementation with magnesium, which have been shown to significantly increase muscle strength in young subjects (Brilla and Haley 1992).

Muscle mass contains the major body pool of potassium and is critical for potassium homeostasis, and consequently depletion affects the activity of muscles (McDonough *et al.* 2002). It has been suggested that long-term supplementation with potassium bicarbonate from fruits and vegetables may benefit anabolism, including improvement of calcium, phosphorus and nitrogen balance, and an increase of bone formation markers, implying that this might be beneficial for muscle wasting too (Frassetto *et al.* 1997, Sebastian *et al.* 1994).

Selenium plays various functions in muscles and deficiency is associated with skeletal myopathy and cardiomyopathy (Keshan's disease) (Rederstorff

et al. 2006). One cross-sectional study found that energy-adjusted selenium intake was associated with significantly higher hand grip strength in 2983 men and women aged 59 to 73 years from the Hertfordshire cohort (UK) (Robinson SM *et al.* 2008). One cross-sectional study in older men and women (≥ 65 years) has also shown that low plasma selenium concentration was independently associated with poor skeletal muscle strength (defined as the lowest quartile of hip flexion, grip and knee extension strength) (Lauretani F *et al.* 2007).

1.3.3 Vitamins C and E, and carotenoids

There has been an increased interest on the associations between dietary intake and serum levels of nutrients that have the potential to reduce the risk of developing sarcopenia, because oxidative stress is involved in the pathogenesis of sarcopenia and has also catabolic effects on skeletal muscle (Jackson *et al.* 2004, Schwartz and Weindruch 1995).

Skeletal muscle contains about 67 % of whole-body's vitamin C content. In muscle cells, ascorbic acid plays a protective role against oxidative stress, and sustains the activity of ε-N-trimethyllysine hydroxylase – a key enzyme for carnitine biosynthesis. Fatigue is an early clinical sign of vitamin C deficiency, which is caused by deregulation of this enzyme's activity triggering depletion of muscle carnitine (Savini *et al.* 2005). Cross-sectional analyses have shown positive associations between lower intakes and /or plasma vitamin C with reduced muscle strength, reduced performance and frailty (Bartali B *et al.* 2006, Cesari *et al.* 2004, Robinson SM *et al.* 2008).

More recently, it has been shown that human skeletal muscle comprises a relatively labile pool of ascorbate and is likely to be affected by ascorbate depletion with inadequate vitamin C intake (Carr *et al.* 2013).

Vitamin E deficiency in experimental animals can affect the skeletal muscle causing necrotizing myopathy. Although dietary vitamin E deficiency is unknown, patients with severe fat malabsorption, cystic fibrosis, some forms of chronic liver disease are unable to absorb or transport the vitamin E around the body, and may suffer from muscle membranes damage (Gibney MJ *et al.* 2009). Cross-sectional studies have not shown any associations between vitamin E intake and muscle strength, however, low plasma levels of a-tocopherol and γ-tocopherol have been associated with low muscle strength in older adults (Cesari *et al.* 2004, Robinson SM *et al.* 2008, Semba *et al.* 2003).

Dietary carotenoids may be protective against reactive oxygen species, which are accumulated with ageing and may also act as preventive agents against oxidative damage which contributes to losses of muscle mass and strength (Kim *et al.* 2010). Recent epidemiological studies in community-dwelling older individuals have shown that low serum and/or plasma carotenoid levels were independently associated with low muscle strength and the development of walking disability over time (Lauretani *et al.* 2008, Semba *et al.* 2007). In addition, in the InCHIANTI study low intake of β-carotene was marginally associated with knee extension strength ($\beta = 0.311$, $P = 0.05$) (Cesari *et al.* 2004). Yet, one cross-sectional study in adults aged

53-73 years showed that grip strength was positively associated with energy-adjusted carotene intake, in both women and men (Robinson SM *et al.* 2008).

1.3.4 Vitamin D

It has been suggested that vitamin D plays an important role in muscle function and physical performance, and that its deficiency is followed by muscle weakness and pain (Hamilton 2010). The underlying mechanism may be mediated by de novo protein synthesis, which affects muscle cell growth through the highly specific nuclear vitamin D receptor which is expressed in human muscle tissue (Bischoff *et al.* 2001). Longitudinal studies have consistently shown an association between vitamin D status and muscle strength, frailty and physical performance among older adults (Scott *et al.* 2010, Visser *et al.* 2003). Also, low serum 25-OH vitamin D level has been suggested as an independent predictor of falls (Dawson-Hughes 2008). In cross-sectional studies a positive association between 25-OHD (a plasma metabolite used to assess vitamin D status) and muscle strength has been reported (Gerdhem *et al.* 2005, Houston *et al.* 2007). Yet, one cross-sectional study has shown that among men and women aged 53-73 years, grip strength was positively associated with energy-adjusted vitamin D intake, but only in women (Robinson SM *et al.* 2008). Evidence from double-blind randomised controlled trials suggests that supplementation with vitamin D increased muscle strength and physical performance, and also associated with a decline in the risk of falls among both community-dwelling older individuals and residents in nursing homes

with low vitamin D status (Annweiler *et al.* 2009, Bischoff-Ferrari *et al.* 2009, Dawson-Hughes 2008, Lisa 2008).

1.3.5 Summary

In summary, there are currently limited population studies examining associations between habitual dietary intake and indexes of muscle mass and muscle strength and the available data are inconsistent (Robinson SM *et al.* 2008, Scott *et al.* 2010). These previous studies have also been conducted in a narrow age range (50 to 79 years), and they have not examined the range of nutrients potentially important for muscle mass. Cross-sectional studies have not shown any associations between protein intake and muscle mass, although positive associations have been found in longitudinal studies (Baumgartner *et al.* 1999, Houston *et al.* 2008, Mitchell *et al.* 2003, Stookey JD *et al.* 2005). However, these studies were also limited to older populations and some of them measured a narrow range of lean mass (appendicular lean mass in the four limbs) and not whole body fat free mass, although appendicular lean mass accounts for more than 75 % of the total skeletal muscle mass (Gallagher *et al.* 1997). Moreover, intervention trials using amino acid supplementation suggest beneficial effects on muscle mass and strength in the elderly (Børsheim *et al.* 2008, Ferrando *et al.* 2010). Indeed, amino acid supplementation (essential and non-essential) ranging from 11 to 16 g/d increased total body lean mass and muscle strength among individuals aged more than 65 years (Borsheim *et al.* 2008, Scognamiglio *et al.* 2004, Solerte *et al.* 2008). However, two of these studies were poorly controlled and pre-intervention data were used as

a control group, limiting the robustness of the study, and in the third intervention study, although there was an increase in total body lean mass, data were not shown. Nevertheless, these data suggest an association between amino acids and muscle mass and strength providing evidence for further long-term randomised controlled trials, including bigger sample size and a wider age range.

There are however limited studies examining the influence of habitual dietary intake of a range of nutrients that might be relevant for muscle mass in cohorts including younger adults, and there are no studies examining the relationship between the overall diet quality and indexes of muscle mass, muscle strength and muscle quality in population-based studies. There is evidence that nutrients, such as protein, essential amino acids, and vitamin D are not only important for muscle biology (Fujita and Volpi 2006, Koopman and van Loon 2009), but have been shown to be beneficial in supplementation studies. However, in relation to protein, supplementation did not further benefit the improvements in muscle mass and strength due to exercise (Campbell 2007), and in relation to amino acids the studies were poorly controlled, short term, and limited in older subjects aged more than 65 years (Borsheim *et al.* 2008, Scognamiglio *et al.* 2004, Solerte *et al.* 2008). Other micronutrients, such as magnesium (Maguire and Cowan 2002), potassium (McDonough *et al.* 2002), and selenium (Rederstorff *et al.* 2006) have been shown to be essential for muscle metabolism, however, little evidence exists for their habitual intake in association with muscle mass and strength in population studies (Robinson *et al.* 2008, Scott *et al.*

2010). In addition, vitamins C (Savini *et al.* 2005), E (Semba *et al.* 2003), and carotenoids (Kim *et al.* 2010) are also important for muscle mass through their potential to act against oxidative stress, which has catabolic effects on skeletal muscle, however, little evidence is available for the influence of intake of these nutrients in association with indexes of muscle mass, muscle strength and muscle quality (Robinson *et al.* 2008, Scott *et al.* 2010).

Therefore, given these gaps in the current literature, it is important to further understand associations between dietary intake of mentioned range of nutrients potentially important for muscle mass in order to appreciate the influence of habitual dietary intake of nutrients and diet quality on muscle mass and muscle strength in a population including younger adults. Notably, it is important to examine dietary associations with muscle mass in individuals of all ages, as changes in skeletal muscle mass start to occur earlier in adult life (between 30 and 45 years of age) (Cesari and Pahor 2008, Janssen *et al.* 2000).

1.4 Sarcopenia and inflammation

As mentioned briefly in section 1.1, ongoing research on ageing and sarcopenia suggests a link between sarcopenia and inflammation (Chung *et al.* 2009). It is well known that the ageing process is associated with a gradual, chronic production and increase of pro-inflammatory cytokines (Roubenoff *et al.* 1998). The age-related cytokine production is a risk factor leading to the predisposition to sarcopenia, as the pro-inflammatory environment triggers catabolism and protein breakdown and therefore leads

to imbalances in the synthesis of muscle tissue (Morley and Baumgartner 2004, Roubenoff and Hughes 2000).

Epidemiological evidence have shown an inverse relationship between muscle mass, strength and function and inflammatory cytokines (Barbieri *et al.* 2003, Cesari *et al.* 2005, Payette *et al.* 2003, Schaap *et al.* 2006, Schaap *et al.* 2009, Visser *et al.* 2002). Indeed, in the Health, Aging and Body Composition (Health ABC) study among men and women aged 70-79 years, it was observed that per standard deviation increase in IL-6, grip strength was 1.1 to 2.4 kg lower, and participants with high levels of IL-6 (> 1.80 pg/ml) and TNF-a (> 3.20 pg/ml) had a smaller muscle area, less appendicular muscle mass, lower knee extensor strength and grip strength (Visser *et al.* 2002). A report from the InCHIANTI cohort found that among men and women aged 20-102 years, IL-6 was an independent predictor of hand grip strength and muscle power (Barbieri *et al.* 2003). In a cohort of older adults aged 72 to 92 years, from the Framingham Heart study, IL-6 was a significant predictor of fat free mass over two years follow-up (Payette *et al.* 2003). In another report from the InCHIANTI cohort among men and women aged more than 65 years it has been shown that there was an independent association between IL-6 and CRP and muscle strength and poor physical performance (Cesari *et al.* 2004). In the Longitudinal Aging Study Amsterdam (LASA) among men and women with a mean age of 74.6 years, high IL-6 and CRP were associated with a two to three fold higher risk of losing greater than 40 % of muscle strength, however no associations were found with appendicular muscle mass (Schaap *et al.* 2006). Similarly,

in the Health, Aging and Body Composition (Health ABC) study among men and women aged 70-79 years, it was observed that TNF-a levels and its soluble receptors were associated with five year decline in cross-sectional muscle area and hand grip strength (Schaap *et al.* 2009).

These findings were also supported by animal studies showing that administration of IL-6 and TNF-a in rats was associated with changes in muscle metabolism, including increased skeletal muscle protein breakdown, decreased protein synthesis rate, decreased amino acid concentration of skeletal muscle, and muscle wasting (Goodman 1991, Hoshino *et al.* 1991).

Chronic low-grade inflammation is considered detrimental to skeletal muscle mass and strength in both humans and animal models (Howard *et al.* 2007, Siu *et al.* 2008). It has been suggested that the underlying mechanism involves enhancement of the redox-sensitive transcription factors NF-kB, and AP-1 from reactive oxygen species (ROS). These two signaling pathways are involved in the regulation of pro-inflammatory cytokines, such as IL-6 and TNF-α, up-regulation of acute phase proteins, such as C-reactive protein, and inflammatory mediators, such as IL-10 (Janssen-Heininger *et al.* 2000). It has also been suggested that redox-sensitive NF-kB (which induces many inflammatory mediator gene expressions) is activated by the ubiquitin-proteasome system causing an imbalance between protein synthesis and degradation in skeletal muscle, leading to loss of muscle mass and muscle atrophy (Chung *et al.* 2009).

The most commonly studied marker of inflammation is C-reactive protein (CRP), which is an acute phase protein and which has the highest increases during an acute phase response in man (Heinrich *et al.* 1990). CRP is synthesised in the liver, and may be mediated by a number of cytokines, but mainly IL-6 (Shoelson *et al.* 2007). In addition to liver derived CRP, data have shown that adipose tissue itself may contribute to obesity associated increase of CRP levels (Berg and Scherer 2005). It has also been reported that CRP levels are higher in obese individuals who are insulin resistant and decrease with weight loss and improvement of insulin sensitivity (Kopp *et al.* 2003, McLaughlin *et al.* 2002). It is well known that a potential mechanism underlying these observations is that IL-6 production by human adipose tissue increases during obesity, thus it may induce the hepatic CRP synthesis and may promote the onset of insulin resistance (Bastard *et al.* 2006, de Luca and Olefsky 2008). The epidemiologic association between elevated levels of circulating CRP and increased cardiovascular risk is quite robust with to date more than 20 prospective epidemiologic studies providing evidence that high-sensitivity CRP is an independent predictor of a number of CVD events, such as myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death, even in apparently healthy subjects (Calabrò *et al.* 2009).

The recent literature refers to the term ‘high-sensitivity’ or ‘highly sensitive’ CRP (hs-CRP). This term is used for the measurement of CRP in plasma or serum using sufficiently sensitive immunoassay methods to quantify CRP throughout its normal range compared to older commercial assays which

were less sensitive and had detection limits in the range of 2-10 mg/L and were used to measure acute phase responses of CRP instead of baseline values (Casas *et al.* 2008). These high sensitivity assays can detect concentrations of <0.3 mg/L accurately and reproducibly (Zaninotto *et al.* 2007).

1.5 Diet and inflammation

Diet is one factor that plays a major role in the regulation of chronic inflammation. Dietary constituents have the potential to exert anti-inflammatory as well as pro-inflammatory effects (Cavicchia *et al.* 2009). Diverse dietary components, such as n-3 fatty acids, antioxidant vitamins and plant flavonoids exert anti-inflammatory effects via a range of mechanisms including decreasing inflammatory mediator production through effects on cell signaling and gene expression and by reducing production of oxidants (Calder *et al.* 2009). Dietary patterns such as the Mediterranean diet which consist of high intake of vegetables, fruits and nuts, legumes, fish and seafood, cereals and whole grains, high ratio of monounsaturated to saturated lipids, and low intake of meat, meat and dairy products may reduce chronic inflammatory status and prevent the occurrence of diseases mediated by inflammation, such as CVD and type II diabetes (Esposito *et al.* 2004, Trichopoulou *et al.* 2009).

In contrast, there are dietary components suggested to have pro-inflammatory effects, such as diets high in saturated and *trans*-fatty acids, sugar and refined starches and low in fruits and vegetables, polyphenols and

other antioxidants (Calder *et al.* 2009). This is most likely a result of increased production of pro-inflammatory cytokines followed by a reduced production of anti-inflammatory cytokines. Available evidence suggests that saturated and *trans* fatty acids have an effect on the acute phase proteins, such as CRP and on inflammatory cytokines, such as IL-6 and TNF-a (Basu *et al.* 2006). Thus, high intake of saturated fatty acids through consumption of a more Western dietary pattern high in fast foods, processed foods, high-fat dairy products, red meat and pork act as a pro-inflammatory factors (Lundman *et al.* 2007).

Although there are a number of studies that have examined the role of diet on chronic inflammation, these were mainly focused on inflammatory status which mediates cardiovascular diseases and diabetes (Esposito *et al.* 2004, Trichopoulou *et al.* 2009). However, there are no population studies in the literature examining whether habitual diet may influence chronic inflammation associated with sarcopenia. Therefore, further understanding potential influences of the habitual diet on inflammation related sarcopenia, associations between the habitual diet and the inflammatory marker C-reactive protein is important.

1.6 Research gaps

Although the importance of healthy eating patterns on mortality and chronic disease risk is well documented in adults (Akbaraly *et al.* 2011, Huijbregts *et al.* 1997, McCullough *et al.* 2002, Patterson *et al.* 1994, Seymour *et al.* 2003, Trichopoulou *et al.* 2005), to date, there are no studies that have

examined the relative importance of diet as a whole on muscle mass, strength and muscle quality and consequently sarcopenia with physiological ageing. In addition, there are limited population-based studies on associations between habitual dietary intake and indexes of muscle mass (Houston *et al.* 2008, Scott *et al.* 2010, Stookey JD *et al.* 2005), muscle strength (Robinson SM *et al.* 2008, Scott *et al.* 2010) and no studies on muscle quality as outlined in Chapter 1, section 1.3.

Also, it is well reported that a diet high in fruit and vegetables, whole grains, nuts and polyunsaturated fatty acids, as well as dietary patterns high in these food components, may exert anti-inflammatory properties and is associated with lower concentrations of inflammatory markers including CRP (Chacko *et al.* 2010, de Oliveira *et al.* 2011, Esmaillzadeh *et al.* 2006, Fung *et al.* 2005, Galland 2010, Lopez-Garcia *et al.* 2005, Lutsey *et al.* 2007, Mozaffarian *et al.* 2004, Song *et al.* 2005). Also, there is evidence that higher levels of inflammatory markers negatively influence muscle mass and strength, potentially increasing the development of sarcopenia (Barbieri *et al.* 2003, Cesari *et al.* 2005, Payette *et al.* 2003, Schaap *et al.* 2006, Schaap *et al.* 2009, Visser *et al.* 2002). However, to our knowledge there are no studies examining whether dietary constituents could influence the association between inflammation and muscle mass, strength or muscle quality, and subsequently sarcopenia.

The current thesis will address these research gaps in a population-based study of women by evaluating associations between the habitual diet and

muscle mass, muscle strength and muscle quality, diet and inflammation, and examining whether diet mediates the relationship between muscle mass and inflammation.

1.7 Aims and hypotheses

Therefore, given the lack of previous research, the overall aim of this thesis was to examine associations between habitual diet and muscle mass, strength and muscle quality in adult women from the TwinsUK cohort. The TwinsUK cohort is an ongoing study examining a wide range of age-related phenotypes, behavioural and disease risk markers, and participants are a national sample of healthy adult twin volunteers from the UK (a detailed description of the cohort follows in Chapter 2) (Spector and Williams 2006). An additional aim was to examine associations between habitual diet and inflammation to determine whether diet influences the relationship between muscle mass, strength, muscle quality and inflammation in women.

It was specifically hypothesised that higher dietary intakes of a range of antioxidant micronutrients, protein and amino acids, and higher adherence to a better diet quality were associated with i) higher indexes of muscle mass, muscle strength and muscle quality and ii) lower inflammation levels (as assessed by CRP). We also hypothesised that higher CRP levels were associated with lower indexes of muscle mass. We additionally hypothesised that higher dietary intakes of a range of antioxidant micronutrients, amino acids, and higher adherence to a better diet quality would attenuate the negative impact of CRP on muscle mass.

1.8 Objectives

1) To examine whether habitual dietary intakes of a range of antioxidant micronutrients and protein and amino acids influence indexes of muscle mass, including fat free mass (FFM, kg), percentage fat free mass (FFM %) and fat free mass index (FFMI, kg/m^2), muscle strength (assessed by hand grip strength (kg), and muscle quality calculated as the ratio of muscle strength divided by fat free mass (kg/kg).
2) To develop five predefined diet quality scores: the Mediterranean Diet Score (MDS), Healthy Diet Indicator (HDI), Diet Quality Index (DQI), Alternate Healthy Eating Index (AHEI), and the Dietary Approach to Stop Hypertension score (DASH-style score) using data on dietary intake from the TwinsUK cohort.
3) To examine associations between adherence to a better diet quality (assessed by the diet quality scores) and indexes of muscle mass, muscle strength and muscle quality.
4) To evaluate whether a range of antioxidant nutrients, protein and amino acids and diet as a whole (assessed by five predefined diet quality scores) influences inflammation markers, specifically C-reactive protein (CRP).
5) To develop an analytical strategy to investigate the relationship between muscle mass and inflammation (assessed by CRP) to evaluate whether dietary constituents and diet quality scores mediate the relationship between muscle mass and inflammation.

Chapter 2

Subjects and Methods

2.0 Subjects and methods

2.1 Subjects

2.1.1 The TwinsUK cohort

The TwinsUK cohort is an ongoing study examining a wide range of age-related phenotypes, behavioural and disease risk markers. The cohort is a national sample of healthy adult twin volunteers from the UK recruited through a series of media campaigns. All the twins were brought up together and lived apart in adult life in the UK. Recruitment started in 1992 and focused on middle-aged females, because the diseases under investigation, including osteoarthritis and osteoporosis, are more prevalent in women (Spector and MacGregor 2002). From 1995 onwards, men were also invited to participate.

Between 1992 and 2004, twins invited for a comprehensive baseline visit. More than 7000 twins responded to annual health questionnaires and 5725 of them (out of about 10000 registered twins) attended a comprehensive visit. One thousand three hundred forty seven twins were added to the TwinsUK registry and all the 6740 active twins on the registry (not deceased, withdrawn or not contactable) attended the first follow-up visit (a 1-day clinical visit) between 2004 and 2007. The second follow-up visit was conducted between 2007 and 2010. This visit included only women aged more than 40 years with at least one previous clinical visit. From the 4610 women that were invited, 3125 attended the clinic, 53 were deceased, 646 declined to attend and 786 were not contactable.

Participants in the second follow-up visit appeared to have higher socio-economic status, lower self-rated health status and be more health aware according to their level of alcohol intake and smoking.

The main objectives of the study were to estimate the heritability for common diseases and traits in adults, discover associated genes, and provide a framework for epidemiological studies using the twin design (Spector and Williams 2006). More recently, the TwinsUK cohort has focused on "healthy ageing", since ageing is a multifactorial complex process, which involves multiple organs and multiple molecular pathways (Viña *et al.* 2007).

The Registry contains now approximately 12,000 twins (51% monozygotic and 49% dizygotic), males and females (83%), aged 18 to 103 years (Moayyeri *et al.* 2012). Ethical approval for the study was obtained from the St. Thomas Hospital Research Ethics committee and informed consent was obtained from all participants.

A variety of data are available from the TwinsUK Registry, including demographic and lifestyle data (such as physical activity, medication history, cognitive function and food frequency questionnaires), clinical outcomes (anthropometrics, whole body dual energy x-ray absorptiometry scans, and grip strength) and biological samples (such as fasting blood and urine samples, DNA extraction, and lipid and metabolic profiles). The cohort also benefits from advanced technologies and available data on genetic measurements (genome wide scans, epigenetic analysis, and gene expression – RNA sequencing) and specialised tests (for example telomere

length analysis, metabolomic profiles, fat and muscle biopsies among others) (Moayyeri *et al.* 2012, Spector and Williams 2006).

Some of the major earlier research findings of the cohort were associated with studies examining the heritability of diseases and associated genes that influence diseases and traits in adults. Indicative research findings have shown that osteoarthritis of knee, hand, hip, and spine were all strongly heritable (Spector and MacGregor 2004). In addition, risk factors that were associated with osteoporotic fractures, including bone density, bone quality, hip axis length, vitamin D levels, bone turnover markers and muscle strength were strongly and independently genetic (Arden *et al.* 1996). Moreover, many studies have found that cardiovascular disease outcomes, such as hypertension, pulse rate, blood pressure, arterial resistance, and cardiovascular disease have shared genetic influences (Snieder *et al.* 2000, Williams *et al.* 2004).

In this framework, the Department of Nutrition at the University of East Anglia in collaboration with the Twins Unit (King's College London) was involved in the analyses of datasets incorporating nutritional data and a number of phenotypes. To date, collected research findings from this collaboration have shown that in female twins from the TwinsUK registry habitual total flavonoids intake was positively associated with bone mineral density, with effects observed for anthocyanins and flavones at both the hip and spine (Welch *et al.* 2012); higher anthocyanin intake was associated with lower arterial stiffness and central blood pressure (Jennings *et al.*

2012); wine intake was positively associated with spine bone mineral density and a traditional English diet pattern had a negative association with hip bone mineral density (Fairweather-Tait *et al.* 2011); and plasma adiponectin concentrations were associated with body composition and plant-based dietary factors (Cassidy *et al.* 2009). The above findings support a role of dietary constituents present in plant-based foods on a number of health related outcomes in female twins from the TwinsUK cohort.

2.1.2 Study population

For the purposes of this thesis, analyses were performed for twins as singletons/individuals, because previous studies have shown that participants from the TwinsUK registry were not different from age-matched singleton women in the distribution of common traits and outcomes and therefore data were well characterised (Andrew *et al.* 2001). Moreover, characteristics and energy intake from the TwinsUK cohort has shown to be similar with those from UK singleton populations (Henderson *et al.* 2004, Sproston and Primatesta 2004). Data from two subsets of this cohort examined at two different time points, initially in 1996 and then again in 2005, were used for my analyses. For the first subset, data on dietary assessment, body composition parameters, and other covariates were available for n = 2570 women, aged 18 to 79 years, representing 792 monozygotic (MZ) females and 1778 dizygotic (DZ) females, who had completed a food frequency questionnaire (FFQ) and attended for dual-energy X-ray absorptiometry (DEXA) scans between 1996 and 2000 (Teucher B 2007). This subset also included those who had fasting blood

samples taken, to measure C-reactive protein (CRP); *n* = 1658 individuals, representing 582 monozygotic (MZ) females and 1076 dizygotic (DZ) females, aged 18 to 79 years (Moayyeri *et al.* 2012). For the second subset focusing on data available for grip strength, data on dietary assessment, body composition parameters, and other covariates were available for *n* = 949 individuals, representing 415 monozygotic (MZ) females and 534 dizygotic (DZ) females, aged 34 to 83 years, who had completed a food frequency questionnaire (FFQ) and attended for hand grip strength measurements and DEXA scans between 2005 and 2008 (Moayyeri *et al.* 2012).

Statistical analysis was subdivided into two subsets as measurements on body composition parameters, including fat free mass and hand grip strength were taken at two different time points. Data on fat free mass were initially collected in 1996, whereas data on hand grip strength were initially collected in 2005. Therefore, the analyses in this thesis included two subsets of the cohort according to the availability of data from the two different visits.

2.2 Methods

2.2.1 Zygosity

Zygosity was derived by questionnaire and confirmed by supplemented multiplex-DNA fingerprinting in cases with disputed or uncertain zygosity (PE Applied Biosystems) (Spector and Williams 2006).

2.2.2 Assessment of anthropometry variables and body composition

Height and weight were measured, BMI was calculated as weight in kilograms (kg) divided by height in meters squared (m^2), and overweight status was defined using categories based on WHO criteria (WHO 1998).

Fat free mass: Fat free mass (FFM, kg) and fat mass (FM, kg) were assessed by dual-energy X-ray absorptiometry scans with a Hologic QDR-2000 DXA scanner (Hologic Inc., Waltham, MA, U.S.A.). The percentage fat free mass (FFM %) was calculated as the ratio of fat free mass in kilograms (kg) divided by total body weight in kilograms (kg) and multiplied by 100. The fat free mass index (FFMI, kg/m^2) was calculated as the ratio of fat free mass in kilograms (kg) divided by height in metres squared (m^2). The fat free mass index is a useful index in evaluating body composition parameters as it eliminates differences in fat free mass associated with height. It also gives the advantage of having one set of recommended ranges, independent of age and height, and is an important marker of nutritional status in health and disease (Kyle *et al.* 2003). Fat free mass in kilograms and percentage fat free mass were also included in the current thesis to enable comparison of the findings with other studies.

Hand grip strength and muscle quality: Grip strength of the dominant arm was assessed using a Jamar hand grip dynamometer (Sammons, Preston, UK). Repeated measurement on 24 individuals was used to assess reproducibility, with a coefficient of variation (CV) of 11.4 % (Arden and

Spector 1997). Muscle quality was calculated as the ratio of hand grip strength in kilograms (kg) divided by muscle mass in kilograms (kg).

2.2.3 Assessment of C-reactive protein (*CRP*)

C-reactive protein was measured using a highly sensitive automated microparticle capture enzyme immunoassay, previously standardized by the World Health Organization International Reference Standard for CRP immunoassay 85/506 (WHO 1987, Wilkins *et al.* 1998).

Subjects were excluded from the analysis if there were differences greater than six kilograms between body weight measured used DEXA and calibrated scales, or if BMI was less than 16 kg/m^2, or if the subject's twin did not have measurement.

2.2.4 Assessment of dietary intake

Participants completed a 131-item FFQ previously used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, which has been validated against urinary biomarkers and plasma biomarkers of intake (urinary nitrogen, potassium, sodium, ascorbic acid and n-3 PUFAs) (Bingham *et al.* 1997, McKeown *et al.* 2001, Welch *et al.* 2006). Daily nutrient intake was determined using nutrient values from McCance and Widdowson food composition tables and the EPIC database of the carotenoid content of foods (McCance *et al.* 1991, Pattison *et al.* 2005). In relation to the amino acid intakes a new database was developed with 78% of the data from McCance and Widdowson food composition tables and the

remainder from the United States Department of Agriculture (USDA) database.

Frequencies of the different food items from the FFQ were transformed to intakes in grams per day by multiplying the frequency of weekly consumption of each food item by the appropriate portion size of each item and then dividing by seven (**APPENDIX I** for portion sizes of different food items). Gram amounts of foods were classified into key food groups, such as fruit and vegetables, whole grains; complex carbohydrates, meat group, fish group, and dairy products group (there were small differences between the two food frequency questionnaires and both sets of classifications are shown in **APPENDICES II** and **III** for food groups' definition). Gram amounts of macronutrients from the FFQ were converted to percentage of total energy intake using the following conversion factors: protein intake: 4 kilocalories per gram (kcal/g), fat intake: 9 kilocalories per gram (kcal/g), and for carbohydrate intake: 3.75 kilocalories per gram (kcal/g) (McCance *et al.* 1991). Subjects' FFQs were excluded if answers for more than 10 food items were left blank; the subject's twin did not complete a FFQ; and if the subject's ratio of the estimated total energy intake (derived from the FFQ) to the subject's estimated basal metabolic rate (based on the Harris-Benedict equation) was two standard deviations away from the mean of this ratio (<0.52 or >2.58) (Frankenfield *et al.* 1998, Teucher B 2007). Therefore, extreme over- and under-reporters were excluded *a priori* to any other exclusion. The numbers that fall into these categories were: for subset 1 (n = 2570 women, aged 18 to 79 years): 172 out of 5415 women (3.2 %) were over-reporters and 29 out of 5415 women

were under-reporters (0.54 %); and for subset 2 (*n* = 949 women, aged 34 to 83 years): 112 out of 3370 women (3.3 %) were over-reporters and 26 out of 3370 women (0.77 %) were under-reporters. To further evaluate whether misreporting was influencing associations between diet and indexes of muscle mass and estimate energy requirements, the ratio of reported energy intake (EI:EER) to estimate energy expenditure was calculated and this was included as a covariate for adjustment in the statistical analyses (Otten *et al.* 2006, Rennie *et al.* 2007) (Chapter 3, section 3.2, p. 87). Estimated energy requirements (EER) for each subject were calculated using the 2002 Institute of Medicine of the National Academies published Default ((2002)). These equations are sex-and age-specific and are based on the age, weight and height of the subject and are derived from collated doubly labelled water energy expenditure data. They also allow for three levels of activity: low activity (PAL $\geq$ 1.4 and <1.6), active (PAL $\geq$ 1.6 and < 1.9) and very active (PAL $\geq$ 1.9 and <2.5) with a corresponding physical activity coefficient in the EER equations. For example, the EER equation for women aged over 19 years was derived by the following formula:

EER = 354 – (6.91 x age) + PA x [(9.36 x weight (kg)) + (726 x height (m)]

Misreporting of energy is a common issue in population studies, and the implications of under-reporting are that they are very likely to attenuate any diet and disease relationships. Previous studies have suggested that the probability of under-reporting increases with increasing BMI (Livingstone and Black 2003). Also, the selective reporting of foods has hampered the definition of food patterns and the subsequent derivation of food based

guidelines (Becker *et al.* 1999). Indeed, assessment of dietary intake using FFQs has shown that foods perceived as "good" are often over-reported, whilst foods perceive as "bad" are under-reported. Socioeconomic and education determinants have also been implicated in under-reporting (Poslusna *et al.* 2009). The most commonly used method to identify under-reporting of energy intake is the use of the Goldberg cut-offs (Goldberg *et al.* 1991), which have been used inappropriately or without a thorough understanding of their principles (Livingstone and Black 2003). However, this method is also subject to limitations and it has been suggested that excluding on the basis of misreporting too much data from a dataset does not remove all or the majority of under-reporters (McCrory *et al.* 2002), and this may distort the data further (Stubbs 2009). Nevertheless, despite the issues with identifying and finding solutions to the issues of misreporting, improvements in methods evaluating misreporting of energy intake will further help the interpretation of diet and health relationships.

An overview of the methodology used to derive the five predefined diet scores used in the current analyses is presented in **Table 2.1**.

Table 2.1 Diet score components, and scoring criteria

Components	Scoring	Criteria for minimum and maximum score
Mediterranean diet score (MDS)		
Fruit and nuts (g/d)	0 / 1	Below median / Above median
Vegetables (g/d)	0 / 1	Below median / Above median
Legumes (g/d)	0 / 1	Below median / Above median
Fish (g/d)	0 / 1	Below median / Above median
Cereals (g/d)	0 / 1	Below median / Above median
Ratio of mono- and poly- to saturated fat	0 / 1	Below median / Above median
Dairy products (g/d)	0 / 1	Above median / Below median
Meat group (g/d)	0 / 1	Above median / Below median
Alcohol (g/d)	0 / 1	<5 and >25 / 5-25
Healthy diet indicator (HDI)		
Fruit and vegetables (g/d)	0 / 1	<400 / >400
Complex carbohydrates (%energy/d)	0 / 1	<50 or >70 / 50-70
Dietary fiber (g/d)	0 / 1	<27 or >40 / 27-40
Pulses and nuts (g/d)	0 / 1	<30 / >30
Mono- and disaccharides (%energy/d)	0 / 1	>10 / 0-10
Protein (%energy/d)	0 / 1	<10 or >15 / 10-15
Saturated fat (%energy/d)	0 / 1	>10 / 0-10
Polyunsaturated fat (%energy/d)	0 / 1	<3 or >7 / 3-7
Cholesterol (mg/d)	0 / 1	>300 / 0-300
Diet quality index (DQI)		
Fruit and vegetables (servings/d)	0 / 1 / 2	0-2 / 3-4 / 5+
Total fat (%energy/d)	0 / 1 / 2	>40 / 30-40 / ≤30
Complex carbohydrates (servings/d)	0 / 1 / 2	0-3 / 4-5 / 6+
Saturated fat (%energy/d)	0 / 1 / 2	>13 / 10-13 / <10
Cholesterol (mg/d)	0 / 1 / 2	>400 / 300-400 / <300>
Protein (% RDA)	0 / 1 / 2	150 / 100-150 / <100
Sodium (mg/d)	0 / 1 / 2	>3400 / 2400-3400 / <2400
Calcium (% RDA)	0 / 1 / 2	<67% / 67-100% / ≥100%
Alternate Healthy Eating Index (AHEI)		
Vegetables (servings/d)	0 / 10	0 / ≥5
Fruit (servings/d)	0 / 10	0 / ≥4
Nuts and soy protein (servings/d)	0 / 10	0 / ≥1
Ratio of white to red meat	0 / 10	0 / ≥4
Dietary fiber (g/d)	0 / 10	0 / ≥18
Trans fat (% energy/d)	0 / 10	≥4 / ≤0.5
Polyunsaturated to saturated fat ratio	0 / 10	≤0.1 / ≥1
Vitamin supplement use (no/yes)	2.5 / 7.5	No / Yes
Alcohol (servings/d)	0 / 10	0 or ≥2.5 / 0.5-1.5
DASH-style score		
Fruits (servings/d)	1/2/3/4/5	Quartile 1/2/3/4/5
Vegetables (servings/d)	1/2/3/4/5	Quartile 1/2/3/4/5
Nuts and legumes (servings/d)	1/2/3/4/5	Quartile 1/2/3/4/5
Whole grains (servings/d)	1/2/3/4/5	Quartile 1/2/3/4/5
Low fat dairy products (servings/d)	1/2/3/4/5	Quartile 1/2/3/4/5
Sodium (mg/d)	5/4/3/2/1	Quartile 1/2/3/4/5

Red and processed meat (servings/d)	5/4/3/2/1	Quartile 1/2/3/4/5
Sweetened beverages (servings/d)	5/4/3/2/1	Quartile 1/2/3/4/5

2.2.4.1 Development of dietary scores

MDS: For the derived MDS, one point was given for intake at or above the median for components considered as healthy (fruits and nuts, vegetables-excluding potatoes, legumes, fish, cereals and monounsaturated + polyunsaturated to saturated fat ratio), otherwise, a value of zero was given. In addition, one point was given for intake less than the median for components considered to be unhealthy (dairy products and meat), otherwise a value of zero was assigned. A value of one was assigned to women with daily alcohol consumption from five grams to 25 grams and a value of zero for consumptions less than five grams or more than 25 grams per day (Trichopoulou *et al.* 2003). In the current analyses, fruit juice was excluded from the fruit and nuts group as fruit juice consumption is not associated with lower risk of CVD, cancer or diabetes (Hung *et al.* 2004, Palmer 2008). Potato products were also excluded from the vegetable group as potatoes (including French fries) are not associated with chronic disease risk and are associated with higher risk of diabetes (Halton *et al.* 2006, WCRF/AICR 2007).

For the MDS, a scale from zero (minimum conformity) to nine (maximum conformity) was constructed from the TwinsUK dataset, indicating the degree of adherence to the derived Mediterranean score. The MDS score is population specific and was generated according to the distribution of foods within the TwinsUK participants.

HDI: For the derived HDI, a dichotomous variable was generated for each food group or nutrient outlined in **Table 2.1**, including intakes of fruit and vegetables, complex carbohydrates (breads, cereals, legumes, and peas), dietary fiber, pulses and nuts, mono- and disaccharides (total sugars), protein, saturated fatty acids, polyunsaturated fatty acids, and cholesterol (Huijbregts *et al.* 1997). If an individual's intake was within the recommended range, the variable was coded as one; otherwise, it was coded as zero. The final HDI score was the sum of all these variables, creating a scale of zero to nine, with a higher score indicating a better diet quality.

DQI: The components of the derived score included fruit and vegetables, total fat, complex carbohydrates, saturated fatty acids, cholesterol, protein, sodium and calcium (Patterson *et al.* 1994). The scoring of the original score was reversed, so intakes were rated as two (good), one (satisfactory), or zero (poor), to enable comparison with the other four scores. The Recommended daily allowances (RDAs) for protein and calcium (dietary components which were based on percentage of recommended intakes) were derived using the UK report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy instead of U.S. recommendations. After summing across all dietary components, scores were ranged from five to sixteen with a higher score indicating higher quality.

AHEI: The original AHEI, included vegetables, fruit, nuts and soy protein, the ratio of white to red meat, cereal fiber, trans fatty acids (as percentage of

total energy intake), the ratio of polyunsaturated to saturated fatty acids, the duration of multivitamin use, and alcohol intake (McCullough *et al.* 2002). In the current analysis, for the derived AHEI, cereal fiber was adapted in the way another UK study did as NSP (as percentage of total energy intake) from our data (Akbaraly *et al.* 2011). Also, our dataset provided data on vitamin supplement use as a dichotomous variable asking for use or non-use. Therefore, we adapted this variable in place of the duration of multivitamin use from the original AHEI.

All components contributed zero to ten points to the total score; with a score of ten indicating that the recommendations were fully met and a score of zero indicating minimum conformity to the recommendations. Intermediate intakes were scored proportionally between zero and ten. For the ratio of white to red meat a value of ten was assigned to those who never consumed red meat or to those who consumed red meat less than once per month, as well as to those who were vegetarians, using the frequencies from the FFQ. For the NSP component we based our scoring on the daily reference value (DRV) for adults in the UK, which is 18 grams per day, and assigned a value of ten to those who had maximum conformity and a value of zero to those who had minimum conformity. For the vitamin supplement use, a value of 7.5 was assigned to those who had used vitamin supplements; otherwise 2.5 points were assigned (non-use). After summing across all dietary components, scores were ranged from 16.5 to 87.5 with a higher score indicating higher quality.

DASH-style score: The derived DASH-style score was based on the original DASH score, which included foods and nutrients, emphasized or minimised in the DASH diet (Appel *et al.* 1997). For the purpose of our study we used the DASH-style score and not the original DASH score, as the DASH-style score highlights the key components of the DASH diet. Moreover, the DASH-style score was used as it ranks individuals according to intake therefore it is population specific and was more suitable to examine potential associations with muscle mass and strength in this cohort. For each of the dietary components, we classified women into quintiles according to their intake for fruits, vegetables, nuts and legumes, low fat dairy products, and whole grains, so that individuals in quintile one were assigned one point and individuals in quintile five were assigned five points. For the remaining components, including sodium, red and processed meats and sweetened beverages, the scoring was reversed so low intake indicated a higher score. We then summed the scores for all components and a total DASH-style score from eight to 40 was constructed, with lower score indicating lower conformity to the DASH diet and higher score indicating higher conformity to the DASH diet, respectively.

The diet scores had a few differences in the way they were constructed. The MDS used the median intake of dietary components as the cut-off between healthy and unhealthy intakes, whereas the DASH-style score used quintiles to classify intakes of dietary components, with a "healthy" point assigned only if the quantity consumed was in the highest quintile. In contrast, the HDI, DQI, and the AHEI were cut-point specific scores based on healthy

eating guidelines. Moreover, different foods were included in the cereal, whole grains and complex carbohydrate groups in the construction of the scores between subset 1 (see **APPENDIX II**) and subset 2 (see **APPENDIX III**) according to availability of data in the two datasets.

2.2.5 Assessment of covariates used for the multivariate statistical analysis models

Age

Associations between diet and muscle mass, muscle strength and muscle quality

As age has a great influence on muscle mass, strength, and muscle quality all analyses were adjusted for age (in years) (Cruz-Jentoft *et al.* 2010, Lynch *et al.* 1999). In the current analysis higher mean age was correlated with lower indexes of muscle mass (for FFM, $r = -0.15$, $P < 0.001$; for percentage FFM, $r = -0.35$, $P < 0.001$; and for FFMI, $r = -0.06$, $P = 0.76$), lower hand grip strength ($r = -0.47$, $P < 0.001$) and lower muscle quality ($r = -0.39$, $P < 0.001$).

Associations between diet and C-reactive protein (CRP); and associations between diet, muscle mass and CRP

Chronic low-grade inflammatory status may contribute to age-associated morbidity and mortality (Franceschi *et al.* 2007). Ageing is also strongly associated with an increased low grade inflammatory status (Chung *et al.* 2009). Therefore, CRP was adjusted for age. Since age was inversely and significantly correlated with indexes of muscle mass, this analysis for

evaluating associations between diet, muscle mass and CRP was stratified by age using two categories, less than 50 years (**Tables 8.2, 8.5 and 8.8**) and more than 50 years (**Tables 8.3, 8.6 and 8.9**) to examine associations in the older age.

Physical activity

Associations between diet and muscle mass, muscle strength and muscle quality

Regular physical activity, involving both aerobic and resistance exercise has been associated with increased muscle mass and strength and decreased rate of fat accumulation, contributing to the improvement of fat free mass, and to the prevention of physical disability and frailty (Frankel *et al.* 2006, Heath and Stuart 2002, Kuh *et al.* 2002, Roubenoff 2007, van Kan *et al.* 2008, Wolfe 2006). For this thesis, lifetime physical activity was assessed using a physical activity questionnaire, which previously shown to strongly correlate with more in-depth assessment of time spent in physical activity (more detailed questionnaire) in the cohort (Cherkas *et al.* 2008). For participants in the second subset who had missing data on physical activity, the General Practice Physical Activity Questionnaire (GPPAQ) was used and coding was performed as shown in **Table 2.2.** The GPPAQ was based on the original physical activity index developed by the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. The EPIC physical activity index was previously validated against heart rate monitoring in two independent studies (Wareham *et al.* 2002, Wareham *et al.* 2003) and was inversely related to all-cause mortality and cardiovascular

disease incidence in men and women of a wide range of age and social class from the EPIC-Norfolk study (Khaw *et al.* 2006).

Participants of this cohort were allocated into three categories: physically inactive (sedentary job and no physical exercise and/or cycling per week), moderately active (sedentary job and one to two point nine hours physical exercise and/or cycling per week, or standing job but less than one hour physical exercise and/or cycling per week, or physical job and no physical exercise or cycling), and physically active (any category with activity levels above the latter).

Table 2.2 Description of physical activity coding based on the GPPAQ[1]

	Occupation			
Physical exercise and / or cycling (hr/wk)	Sedentary	Standing	Physical	Heavy Manual
0	Inactive	Moderately Inactive	Moderately Active	Active
Some but < 1	Moderately Inactive	Moderately Active	Active	Active
1-2.9	Moderately Active	Active	Active	Active
≥ 3	Active	Active	Active	Active

[1] Questions concerning walking, Housework and Gardening have been included to allow participants to record their physical activity in these categories; however these questions have not been shown to yield data of a sufficient reliability to contribute to an understanding of overall physical activity levels. http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/@ps/documents/digitalasset/dh_112134.pdf

In the current data those who were active had a greater mean FFM ($P = 0.64$), percentage FFM ($P < 0.001$) and FFMI ($P = 0.79$) compared to those who were moderately active or inactive. Also, those who were active had a greater mean hand grip strength ($P < 0.001$) and muscle quality ($P = 0.0006$) compared to those who were moderately active or inactive.

Associations between diet and C-reactive protein (CRP); and associations between diet, muscle mass and CRP

Evidence has shown that physical activity induces a decrease in the systemic levels of a number of cytokines with anti-inflammatory properties (Gleeson *et al.* 2011, Petersen and Pedersen 2005). This is more likely because inflammation is lower in physically active individuals primarily because of lower absolute amount of total and visceral body fat. However, more studies that accounted for this by using a direct measure of fat mass are needed before definitive conclusions (Beavers *et al.* 2010).

Smoking status

Associations between diet and muscle mass, muscle strength and muscle quality

It has been suggested that smoking may increase the risk of sarcopenia (Morse *et al.* 2007, Petersen *et al.* 2007, Wüst *et al.* 2008). Interestingly, in this analysis those who never smoked had lower mean FFM ($P < 0.001$) and FFMI ($P = 0.0003$) compared to those who were current and former smokers; and those who were former smokers had lower percentage FFM compared with those who had never smoked or were current smokers ($P < 0.001$). Also, those who were current smokers had higher mean hand grip strength and muscle quality compared to those who were not current smokers, although associations were not significant ($P = 0.26$ and 0.16, respectively). However, as smoking may influence muscle mass and strength, smoking status was taken into account in the analyses. Data on

smoking status was collected by questionnaire and defined as never, current, and former smoking.

Associations between diet and C-reactive protein (CRP); and associations between diet, muscle mass and CRP

Cigarette smoking increases oxidative stress and inflammation (Lee *et al.* 2012). Therefore, analyses were adjusted for smoking.

Energy intake

Associations between diet and muscle mass, muscle strength and muscle quality; and C-reactive protein (CRP); and associations between diet, muscle mass and CRP

In free-living human populations, total energy intake is attributed largely in variations to physical activity, body size and metabolic efficiency (Willett 1986). In epidemiological studies, total energy intake is often associated with disease risk because of associations between physical activity or body size and the probability of disease. As, intakes of most nutrients, particularly macronutrients, are correlated with total energy intake they may be non-causally associated with disease as a result of confounding by total energy intake. Also, extraneous variation in nutrient intake resulting from variation in total energy intake that is unrelated to disease risk may weaken associations if energy intake is not taken into account (Jakes *et al.* 2004). Moreover, individuals or populations must alter their intake of specific nutrients primarily by altering the composition of their diets rather than by changing their total energy intake, unless physical activity or body weight is

changed substantially (Willett *et al.* 1997). In addition, measurement errors are inevitable in epidemiological studies and errors in reporting food intake translates into errors in energy and nutrient intake (Poslusna *et al.* 2009). Thus, adjustment for total energy intake is important in epidemiological studies to control for confounding, and reduce extraneous variation induced by physical activity, body size, and metabolic efficiency and partially misreporting.

Most nutrients are associated with total energy intake either because they contribute directly to energy intake or because individuals who consume more total energy also eat, on average, more of all specific nutrients (Mackerras 1996). So, controlling for confounding by total energy intake forms an important issue for the statistical analysis plans (Willett *et al.* 1997).

As energy is not derived from micronutrients, most micronutrients are not highly correlated with energy intake. However, in this cohort some micronutrients were highly correlated to energy intake, such as vitamin D ($r = 0.51$, $P < 0.001$), vitamin E ($r = 0.74$, $P < 0.001$), selenium ($r = 0.54$, $P < 0.001$), magnesium ($r = 0.77$, $P < 0.001$) and potassium ($r = 0.75$, $P < 0.001$). It is likely that these correlations were due to the fact that some of the main sources of these nutrients were relatively high in energy. Vitamin E, for example, is found in olive oil, nuts (almonds, hazelnuts), mackerel, salmon; selenium is found in Brazil nuts, wheat products-cereals, bread; magnesium is found in whole grain products, and nuts; potassium is found in nuts, banana, melon, avocado, cereals and dairy products. Although, some other micronutrients were not very strongly associated with total

energy intake, such as carotenoids (α-carotene, r = 0.18, P < 0.001; β-carotene, r = 0.18, P < 0.001; total carotene, r = 0.27, P < 0.001; β-cryptoxanthin, r = 0.19, P < 0.001; lycopene, r = 0.33, P < 0.001; lutein, r = 0.21, P < 0.001; zeaxanthin, r = 0.22, P < 0.001), and vitamin C (r = 0.35, P < 0.001), energy intake was included in the multivariate model in order to control for any additional confounding. Energy intake (kcal/d) was also included in the multivariate models for the MDS (Trichopoulou *et al.* 1995), AHEI (McCullough *et al.* 2002) and the DASH-style scores (Fung *et al.* 2008) as these scores do not take into account energy intake, in contrast to the HDI and DQI which do (Huijbregts *et al.* 1997, Patterson *et al.* 1994).

Total body mass

Associations between diet and muscle mass

In order to undertake informative comparisons of a population's body composition, normalization for total body mass is required. It is suggested that lean mass has a linear relation with body mass (Harris 1997). There is also evidence that heavier people are taller, and because an increase in fat mass is associated with an increase in fat free mass, total body mass was considered as a confounding variable for both fat free mass and fat free mass index (Wells JCK *et al.* 2002, Wolfe 2006). The percentage fat free mass was not adjusted for total body mass, because this means it already takes into account total body mass in its calculation.

Height

Associations between diet and muscle strength and muscle quality

Height has been previously suggested as a determinant of grip strength and isokinetic muscle strength (Jerome *et al.* 1991, Neder *et al.* 1999). Higher hand grip strength has also been associated with increased height in epidemiological studies (Inskip HM *et al.* 2007, Robinson SM *et al.* 2008). In this study those who were taller had significantly higher mean hand grip strength ($r = 0.36$, $P < 0.001$). Therefore, the analyses were adjusted for height.

Body mass index (BMI)

Associations between diet and C-reactive protein (CRP); and associations between diet, muscle mass and CRP

There is evidence that increased adipose tissue is related to increased chronic systemic inflammation through the increased release of cytokines (Festa *et al.* 2001). Specifically, increases in fat mass, and particularly in visceral fat, may trigger the secretion of a number of pro-inflammatory cytokines, indicating that central obesity is more important when studying obesity in association with inflammation, because it is in general considered more pro-inflammatory (Santos *et al.* 2005, Tilg and Moschen 2006, You *et al.* 2008).

There is strong evidence from epidemiological studies on the association between markers of obesity and CRP (Festa *et al.* 2001, Hodge *et al.* 2010, Khoo *et al.* 2011, Pannacciulli *et al.* 2001). These studies have evaluated obesity including a variety of obesity indices, such as BMI and total body

fat and central obesity including waist and hip circumferences, and waist to hip ratio. There is evidence of a strong relationship between plasma levels of CRP and both total and central abdominal fat (CAF) measured by DEXA scans in the Twins UK cohort (Greenfield *et al.* 2004). In the current subset there was a significant positive correlation between plasma levels of CRP with both total obesity (assessed by BMI) and central obesity (assessed by % central fat of regional mass), and % central fat of regional mass and BMI were highly correlated. Therefore, BMI was included in the multivariate adjusted model.

Menopausal status

Associations between diet and muscle strength and muscle quality

Menopause is followed by a natural decline in estrogens and it is likely that hormonal changes due to menopause contribute to muscle weakness. A number of studies have suggested that a loss in muscle strength has been associated with menopause (Carville *et al.* 2006, Cooper *et al.* 2008, Greeves *et al.* 1999, Kurina *et al.* 2004). Although the underlying mechanisms have not been fully elucidated, it has been proposed that estrogens have anabolic effects on muscle because they stimulate insulin growth factor 1 (IGF-1) receptors (Sitnick *et al.* 2006). Evidently human skeletal muscle contains the largest amount of estrogen receptors (expressed in the mRNA level) (Lemoine *et al.* 2003), however, not only circulating estrogen but also IGF-1 can also activate estrogen receptors (Ciana *et al.* 2002). During the menopause both estrogen and IGF-1 decrease, which is likely to negatively influence muscle strength. In the current cohort, data on

menopausal status was collected by questionnaire and recorded as premenopausal or postmenopausal. Premenopausal women had significantly higher mean hand grip strength and muscle quality compared with postmenopausal women ($P < 0.001$, respectively). However, it needs to be acknowledged that although menopause is related to muscle strength, it is also strongly associated with age making it difficult to know if differences in muscle strength are due to the menopause or age.

Hormone replacement therapy (HRT)

Associations between diet and muscle strength and muscle quality

Following the argument from the menopausal status section above, there is a working hypothesis that estrogens benefit muscle strength loss (Lowe *et al.* 2010). Results from a systematic review from 23 studies on muscle strength that included 10.000 post-menopausal women who were or were not on HRT showed that women who had received HRT had approximately five percent greater strength compared with those who had not received HRT (effect size = 0.23, $P = 0.003$). This effect might be due to the improvement of muscle quality rather or more than quantity or muscle hypertrophy (Greising *et al.* 2009). In the TwinsUK cohort data on hormone replacement therapy (HRT) was collected by questionnaire and recorded as no or yes. For participants with missing data on HRT the following assumptions were considered when imputing values: women aged less than 50 years were assumed to be not taking HRT, premenopausal women aged over 50 years were assumed to be not taking HRT, and postmenopausal women aged over 50 years were assumed to be taking HRT. The age of 50 years was used,

because this was the median age of menopause in this cohort (Aviv *et al.* 2006). In the current analysis, women on HRT had a higher mean hand grip strength and muscle quality compared with women who were not in HRT, although the trend was not significant (P = 0.30 and 0.43, respectively). Although HRT alone is not likely to be beneficial on muscle strength it was included in the overall model in combination with other potential factors that influence muscle strength, as there is evidence that combined with physical exercise it has shown to improve physical function (Sipila *et al.* 2001).

Associations between diet and C-reactive protein (CRP); and associations between diet, muscle mass and CRP

A number of studies in the literature have shown that hormone replacement therapy has been associated with higher CRP, and may contribute to higher levels of CRP seen in women (Ford *et al.* 2004, Hung *et al.* 2008, Khera *et al.* 2005, Woodward *et al.* 2003). In the TwinsUK cohort it has been shown previously that HRT users had higher plasma CRP levels compared with nonusers, which supports findings from epidemiological and randomised controlled trials showing that oral HRT, and particularly estrogen, increases CRP levels (Davison and Davis 2003, Fröhlich *et al.* 2003, Greenfield *et al.* 2004, Manns *et al.* 2003). In the same cohort HRT use remained a significant determinant of increased CRP, even after adjusting for adiposity in multiple regression models. The mechanism through which HRT use influences plasma CRP concentrations has not yet been established. However, results from oral HRT use have shown that there is a

consequential increased synthesis of CRP by the liver and therefore increased plasma concentrations (Luyer *et al.* 2001).

Medication

Associations between diet and C-reactive protein (CRP)

Anti-inflammatory medication (including statins, non-steroid anti-inflammatory drugs - NSAIDS, and aspirin) was used as a covariate to control for its influence on inflammation.

2.3 Statistical analysis

All analyses were performed in STATA statistical software (version 11.0; STATA Corp, USA). The analyses were performed treating twins as individuals as previous studies have shown that participants from the TwinsUK registry were similar to age-matched singleton women in the distribution of common traits and outcomes (Andrew *et al.* 2001). The normality of the variables was assessed and CRP concentrations were subsequently natural log-transformed. Quintiles of each nutrient intake and quartiles of each diet quality score were derived. The distribution of nutrient intake was divided into quintiles, and the diet quality scores into quartiles to ensure equal numbers of participants in each of the categories. One-way ANOVA was applied for continuous variables (such as age, BMI, FFM) and Chi-squared test for categorical variables (such as physical activity and smoking history) to examine differences in anthropometric characteristics, body composition and lifestyle factors by each quartile of the diet quality scores. In addition to unadjusted analysis, multivariate robust cluster

regression models were used to assess indexes of muscle mass, hand grip strength and muscle quality (dependent variables) across quintiles of micronutrient, protein and amino acid intakes and quartiles of diet quality scores to evaluate statistical differences in muscle mass, muscle strength and muscle quality after adjustment for relevant confounders outlined in Chapter 3 (section 3.2, p. 84) and Chapter 4 (section 4.2, p. 129), respectively. Age was strongly and inversely correlated with hand grip strength (r = -0.5, P < 0.001) and muscle quality (r = -0.4, P < 0.001) analysis in the second subset of 949 women was further stratified by age using two categories, less than 50 years and more than 50 years. To assess whether associations were determined by the genetic background, the statistical analyses for the association between one nutrient intake that was strongly and another nutrient intake that was not strongly associated with indexes of muscle mass was performed using the within twins approach (Chapter 3). To examine associations within-twin pairs, the model was extended to take into account the twin pair mean:

$E(Y_{ij}) = \beta_0+\beta_i(X_{ij})+\beta_t X_i$, where Ẋi is the mean value of X for twin pair i. The β_i gives the expected mean in Y for a 1-unit deviation from the pair change in the outcome variable (within-pair). The β_t gives the expected change in Y for a 1-unit change in the twin pair average X (between-pair) while holding constant the individual deviation from the average. Thus, β_i (the within-pair effect) represents the strength of the association within pairs and is free from the confounding influence of factors that are common to the twin pair. β_t (the between-pair effect) reflects further variation in Y that is accounted for by factors in the shared environment or genetic background of

twins and which do not account for individual differences. In general, a strong within-pair effect with a small or absent between-pair effect is consistent with an individual specific mechanism/causal mechanism or a direct/real effect (Carlin *et al.* 2005). Moreover, a strong between-pair association, with a less strong or no within-pair effect, suggests that potential shared lifestyle and genetic factors confound associations between an exposure and an outcome (Carlin *et al.* 2005).

Multivariate robust cluster regression models were also fitted between the inflammation marker CRP across quintiles of different nutrient intakes and quartiles of diet quality scores to evaluate statistical differences in CRP in these groups after accounting for confounding factors outlined in Chapters 6 (section 6.2, p. 206) and 7 (section 7.2, p. 237). Multivariate adjusted analysis was further used to examine whether diet mediates the association between muscle mass and inflammation assessed by CRP (see Chapter 8, section 8.2, p. 265). This analysis was further stratified by age using two categories, less than 50 years and more than 50 years to investigate whether diet mediates the association between muscle mass and inflammation assessed by CRP in the older group of women. Multivariate model analyses were performed using the robust cluster regression option in STATA. These models take into account clustering of individuals when calculating standard errors of the mean (Richards *et al.* 2007) to ensure familial aggregation within twin pairs was accounted for.

2.3.1 Sensitivity analysis

Secondary or sensitivity analyses were performed for the association between nutrient intakes and fat free mass index (FFMI) in order to: i) assess the collinearity of the various nutrients in this analysis, and ii) to model the nutrients that were significantly associated with fat free mass index in a single multivariate regression analysis, to assess whether each was independently predictive (Chapter 3, p.89).

2.4 Comparison with other cohorts (representativeness of the cohort)

There is a concern that association studies may not be representative of the general population, and therefore it is important that sample populations from cohort studies are representative of the general population to ensure generalisability of the results (Paolo 2011). Data from the TwinsUK cohort has shown that participants were not different from age-matched singletons for measures or disease outcomes, such as height, bone mineral density, osteoarthritis, blood pressure, use of hypertensive medication, alcohol consumption, menopausal, hysterectomy and ovariectomy status, supporting that twins are representative of singleton populations (Andrew *et al.* 2001, Henderson *et al.* 2004, Sproston and Primatesta 2004). To assess the representativeness of the participants in the current analysis, the baseline characteristics and body composition parameters of the two subsets outlined in section **2.1.2** are presented in **Table 2.3**, and were compared with data from the National Diet and Nutrition Survey (NDNS 2009/10).

Table 2.3 Characteristics of two subsets of 2570 and 949 women from the TwinsUK cohort examined in 1996 and 2005, respectively[1]

Characteristic	1996-2000 (*n*=2570) 18-79 yrs	2005-2008 (*n*=949) 34-83 yrs	NDNS 2009/10 Women 19-64 yrs
Age (yrs)	48.3±12.7 (18-79)	59.1±9.3 (34-83)	41.5
Height (cm)	162±6.1 (143-183)	162±5.9 (143-182)	
Weight (kg)	65.6±11.2 (39-118)	69.2±12.5 (37-121)	
BMI (kg/m^2)	24.9±4.1 (16.3-46.1)	26.5±4.7 (17.4-49.7)	27.0±0.34 (SE)
Fat mass (kg)	22.7±7.9 (6.37-64.9)		
Fat mass percentage (%)	33.9±7.2 (13.1-56.9)		
Fat free mass (kg)	39.6±5.3 (20.9-66.9)		
Fat free mass percentage (%)	61.1±6.5 (38.8-87.3)		
Fat free mass index (kg/m^2)	15.0±1.7 (9.79-24.6)		
Hand grip strength (kg)		28.8±5.9 (6-51)	
Muscle quality (kg/kg)		0.69±0.14 (0.15-1.08)	
Physical activity %			
Inactive	21.9	39.6	30
Moderate	53.9	34.2	52
Active	24.2	26.2	17
Smoking history %			
Never	50.2		59.6
Current	18.2	90.21 (yes)	20.6
Former	31.6	9.79 (no)	19.6
Hormone replacement therapy %			
Yes		9.38	
No		90.63	
Menopausal status %			
Premenopausal		10.3	
Postmenopausal		89.7	
Anti-inflammatory medication %[2]			
Yes		6.15	
No		93.85	

[1] Values are means and standard deviations (means ± SD) unless indicated; interquartile range in parentheses

[2] Subset analysis for 1658 participants

2.4.1 Results

The results indicated that the cohort was similar to the NDNS regarding physical activity and smoking status, although there were differences in BMI, which might be explained by the age difference between the cohorts, as the NDNS is a younger cohort. Notably, obesity based on BMI is shown to more prevalent in older adults aged more than 65 years (Bouchard *et al.* 2009). Fat free mass index was 15.0 kg/m^2 ± 1.7 (range 9.79-24.6) in the

TwinsUK cohort, which was within the normal range of 14.6 – 16.8 kg/m^2, reported for healthy Caucasian women with normal BMI ranges (Kyle *et al.* 2003).

To further assess representativeness of this cohort data on body composition parameters, including fat free mass (kg), fat free mass index (kg/m^2), hand grip strength (kg) and muscle quality (kg/kg) were stratified by 10 year age bands, to enable comparison with other cohorts assessing body composition differences with age.

Differences in body composition parameters, such as fat free mass – FFM (kg) and fat free mass index – FFMI (kg/m^2) by 10 years age bands are presented in **Figures 2.1** and **2.2.** Mean FFM was significantly lower by 7.23 % ($P < 0.001$) in the 70th compared with the 20th decade (**Figure 2.1**). Mean FFMI showed a peak at the 30th decade, but it was generally similar across age categories and was higher by 1.35 % ($P = 0.01$) in the 70th decade compared with the 20th decade (**Figure 2.2**).

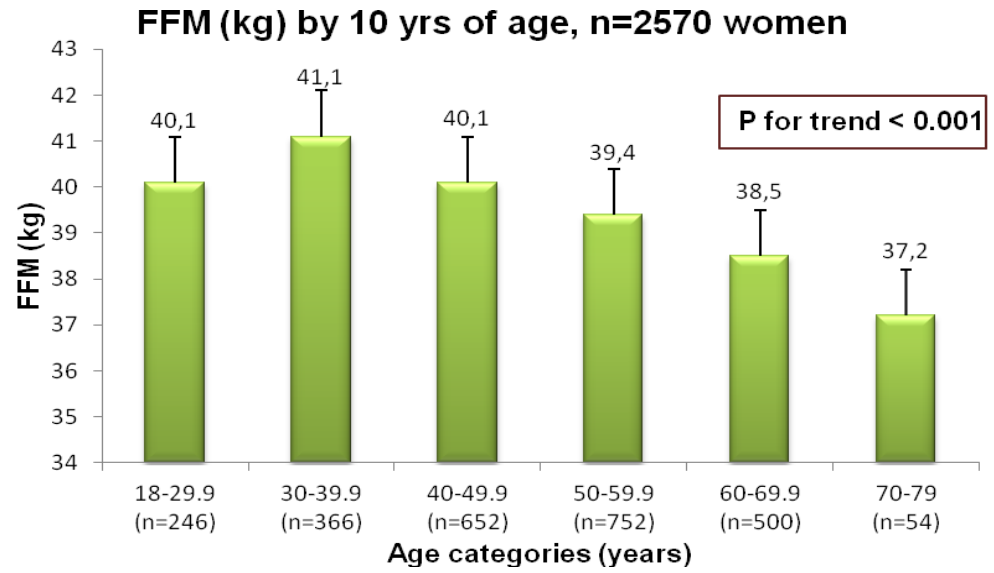


Figure 2.1: Fat free mass - FFM (kg) by 10 years of age in 2570 women from the TwinsUK cohort

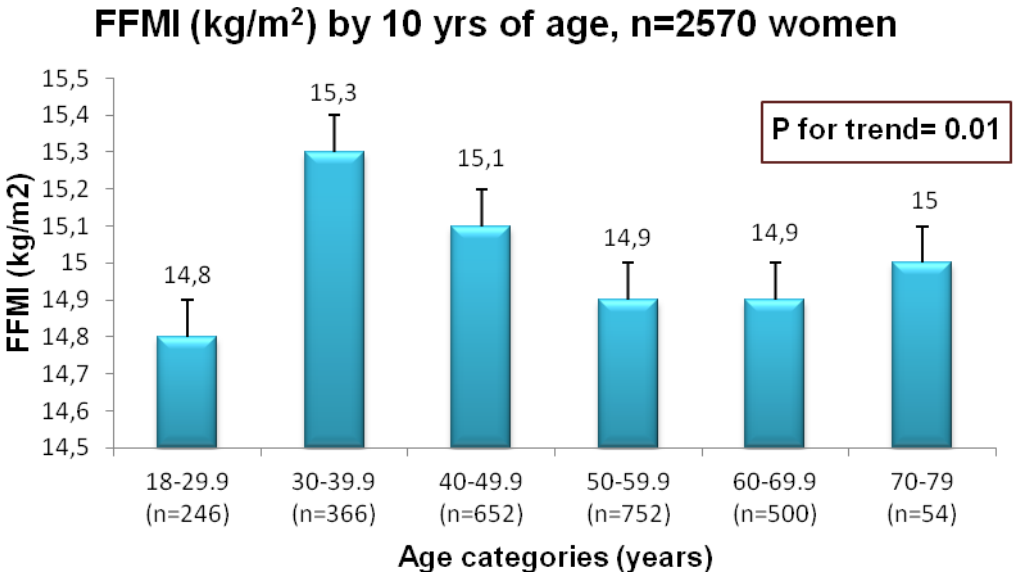


Figure 2.2: Fat free mass index - FFMI (kg/m^2) by 10 years of age in 2570 women from the TwinsUK cohort

Differences in hand grip strength (kg) and muscle quality (kg/kg) by 10 years age bands are presented in **Figures 2.3** and **2.4**. Mean hand grip strength was lower by 30.1 % between the 70th and the 30th decade (P < 0.001) (**Figure 2.3**). Mean muscle quality was lower by 27.7 % (P < 0.001), in the 70th compared with the 30th decade (**Figure 2.4**).

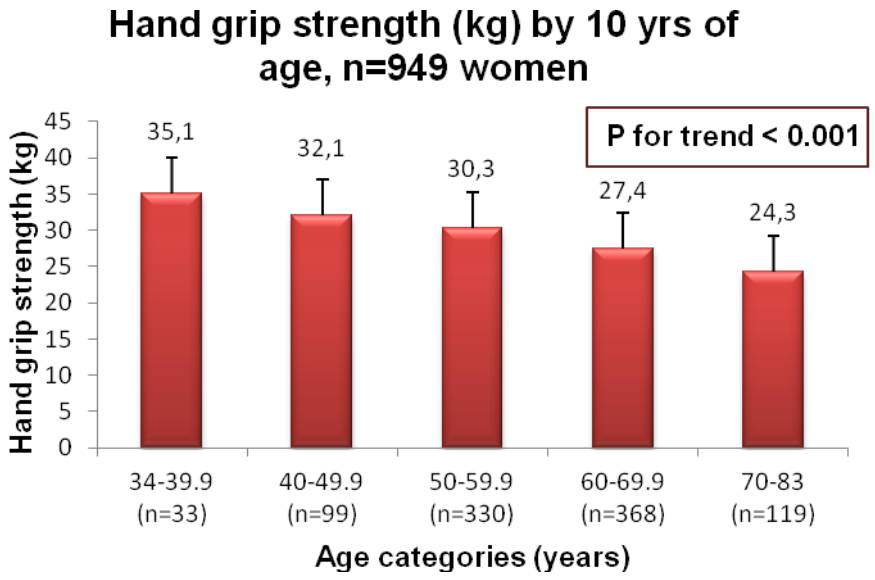


Figure 2.3: Hand grip strength (kg) by 10 years of age in 949 women from the TwinsUK cohort

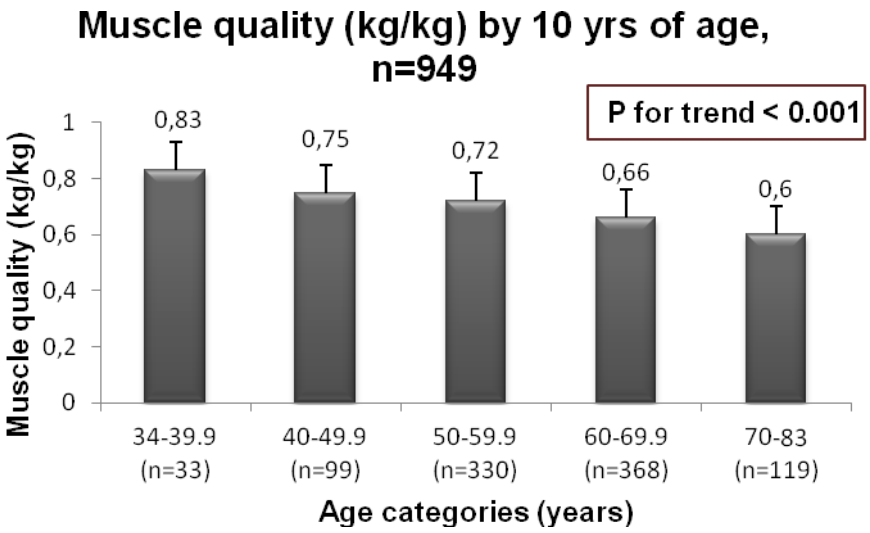


Figure 2.4: Muscle quality (kg/kg) by 10 years of age in 949 women from the TwinsUK cohort

2.4.2 Discussion

Findings from this analysis showed that older age was associated with progressively lower FFM (kg). However, differences in FFMI (kg/m^2) were small across age categories. These findings were similar to observations from previous cross-sectional studies and longitudinal studies in women (Coin *et al.* 2008, Ding *et al.* 2007, Guo *et al.* 1999, Janssen *et al.* 2000, Kyle *et al.* 2001, Kyle *et al.* 2006).

In one of two previous cross-sectional studies that have examined age-related body composition differences, among women aged 60-94 years, body composition parameters were measured using dual-energy X-ray absorptiometry scans (DEXA) (Kyle *et al.* 2001) as in the TwinsUK participants. The study found that mean fat free mass was 2.9 kilograms (6.8

%) lower in women 80 years and over compared to those aged 60-70 years. In the TwinsUK cohort mean fat free mass was 1.3 kilograms (3.38 %) lower in women aged 70-79 compared to that aged 60-69.9 (**Figure 2.1**). This difference might suggest that after the age of 80 years further loss of fat free mass occurs, and therefore because the maximum age of the first subset of the Twins data was 79 years we were not able to detect a difference as high as Kyle *et al*. (2001), who included subjects aged up to 94 years.

In a second cross-sectional study, among women aged 18-88 years body composition was measured by Magnetic Resonance Imaging (MRI) (Janssen *et al.* 2000). Mean skeletal muscle mass in this study was 3.8 kilograms lower in women aged more than 70 years compared to those aged between 18 to 29 years. This study also showed that absolute skeletal muscle mass was lower after the fifth decade in their cohort of women (Janssen *et al.* 2000). Data from TwinsUK cohort showed similar trends in the association between muscle mass indexes and age in women aged 18 to 79 years that had their body composition measured by DEXA scans. Mean fat free mass was found to be 2.9 kilograms lower in women aged 70-79 years compared to those aged 18-29.9 years. Janssen *et al*. (2000), showed that absolute skeletal muscle mass was lower after the fifth decade in their cohort of women, confirming findings from TwinsUK cohort showing that muscle mass started to decline from the fifth decade (**Figure 2.1**).

Results of a multicentre, retrospective study among a representative healthy Italian population of men and women, between 20 and 80 years, showed

that mean fat free mass index was 16.1 ± 1.8 (kg/m^2), which was lower in the oldest group of women (70-80 years) (16.0 ± 1.5, kg/m^2) (Coin *et al.* 2008). In the TwinsUK cohort among women, mean fat free mass index was 15.0 ± 1.7 (kg/m^2) (**Table 2.1**). Body composition was assessed by DEXA scans, in both studies. The trend for fat free mass index was also very similar across the age categories in the TwinsUK cohort (**Figure 2.2**), and this was probably due to age-related height reductions occurring with a decrease in fat free mass and an increase in body weight.

The inverse associations between indexes of muscle mass and age that observed in the TwinsUK participants were also supported by longitudinal studies showing that greater age range was associated with lower fat free mass and higher fat mass over time of follow-up (Ding *et al.* 2007, Guo *et al.* 1999, Kyle *et al.* 2006).

The results also indicated that muscle strength was lower with higher age categories (**Figure 2.3**), although the percentage difference between extreme age bands was different to that of other studies (Lynch *et al.* 1999). This might be because muscle strength was assessed by a different method in the TwinsUK study (muscle strength was defined as hand grip strength measured by hand dynamometer) compared with other cross-sectional studies that have defined muscle strength as concentric and eccentric peak torque measured in the elbow and the knee flexors and extensors and measured by isokinetic dynamometer (Lynch *et al.* 1999).

Muscle quality was included as an additional measurement in this thesis to further understand the association between muscle mass and strength, and was considerably lower by 27.7 % in the 70-83 years group compared to individuals aged 34-39.9 years (**Figure 2.4**).

Findings from this cohort showed that it is representative of national studies and further confirmed other longitudinal and cross-sectional studies (using different methods to assess body composition and different populations and age ranges) showing that fat free mass was lower with age in women, with a peak at the age of 30 years (Coin *et al.* 2008, Kyle *et al.* 2003) and considerable differences from the 50th decade of life, as previously reported (Hughes *et al.* 2002).

Overall, this analysis highlighted the great influence of age on body composition, with significant differences between lower indexes of muscle mass, muscle strength and muscle quality and higher age bands (Baumgartner 2000). However, age is a natural process which cannot be modified, and to date, current literature poorly describes the influence of factors other than age on muscle mass, muscle strength and muscle quality in population-based studies. Thus, in order to appreciate the influence of other important factors that may be easily modifiable, the role of diet and inflammation on muscle mass, muscle strength and muscle quality, after accounting for age and other confounding factors will be investigated in the following chapters. Therefore, the next chapter will be focused on associations between a number of dietary components and indexes of

muscle mass, muscle strength and muscle quality in women from the TwinsUK cohort. Within this chapter a review of the current literature will be provided, with some narrative highlighting the gaps in present knowledge, followed by results, discussion and concluding remarks.

Chapter 3

Associations between micronutrient intakes and indexes of muscle mass, muscle strength and muscle quality

3.0 Introduction

Emerging evidence suggests that a number of nutrients are associated with muscle mass and strength due to their potential to act against oxidative stress and inflammation, such as vitamins C and E, selenium, and carotenoids (Ferrucci *et al.* 2005, Helmersson *et al.* 2005, Young *et al.* 2004). Moreover, other nutrients have been shown to be beneficial in supplementation studies such as vitamin D (Dawson-Hughes 2008), or they play a role in muscle metabolism, such as magnesium and potassium (Frassetto *et al.* 1998, Song *et al.* 2007, Song *et al.* 2005). Recent data showed that although several observational studies have examined associations between plasma/serum nutrient levels and different measurements of muscle strength (Cesari *et al.* 2004, Dominguez *et al.* 2006, Lauretani *et al.* 2008, Semba *et al.* 2007) there are limited studies on associations between habitual dietary intake and muscle mass and strength and no studies on muscle quality (Robinson SM *et al.* 2008, Scott *et al.* 2010). This might be due to a lack of consensus on the definition of sarcopenia. Notably, only recently the European Working Group on Sarcopenia in Older People developed a clinical definition for age-related sarcopenia (Cruz-Jentoft *et al.* 2010).

One previous prospective study found that energy-adjusted iron, magnesium, phosphorus and zinc intakes were positive predictors of muscle mass over 2.6 years follow-up in participants aged 50 to 79 years. A positive association was found for vitamin C intake and muscle mass only at follow up and not at baseline; however, the authors did not offer an explanation for

this finding. No associations were observed between any nutrient intakes and muscle strength in this study (Scott *et al.* 2010). Another cross-sectional study found that energy-adjusted carotene and selenium were associated with higher grip strength in men, and energy-adjusted vitamin C, carotene, selenium and vitamin D were associated with higher grip strength in women aged 59 to 73 years (Robinson *et al.* 2008). However, to our knowledge, there are currently no studies examining associations between a range of nutrients and muscle mass in cohort studies specifically including also younger adults. It is likely that this would provide a useful insight into the potential role of a range of nutrients contributing to the overall diet quality (highlighted in Chapter 1, section 1.3, p. 21), to potentially maintain muscle mass, strength and muscle quality.

3.1 Aims

The aim of this study was to test the hypothesis that habitual dietary intake of a range of micronutrients with the potential to act against oxidative stress and with potential anti-inflammatory properties (shown in **Table 3.1**) or are known to be involved in the biology of human muscle are associated with muscle mass as assessed by 3 indexes, fat free mass (FFM, kg), percentage fat free mass (FFM%) and fat free mass index (FFMI, kg/m^2), among women aged 18-79 years from the TwinsUK cohort (subset 1). An additional aim of this study was to examine the hypothesis that habitual dietary intake of these nutrients is associated with muscle strength assessed by hand grip strength (kg) and muscle quality (muscle strength divided by muscle mass) (kg/kg) in women aged 34-83 years from the TwinsUK cohort

(subset 2). Moreover, in secondary analyses, additional aims were to examine the hypotheses that i) the nutrients that were significantly associated with indexes of muscle mass (subset 1) were independently predictive when analyses were performed in a single regression model, and ii) the genetic background determines the association between nutrient intake and indexes of muscle mass.

The nutrients included are presented in (**Table 3.1**).

Table 3.1 Nutrients included in the analysis

Vitamins	***Minerals***	***Carotenoids***
Vitamin C (mg/d)	Magnesium (mg/d)	α-carotene (µg/d)
Vitamin E (mg/d)	Potassium (mg/d)	ß-carotene (µg/d)
Vitamin D (µg/d)	Selenium (µg/d)	Total carotene (µg/d)
		ß-cryptoxanthin (µg/d)
		Lycopene (µg/d)
		Lutein (µg/d)
		Zeaxanthin (µg/d)

3.2 Covariate plan and statistical analysis

All analyses were performed using STATA statistical software (version 11.0; STATA Corp, USA). Means and standard deviations for the two subsets of the cohort were calculated (**Table 3.2)** and quintiles of each micronutrient intake were derived. Pearson correlation coefficients were used to evaluate associations between age and indexes of muscle mass, hand grip strength and muscle quality; nutrient intakes and energy intake; height and hand grip strength; and between the various nutrients that were included in this analysis. The analyses were performed treating twins as individuals as previous studies have shown that participants from the TwinsUK registry were similar to age-matched singleton women in the distribution of common traits and outcomes (Andrew *et al.* 2001). Muscle quality was calculated as

the ratio of hand grip strength (kg) to lean mass (kg). Data on muscle mass were collected in 1996 (subset 1) and data on hand grip strength ten years later in 2005 (subset 2). For the first subset, data on dietary intake (FFQ), body composition parameters, and other covariates were available for *n* = 2570 women, aged 18 to 79 years, who had completed a food frequency questionnaire (FFQ) and attended for dual-energy X-ray absorptiometry (DEXA) scans between 1996 and 2000. For the second subset, data on dietary intake (FFQ), body composition parameters, and other covariates were available for *n* = 949 women, aged 34 to 83 years, who had completed a food frequency questionnaire (FFQ) and attended for hand grip strength measurements and DEXA scans between 2005 and 2008. Secondary analyses were also performed using a co-twin design within-twin pair analyses to assess whether the shared environment of the twins influences the associations between nutrients that were previously associated with indexes of muscle mass in our analyses where twins were considered as singletons in women aged 18 to 79 years (subset 1). For that an example of a strong and an example of a poor association was illustrated.

Prior to conducting statistical analysis, an analysis plan with justification for each confounding factor in the multivariate model was developed as described in Chapter 2, section 2.2.5, p. 57. Multivariate regression analysis was used to assess associations between micronutrient intakes and indexes of muscle mass, hand grip strength and muscle quality using the robust cluster regression option in STATA. These models take into account clustering of individuals when calculating standard errors of the mean

(Richards *et al.* 2007) to ensure familial aggregation within twin pairs was accounted for.

As age has a great influence on muscle mass, strength, and muscle quality all analyses were adjusted for age (in years) (Cruz-Jentoft *et al.* 2010, Lynch *et al.* 1999). Models were then also adjusted for physical activity and smoking status, as regular physical activity has been associated with increased muscle mass and strength (Frankel *et al.* 2006, Heath and Stuart 2002, Kuh *et al.* 2002, Roubenoff 2007, van Kan *et al.* 2008, Wolfe 2006), and smoking has been shown to increase the risk of sarcopenia (Morse *et al.* 2007, Petersen *et al.* 2007, Wüst *et al.* 2008). Energy intake (kcal/d) was also included in the multivariate models. Total body mass was included as a covariate in the evaluation of associations between diet and FFM and FFMI as described in Chapter 2, section 2.2.5, p. 63 (Subset 1) (**Tables 3.3, 3.4, 3.5**). Additional adjustments were made for hand grip strength and muscle quality including height, menopausal status (premenopausal or postmenopausal), and hormone replacement therapy use (HRT) (no or yes), as these factors have been previously shown to affect muscle strength and muscle quality (Subset 2) (**Tables 3.6, 3.7, 3.8, 3.9, 3.10, 3.11**) (Carville *et al.* 2006, Cooper *et al.* 2008, Greeves *et al.* 1999, Greising *et al.* 2009, Jerome *et al.* 1991, Kurina *et al.* 2004, Neder *et al.* 1999, Sipila *et al.* 2001). Since age was inversely correlated with hand grip strength and muscle quality, this analysis was stratified by age using two categories, less than 50 years and more than 50 years (**Tables 3.7, 3.8, 3.10 and 3.11**). Further adjustments were made to account for the impact of protein intake on the

association between diet and muscle mass, muscle strength and muscle quality, as protein is a nutrient which is strongly associated with muscle mass, and for misreporting using the ratio of reported energy intake to estimate energy expenditure.

3.3 Results

Descriptive characteristics of the two subsets of the TwinsUK cohort and the mean intake of micronutrients are shown in **Table 3.2**. For the first subset (*n* = 2570 women) the mean (±SD) age of participants was 48.3 ± 12.7 years and mean (±SD) BMI was 24.9 ± 4.14 kg/m^2. Mean FFM (±SD) was 39.6 ± 5.30 kg, percentage FFM 61.1 ± 6.49 %, and FFMI 15 ± 1.71 kg/m^2. More than one-half of the participants were moderately active (54 %), and in relation to smoking status, 18.2 % were current smokers. Mean (±SD) energy intake was 1979 ± 524 kcal/d (**Table 3.2**).

For the second subset (*n* = 949 women) the mean (±SD) age of participants was 59.1 ± 9.30 years and the mean (±SD) BMI was 26.5 ± 4.73 kg/m^2. Mean hand grip strength was 28.8 ± 5.95 kg, and muscle quality [calculated as the ratio of hand grip strength (kg) to lean mass (kg)] was 0.69 ± 0.14 kg/kg. Mean (±SD) percentage FM was 35.7 ± 6.28 %, and percentage FFM was 61.4 ± 6.18 %. In relation to physical activity, 39.6 % of participants were inactive and few of them were current smokers (9.8 %). Mean energy intake (±SD) was 1917 ± 637 kcal/d (**Table 3.2**).

Notable differences were observed in body composition between the two subsets of this cohort, participants in the second subset were heavier, had

higher BMI and fat mass, percentage fat mass and fat free mass compared to the first subset of the cohort which might be because increases in fat mass are associated with increases in fat free mass (Wells JCK *et al.* 2002, Wolfe 2006) (**Table 3.2**). Concerning percentage fat free mass there were no differences in the two subsets measured ten years apart. This finding might be because although fat free mass and body weight were higher in the second subset compared to the first subset of the cohort the ratio which defines the percentage fat free mass was similar.

In this cohort fat free mass was positively correlated with hand grip strength ($r = 0.35$, $P < 0.001$). The relationships between indexes of muscle mass (FFM, percentage FFM, and FFMI) **(Tables 3.3, 3.4, 3.5)**, hand grip strength (**Table 3.6**) and muscle quality (**Table 3.9**) with different nutrient intakes were also examined in unadjusted and multivariate adjusted models.

Subset 1 (2570 women, 18-79 years)

Fat free mass (FFM, kg)

In multivariate analyses, when comparing extreme quintiles of nutrient intakes, FFM was positively and significantly associated with vitamin C intake by 1.2 kg, magnesium by 1.8 kg, potassium by 1.1 kg, ß-carotene by 0.8 kg, total carotene by 0.6 kg, lutein by 0.8 kg, and ß-cryptoxanthin by 0.7 kg, compared to the lowest quintile of each nutrient (P for difference < 0.05 for all). No significant associations were found for the other nutrients (**Table 3.3**).

Percentage fat free mass (FFM %)

In the multivariate model, when compared extreme quintiles of nutrient intake, percentage FFM was positively and significantly associated with vitamin E by 2.18 %, magnesium by 1.58 %, and lutein by 0.93 %, compared to the lowest quintile of each nutrient (P for difference < 0.05 for all). However, intakes of vitamin D and lycopene were significantly associated with lower mean percentage FFM by 0.9 % (P for difference = 0.037 and 0.022, respectively). No associations were observed for the other nutrients (**Table 3.4**).

Fat free mass index (FFMI, kg/m^2)

After multivariate adjustment, vitamin C, magnesium and potassium were significantly associated with mean FFMI with a difference of association of 0.5 kg/m^2 between extreme quintiles of intake (P for difference < 0.001 for all). Higher FFMI was also significantly associated with intakes of α-carotene, ß-carotene, total carotene, ß-cryptoxanthin, and lycopene with differences of association of 0.3 kg/m^2 between extreme quintiles of intake (P for difference < 0.05 for all). No associations were observed for the other nutrients (**Table 3.5**).

Sensitivity analysis

After assessing the collinearity of the all the nutrients included in this chapter, the strongest correlation coefficients (> 0.8) were found between magnesium and potassium (r = 0.91), a-carotene and β-carotene (r = 0.88),

a-carotene and total carotene (r = 0.91), β-carotene and total carotene (r = 0.99), and zeaxanthin and ß-cryptoxanthin (r = 0.71).

Therefore, the nutrients that included in the single multivariate regression model were those which were not collinear (i.e. magnesium, vitamin C, total carotene and ß-cryptoxanthin). After multivariate adjustment only magnesium intake was significantly and independently associated with all indexes of muscle mass. The difference in FFM, percentage FFM and FFMI per quintile of magnesium intake was 0.30 (kg), 0.39 %, and 0.08 (kg/m^2) - [ß coefficient (95 % CI): 0.30 (0.08, 0.52), P = 0.007], [ß coefficient (95 % CI): 0.39 (0.09, 0.68), P = 0.009], and [ß coefficient (95 % CI): 0.08 (0.006, 0.15), P = 0.033], respectively.

Within twins analysis

After multivariate adjustment for age, physical activity, smoking, energy intake and total body fat, within-pair associations between magnesium intake and the fat free mass index [ß coefficient (95 % CI): 0.06 (-0.003, 0.12), P = 0.064] were towards significance, and between pair associations were strongly significant [ß coefficient (95 % CI): 0.12 (0.05, 0.20), P = 0.0014]. There were no significant within-pair associations between selenium intake and the fat free mass index [ß coefficient (95 % CI): 0.03 (-0.02, 0.08), P = 0.305].

Subset 2 (949 women, 34-83 years)

Hand grip strength

In the multivariate model, although there was a positive trend between vitamin D, magnesium, selenium, potassium, ß-carotene, and lycopene intakes and hand grip strength, however the associations were not significant (P > 0.05, for all). Intakes of vitamin C, vitamin E, α-carotene, total carotene, lutein, zeaxanthin, and ß-cryptoxanthin were not significantly associated with lower mean hand grip strength (P > 0.05, for all) (**Table 3.5**). Even after stratification for age, associations between nutrient intakes and hand grip strength remained non-significant (**Tables 3.7 and 3.8**).

Muscle quality

In multivariate analyses, although there was a positive trend between vitamin E, selenium, ß-carotene, and lycopene intakes and muscle quality (kg/kg), the associations were not significant (P > 0.05, for all) (**Table 3.9**). After stratification for age, selenium intake was positively and significantly associated with muscle quality by 0.13 kg/kg between extreme quintiles of intake (P for difference = 0.023) in participants aged less than 50 years (**Table 3.10**). However, no associations were observed in participants aged more than 50 years (**Table 3.11**).

3.4 Discussion

In this study using two subsets of a large population-based sample of women aged 18 to 79 years and 34 to 83 years, respectively, we evaluated

the association between a range of micronutrients present in the habitual diet and indexes of muscle mass, hand grip strength and muscle quality. This is one of the first cross-sectional studies to show that higher consumption of vitamins C and E, magnesium, potassium, α-carotene, ß-carotene, total carotene, lutein, ß-cryptoxanthin and lycopene were significantly associated with muscle mass in a cohort of women across a wide age range. In addition, magnesium intake was significantly and independently associated with all indexes of muscle mass, even after taking into account intakes of other nutrients (i.e. vitamin C, total carotene and ß-cryptoxanthin), and adjusting for misreporting. Associations were towards significance in the within-pair analysis between magnesium intake and fat free mass index, suggesting that potential shared lifestyle and genetic factors were driving this apparent relationship. Few previous studies have examined this range of micronutrients in association with measurements of muscle mass and have been focused in older cohorts (Robinson SM *et al*. 2008, Scott *et al.* 2010). However, the current study highlighted the influence of a range of micronutrients on estimates of muscle mass in a cohort of young to older aged women.

Micronutrients and indexes of muscle mass

Vitamins

The current study observed positive associations between vitamin C intake and FFM and FFMI; and between vitamin E intake and percentage fat free mass (FFM %). The greatest association was observed between vitamin C intake and FFMI, with a difference of 3.11 % of the population mean for

FFMI, between extreme quintiles of intake. The difference in FFMI per quintile of vitamin C intake was 0.09 (kg/m^2) [ß coefficient (95 % CI): 0.09 (0.05, 0.14), $P < 0.001$]. The magnitude of association was similar to the only previous prospective study, in which, vitamin C intake was positively associated with muscle mass (ß coefficient: 0.06, $P = 0.002$) but only at follow up (2.6 years) in men and women aged 50 to 79 years (Scott *et al.* 2010). The difference of 3.11 % in FFMI observed in the current study between extreme quintiles of vitamin C intake equated to a 207 mg/d mean difference in vitamin C intake between the lower (68 mg/d) and the higher (275 mg/d) quintile. This finding corresponded to consumption of two oranges (assuming one portion of oranges to be 120 g for adults) and one portion of strawberries (100 g/d), highlighting the importance of incorporating more plant-based food components into the habitual diet.

Even though findings from this study showed modest cross-sectional associations between vitamin C and indexes of muscle mass, the results could provide a further explanation for the role of vitamin C on sarcopenia, through its potential to act against reactive oxygen species (ROS) and oxidative stress, which directly mediates muscle damage (Meng and Yu 2010). The observed association between vitamin C intake and muscle mass is supported by *in vitro* (Silveira *et al.* 2006), animal (Gomez-Cabrera *et al.* 2005), and human study findings (Gomez-Cabrera *et al.* 2006, Ristow *et al.* 2009), in which supplementation with vitamin C has shown to suppress some adaptive responses of skeletal muscle mass to oxidants during exercise (Khassaf *et al.* 2003). In addition, vitamin C has been shown to exert anti-

inflammatory properties. Indeed, one randomised controlled trial has shown that supplementation with 1000 mg/d vitamin C for two months reduced the inflammation marker C-reactive protein (CRP) by 25.27 % among participants with elevated baseline CRP more than 1 mg/L (Block *et al.* 2009). Findings from the current study are also in contrast with one cross-sectional study from the InCHIANTI cohort where higher vitamin C intake was significantly associated with knee extension strength ($\beta = 0.383$, $P = 0.02$) and physical performance ($\beta = 0.029$, $P = 0.04$) (Cesari *et al.* 2004). Although dietary interventions on muscle mass and sarcopenia have not addressed this important issue yet, these findings may suggest that adequate intake of nutrients with potential anti-inflammatory properties may play a critical role in the maintenance of healthy muscles.

In the current study vitamin D intake was not associated with muscle strength and was negatively related to FFM %. This finding contrasts with other cross-sectional and supplementation studies reporting that low vitamin D intake and status were associated with low muscle strength, physical performance and frailty, although supplementation studies have not always shown improvements in muscle function (Annweiler *et al.* 2009, Bartali B *et al.* 2006, Lisa 2008). There is currently a lack of high-quality observational data from large cohorts on associations between vitamin D status and physical performance (Institute of Medicine 2010). In addition, evidence from meta-analysis of randomised controlled trials (RCTs) suggested that there was a non-significant dose-response relationship between supplementation of vitamin D (even in high doses at or more than 20 µg/d)

or achieved serum 25(OH)D and the risk of sustaining at least one fall (Millward 2012).

Nevertheless, the relative contribution of sun exposure on vitamin D status should be acknowledged, as sun exposure is the most important contributor to vitamin D status with 80-90 % vitamin D obtained from the sunlight and diet contributing to a much lesser extent (Holick 1994). Longitudinal data suggest that UVB exposure (according to season) contributes mainly to changes in plasma 25-hydroxy vitamin D (25-OHD) levels (a plasma metabolite used to assess vitamin D status), with measurements being lower among two groups of Caucasian women in the north compared with those in the south of the UK (Macdonald *et al.* 2011). It is possible that the lack of association in the current study was the result of the fact that the sunlight contribution was not considered in the current analysis. Additionally, the lack of association might be because the mean (±SD) vitamin D intake was only 2.61 (±1.42) µg/d, which is lower compared to the usual daily intake of vitamin D in the UK (which is relatively low), of around 3.5 µg/d (Scientific Advisory Committee on Nutrition, 2007).

Minerals

In relation to minerals, the current analysis found that magnesium intake was positively associated with all three indexes of muscle mass, when women were considered as individuals, and were towards significance in the within-pair analysis, suggesting that potential shared lifestyle and genetic factors were driving this apparent association. These factors are not yet known and it would be interesting to be investigated in future genetic

studies. When twins were considered as singletons, the greatest associations were observed for FFM with differences of 4.63 % ($P < 0.001$) of the population mean, and 3.39 % ($P = 0.001$) of the population mean for FFMI, between extreme quintiles of intake after adjustment for all the other confounders. Alternatively, the difference in FFM per quintile of magnesium intake was 0.41 (kg) [ß coefficient (95 % CI): 0.41 (0.21, 0.61), $P < 0.001$], and the difference in FFMI per quintile of magnesium intake was 0.12 (kg/m^2) [ß coefficient (95 % CI): 0.12 (0.05, 0.19), $P < 0.001$]. Findings were of a similar trend although of a greater magnitude to a previous prospective study reporting that energy-adjusted magnesium intake was a positive predictor of change in appendicular lean mass over 2.6 years follow-up among older people (ß coefficient = 0.07, $P = 0.02$) (Scott *et al.* 2010). This difference may be explained by the fact that the study from Scott *et al.* was longitudinal, included fewer participants ($n = 740$ men and women) compared with the current study ($n = 2570$) and a narrower age range (50 to 79 years). It also used a much more limited FFQ (74 food items) and it measured only appendicular lean mass (although this accounts for more than 75 % of the total skeletal muscle mass) (Gallagher *et al.* 1997), whereas the current cohort measured total body fat free mass. A difference of 3.4 % in FFMI reported between extreme quintiles of magnesium intake equated to a 256 mg/d mean difference in magnesium intake between the lower (225 mg/d) and the higher (481 mg/d) quintile. In food terms, this difference corresponded to consumption of 1.8 portions of all bran per day (108 g/d) from the habitual diet (assuming 1 portion of all bran to be 60 g for adults). Interestingly, magnesium intake was

significantly and independently associated with all indexes of muscle mass, even after taking into account intakes of vitamin C, total carotene and ß-cryptoxanthin, indicating its importance for muscle mass.

The observed associations between magnesium intake and indexes of muscle mass is supported by previous studies in humans showing a strong effect of magnesium status on muscle performance, possibly through its role in energetic metabolism and trans-membrane transport in muscles (Lukaski 2004). Indeed, adequate availability of magnesium may lead to increased muscle mitochondrial efficiency for which magnesium is critical (Wolf and Cittadini 2003), and also to reduced production of reactive oxygen species, which mediate muscle damage (Meng and Yu 2010). Therefore, magnesium availability is important for the function of muscle mitochondria and for the control of oxidative stress. In addition, results from the current study provide a potential explanation to support the anti-inflammatory properties of magnesium. Poor magnesium status may trigger a pro-inflammatory state accompanied by production and release of pro-inflammatory cytokines, such as interleukin 1ß (IL-1ß) and tumor necrosis factor-α (TNF-α), which activates a low-grade chronic inflammation, which has been suggested as a major pathway in the aetiology of age-related muscle loss (Meng and Yu 2010) (Kramer *et al.* 2003, Lukaski 2004). In previous cross-sectional studies dietary magnesium intake was modestly and inversely associated with C-reactive protein (a marker of systemic inflammation) in women ≥45 years (Song *et al.* 2007, Song *et al.* 2005). Therefore, adequate magnesium

intake is not only essential for muscle metabolism (Maguire and Cowan 2002), but it is also important due to its anti-inflammatory properties.

An interesting finding of this study was the significant association between higher potassium intake and indexes of muscle mass with percentage differences in FFM of 2.80 % and in FFMI of 3.32 % between extreme quintiles of intake ($P = 0.004$ and < 0.001, respectively). There are no studies in the literature examining associations between habitual potassium intake and muscle mass, however, potassium may be an important micronutrient as muscle mass is involved in potassium homeostasis, and therefore inadequate dietary potassium intake may have a subsequent effect on the activity of muscles (McDonough *et al.* 2002). Moreover, dietary potassium may protect against acidosis, which has strong catabolic influences on muscle (May *et al.* 1987). From previous studies it is evident that consumption of a Western-style diet leads to a chronic low-grade acidosis through the release of noncarbonic acids into the systemic circulation (e.g. protein metabolism releases sulphuric acid) in greater amounts than that of the simultaneous release of bases (e.g. bicarbonate from the oxidization of organic salts of potassium from vegetable sources) (Lennon *et al.* 1966). Potassium bicarbonate has been suggested to neutralise net acid load and therefore may also neutralize the diet and age dependent metabolic acidosis. Potassium bicarbonate is a natural base generated endogenously from the metabolism of organic salts of potassium, such as potassium citrate, which is found mainly in fruits and vegetables. It has been suggested that long-term supplementation of the diet with potassium bicarbonate may benefit anabolism and may be beneficial to

muscle wasting (Frassetto *et al.* 1997, Sebastian *et al.* 1994). Therefore, it is important to incorporate foods high in potassium, such as fruits and vegetables, in the habitual diet.

In the current analysis an association between habitual selenium intake and indexes of muscle mass was not observed although there are previous cross-sectional studies suggesting positive associations between low plasma selenium and poor skeletal muscle strength among older adults with selenium intake found to associated with higher grip strength in women 59 to 73 years (Lauretani F *et al.* 2007, Robinson SM *et al.* 2008). The lack of associations in the current study may be because the selenium content of foods varies depending on the soil content and other environmental conditions (especially the quantity and species of selenium to which the animal/plant is exposed) (Fairweather-Tait *et al.* 2010). This affects the form of selenium present in plant and animal food sources. This may impact on the absorption and use of selenium and consequently influence selenium's protective role in muscle functions (Rederstorff *et al.* 2006).

Carotenoids

In relation to carotenoids, findings from the current analysis showed positive associations between carotenoid intakes and indexes of muscle mass. The greatest associations were observed between FFMI and higher intakes of total carotene, α-carotene, ß-carotene, and ß-cryptoxanthin with associations of approximately 2 %. For lycopene a difference of 1.54 % of the population mean for FFMI was seen between extreme quintiles of

intake. Associations were not observed for lutein and zeaxanthin in association with FFMI and this might be explained by the fact that higher sources of lutein and zeaxanthin, such as kale and tangerines were not included in the FFQ used to assess dietary intake.

These findings are supported by recent epidemiological studies in community-dwelling older individuals which have shown that low serum and/or plasma carotenoid levels were independently associated with low muscle strength and the development of walking disability over time (Lauretani *et al.* 2008, Semba *et al.* 2007). In addition, in the InCHIANTI study low intake of β-carotene was marginally associated with knee extension strength ($\beta = 0.311$, $P = 0.05$) (Cesari *et al.* 2004). The underlying mechanisms which explain these associations are speculated to be oxidative stress and inflammation, which are implicated in the ageing process and the pathogenesis of sarcopenia (Meng and Yu 2010). Although, mechanistic studies have not shown beneficial effects of β-carotene supplementation on the responses of skeletal muscle to exercise induced oxidative stress (Jackson *et al.* 2004) population-based studies have shown that low serum carotenoid levels were inversely associated with serum protein carbonyl concentrations (indicator of oxidative protein damage) (Alipanah *et al.* 2007). Moreover, although there is a lack of mechanistic studies regarding the effects of carotenoids on inflammation associated sarcopenia, there is evidence from population-based cohorts that low serum carotenoid levels were associated with higher levels of the inflammatory marker IL-6, and women with low levels of a-carotene, β-carotene, total carotene, lutein/zeaxanthin were more likely to have increased IL-6 over a period of

two years (Walston *et al.* 2006). However, this is the first study to examine associations between dietary intake of a range of carotenoids and indexes of muscle mass, giving an insight to their potential role in sarcopenia.

Of interest, the proportion of women aged 18 to 79 years (subset 1) with intakes below the Reference Nutrient Intake (RNI) was 1.2 % for vitamin C (RNI = 40 mg/d), 21.4 % for magnesium (RNI = 270 mg/d), and 33.4 % for potassium (RNI = 3500 mg/d). In contrast the proportion of women of the same subset with intakes below the RNI was 73.1 % for selenium (RNI = 60 µg/d). The proportion of women below the Estimated Average Requirements (EAR) for vitamin D was 99.8 % (EAR = 10 µg/d); and the proportion of women below the Recommended Daily Amount of vitamin E was 81.2 % (RDA = 15 mg/d) (National Research Council 2011). These proportions were very similar for subset 2 (women aged 34 to 83 years).

Micronutrients and hand grip strength and muscle quality

In the current study we did not observe any cross-sectional associations between habitual nutrient intake and hand grip strength. Although, mean hand grip strength was higher with higher intakes of vitamin D, magnesium, selenium, potassium, ß-carotene, and lycopene, the associations were not significant. Mean muscle quality was also higher with higher vitamin E, ß-carotene, and lycopene intakes, but the associations were not significant. Even after stratification by age, associations between nutrient intakes and hand grip strength and muscle quality did not reach statistical significance.

To date, there are only two previous epidemiological studies examining associations between nutrient intakes and muscle strength measurements with conflicting results (Robinson SM *et al.* 2008, Scott *et al.* 2010). One of these studies was a prospective cohort study among 740 older women and men (mean age 62 ± 7 years), and muscle strength was assessed by a seated isometric contraction of the knee extensors at baseline and after 2.6 years of follow-up. Findings from this study showed that there was no association between nutrient intake (protein, saturated fat, calcium, magnesium, niacin, niacin equivalents, phosphorus, potassium, riboflavin, zinc) and muscle strength of the knee extensors over 2.6 years of follow-up (Scott *et al.* 2010).

The second study was a cross-sectional examination within the Hertfordshire UK cohort among 2983 women and men aged between 59 to 73 years, in which muscle strength was assessed by hand grip strength. Results from this study showed that in women, after adjustment for energy intake, the proportional difference in hand grip strength per unit of nutrient intake was: 14.75 kg for selenium intake [ß coefficient (95 % CI): 14.75 (4.27, 50.93), P < 0.001], 4.17 kg for vitamin D intake [ß coefficient (95 % CI): 4.17 (2.15, 8.07), P < 0.001], 3.44 kg for vitamin C intake [ß coefficient (95 % CI): 3.44 (1.70, 6.96), P = 0.001], and 2.25 kg for carotene intake [ß coefficient (95 % CI): 2.25 (1.16, 4.36), P = 0.016] (Robinson SM *et al.* 2008).

Notably, the assessment measurements used for muscle strength were different between these two previous cohorts. In the first study the lack of associations might be due to the fact that there was an increase in muscle strength over the 2.6 years of follow-up compared with baseline, most likely because of inadequate use of the test as it was not previously validated (Phillips *et al.* 2004). In the second study, muscle strength was assessed by hand grip strength, which has been suggested as a commonly used proxy for global muscle strength (Abellan Van Kan *et al.* 2009, Lauretani *et al.* 2003).

The highest association observed in the current study was between selenium intake and hand grip strength although the association was not statistically significant. The magnitude of association in this study was smaller compared with that from the Hertfordshire cohort with a difference in hand grip strength per quintile of selenium intake of 0.07 kg [ß coefficient (95 % CI): 0.07 (- 0.21, 0.34), P = 0.64] compared with 14.75 kg [ß coefficient (95 % CI): 14.75 (4.27, 50.93), P < 0.001] in the Hertfordshire cohort. Although both studies use the same method used to assess muscle strength and a number of covariates that may mediate the relationship between nutrient intake and muscle strength and muscle quality were considered in the current analysis, the findings did not reach significance. It should be acknowledged that the Hertfordshire cohort was an older cohort (59-73 years), with lower mean hand grip strength (26.5 kg) compared to the second subset of the current analysis which included younger individuals (34-83 years), with subsequently higher hand grip strength (28.8 kg). Our

cohort was younger and therefore might be less likely to observe changes in muscle strength in association with diet.

The lack of significant associations observed in the current study might be due to the fact that other lifestyle factors than diet have a greater influence on muscle strength and muscle quality. Interestingly, in this cohort, physical activity had a greater effect on hand grip strength, and age had a greater effect on muscle quality for all the nutrients. For example, after multivariate adjustment for relevant confounders, the effect of physical activity [ß coefficient (SEM): 1.05 (0.414), P = 0.01] was 5.25 times greater of that of vitamin C intake [ß coefficient (SEM): - 0.2 (0.122), P = 0.14] on hand grip strength. This finding was observed even after stratification for age. Notably, intervention programs have shown that exercise and strength training was beneficial for muscle strength, and epidemiological studies suggested that increased customary physical activity was associated with improved muscle strength and physical performance (Haight *et al.* 2005, Martin *et al.* 2008, McDermott *et al.* 2002, Puggaard 2003). The observed effect of age [ß coefficient (SEM): - 0.006 (0.0006), P < 0.001] was two-fold greater than the effect of vitamin C intake [ß coefficient (SEM): - 0.003 (0.003), P = 0.27] on muscle quality and these findings are supported by a number of studies which have previously described decreases in muscle quality with age (Newman *et al.* 2003).

Overall, this study showed that higher consumption of vitamins C and E, magnesium, potassium, α-carotene, ß-carotene, total carotene, lutein, ß-

cryptoxanthin and lycopene were significantly associated with increased muscle mass in a cohort of women including younger adults, after accounting for age and other lifestyle factors, such as physical activity and smoking status. Findings remained significant with no substantial differences between the association of the above mentioned nutrient intakes and indexes of muscle mass, when protein intake was added in the multivariate model. This finding suggested that although protein intake is important for maintenance of muscle mass, other nutrients in the diet with the potential to act against oxidative stress and exert anti-inflammatory properties, such as vitamin C and E, magnesium and carotenoids, and also nutrients with the potential to benefit age-dependent metabolic acidosis and muscle wasting, such as potassium, may also be relevant for muscle mass. Among the nutrients studied, magnesium appeared to be independently predictive for all three indexes of muscle mass on top of intakes of vitamin C, total carotene and ß-cryptoxanthin, indicating its importance for muscle mass. Findings were not significant for associations between nutrient intake and muscle strength and quality possibly because other lifestyle factors, such as physical activity and the ageing process itself, had a greater effect in this cohort.

3.5 Strengths and limitations

The strengths of this study include the large sample size, and the wide age range of participants as most previous studies on sarcopenia examined only older individuals (Robinson SM *et al.* 2008, Scott *et al.* 2010). Moreover, this is the first study, to our knowledge, that accounted for familial or

genetic influences that may be shared. An additional strength was that body composition was objectively assessed by DEXA scans and an objective measurement was used to assess muscle strength, which allowed calculation of muscle quality. Furthermore, this study was focused in women, although men have on average greater amounts of muscle mass than women, but they have shorter survival, which implies that sarcopenia may potentially become a greater public health concern in women than men (Abellan Van Kan 2009). Moreover, this was the first study that examined associations between intakes of an extended range of carotenoids, using the EPIC database of the carotenoid content of food (Pattison *et al.* 2005). Data from the TwinsUK cohort has shown that participants were representative of the general population in terms of measures or disease outcomes, such as height, bone mineral density, osteoarthritis, blood pressure, use of hypertensive medication, alcohol consumption, menopausal, hysterectomy and ovariectomy status and diet (Andrew *et al.* 2001, Henderson *et al.* 2004, Sproston and Primatesta 2004, Teucher *et al.* 2007). Also, the FFQ used in the current study was previously compared and validated against a 7-day weighed record in the EPIC Norfolk study, and although the two approaches to measure dietary intake were different, both methods identified similar intakes of macronutrients when these expressed as percentage of total energy intake (Bingham *et al.* 2001). Moreover, this FFQ was previously validated against urinary and plasma biomarkers of intake, such as urinary nitrogen for protein intake, urinary potassium and sodium, plasma ascorbic acid and plasma n-3 PUFAs, and in addition, it has been shown previously that serum ß-carotene, ß-cryptoxanthin, and zeaxanthin concentrations were

moderately correlated with dietary carotenoid intakes measured by an FFQ (McKeown *et al.* 2001, Tucker *et al.* 1999, Welch *et al.* 2006). Furthermore, in order to examine associations between dietary intake and health outcomes participants need to be ranked according to their usual dietary intake and FFQs have been shown to rank individuals well (Molag *et al.* 2007).

In the current analyses multiple corrections were not performed as the hypothesis of the study was well defined *a priori*, and the mechanisms linking the associations between different micronutrients and indexes of muscle mass, strength and muscle quality were independent. Multiple testing is an important consideration in exploratory studies is (Benjamini 2010). However, since our hypotheses were well defined *a priori*, and the mechanisms linking the different exposures and the outcome were independent, then correction for multiple testing is not strictly necessary (Bender and Lange 2001).

This study also has a number of limitations. The cross-sectional nature of the study limits causal inference, but enables us to generate hypotheses regarding associations between specific nutrient intakes and indexes of muscle mass, strength and muscle quality based on plausible mechanisms. High intakes of vitamins C and E, magnesium, potassium and carotenoids may be indicators of a healthy lifestyle, and even though all analyses were adjusted for lifestyle and dietary protein intake, residual confounding may still be present because of the observational nature of the study. In addition, as in all observational studies, measurement error in self-reported dietary

intakes is inevitable. It is also widely acknowledged that the use of FFQs for measuring dietary intake has its own limitations related to memory errors, they provide relative than absolute intake, they introduce bias due to over- or under-reporting, and they may introduce systematic errors as preparation methods are inadequately considered (McNeill *et al.* 2009). Also, FFQs measure food intake over a period of one year and reflect the habitual diet over this period of time but not lifetime dietary habits (Teucher *et al.* 2007). The observed findings relate to women and further work is needed to investigate if these findings are replicated in a male population of the same country or in populations from different ethnic backgrounds.

3.6 Concluding remarks

This is one of the first studies to our knowledge to show that consumption of a range of micronutrients is associated with muscle mass in women of different ages, and to account for familial or genetic influences that may be shared. This is also one of the first studies to examine cross-sectional associations between a range of micronutrients and muscle strength and muscle quality. Our findings extended the results from few previous studies by examining simultaneously associations between a range of nutrients relevant for muscle mass in a cohort including younger adults. It is evident that notable changes in skeletal muscle mass may occur earlier in adult life (between 30 and 45 years of age), and it is important to examine dietary associations with muscle mass in individuals of all ages (Cesari and Pahor 2008, Janssen *et al.* 2000). Additionally, our findings extended the existing literature by evaluating the relative importance of diet in terms of nutrients

and muscle quality to further understand the association between muscle strength and muscle mass with diet.

In conclusion, there was a positive association between intakes of vitamin C, magnesium, potassium, ß-carotene, total carotene, lutein, and ß-cryptoxanthin and fat free mass (FFM, kg); vitamin E, magnesium, and lutein and percentage fat free mass (FFM %); and vitamin C, magnesium, potassium, α-carotene, ß-carotene, total carotene, ß-cryptoxanthin, and lycopene and fat free mass index (FFMI, kg/m^2). Also, although there was a positive association between intakes of vitamin D, magnesium, potassium, selenium, ß-carotene, and lycopene intakes with muscle strength (kg), and vitamin E, ß-carotene, and lycopene intakes with muscle quality (kg/kg), these associations were not statistically significant.

A consistent significant association was observed between higher magnesium intakes with all three indexes of muscle mass when women were considered as singletons. These associations were observed after adjustments for age, physical activity, smoking, energy intake and total body mass. Additionally, among the nutrients studied, magnesium appeared to be independently predictive for all three indexes of muscle mass on top of intakes of vitamin C, total carotene and ß-cryptoxanthin, indicating its importance for muscle mass. Of interest, the effect of different categories of physical activity (moderately active vs. inactive and active vs. inactive) on FFMI was [ß coefficient (95% CI): 0.16 (0.06-0.26), P = 0.002], which was similar to the effect of each quintile of magnesium intake on FFMI [ß

coefficient (95% CI): 0.12 (0.05-0.19), P < 0.001]. Even though the effect of physical activity (a factor important for muscle mass) was similar to that of diet, our findings regarding magnesium intake showed that there was a significant influence of micronutrient intake on FFMI after adjusting for all relevant confounders (including physical activity). Therefore, the magnitude of association of magnesium intake was comparable to that of physical activity, indicating its importance for muscle mass after accounting for all other confounders. Although the strength of the associations was modest, our results supported the initial hypothesis that higher intake of nutrients relevant for muscle mass may be important in maintaining fat free mass. Associations were towards significance in the within-pair analysis, suggesting that potential shared lifestyle and genetic factors other than dietary intake of magnesium were driving this apparent association. However, these findings need to be verified in other twin studies.

Nevertheless, not all the nutrients under study were positively associated with indexes of muscle mass, strength and muscle quality in this study. This was possibly due to the fact that other factors, such as physical activity may be more important than diet in terms of nutrients to preserve or maintain muscle mass (as assessed by the relevant indexes) and improve or maintain muscle strength and quality. Yet, it is possible that a longitudinal study design might be a more suitable approach to detect associations between nutrient intake and muscle mass indexes. Although further work is needed to examine associations between overall diet quality (assessed by diet quality scores) and muscle mass, muscle strength and muscle quality, results

from the current analysis suggest that it is important for adult women to consume a variety of foods high in vitamins C and E, magnesium, potassium and a range of carotenoids to ensure greater muscle mass and strength. From a public health perspective, these findings will help to improve the knowledge and understanding of the effects of dietary and lifestyle factors on muscle mass and strength and also provide useful information in the development and planning dietary intervention trials to improve conservation of muscle mass and strength in adult life.

TABLES

Table 3.2 Baseline characteristics of female participants from the TwinsUK cohort[1]

Characteristic	Subset 1 *N*=2570, 18-79 yrs	Subset 2 *N*=949, 34-83 yrs
Age (y)	48.3±12.7	59.1±9.30
Weight (kg)	65.6±11.2	69.2±12.5
Height (cm)	162±6.07	162±5.93
BMI (kg/m^2)	24.9±4.14	26.5±4.73
Fat mass (kg)	22.7±7.87	25.2±8.30
Fat mass %	33.9±7.17	35.7±6.28
Fat free mass (kg)	39.6±5.30	42.0±5.47
Fat free mass %	61.1±6.49	61.4±6.18
Fat free mass index (kg/m^2)	15.0±1.71	
Hand grip strength (kg)		28.8±5.95
Muscle quality (kg/kg)		0.69±0.14
Physical activity %		
Inactive	21.9	39.6
Moderate	53.9	34.2
Active	24.2	26.2
Smoking history %		
Never	50.2	
Current	18.2	
Former	31.6	
Current smoking %		
Yes		9.79
No		90.21
Menopausal status %		
Premenopausal		10.3
Postmenopausal		89.7
Hormone replacement therapy %		
No		90.63
Yes		9.38
Dietary components		
Total energy intake (kcal/d)	1979±524	1917±637
Vitamins		
Vitamin C intake (mg/d)	155±80.2	177±87.9
Vitamin E intake (mg/d)	11.4±4.57	11.5±5.46
Vitamin D intake (µg/d)	2.61±1.42	2.63±1.67
Minerals		
Magnesium intake (mg/d)	344±92.3	351±110
Potassium intake (mg/d)	3973±1004	4005±1177
Selenium intake (µg/d)	44.9±16.0	51.8±24.5
Carotenoids		
α-carotene intake (µg/d)	559±416	633±542
ß-carotene intake (µg/d)	3091±1757	3767±2630
Total carotene intake (µg/d)	3448±1944	4227±2907
Lycopene intake (µg/d)	1347±958	1896±1549
Lutein intake (µg/d)	2183±1450	2906±2401
Zeaxanthin intake (µg/d)	83.4±70.9	78.8±66.4
ß-cryptoxanthin intake (µg/d)	200±194	286±320

[1] Values are unadjusted means ± standard deviations (mean ± SDs) unless indicated

Table 3.3 The associations between nutrient intakes and fat free mass (kg) in 2570 women from the TwinsUK cohort aged 18-79 years[1]

Quintiles of nutrient intake	Fat free mass (kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	38.9±0.3	39.7±0.3	39.6±0.2	39.9±0.3	40.1±0.2	0.001
Adjusted model[2]	39.0±0.2	39.6±0.2	39.6±0.2	39.9±0.2	40.2±0.2	0.001
Vitamin D (µg/d)						
Unadjusted	39.6±0.3	39.8±0.3	39.3±0.2	39.8±0.3	39.8±0.3	0.71
Adjusted model	39.9±0.2	39.9±0.2	39.4±0.2	39.6±0.2	39.4±0.2	0.057
Vitamin E (mg/d)						
Unadjusted	39.2±0.3	39.5±0.3	39.9±0.2	39.9±0.3	39.7±0.2	0.06
Adjusted model	39.2±0.3	39.5±0.2	39.9±0.2	39.8±0.2	39.7±0.3	0.23
Magnesium (mg/d)						
Unadjusted	39.1±0.3	39.3±0.3	39.6±0.2	39.7±0.2	40.5±0.3	<0.001
Adjusted model	38.9±0.3	39.2±0.2	39.6±0.2	39.8±0.2	40.7±0.3	<0.001
Selenium (µg/d)						
Unadjusted	39.1±0.3	39.5±0.3	39.5±0.3	39.9±0.2	40.3±0.3	0.001
Adjusted model	39.5±0.3	39.5±0.2	39.5±0.2	39.9±0.2	39.9±0.2	0.11
Potassium (mg/d)						
Unadjusted	39.3±0.3	39.2±0.2	39.4±0.2	40.0±0.2	40.4±0.3	<0.001
Adjusted model	39.3±0.3	39.2±0.2	39.4±0.2	39.9±0.2	40.4±0.3	0.004
Total carotene (µg/d)						
Unadjusted	39.1±0.3	39.4±0.2	39.8±0.3	40.2±0.3	39.7±0.3	0.012
Adjusted model	39.2±0.2	39.4±0.2	39.7±0.2	40.2±0.2	39.8±0.2	0.008
α-carotene (µg/d)						
Unadjusted	39.4±0.3	39.6±0.2	39.4±0.3	39.9±0.2	40.0±0.3	0.09
Adjusted model	39.4±0.2	39.6±0.2	39.6±0.2	39.7±0.2	40.0±0.2	0.17
ß-carotene (µg/d)						
Unadjusted	39.1±0.3	39.3±0.2	40.0±0.3	40.1±0.3	39.8±0.3	0.006
Adjusted model	39.1±0.2	39.5±0.2	39.8±0.2	40.0±0.2	39.9±0.2	0.007
Lutein (µg/d)						
Unadjusted	39.0±0.3	39.4±0.2	40.3±0.3	39.9±0.3	39.7±0.2	0.019
Adjusted model	39.1±0.2	39.3±0.2	40.1±0.2	39.9±0.2	39.9±0.2	0.004
Zeaxanthin (µg/d)						
Unadjusted	39.0±0.3	39.7±0.2	40.0±0.2	39.5±0.3	40.1±0.3	0.010
Adjusted model	39.1±0.2	39.8±0.2	40.1±0.2	39.7±0.2	39.7±0.2	0.17
ß-cryptoxanthin (µg/d) *Unadjusted*	39.0±0.3	39.8±0.3	39.7±0.3	39.8±0.3	40.0±0.2	0.015
Adjusted model	39.2±0.2	39.7±0.2	39.7±0.2	39.8±0.2	39.9±0.2	0.032
Lycopene (µg/d)						
Unadjusted	38.9±0.3	39.3±0.3	40.0±0.3	39.7±0.2	40.3±0.3	<0.001
Adjusted model	39.3±0.2	39.6±0.2	39.9±0.2	39.7±0.2	39.7±0.2	0.34

[1] All values are means and SEM for fat free mass (kg) by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking history (never, former, current), energy intake (kcal/d), and total body mass (kg)

Table 3.4 The associations between nutrient intakes and percentage fat free mass in 2570 women from the TwinsUK cohort aged 18-79 years[1]

Quintiles of nutrient intake	Percentage fat free mass					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	61.09±0.3	61.09±0.3	61.18±0.3	60.93±0.3	61.17±0.3	0.99
Adjusted model[2]	60.63±0.3	60.99±0.3	61.14±0.3	61.14±0.3	61.57±0.3	0.07
Vitamin D (µg/d)						
Unadjusted	61.79±0.3	61.11±0.3	61.28±0.3	60.98±0.3	60.30±0.3	0.002
Adjusted model	61.55±0.3	61.16±0.3	61.14±0.3	61.02±0.3	60.61±0.3	0.037
Vitamin E (mg/d)						
Unadjusted	60.71±0.3	60.92±0.3	61.36±0.3	61.05±0.3	61.42±0.3	0.11
Adjusted model	59.99±0.4	60.62±0.3	61.36±0.3	61.33±0.3	62.17±0.4	<0.001
Magnesium (mg/d)						
Unadjusted	61.08±0.3	60.66±0.3	61.21±0.3	61.26±0.3	61.25±0.3	0.34
Adjusted model	60.35±0.4	60.49±0.3	61.17±0.3	61.53±0.3	61.93±0.4	0.004
Selenium (µg/d)						
Unadjusted	61.51±0.3	60.84±0.3	60.59±0.3	61.58±0.3	60.94±0.3	0.68
Adjusted model	61.44±0.3	60.77±0.3	60.82±0.3	61.56±0.3	60.88±0.3	0.79
Potassium (mg/d)						
Unadjusted	61.45±0.3	60.93±0.3	61.34±0.3	61.14±0.3	60.61±0.3	0.13
Adjusted model	61.04±0.4	60.85±0.3	61.37±0.3	61.28±0.3	60.94±0.4	0.93
α-carotene (µg/d)						
Unadjusted	61.77±0.3	61.54±0.3	60.37±0.3	60.92±0.3	60.86±0.3	0.011
Adjusted model	61.36±0.3	61.24±0.3	60.76±0.3	60.88±0.3	61.24±0.3	0.40
ß-carotene (µg/d)						
Unadjusted	61.50±0.3	61.53±0.3	60.72±0.3	60.97±0.3	60.78±0.3	0.049
Adjusted model	60.96±0.3	61.42±0.3	60.70±0.3	61.15±0.3	61.24±0.3	0.95
Total carotene (µg/d)						
Unadjusted	61.58±0.3	61.29±0.3	60.69±0.3	61.16±0.3	60.76±0.3	0.07
Adjusted model	61.06±0.3	61.09±0.3	60.76±0.3	61.32±0.3	61.25±0.3	0.70
Lutein (µg/d)						
Unadjusted	61.21±0.3	60.99±0.3	60.96±0.3	60.95±0.3	61.36±0.3	0.79
Adjusted model	60.77±0.3	60.75±0.3	61.06±0.3	61.19±0.3	61.70±0.3	0.031
Zeaxanthin (µg/d)						
Unadjusted	60.32±0.3	60.94±0.3	61.15±0.3	61.24±0.3	61.82±0.3	0.001
Adjusted model	60.59±0.3	61.14±0.3	61.29±0.3	61.51±0.3	60.93±0.3	0.35
ß-cryptoxanthin (µg/d)						
Unadjusted	60.84±0.3	60.91±0.3	61.19±0.3	61.45±0.3	61.09±0.3	0.29
Adjusted model	60.86±0.3	60.92±0.3	61.09±0.3	61.41±0.3	61.18±0.3	0.32
Lycopene (µg/d)						
Unadjusted	60.89±0.3	61.06±0.3	61.22±0.3	61.29±0.3	60.99±0.3	0.65
Adjusted model	61.25±0.3	61.42±0.3	61.26±0.3	61.19±0.3	60.35±0.3	0.022

[1] All values are means and SEM for percentage fat free mass by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking history (never, former, current), and energy intake (kcal/d)

Table 3.5 The associations between nutrient intakes and fat free mass index (kg/m^2) in 2570 women from the TwinsUK cohort aged 18-79 years[1]

Quintiles of nutrient intake	Fat free mass index (kg/m^2)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	14.79±0.1	15.05±0.1	14.97±0.1	15.09±0.1	15.22±0.1	<0.001
Adjusted model[2]	14.79±0.1	15.01±0.1	14.98±0.1	15.07±0.1	15.25±0.1	<0.001
Vitamin D (µg/d)						
Unadjusted	14.98±0.1	15.06±0.1	14.93±0.1	15.09±0.1	15.06±0.1	0.46
Adjusted model	15.05±0.1	15.06±0.1	14.97±0.1	15.07±0.1	14.96±0.1	0.40
Vitamin E (mg/d)						
Unadjusted	14.97±0.1	15.06±0.1	15.04±0.1	15.06±0.1	14.99±0.1	0.84
Adjusted model	14.94±0.1	15.04±0.1	15.07±0.1	15.04±0.1	15.02±0.1	0.88
Magnesium (mg/d)						
Unadjusted	14.87±0.1	14.97±0.1	15.03±0.1	15.08±0.1	15.17±0.1	0.008
Adjusted model	14.77±0.1	14.91±0.1	15.03±0.1	15.13±0.1	15.27±0.1	0.001
Selenium (µg/d)						
Unadjusted	14.89±0.1	15.01±0.1	14.99±0.1	15.05±0.1	15.17±0.1	0.027
Adjusted model	14.95±0.1	14.99±0.1	14.98±0.1	15.08±0.1	15.11±0.1	0.12
Potassium (mg/d)						
Unadjusted	14.85±0.1	14.88±0.1	15.01±0.1	15.14±0.1	15.21±0.1	<0.001
Adjusted model	14.78±0.1	14.87±0.1	15.03±0.1	15.06±0.1	15.27±0.1	<0.001
α-carotene (µg/d)						
Unadjusted	14.85±0.1	14.98±0.1	15.05±0.1	15.05±0.1	15.18±0.1	0.004
Adjusted model	14.89±0.1	14.99±0.1	15.03±0.1	15.03±0.1	15.17±0.1	0.019
ß-carotene (µg/d)						
Unadjusted	14.81±0.1	14.92±0.1	15.09±0.1	15.19±0.1	15.10±0.1	0.001
Adjusted model	14.82±0.1	14.98±0.1	15.03±0.1	15.18±0.1	15.10±0.1	0.003
Total carotene (µg/d)						
Unadjusted	14.82±0.1	14.94±0.1	15.08±0.1	15.18±0.1	15.09±0.1	0.001
Adjusted model	14.84±0.1	14.96±0.1	15.03±0.1	15.19±0.1	15.10±0.1	0.003
Lutein (µg/d)						
Unadjusted	14.84±0.1	14.99±0.1	15.15±0.1	15.09±0.1	15.03±0.1	0.07
Adjusted model	14.88±0.1	14.98±0.1	15.09±0.1	15.09±0.1	15.07±0.1	0.08
Zeaxanthin (µg/d)						
Unadjusted	14.93±0.1	14.98±0.1	15.09±0.1	14.99±0.1	15.12±0.1	0.14
Adjusted model	14.91±0.1	14.97±0.1	15.09±0.1	15.05±0.1	15.08±0.1	0.12
ß-cryptoxanthin (µg/d)						
Unadjusted	14.87±0.1	14.97±0.1	15.08±0.1	15.05±0.1	15.15±0.1	0.014
Adjusted model	14.87±0.1	14.97±0.1	15.08±0.1	15.05±0.1	15.15±0.1	0.014

Lycopene (µg/d)						
Unadjusted	14.87±0.1	14.91±0.1	15.03±0.1	15.05±0.1	15.26±0.1	<0.001
Adjusted model	14.92±0.1	14.95±0.1	15.01±0.1	15.08±0.1	15.15±0.1	0.027

[1] All values are means and SEM for fat free mass index by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking history (never, former, current), energy intake (kcal/d), and total body mass (kg)

Table 3.6 The associations between nutrient intakes and hand grip strength (kg) in 949 women from the TwinsUK cohort aged 34-83 years[1]

Quintiles of nutrient intake	Hand grip strength (kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	28.76±0.5	29.48±0.5	28.82±0.5	27.80±0.4	27.80±0.4	0.44
Adjusted model[2]	29.02±0.4	29.47±0.4	28.50±0.4	28.07±0.4	28.85±0.4	0.14
Vitamin D (µg/d)						
Unadjusted	29.19±0.5	28.83±0.5	29.19±0.4	27.91±0.4	28.80±0.4	0.24
Adjusted model	29.07±0.4	28.52±0.4	29.09±0.4	28.13±0.4	29.11±0.4	0.75
Vitamin E (mg/d)						
Unadjusted	28.44±0.5	29.43±0.5	28.94±0.5	28.52±0.4	28.58±0.4	0.66
Adjusted model	29.00±0.5	29.42±0.4	28.56±0.4	28.73±0.4	28.20±0.4	0.18
Magnesium (mg/d)						
Unadjusted	25.50±0.4	28.79±0.5	28.51±0.4	28.77±0.5	29.34±0.4	0.24
Adjusted model	28.74±0.4	28.77±0.4	28.44±0.4	29.16±0.4	28.80±0.5	0.71
Selenium (µg/d)						
Unadjusted	28.16±0.5	29.34±0.5	27.95±0.5	28.80±0.4	29.66±0.4	0.08
Adjusted model	28.47±0.4	29.22±0.4	28.39±0.4	28.69±0.3	29.15±0.4	0.64
Potassium (mg/d)						
Unadjusted	29.05±0.5	28.93±0.5	28.06±0.5	28.96±0.5	28.91±0.4	0.86
Adjusted model	28.81±0.4	29.11±0.4	27.99±0.4	28.96±0.4	29.03±0.5	0.99
α-carotene (µg/d)						
Unadjusted	29.22±0.5	28.75±0.4	28.96±0.5	28.70±0.5	28.29±0.5	0.20
Adjusted model	29.36±0.4	28.39±0.3	28.91±0.4	28.56±0.4	28.69±0.4	0.38
ß-carotene (µg/d)						
Unadjusted	29.11±0.5	28.60±0.4	29.09±0.5	28.38±0.5	28.72±0.5	0.51
Adjusted model	28.88±0.4	28.57±0.4	28.89±0.4	28.65±0.4	28.92±0.4	0.91
Carotene (µg/d)						
Unadjusted	28.86±0.5	28.84±0.4	29.06±0.4	28.38±0.5	28.78±0.5	0.67
Adjusted model	28.92±0.4	28.68±0.4	28.99±0.4	28.44±0.4	28.88±0.4	0.80
Lutein (µg/d)						
Unadjusted	29.04±0.5	29.05±0.5	28.93±0.4	28.19±0.5	28.70±0.5	0.29
Adjusted model	29.29±0.4	28.70±0.4	28.67±0.4	28.55±0.4	28.71±0.4	0.31
Zeaxanthin (µg/d)						
Unadjusted	28.41±0.5	28.98±0.4	28.84±0.5	28.11±0.4	29.57±0.5	0.32
Adjusted model	28.84±0.4	29.25±0.4	28.78±0.4	28.30±0.4	28.74±0.4	0.38
ß-cryptoxanthin (µg/d)						
Unadjusted	28.16±0.5	29.21±0.5	28.91±0.4	28.43±0.4	29.19±0.5	0.36
Adjusted model	28.69±0.4	29.40±0.4	28.94±0.4	28.48±0.4	28.40±0.4	0.21
Lycopene (µg/d)						
Unadjusted	27.81±0.5	29.03±0.5	29.45±0.4	28.24±0.5	29.38±0.4	0.09
Adjusted model	28.39±0.4	29.43±0.4	28.98±0.4	28.02±0.4	29.09±0.4	0.98

[1] All values are means and SEM for hand grip strength (kg) by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), current smoking (yes/no), energy intake (kcal/d), height (m), menopausal status (premenopausal/postmenopausal) and for HRT use (no/yes)

Stratification by age

Table 3.7 The associations between nutrient intakes and hand grip strength (kg) in 132 women, aged less than 50 years, from the TwinsUK cohort[1]

Quintiles of nutrient intake	Hand grip strength (kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	32.87±0.9	33.21±1.3	31.00±0.9	32.19±1.2	34.72±1.2	0.40
Adjusted model[2]	33.41±0.9	32.61±1.2	31.25±0.9	32.59±1.1	34.07±0.9	0.56
Vitamin D (µg/d)						
Unadjusted	34.28±0.9	31.77±1.1	33.66±0.9	31.72±0.9	32.95±1.5	0.48
Adjusted model	34.13±1.0	31.49±0.9	33.50±0.9	31.42±0.9	34.16±1.4	0.94
Vitamin E (mg/d)						
Unadjusted	33.73±0.9	33.58±0.8	32.69±1.2	34.05±1.1	30.36±1.2	0.05
Adjusted model	34.75±1.0	33.81±0.9	32.92±1.1	32.85±1.0	29.90±1.2	0.02
Magnesium (mg/d)						
Unadjusted	33.37±0.7	33.96±1.0	32.07±0.9	32.09±1.8	32.57±1.4	0.37
Adjusted model	33.09±1.2	33.72±0.9	31.60±0.9	33.64±1.5	32.41±1.7	0.60
Selenium (µg/d)						
Unadjusted	32.32±1.1	34.85±1.3	31.18±1.0	32.48±1.2	33.09±1.0	0.83
Adjusted model	31.51±1.2	33.66±1.0	31.25±0.9	33.71±1.1	33.64±1.1	0.36
Potassium (mg/d)						
Unadjusted	34.00±0.8	33.48±0.9	31.95±1.1	31.80±1.2	33.12±1.5	0.24
Adjusted model	34.08±1.0	33.11±0.9	31.47±1.1	32.37±1.2	33.05±1.7	0.46
α-carotene (µg/d)						
Unadjusted	34.31±1.2	31.26±0.8	34.00±1.1	32.96±1.6	32.43±1.2	0.71
Adjusted model	33.97±0.9	31.75±0.7	32.97±1.2	32.55±1.4	33.58±1.0	0.93
ß-carotene (µg/d)						
Unadjusted	32.49±1.0	32.55±0.8	33.40±1.3	32.82±1.2	33.30±1.3	0.59
Adjusted model	32.50±0.8	32.88±0.6	32.40±1.2	32.64±1.2	34.12±1.1	0.39
Carotene (µg/d)						
Unadjusted	32.47±1.1	32.22±0.8	33.04±1.1	33.23±1.4	33.54±1.3	0.42
Adjusted model	32.74±0.9	32.35±0.6	32.74±1.0	32.91±1.2	33.69±1.1	0.49
Lutein (µg/d)						
Unadjusted	32.88±0.8	33.03±0.9	32.25±1.1	33.46±1.5	32.70±1.3	0.98
Adjusted model	33.44±0.9	32.96±0.7	32.15±1.0	33.56±1.4	32.23±1.1	0.67
Zeaxanthin (µg/d)						
Unadjusted	33.30±0.9	33.80±1.2	31.60±1.0	31.75±1.4	33.47±1.1	0.98
Adjusted model	32.15±0.8	34.19±1.1	31.19±1.0	33.33±1.3	33.59±1.0	0.43
ß-cryptoxanthin (µg/d)						
Unadjusted	32.40±1.0	32.45±1.4	31.88±0.9	32.33±1.2	34.20±1.0	0.21
Adjusted model	32.53±0.8	34.11±1.1	30.49±1.0	32.54±1.0	33.64±0.8	0.56
Lycopene (µg/d)						
Unadjusted	33.14±1.2	33.58±1.3	32.79±1.2	33.58±1.0	31.71±1.1	0.40
Adjusted model	33.34±1.2	33.07±0.7	31.95±1.0	32.23±1.0	33.78±1.1	0.89

[1] All values are means and SEM for hand grip strength (kg) by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), current smoking (yes/no), energy intake (kcal/d), height (m), menopausal status (premenopausal/postmenopausal) and for HRT use (no/yes)

Table 3.8 The associations between nutrient intakes and hand grip strength (kg) in 817 women, aged more than 50 years, from the TwinsUK cohort[1]

Quintiles of nutrient intake	Hand grip strength (kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	27.97±0.5	28.95±0.5	28.51±0.5	27.11±0.4	28.04±0.4	0.23
Adjusted model[2]	28.32±0.4	28.90±0.4	28.01±0.4	27.43±0.4	27.93±0.4	0.08
Vitamin D (µg/d)						
Unadjusted	28.43±0.5	28.26±0.5	28.29±0.5	27.34±0.5	28.29±0.4	0.44
Adjusted model	28.18±0.5	28.06±0.5	28.39±0.4	27.61±0.4	28.37±0.4	0.90
Vitamin E (mg/d)						
Unadjusted	27.76±0.5	28.57±0.5	28.19±0.5	27.80±0.4	28.32±0.4	0.80
Adjusted model	28.13±0.5	28.61±0.5	27.91±0.4	28.07±0.4	27.90±0.4	0.57
Magnesium (mg/d)						
Unadjusted	27.59±0.5	27.91±0.5	27.92±0.5	28.36±0.5	28.79±0.4	0.05
Adjusted model	27.94±0.5	27.91±0.5	27.88±0.4	28.60±0.4	28.25±0.5	0.39
Selenium (µg/d)						
Unadjusted	27.54±0.5	28.44±0.5	27.53±0.5	28.20±0.4	28.95±0.4	0.08
Adjusted model	27.90±0.4	28.40±0.4	27.90±0.5	27.95±0.4	28.46±0.4	0.74
Potassium (mg/d)						
Unadjusted	28.09±0.5	28.12±0.5	27.55±0.5	28.33±0.5	28.50±0.5	0.49
Adjusted model	27.91±0.5	28.40±0.5	27.41±0.4	28.58±0.4	28.33±0.5	0.62
α-carotene (µg/d)						
Unadjusted	28.42±0.5	28.19±0.4	28.27±0.5	28.00±0.5	27.72±0.5	0.29
Adjusted model	28.59±0.5	27.94±0.4	28.20±0.4	27.86±0.4	27.99±0.4	0.37
ß-carotene (µg/d)						
Unadjusted	28.36±0.6	27.90±0.5	28.45±0.5	27.81±0.5	28.10±0.5	0.70
Adjusted model	28.34±0.5	27.89±0.4	28.24±0.4	27.99±0.4	28.15±0.4	0.85
Carotene (µg/d)						
Unadjusted	28.14±0.6	28.28±0.4	28.47±0.5	27.61±0.5	28.09±0.5	0.62
Adjusted model	28.34±0.5	28.09±0.4	28.33±0.4	27.76±0.4	28.09±0.4	0.56
Lutein (µg/d)						
Unadjusted	28.49±0.5	28.23±0.5	28.36±0.4	27.37±0.5	28.16±0.5	0.33
Adjusted model	28.66±0.4	28.00±0.4	28.10±0.4	27.74±0.4	28.09±0.4	0.33
Zeaxanthin (µg/d)						
Unadjusted	27.75±0.5	28.42±0.4	28.33±0.5	27.78±0.4	28.37±0.5	0.71
Adjusted model	28.27±0.4	28.47±0.4	28.31±0.4	27.60±0.4	27.94±0.5	0.27
ß-cryptoxanthin (µg/d)						
Unadjusted	27.53±0.5	28.80±0.5	28.62±0.5	27.71±0.5	27.88±0.4	0.80
Adjusted model	28.05±0.4	28.76±0.4	28.47±0.4	27.82±0.4	27.38±0.4	0.10
Lycopene (µg/d)						
Unadjusted	27.12±0.5	28.53±0.5	28.88±0.5	27.21±0.5	28.88±0.4	0.12
Adjusted model	27.59±0.4	28.80±0.4	28.47±0.4	27.20±0.4	28.51±0.4	0.85

[1] All values are means and SEM for hand grip strength (kg) by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), current smoking (yes/no), energy intake (kcal/d), height (m), menopausal status (premenopausal/postmenopausal) and for HRT use (no/yes)

Table 3.9 The associations between nutrient intakes and muscle quality (kg/kg) in 949 women from the TwinsUK cohort aged 34-83 years[1]

Quintiles of nutrient intake	Muscle quality (kg/kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	0.69±0.01	0.71±0.01	0.69±0.01	0.67±0.01	0.70±0.01	0.45
Adjusted model[2]	0.69±0.01	0.71±0.01	0.68±0.01	0.67±0.01	0.69±0.01	0.27
Vitamin D (µg/d)						
Unadjusted	0.69±0.01	0.70±0.01	0.70±0.01	0.68±0.01	0.69±0.01	0.25
Adjusted model	0.69±0.01	0.69±0.01	0.70±0.01	0.68±0.01	0.69±0.01	0.86
Vitamin E (mg/d)						
Unadjusted	0.68±0.01	0.71±0.01	0.69±0.01	0.68±0.01	0.70±0.01	0.99
Adjusted model	0.68±0.01	0.70±0.01	0.69±0.01	0.69±0.01	0.70±0.01	0.77
Magnesium (mg/d)						
Unadjusted	0.69±0.01	0.69±0.01	0.68±0.01	0.69±0.01	0.69±0.01	0.80
Adjusted model	0.70±0.01	0.69±0.01	0.68±0.01	0.69±0.01	0.69±0.01	0.51
Selenium (µg/d)						
Unadjusted	0.68±0.01	0.71±0.01	0.67±0.01	0.70±0.01	0.70±0.01	0.28
Adjusted model	0.68±0.01	0.70±0.01	0.68±0.01	0.70±0.01	0.69±0.01	0.69
Potassium (mg/d)						
Unadjusted	0.70±0.01	0.71±0.01	0.67±0.01	0.69±0.01	0.68±0.01	0.20
Adjusted model	0.70±0.01	0.71±0.01	0.67±0.01	0.69±0.01	0.68±0.01	0.18
α-carotene (µg/d)						
Unadjusted	0.69±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.68±0.01	0.66
Adjusted model	0.70±0.01	0.68±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.97
ß-carotene (µg/d)						
Unadjusted	0.69±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.82
Adjusted model	0.69±0.01	0.68±0.01	0.69±0.01	0.69±0.01	0.70±0.01	0.54
Carotene (µg/d)						
Unadjusted	0.69±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.99
Adjusted model	0.69±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.66
Lutein (µg/d)						
Unadjusted	0.70±0.01	0.69±0.01	0.70±0.01	0.69±0.01	0.69±0.01	0.57
Adjusted model	0.70±0.01	0.68±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.74
Zeaxanthin (µg/d)						
Unadjusted	0.69±0.01	0.69±0.01	0.70±0.01	0.68±0.01	0.70±0.01	0.64
Adjusted model	0.70±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.68±0.01	0.21
ß-cryptoxanthin (µg/d)						
Unadjusted	0.68±0.01	0.70±0.01	0.69±0.01	0.69±0.01	0.70±0.01	0.28
Adjusted model	0.68±0.01	0.70±0.01	0.70±0.01	0.69±0.01	0.68±0.01	0.59
Lycopene (µg/d)						
Unadjusted	0.68±0.01	0.70±0.01	0.70±0.01	0.68±0.01	0.70±0.01	0.25
Adjusted model	0.68±0.01	0.71±0.01	0.70±0.01	0.67±0.01	0.70±0.01	0.84

[1] All values are means and SEM for muscle quality (kg/kg) by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), current smoking (yes/no), energy intake (kcal/d), height (m), menopausal status (premenopausal/postmenopausal) and for HRT use (no/yes)

Stratification by age

Table 3.10 The associations between nutrient intakes and muscle quality (kg/kg) in 132 women aged less than 50 years from the TwinsUK cohort[1]

Quintiles of nutrient intake	Muscle quality (kg/kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	0.76±0.02	0.82±0.03	0.74±0.02	0.74±0.03	0.80±0.03	0.88
Adjusted model[2]	0.76±0.02	0.80±0.04	0.75±0.02	0.76±0.03	0.79±0.02	0.70
Vitamin D (µg/d)						
Unadjusted	0.78±0.03	0.75±0.03	0.80±0.03	0.78±0.02	0.73±0.04	0.57
Adjusted model	0.77±0.03	0.75±0.02	0.79±0.03	0.78±0.02	0.75±0.04	0.87
Vitamin E (mg/d)						
Unadjusted	0.81±0.02	0.79±0.02	0.77±0.03	0.74±0.03	0.74±0.03	0.026
Adjusted model	0.84±0.03	0.78±0.03	0.78±0.03	0.74±0.02	0.71±0.03	0.013
Magnesium (mg/d)						
Unadjusted	0.82±0.02	0.77±0.03	0.77±0.02	0.73±0.03	0.73±0.04	0.017
Adjusted model	0.84±0.03	0.78±0.03	0.77±0.02	0.75±0.03	0.70±0.04	0.043
Selenium (µg/d)						
Unadjusted	0.70±0.04	0.80±0.03	0.77±0.03	0.76±0.02	0.82±0.03	0.027
Adjusted model	0.69±0.03	0.80±0.03	0.77±0.03	0.76±0.02	0.82±0.03	0.023
Potassium (mg/d)						
Unadjusted	0.81±0.02	0.81±0.02	0.73±0.03	0.74±0.03	0.73±0.04	0.018
Adjusted model	0.84±0.03	0.79±0.02	0.73±0.03	0.74±0.03	0.71±0.05	0.026
α-carotene (µg/d)						
Unadjusted	0.77±0.04	0.76±0.02	0.77±0.03	0.78±0.03	0.77±0.03	0.93
Adjusted model	0.78±0.03	0.76±0.02	0.75±0.03	0.78±0.03	0.79±0.03	0.58
ß-carotene (µg/d)						
Unadjusted	0.76±0.03	0.79±0.02	0.76±0.02	0.78±0.03	0.77±0.04	0.84
Adjusted model	0.76±0.03	0.78±0.02	0.74±0.03	0.76±0.03	0.80±0.03	0.54
Carotene (µg/d)						
Unadjusted	0.75±0.03	0.78±0.02	0.76±0.02	0.79±0.03	0.77±0.04	0.67
Adjusted model	0.76±0.03	0.77±0.01	0.75±0.02	0.78±0.03	0.79±0.03	0.50
Lutein (µg/d)						
Unadjusted	0.78±0.03	0.76±0.02	0.76±0.03	0.78±0.03	0.76±0.04	0.74
Adjusted model	0.80±0.03	0.77±0.02	0.76±0.03	0.77±0.03	0.75±0.03	0.35
Zeaxanthin (µg/d)						
Unadjusted	0.77±0.03	0.80±0.03	0.76±0.02	0.74±0.03	0.77±0.03	0.86
Adjusted model	0.77±0.03	0.81±0.03	0.74±0.02	0.77±0.04	0.77±0.02	0.85
ß-cryptoxanthin (µg/d)						
Unadjusted	0.75±0.03	0.78±0.03	0.73±0.03	0.78±0.03	0.78±0.02	0.37
Adjusted model	0.75±0.03	0.79±0.03	0.72±0.02	0.78±0.03	0.78±0.02	0.51
Lycopene (µg/d)						
Unadjusted	0.76±0.03	0.79±0.03	0.75±0.03	0.77±0.02	0.77±0.03	0.88
Adjusted model	0.75±0.03	0.79±0.03	0.74±0.02	0.76±0.02	0.80±0.01	0.35

[1] All values are means and SEM for muscle quality (kg/kg) by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), current smoking (yes/no), energy intake (kcal/d), height (m), menopausal status (premenopausal/postmenopausal) and for HRT use (no/yes)

Table 3.11 The associations between nutrient intakes and muscle quality (kg/kg) in 817 women aged more than 50 years from the TwinsUK cohort[1]

Quintiles of nutrient intake	Muscle quality (kg/kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	0.68±0.01	0.70±0.01	0.68±0.01	0.66±0.01	0.68±0.01	0.39
Adjusted model[2]	0.68±0.01	0.70±0.01	0.67±0.01	0.66±0.01	0.68±0.01	0.20
Vitamin D (µg/d)						
Unadjusted	0.68±0.01	0.69±0.01	0.68±0.01	0.66±0.01	0.68±0.01	0.42
Adjusted model	0.68±0.01	0.68±0.01	0.68±0.01	0.67±0.01	0.68±0.01	0.95
Vitamin E (mg/d)						
Unadjusted	0.67±0.01	0.69±0.01	0.67±0.01	0.67±0.01	0.69±0.01	0.41
Adjusted model	0.66±0.01	0.68±0.01	0.67±0.01	0.68±0.01	0.69±0.01	0.26
Magnesium (mg/d)						
Unadjusted	0.67±0.01	0.68±0.01	0.68±0.01	0.68±0.01	0.69±0.01	0.32
Adjusted model	0.68±0.01	0.68±0.01	0.67±0.01	0.69±0.01	0.68±0.01	0.63
Selenium (µg/d)						
Unadjusted	0.69±0.01	0.67±0.01	0.66±0.01	0.67±0.01	0.70±0.01	0.62
Adjusted model	0.69±0.01	0.67±0.01	0.66±0.01	0.67±0.01	0.69±0.01	0.98
Potassium (mg/d)						
Unadjusted	0.68±0.01	0.69±0.01	0.67±0.01	0.68±0.01	0.68±0.01	0.95
Adjusted model	0.68±0.01	0.69±0.01	0.66±0.01	0.68±0.01	0.68±0.01	0.72
α-carotene (µg/d)						
Unadjusted	0.68±0.01	0.67±0.01	0.68±0.01	0.68±0.01	0.67±0.01	0.76
Adjusted model	0.68±0.01	0.67±0.01	0.68±0.01	0.67±0.01	0.68±0.01	0.89
ß-carotene (µg/d)						
Unadjusted	0.68±0.01	0.67±0.01	0.68±0.01	0.68±0.01	0.68±0.01	0.90
Adjusted model	0.68±0.01	0.67±0.01	0.68±0.01	0.68±0.01	0.68±0.01	0.71
Carotene (µg/d)						
Unadjusted	0.68±0.01	0.68±0.01	0.68±0.01	0.68±0.01	0.68±0.01	0.94
Adjusted model	0.68±0.01	0.67±0.01	0.68±0.01	0.68±0.01	0.68±0.01	0.88
Lutein (µg/d)						
Unadjusted	0.68±0.01	0.67±0.01	0.69±0.01	0.67±0.01	0.68±0.01	0.72
Adjusted model	0.68±0.01	0.67±0.01	0.68±0.01	0.68±0.01	0.68±0.01	0.88
Zeaxanthin (µg/d)						
Unadjusted	0.68±0.01	0.67±0.01	0.69±0.01	0.68±0.01	0.67±0.01	0.95
Adjusted model	0.69±0.01	0.68±0.01	0.68±0.01	0.68±0.01	0.66±0.01	0.23
ß-cryptoxanthin (µg/d)						
Unadjusted	0.67±0.01	0.69±0.01	0.69±0.01	0.67±0.01	0.68±0.01	0.82
Adjusted model	0.67±0.01	0.69±0.01	0.69±0.01	0.68±0.01	0.66±0.01	0.40
Lycopene (µg/d)						
Unadjusted	0.66±0.01	0.69±0.01	0.69±0.01	0.66±0.01	0.69±0.01	0.46
Adjusted model	0.67±0.01	0.69±0.01	0.69±0.01	0.66±0.01	0.68±0.01	0.70

[1] All values are means and SEM for muscle quality (kg/kg) by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), current smoking (yes/no), energy intake (kcal/d), height (m), menopausal status (premenopausal/postmenopausal) and for HRT use (no/yes)

Chapter 4

Associations between protein, essential amino acid intakes and indexes of muscle mass, muscle strength and muscle quality

4.0 Introduction

Muscle is the main pool of protein in humans (containing 50 to 75 % of all proteins in the body) (Matthews 1999) and also serves as the main store of amino acids (Welle 1999). Moreover, it is evident that higher essential amino acid or protein availability can increase muscle protein synthesis and anabolic processes in both younger and older individuals (regardless of the fact that muscle mass decreases with age) (Paddon-Jones *et al.* 2004, Symons *et al.* 2007, Volpi *et al.* 2003). Among the essential amino acids, the branched-chain amino acids (BCAAs) (leucine, isoleucine and valine) have been suggested to regulate muscle protein synthesis in acute studies in both young and older adults (Fujita and Volpi 2006). Leucine, isoleucine and valine appear to directly stimulate muscle protein synthesis as they act as major carriers of nitrogen in the skeletal muscle tissue (Laviano *et al.* 2005). Among the BCAAs leucine has been suggested as the most efficient for muscle protein synthesis. It has been shown that acutely, leucine (a insulin secretagogue) activates the mammalian target of rapamycin (mTOR) signalling pathway in skeletal muscle (Fujita *et al.* 2007). The mammalian target of rapamycin (mTOR) pathway is affected by amino acid deprivation. Therefore, BCAAs supplementation, specifically leucine, through activation of several proteins that are involved in the mTOR signalling pathway is able to modulate protein translation initiation and synthesis in muscle mass (Anthony *et al.* 2002, Dickinson *et al.* 2011). As decreased protein synthesis is a major risk factor in the aetiology of sarcopenia and muscle protein synthesis can be stimulated by dietary protein and essential amino acid intake (mainly BCAAs), it is of particular interest that studies examine the

influence of protein and essential amino acid intake on muscle mass, strength and muscle quality.

Observational studies have focused on associations between total protein intake and muscle mass mainly in populations aged more than 50 years. One previous prospective study among women aged 75 ± 3 years found that those in the highest tertile of protein intake (> 87 g/d) had 5.3% and 6.6% higher whole body and appendicular lean mass, respectively compared with those in the lowest tertile (< 66g/d) after five years follow-up (Meng *et al.* 2009). A second prospective study among participants aged 70-79 years from the Health, Aging and Body Composition study (Health ABC) showed that those in the highest quintile of energy-adjusted protein intake (18.2% protein as a percentage of energy) lost ≈ 40% less lean mass and appendicular lean mass over three years follow-up, than did those in the lowest quintile (11.2% protein as a percentage of energy) (Houston *et al.* 2008). Similarly, an analysis of the China Health and nutrition Survey among older adults aged 50-70 years showed that those with higher protein intake (> 12.1% of energy) lost less midarm muscle area compared with those who had lower protein intake (< 10.4% of energy) over four years follow-up, although midarm muscle area is considered as an imprecise measure of lean mass (Stookey *et al.* 2005). Another previous prospective study found that energy-adjusted protein intake was a positive predictor of muscle mass over 2.6 years follow-up in participants aged 50 to 79 years, however no association was observed for muscle strength in this study (Scott *et al.* 2010). Only one previous cross-sectional study found that energy-adjusted protein intake was positively associated with grip strength

in women aged 59 to 73 years, however in this study in the multivariate models limited adjustment for confounding factors was considered, as only age, height and gender were included in the regression models but not other lifestyle factors that potentially influence the relationship (Robinson *et al.* 2008).

Two short-term intervention studies have examined the effects of protein intake on muscle mass and strength. One small study in 12 elderly women reported that after nine weeks those who consumed the higher protein diet 0.92 g/kg body weight/day had maintained muscle mass and function compared with those with an intake of 0.45 g/kg body weight/day (Castaneda *et al.* 1995). The other small study was a 14-week controlled dietary intake trial (where all feeds were supplied to the volunteers) providing the RDA for protein intake of 0.8 g protein/kg body weight/d) among 10 men and women aged 55 to 77 years. The intervention had no effect on whole body composition (% body fat, fat free mass, protein and mineral mass) although mid-thigh muscle area was decreased at the end of the 14 week intervention (Campbell *et al.* 2001). Nevertheless, supplementation with protein (after or separate from physical activity) in older subjects who frequently consumed adequate amounts of protein ($\geq$ 0.8 g/kg body weight/day) did not further benefit the improvements in muscle mass and strength due to exercise (Campbell 2007).

However, supplementation studies that used essential amino acids were beneficial for muscle mass and muscle strength (Borsheim *et al.* 2008, Scognamiglio *et al.* 2004, Solerte *et al.* 2008). Indeed, amino acid supplementation (essential and non essential mix consisting of: 3.8 g/d leucine, 2 g/d lysine, 1.9 g/d isoleusine, 1.9 g/d valine, 1.1 g/d threonine, 0.4 g/d cysteine, 0.4 g/d histidine, 0.3 g/d phenylalanine, 0.2 g/d Methionine, 0.1 g/d tyrosine, 0.1 g/d tryptophan) at 12.2 g/d, three times per day for three months improved walking function by 48 m, and isometric hand grip strength by 2.6 kg in 44 sedentary and frail individuals aged > 65 years (Scognamiglio *et al.* 2004). Another small intervention study (*n*=12, males and females) showed that supplementation with two daily doses of 11 g/day of essential amino acids (including: 0.36 g/d histidine, 0.94 g/d isoleucine, 3.95 g/d leucine, 1.88 g/d lysine, 0.39 g/d methionine, 0.51 g/d phenylalanine, 1.05 g/d threonine, 0.82 g/d valine) plus 1.10 g/d arginine, in the form of capsules, for 16 weeks, improved lean body mass by 0.60 kg, and muscle strength by 22.2 % on average, in glucose intolerant subjects with mean (±SD) age 67 (±5.6) years (Borsheim *et al.* 2008). However, these two studies were poorly controlled and were not randomised as pre-intervention/baseline data were used as a "control" group, limiting the robustness of the study. In a third intervention study, among 41 subjects with sarcopenia aged 66-88 years, after supplementation with 8 g of essential amino acids twice per day for 18 months, significant increases in whole body lean mass were observed, although the magnitude of the effect was not reported (Solerte *et al.* 2008). These data suggest an association between amino acids and muscle mass and strength and provide evidence

for further long-term randomised controlled trials, with large sample sizes, a wider age range and appropriately powered for the end points of interest.

Although prospective studies have shown associations between protein intake and muscle mass in older participants, available small scale intervention studies involving supplementation of protein have produced limited evidence of efficacy but supplementation studies involving essential amino acids have shown interesting preliminary results. Therefore, more long-term well designed dietary intervention trials needed to confirm and extend existing findings on the effectiveness of protein and amino acid supplementation on sarcopenia. Currently there is only one previous cross-sectional study on associations between habitual dietary protein intake and muscle strength which also adjusted for a limited number of potential confounding factors, including age, height and birth weight (Robinson *et al.* 2008). Moreover, to our knowledge, there are currently no population studies on associations between habitual essential amino acid intake, including BCCAs, and muscle mass, strength and muscle quality in adult women. It is likely that this would provide a useful insight into the potential role of protein and essential amino acid intakes, to potentially maintain muscle mass, strength and muscle quality.

4.1 Aims

The aim of this study was to test the hypothesis that habitual dietary intake of protein and essential amino acids including BCCAs are associated with muscle mass as assessed by 3 indexes, fat free mass (FFM, kg), percentage

fat free mass (FFM%) and fat free mass index (FFMI, kg/m^2), among women aged 18-79 years from the TwinsUK cohort (subset 1). An additional aim of this study was to examine the hypothesis that habitual dietary intake of these nutrients is associated with muscle strength assessed by hand grip strength (kg) and muscle quality (muscle strength divided by muscle mass) (kg/kg) in women aged 34-83 years from the TwinsUK cohort (subset 2).

The nutrients included are presented in (**Table 4.1**).

Table 4.1 Nutrients included in the analysis

Macronutrients	***Amino acids***
Protein (% Energy)	Arginine (% Energy)
	Glutamine (% Energy)
	Histidine (% Energy)
	Isoleucine (% Energy)
	Leucine (% Energy)
	Lysine (% Energy)
	Methionine (% Energy)
	Phenylalanine (% Energy/d)
	Threonine (% Energy/d)
	Tryptophan (% Energy/d)
	Valine (% Energy)

4.2 Covariate plan and statistical analysis

All analyses were performed using STATA statistical software (version 11.0; STATA Corp, USA). Means and standard deviations were calculated (**Table 4.2)** and quintiles of each nutrient intake were derived. Pearson correlation coefficients were used to evaluate associations between age and indexes of muscle mass, hand grip strength and muscle quality; protein and amino acid intakes and energy intake; and between height and hand grip strength. The analysis were performed treating twins as individuals as

previous studies have shown that participants from the TwinsUK registry were similar to age-matched singleton women in the distribution of common traits and outcomes (Andrew *et al.* 2001). Data on muscle mass were collected in 1996 (subset 1) and data on hand grip strength ten years later in 2005 (subset 2). For the first subset, data on dietary intake (FFQ), body composition parameters, and other covariates were available for n = 2570 women, aged 18 to 79 years, who had completed a food frequency questionnaire (FFQ) and attended for dual-energy X-ray absorptiometry (DEXA) scans between 1996 and 2000. For the second subset, data on dietary intake (FFQ), body composition parameters, and other covariates were available for n = 960 women, aged 34 to 83 years, who had completed a food frequency questionnaire (FFQ) and attended for hand grip strength measurements and DEXA scans between 2005 and 2008.

Prior to conducting statistical analysis, an analysis plan with justification for each confounding factor in the multivariate model was developed as described in Chapter 2, section 2.2.5, p. 57. Multivariate regression analysis was used to assess associations between protein, essential amino acid intakes and indexes of muscle mass, hand grip strength and muscle quality using the robust cluster regression option in STATA. These models take into account clustering of individuals when calculating standard errors of the mean (Richards *et al.* 2007) to ensure familial aggregation within twin pairs was accounted for. Amino acid intakes were expressed as percentage of energy intake rather than total intakes to enable comparisons between people with different total food and energy intakes.

As age has a great influence on muscle mass, strength, and muscle quality all analyses were adjusted for age (in years) (Cruz-Jentoft *et al.* 2010, Lynch *et al.* 1999). Even though we found no associations with the age stratified analyses in the previous chapter (Chapter 3) we chose *a priori* not to stratify by age for these analyses. Models were then also adjusted for physical activity and smoking status, as regular physical activity has been associated with increased muscle mass and strength (Frankel *et al.* 2006, Heath and Stuart 2002, Kuh *et al.* 2002, Roubenoff 2007, van Kan *et al.* 2008, Wolfe 2006), and smoking has been shown to increase the risk of sarcopenia (Morse *et al.* 2007, Petersen *et al.* 2007, Wüst *et al.* 2008). Protein and amino acid intakes were expressed as percentage of total energy intake as protein and amino acid intakes are highly correlated with energy intake. Total body mass was included as a covariate in the evaluation of associations between diet and FFM and FFMI as described in Chapter 2, section 2.2.5 (Subset 1) (**Tables 4.3, 4.4, 4.5**). Additional adjustments were made for hand grip strength and muscle quality including height as it has been previously suggested as a determinant of grip strength (Jerome *et al.* 1991, Neder *et al.* 1999); menopausal status (premenopausal or postmenopausal), as a number of studies have suggested that a loss in muscle strength has been associated with ageing in women (Carville *et al.* 2006, Cooper *et al.* 2008, Greeves *et al.* 1999, Kurina *et al.* 2004); and hormone replacement therapy (HRT) (no or yes), as it has been previously shown that HRT use in postmenopausal women has been associated with improvements in muscle strength and muscle quality (Greising *et al.* 2009) (Subset 2) (**Tables 4.6, 4.7)**. Moreover HRT use combined with physical

exercise has been suggested to improve physical function (Sipila *et al.* 2001).

For Subset 1 (2570 women, aged 18-79 years), age was inversely correlated with FFM (r = -0.2, P < 0.001) and percentage FFM (r = -0.4, P < 0.001), but not with FFMI (r = -0.006, P = 0.76). Therefore, this analysis was not further stratified by age. However, since age was highly inversely correlated with hand grip strength (r = -0.5, P < 0.001) and muscle quality (r = -0.4, P < 0.001) analysis in Subset 2 (949 women, aged 34-83 years) was further stratified by age using two categories, less than 50 years and more than 50 years.

4.3 Results

Descriptive characteristics of the two subsets of the TwinsUK cohort were very similar (**Table 4.2**) and were described in previous chapter (see Chapter 3, section 3.3, p. 82). Briefly, for the first subset (n = 2570 women) the mean (±SD) age of participants was 48.3 ± 12.7 years and mean (±SD) BMI was 24.9 ± 4.14 kg/m^2. Mean FFM (±SD) was 39.6 ± 5.30 kg, percentage FFM 61.1 ± 6.49 %, and FFMI 15 ± 1.71 kg/m^2 (**Table 4.2**).

For the second subset (n = 960 women) the mean (±SD) age of participants was 59.1 ± 9.30 years and the mean (±SD) BMI was 26.5 ± 4.73 kg/m^2. Mean hand grip strength was 28.8 ± 5.95 kg, and muscle quality [calculated as the ratio of hand grip strength (kg) to lean mass (kg)] was 0.69 ± 0.14 kg/kg. Mean (±SD) percentage FM was 35.7 ± 6.28 %, and percentage FFM was 61.4 ± 6.18 % (**Table 4.2**).

Subset 1 (2570 women, 18-79 years)

In the multivariate analyses, no significant associations were found between protein and essential amino acid intakes and FFM (**Table 4.3**) and FFMI (**Table 4.5**). Mean percentage FFM was inversely and significantly associated with protein and all essential amino acid intakes ($P < 0.001$ for all) (**Table 4.4**).

Subset 2 (949 women, 34-83 years)

After multivariate adjustment, no significant associations were found between protein and essential amino acid intakes and hand grip strength and muscle quality (**Tables 4.6 and 4.7**). Stratification by age did not alter these findings.

4.4 Discussion

In this study using two subsets from a large population-based sample of women aged 18 to 83 years, we evaluated the association between protein intake and essential amino acids present in the habitual diet and indexes of muscle mass, hand grip strength and muscle quality. Only one previous cross-sectional study has examined associations between habitual dietary protein intake and muscle strength which only adjusted for a few potential confounding factors including age, height and birth weight, and has focused only on older adults (Robinson *et al.* 2008). The current study cross-sectionally evaluated the relationship between protein intake and a range of essential amino acids present in the habitual diet and estimates of muscle mass, hand grip strength and muscle quality in a cohort across a wide age

range. Significant negative associations were observed between protein and essential amino acid intakes and percentage FFM, even after adjustment for age, physical activity and smoking. However, although positive trends were observed between protein and essential amino acid intakes and FFMI the associations were not significant after multivariate adjustment for relevant confounders.

Findings from the current study in relation to protein and FFMI were non-significant. Nevertheless, previous prospective studies have reported that higher protein intake was associated with high muscle mass in people older than 50 years, even though supplementation studies have not always shown improvements on muscle mass (Campbell *et al.* 2001, Campbell 2007, Castaneda *et al.* 1995, Houston *et al.* 2008, Meng *et al.* 2009, Scott *et al.* 2010, Stookey *et al.* 2005). It has been suggested that this might be partly explained due to the fact that in supplementation studies providing protein-containing supplements older individuals tended to simultaneously reduce their energy intake to accommodate the supplement (Fiatarone Singh *et al.* 2000).

The recommended dietary allowance for protein intake (0.8/kg body weight/d) in US studies may not be sufficient for the preservation of muscle mass (Campbell *et al.* 2001, Campbell 2007). Therefore, the Society for Sarcopenia, Cachexia, and Wasting Disease in their review in 2010 has recommended that older adults may ingest between 1.0 and 1.5 g/kg/d of protein for the preservation of muscle mass, however this report does not

clarify the corresponding age range for the suggested dietary protein intake (Morley *et al.* 2010). In the TwinsUK cohort the average protein intake per kg body weight per day was 1.3 g protein/kg/d which was higher compared to the average recommended nutrient intake (0.8 g protein/kg/d) for the UK population in accordance with the UK report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. Therefore there is a need of more robust recommendations on protein intake for optimal skeletal muscle health in both young and older adults.

In the TwinsUK study the range of protein intake was higher compared to previous studies. The mean daily protein intake in the TwinsUK participants in the lowest quintile was 13.1 ± 1.1 % energy (or 69.9 ± 19.3 g/d) and in the highest quintile 20.5 ± 1.6 % energy (or 88.2 ± 22.8 g/d). These intakes were higher than previous prospective cohort studies where protein intake in the lowest tertile was 66 g/d and in the highest tertile 87 g/d (Meng *et al.* 2009), or protein intake in the lowest quintile was 11.2 % and in the highest quintile 18.2 % (Houston *et al.* 2008), or lower protein intake was < 10.4 % energy and higher protein intake was > 12.1 % energy (Stookey *et al.* 2005) highlighting the differences in dietary protein intake between different populations. Moreover, all the above cohorts focused on older participants (aged 70 years and over) compared to the first subset of the TwinsUK cohort which included younger individuals (18-79 years), with subsequently higher muscle mass. Therefore, the wide age range of our cohort including younger adults may explain the lack of associations between muscle mass and protein intake in the current analysis.

The mechanisms involved in the relationship between protein intake and FFMI cannot be specifically identified due to the nature of our study which assessed associations. Nevertheless, current recommendations for the preservation of muscle mass, as mentioned above, are limited to older adults (Morley *et al.* 2010), and studies that have shown that protein intake was associated with higher muscle mass were limited to participants older than 50 years. These data suggest that higher protein intake may be more beneficial for individuals with very low protein intakes, as in older persons the metabolic efficiency is decreased and they require higher protein intake for protein synthesis compared to younger persons (Rattan 2010).

The lack of association observed in the current study in relation to percentage FFM may be due to the fact that lifestyle factors other than protein intake may have a greater influence on muscle mass. Interestingly, in this cohort, the effect of age per 10 years [β coefficient (SEM): - 1.8 (0.11), $P < 0.001$] and of physical activity [β coefficient (SEM): 1.4 (0.19), $P < 0.001$] was four and three times greater respectively of that of protein intake [β coefficient (SEM): - 0.5 (0.09), $P < 0.001$] on percentage FFM. Also, although protein intake was positively associated with FFMI when adjusted for age, physical activity, smoking and total body mass, the association was not significant. This might be because the effect of age per 10 years [β coefficient (SEM): - 0.2 (0.03), $P < 0.001$] and of physical activity [β coefficient (SEM): 0.2 (0.05), $P < 0.001$] was 10 times greater of that of protein intake [β coefficient (SEM): 0.02 (0.02), $P = 0.389$] on FFMI. These findings are supported by a number of studies that have previously

described negative associations between age and fat free mass (Coin *et al.* 2008, Ding *et al.* 2007, Guo *et al.* 1999, Janssen *et al.* 2000, Kyle *et al.* 2001, Kyle *et al.* 2006), and positive associations between regular physical activity and fat free mass (Frankel *et al.* 2006, Roubenoff 2007, van Kan *et al.* 2008, Wolfe 2006).

Yet, although protein is important for maintenance of muscle mass, it is likely that other dietary factors such as a more alkaline diet may also be relevant. Indeed, a recent cross-sectional study in a subset of women from the TwinsUK cohort has reported significant positive associations between a more alkaline diet consisting of fruits and vegetables that supply adequate amounts of potassium and magnesium and muscle mass indexes as opposed to foods with potential acidogenic profile (including meats, fish, eggs, dairy and cereals) (Welch *et al.* 2012).

In the second subset (949 women, 34-83 years) of the current study no associations were observed between protein intake and hand grip strength and muscle quality. Only one previous cross-sectional study found that energy-adjusted protein intake was associated with higher grip strength in women aged 59 to 73 years of the Hertfordshire cohort, however this study was adjusted for limited confounding factors and included only age, height and gender in the multivariate models and no other lifestyle factors that potentially influence the relationship between protein intake and grip strength, such as physical activity, smoking, menopausal status and use of HRT (Robinson *et al.* 2008). It should be acknowledged that the

Hertfordshire cohort was an older cohort with lower mean hand grip strength (26.5 kg) compared to the second subset (see above) of the current analysis which included younger individuals (34-83 years), with as expected higher hand grip strength (28.8 kg). The TwinsUK cohort was younger and therefore it might be less likely to observe changes in muscle strength in association with diet. This might be due to the fact that other lifestyle factors than protein intake may be more important to muscle strength and muscle quality. Interestingly, in our cohort, age and physical activity had a greater effect on hand grip strength compared to protein intake. For example, after multivariate adjustment for relevant confounders, the effect of age [ß coefficient (SEM): -0.27 (0.02), $P < 0.001$] was 135 times greater of that of protein intake [ß coefficient (SEM): 0.002 (0.12), $P = 0.985$] on hand grip strength. The size of the association was similar for muscle quality and these findings are supported by a number of studies which have previously described decreases in muscle strength and muscle quality with age (as outlined in Chapter 1, sections 1.2.3, p.14 and 1.2.5, p.16).

Although protein supplementation has not always been proven to be successful for the preservation of muscle mass, there is a growing body of evidence suggesting that essential amino acids are more efficient at acutely stimulating muscle protein synthesis not only in older but in younger adults (Fujita and Volpi 2006). Indeed, in an acute (single dose) study among 10 younger (41.1 ± 8.0 years) and 10 older (70.2 ± 5.1 years) apparently healthy adults, ingestion of 113 g lean beef containing 30 g protein and 12 g of essential amino acids (1.17 g histidine, 1.04 g isoleucine, 1.98 g leucine,

2.40 g lysine, 0.85 g phenylalanine, 1.33 g threonine, 1.37 g valine and 0.95 g arginine), increased muscle protein synthesis by 50% in both the younger and older adult groups (Symons *et al.* 2007). Among the essential amino acids BCAAs (leucine, isoleucine and valine) appear to directly stimulate muscle protein synthesis as they act as major carriers of amino nitrogen in the skeletal muscle tissue (Laviano *et al.* 2005). Among the BCAAs leucine has been suggested as the most efficient for muscle protein synthesis. Leucine is able to activation signalling pathways (such as the mTOR signalling pathway) that lead to increased protein translation initiation and synthesis in muscle mass (Anthony *et al.* 2002, Dickinson *et al.* 2011, Fujita *et al.* 2007). Although *in vitro* studies suggested that with ageing rat muscle may not be stimulated by the effects of a normal postprandial concentration of leucine (200 µmol/L) (Dardevet *et al.* 2000), more recently it has been shown that leucine supplementation as part of a normal mixed nutrient meal may improve muscle protein synthesis in both animal (Rieu *et al.* 2007) and human studies (Rieu *et al.* 2006). Notably, mechanistic studies in old rats have shown that supplementation of milk protein with high leucine content (β-lactoglobulin, 14.5 % leucine) significantly increased muscle protein synthesis compared to supplementation of milk protein with low leucine content (casein, 10 % leucine) (Rieu *et al.* 2007). Also, an acute study among 20 healthy males aged 69.9 ± 0.8 years showed that after ingestion of a meal supplemented with leucine postprandial muscle protein synthesis was improved. The supplemented leucine diet contained 10.2 kcal, 0.4 g protein (casein), 1.3 g carbohydrate (dextrine maltose) and 0.36 g fat (vegetable oil) per kg body weight, and supplemented with leucine (0.052 g/kg) in order to

increase plasma leucine to twice the normal postprandial leucine concentration, and isoleucine (0.0116 g/kg) and valine (0.0068 g/kg) in order to maintain plasma levels of isoleucine and valine at normal postprandial levels. The control diet was supplemented only with alanine (0.071 g/kg) which did not affect protein synthesis, but provided the same amount of nitrogen as the leucine diet (Rieu *et al.* 2006). Given the existing evidence, the Society for Sarcopenia, Cachexia, and Wasting Disease in their review in 2010 has recommended that older adults may consume a leucine-enriched balanced amino acid supplement for the preservation of muscle mass, however this report does not clarify a suggested dose (Morley *et al.* 2010).

Surprisingly, we found a significant negative associations between essential amino acid intake and percentage FFM and positive associations were observed between essential amino acid intake and FFMI that were not significant after multivariate adjustments for a number of relevant confounders, although the FFQ included a range of foods that contribute to essential amino acid intake, such as soy products (tofu, soy milk), meat and fish, dairy and eggs, as well as legumes. However, it needs to be considered that the range of protein intake in this analysis was higher compared to other studies that included older participants (Houston *et al.* 2008, Meng *et al.* 2009) than in our study. This may suggest that higher protein intake may be more beneficial for individuals with very low protein intakes, as in older persons the metabolic efficiency is decreased and they require higher protein intake for protein synthesis compared to younger persons (Rattan

2010) as mentioned earlier. It is not clear why the significant associations that were found were in opposite directions and further investigation is required. However, there are currently no observational studies in the population on associations between habitual amino acid intake and measures of muscle mass, strength and muscle quality. Moreover, there is a paucity of studies assessing recommended amino acid dietary intakes in the UK. Therefore, comparison of our results with other observational studies was not possible.

4.5 Strengths and limitations

The strengths of this study include the large sample size and the wide age range of participants, as most previous studies on sarcopenia examined older individuals (Robinson SM *et al.* 2008, Scott *et al.* 2010). Moreover, this was the first study that directly compared associations between protein and essential amino acid intake and indexes of muscle mass, muscle strength and muscle quality in a large cohort. Body composition was objectively assessed by DEXA scans and an objective measurement was used to assess muscle strength, which allowed calculation of muscle quality. A further strength is that it is evident that notable changes in skeletal muscle mass may occur earlier in adult life (between 30 and 45 years of age), and it is important to examine dietary associations with muscle mass in individuals of all ages (Cesari and Pahor 2008, Janssen *et al.* 2000).

Our study also has a number of limitations. The cross-sectional nature of the study limits causal inference, but enables us to generate hypotheses regarding associations between protein and amino acid intakes and indexes

of muscle mass, strength and muscle quality based on plausible mechanisms. Although all analyses were adjusted for potential lifestyle factors that influence the relationship between intake of protein, amino acids and muscle mass, residual confounding could not be ruled out because of the observational nature of the study. In addition, as in all observational studies, measurement error in self-reported dietary intakes is inevitable. It is also widely acknowledged that the use of FFQs for measuring dietary intake has its own limitations related to memory errors, they provide relative than absolute intake, they introduce bias due to over- or under-reporting, and they may introduce systematic errors as preparation methods are inadequately considered (McNeill *et al.* 2009). Also, FFQs measure food intake over a period of one year and reflect the habitual diet over this period of time but not lifetime dietary habits (Teucher *et al.* 2007). Nevertheless, the FFQ used in the current study was previously compared with a 7-day weighed record in the EPIC Norfolk study, and although the two approaches to measure dietary intake were different, both methods identified similar intakes of macronutrients when these expressed as percentage of total energy intake (Bingham *et al.* 2001). Also, the food frequency questionnaire (FFQ) used was previously validated in the EPIC cohort against urinary nitrogen for protein intake (McKeown *et al.* 2001, Tucker *et al.* 1999, Welch *et al.* 2006). The aim of the current study design was to examine associations between dietary intake and a health outcomes, therefore ranking individuals according to dietary intake was the most important factor and for this the use of FFQ was a suitable approach (Molag *et al.* 2007). However, further work is needed to investigate if our findings are

replicated in a male population of the same country or in populations from different ethnic backgrounds.

4.6 Concluding remarks

This is one of the first studies to examine simultaneously cross-sectional associations between protein and essential amino acid and muscle mass, muscle strength and muscle quality in a cohort including younger adults. We believe this to be the first study to investigate cross-sectional associations between muscle mass and amino acid intake. Additionally, our findings extended the existing literature by evaluating the relative importance of diet in terms of nutrients and muscle quality to further understand the association between muscle strength and muscle mass with diet.

In conclusion, a significant negative association was observed between protein and essential amino acid intake and percentage FFM. However, positive associations were observed between protein and essential amino acid intake and FFMI that were not significant after multivariate adjustments for a number of relevant confounders. This was possibly due to the fact that other factors, such as physical activity may be more important than diet in terms of protein and essential amino acids to preserve or maintain muscle mass (as assessed by the relevant indexes) and improve or maintain muscle strength and quality. Yet, it is likely that other nutrients in the diet associated with more alkalinogenic foods may also be relevant for muscle mass as recently shown in a subset of the TwinsUK cohort (Welch *et*

al. 2012). Also, it is possible that a longer period of study (such as a longitudinal study design) might be a more suitable approach to detect associations between dietary intake of protein and amino acids and estimates of muscle mass over time. Although in prospective studies higher protein intake has been associated with increased lean mass (Houston *et al.* 2008, Meng *et al.* 2009, Scott *et al.* 2010, Stookey *et al.* 2005) and muscle strength (Robinson *et al.* 2008) in older people, it is not yet clear whether supplementation studies with protein alone or in combination with physical activity may enhance muscle mass and strength as a number of studies have not been successful (Borst 2004, Campbell *et al.* 2001, Campbell 2007). However, both short-term (Borsheim *et al.* 2008, Scognamiglio *et al.* 2004, Solerte *et al.* 2008) and acute studies using essential amino acid supplementation (Symons *et al.* 2007) and particularly additional supplementation with the BCAA leucine (Rieu *et al.* 2007, Rieu *et al.* 2006) have proven promising for muscle protein synthesis and the maintenance of muscle mass and muscle strength.

However, further work is needed from both epidemiological and long-term randomised controlled dietary trials to confirm and extend existing findings. From a public health perspective, findings from the current study may help to improve the knowledge and understanding of the effects of protein and amino acids on muscle mass and strength in a healthy population. These findings contribute towards the debate on protein and amino acid requirements in healthy non frail individuals. Although no associations observed for protein, amino acids and muscle mass, strength and quality in

this study we wanted to further explore interactions between micronutrients, protein and amino acid intake and markers of chronic inflammation including CRP in the TwinsUK cohort (see Chapter 6).

Table 4.2 Baseline characteristics of female participants from the TwinsUK cohort[1]

Characteristic	Subset 1 *N*=2570, 18-79 yrs	Subset 2 *N*=949, 34-83 yrs
Age (y)	48.3±12.7	59.1±9.30
Weight (kg)	65.6±11.2	69.2±12.5
Height (cm)	162±6.07	162±5.93
BMI (kg/m^2)	24.9±4.14	26.5±4.73
Fat mass (kg)	22.7±7.87	25.2±8.30
Fat mass %	33.9±7.17	35.7±6.28
Fat free mass (kg)	39.6±5.30	42.0±5.47
Fat free mass %	61.1±6.49	61.4±6.18
Fat free mass index (kg/m^2)	15.0±1.71	16.0±1.82
Hand grip strength (kg)		28.8±5.95
Muscle quality (kg/kg)		0.69±0.14
Physical activity %		
Inactive	21.9	39.6
Moderate	53.9	34.2
Active	24.2	26.2
Smoking history %		
Never	50.2	
Current	18.2	
Former	31.6	
Current smoking %		
Yes		9.79
No		90.21
Menopausal status %		
Premenopausal		10.3
Postmenopausal		89.7
Hormone replacement therapy %		
Yes		9.38
No		90.63
Dietary components		
Total energy intake (kcal/d)	1979±524	1917±637
Protein intake (g/d)	81.3±21.6	83.6±27.4
Protein intake (% Energy)	16.6±2.62	17.7±2.76
Amino acids		
Arginine (g/d)	4.44±1.27	4.78±1.63
Arginine (% Energy)	0.91±0.19	1.01±0.19
Glutamine (g/d)	16.1±4.43	15.9±5.21
Glutamine (% Energy)	3.30±0.58	3.35±0.47
Histidine (g/d)	2.29±0.65	2.39±0.80
Histidine (% Energy)	0.47±0.09	0.51±0.09
Isoleucine (g/d)	3.83±1.06	3.94±1.31
Isoleucine (% Energy)	0.79±0.16	0.83±0.15
Leucine (g/d)	6.49±1.78	6.63±2.18
Leucine (% Energy)	1.33±0.26	1.40±0.23
Lysine (g/d)	5.52±1.63	5.88±2.04
Lysine (% Energy)	1.14±0.27	1.25±0.27
Methionine (g/d)	1.87±0.53	1.95±0.66
Methionine (% Energy)	0.38±0.08	0.41±0.08
Phenylalanine (g/d)	3.83±1.04	3.88±1.26
Phenylalanine (% Energy/d)	0.78±0.15	0.82±0.13
Threonine (g/d)	3.34±0.94	3.46±1.16
Threonine (% Energy/d)	0.69±0.14	0.73±0.13
Tryptophan (g/d)	1.05±0.29	1.07±0.35
Tryptophan (% Energy/d)	0.22±0.04	0.23±0.04
Valine (g/d)	4.53±1.24	4.62±1.52
Valine (% Energy)	0.93±0.18	0.98±0.16

[1] Values are means ± standard deviations (mean ± SDs) unless indicated

Table 4.3 The associations between protein, essential amino acid intakes and fat free mass (kg) in 2570 women from the TwinsUK cohort aged 18-79 years[1]

Quintiles of nutrient intake	Fat free mass (kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Energy intake (kcal/d)						
Unadjusted	39.1±0.3	39.8±0.3	39.5±0.2	39.4±0.2	40.4±0.3	0.010
Adjusted model[2]	38.9±0.2	39.8±0.2	39.7±0.2	39.7±0.2	40.1±0.2	0.002
Protein (%E)						
Unadjusted	39.5±0.3	39.9±0.3	39.6±0.3	39.5±0.3	39.6±0.3	0.76
Adjusted model	39.6±0.2	40.0±0.2	39.8±0.2	39.4±0.2	39.4±0.2	0.12
Arginine (%E)						
Unadjusted	39.6±0.3	39.8±0.3	39.7±0.2	39.6±0.2	39.6±0.3	0.86
Adjusted model	39.8±0.2	39.7±0.2	39.8±0.2	39.6±0.2	39.4±0.2	0.23
Glutamine (%E)						
Unadjusted	39.4±0.3	39.7±0.2	39.5±0.2	39.8±0.3	39.7±0.2	0.27
Adjusted model	39.5±0.2	39.9±0.2	39.6±0.2	39.7±0.2	39.5±0.2	0.68
Histidine (%E)						
Unadjusted	39.5±0.2	39.6±0.3	39.6±0.2	39.7±0.2	39.7±0.3	0.57
Adjusted model	39.8±0.2	39.6±0.2	39.8±0.2	39.7±0.2	39.4±0.2	0.41
Isoleucine (%E)						
Unadjusted	39.4±0.3	39.7±0.3	39.7±0.2	39.7±0.3	39.7±0.3	0.59
Adjusted model	39.7±0.2	39.8±0.2	39.7±0.2	39.7±0.2	39.4±0.2	0.31
Leucine (%E)						
Unadjusted	39.5±0.3	39.6±0.3	39.7±0.2	39.8±0.3	39.6±0.3	0.64
Adjusted model	39.7±0.2	39.8±0.2	39.6±0.2	39.7±0.2	39.4±0.2	0.30
Lysine (%E)						
Unadjusted	39.5±0.3	39.9±0.3	39.6±0.2	39.6±0.2	39.6±0.3	0.72
Adjusted model	39.7±0.2	40.0±0.2	39.7±0.2	39.5±0.2	39.3±0.2	0.09
Methionine (%E)						
Unadjusted	39.6±0.3	39.6±0.3	39.7±0.2	39.8±0.3	39.6±0.3	0.86
Adjusted model	39.7±0.2	39.8±0.2	39.7±0.2	39.7±0.2	39.3±0.2	0.25
Phenylalanine (%E)						
Unadjusted	39.4±0.3	39.7±0.3	39.7±0.2	39.8±0.3	39.6±0.2	0.60
Adjusted model	39.6±0.2	39.8±0.2	39.8±0.2	39.7±0.2	39.3±0.2	0.41
Threonine (%E)						
Unadjusted	39.6±0.3	39.7±0.3	39.7±0.2	39.5±0.2	39.7±0.3	0.82
Adjusted model	39.7±0.2	39.8±0.2	39.8±0.2	39.5±0.2	39.4±0.2	0.20
Tryptophan (%E)						
Unadjusted	39.3±0.2	39.7±0.2	39.6±0.2	39.8±0.3	39.8±0.3	0.25
Adjusted model	39.5±0.2	39.9±0.2	39.5±0.2	39.8±0.2	39.4±0.2	0.76
Valine (%E)						
Unadjusted	39.4±0.3	39.8±0.3	39.8±0.2	39.7±0.3	39.5±0.2	0.76
Adjusted model	39.6±0.2	39.9±0.2	39.8±0.2	39.7±0.2	39.3±0.2	0.33

[1] Values are means and SEM for fat free mass (kg) by quintiles of nutrient intake.

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking history (never, former, current), and total body mass (kg).

Table 4.4 The associations between protein, essential amino acid intakes and percentage fat free mass in 2570 women from the TwinsUK cohort aged 18-79 years[1]

Quintiles of nutrient intake	Percentage fat free mass					
	Q1	Q2	Q3	Q4	Q5	P for trend
Energy intake (kcal/d)						
Unadjusted	60.47±0.3	61.09±0.3	61.15±0.3	61.49±0.3	61.27±0.3	0.04
Adjusted model[2]	60.48±0.3	61.17±0.3	61.28±0.3	61.64±0.3	60.90±0.3	0.19
Protein (%E)						
Unadjusted	62.36±0.3	61.76±0.3	61.31±0.3	60.42±0.3	59.61±0.3	<0.001
Adjusted model	61.87±0.3	61.53±0.3	61.31±0.3	60.65±0.3	60.11±0.3	<0.001
Arginine (%E)						
Unadjusted	62.44±0.3	61.64±0.3	61.15±0.3	60.78±0.3	59.46±0.3	<0.001
Adjusted model	61.98±0.3	61.30±0.3	61.19±0.3	60.95±0.3	60.05±0.3	<0.001
Glutamine (%E)						
Unadjusted	61.92±0.3	61.83±0.3	61.34±0.3	60.58±0.3	59.80±0.3	<0.001
Adjusted model	61.73±0.3	61.62±0.3	61.24±0.3	60.66±0.3	60.21±0.3	<0.001
Histidine (%E)						
Unadjusted	62.79±0.3	61.44±0.3	61.20±0.3	60.59±0.3	59.44±0.3	<0.001
Adjusted model	62.26±0.3	61.18±0.3	61.27±0.3	60.74±0.3	60.02±0.3	<0.001
Isoleucine (%E)						
Unadjusted	62.53±0.3	61.94±0.3	61.08±0.3	60.62±0.3	59.30±0.3	<0.001
Adjusted model	62.09±0.3	61.60±0.3	61.06±0.3	60.80±0.3	59.92±0.3	<0.001
Leucine (%E)						
Unadjusted	62.63±0.3	61.57±0.3	61.14±0.3	60.66±0.3	59.48±0.3	<0.001
Adjusted model	62.16±0.3	61.42±0.3	61.07±0.3	60.77±0.3	60.05±0.3	<0.001
Lysine (%E)						
Unadjusted	62.65±0.3	61.76±0.3	61.06±0.3	60.42±0.3	59.58±0.3	<0.001
Adjusted model	61.99±0.3	61.62±0.3	61.16±0.3	60.57±0.3	60.13±0.3	<0.001
Methionine (%E)						
Unadjusted	62.47±0.3	61.99±0.3	60.89±0.3	60.62±0.3	59.49±0.3	<0.001
Adjusted model	61.93±0.3	61.75±0.3	61.00±0.3	60.74±0.3	60.06±0.3	<0.001
Phenylalanine (%E)						
Unadjusted	62.21±0.3	61.80±0.3	61.42±0.3	60.59±0.3	59.45±0.3	<0.001
Adjusted model	61.83±0.3	61.54±0.3	61.33±0.3	60.74±0.3	60.02±0.3	<0.001
Threonine (%E)						
Unadjusted	62.71±0.3	61.82±0.3	61.01±0.3	60.55±0.3	59.37±0.3	<0.001
Adjusted model	62.14±0.3	61.57±0.3	61.07±0.3	60.72±0.3	59.96±0.3	<0.001
Tryptophan (%E) *Unadjusted*	62.37±0.3	62.11±0.3	60.96±0.3	60.71±0.3	59.33±0.3	<0.001
Adjusted model	61.88±0.3	61.88±0.3	60.89±0.3	60.87±0.3	59.96±0.3	<0.001
Valine (%E)						
Unadjusted	62.46±0.3	61.93±0.3	61.06±0.3	60.52±0.3	59.49±0.3	<0.001
Adjusted model	61.98±0.3	61.62±0.3	61.03±0.3	60.72±0.3	60.11±0.3	<0.001

[1] Values are means and SEM for percentage fat free mass by quintiles of nutrient intake.

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), and smoking history (never, former, current).

Table 4.5 The associations between protein, essential amino acid intakes and fat free mass index (kg/m^2) in 2570 women from the TwinsUK cohort aged 18-79 years[1]

Quintiles of nutrient intake	Fat free mass index (kg/m^2)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Energy intake (kcal/d)						
Unadjusted	14.97±0.1	15.09±0.1	14.98±0.1	14.99±0.1	15.07±0.1	0.68
Adjusted model[2]	14.92±0.1	15.09±0.1	15.01±0.1	15.08±0.1	15.02±0.1	0.50
Protein (%E)						
Unadjusted	14.87±0.1	15.01±0.1	14.95±0.1	15.07±0.1	15.21±0.1	0.004
Adjusted model	14.82±0.1	14.98±0.1	14.96±0.1	15.09±0.1	15.27±0.1	0.37
Arginine (%E)						
Unadjusted	14.95±0.1	14.93±0.1	15.03±0.1	14.98±0.1	15.24±0.1	0.02
Adjusted model	15.05±0.1	14.95±0.1	15.05±0.1	14.97±0.1	15.10±0.1	0.62
Glutamine (%E)						
Unadjusted	14.85±0.1	14.99±0.1	14.91±0.1	15.12±0.1	15.25±0.1	<0.001
Adjusted model	14.91±0.1	15.06±0.1	14.95±0.1	15.07±0.1	15.12±0.1	0.07
Histidine (%E)						
Unadjusted	14.90±0.1	14.94±0.1	14.95±0.1	15.09±0.1	15.23±0.1	0.002
Adjusted model	15.03±0.1	14.96±0.1	14.99±0.1	15.06±0.1	15.08±0.1	0.37
Isoleucine (%E)						
Unadjusted	14.86±0.1	14.94±0.1	15.00±0.1	15.12±0.1	15.22±0.1	0.001
Adjusted model	14.98±0.1	15.00±0.1	15.00±0.1	15.09±0.1	15.07±0.1	0.29
Leucine (%E)						
Unadjusted	14.88±0.1	14.93±0.1	15.00±0.1	15.15±0.1	15.21±0.1	0.001
Adjusted model	14.99±0.1	14.99±0.1	15.00±0.1	15.09±0.1	15.07±0.1	0.26
Lysine (%E)						
Unadjusted	14.94±0.1	14.95±0.1	15.01±0.1	14.99±0.1	15.22±0.1	0.022
Adjusted model	15.05±0.1	14.99±0.1	15.03±0.1	14.95±0.1	15.08±0.1	0.93
Methionine (%E)						
Unadjusted	14.91±0.1	14.92±0.1	15.00±0.1	15.05±0.1	15.23±0.1	0.004
Adjusted model	15.01±0.1	14.99±0.1	14.99±0.1	15.02±0.1	15.09±0.1	0.42
Phenylalanine (%E)						
Unadjusted	14.89±0.1	14.96±0.1	14.91±0.1	15.15±0.1	15.20±0.1	0.002
Adjusted model	14.98±0.1	15.01±0.1	14.95±0.1	15.11±0.1	15.06±0.1	0.29
Threonine (%E)						
Unadjusted	14.90±0.1	14.94±0.1	14.96±0.1	15.05±0.1	15.26±0.1	0.002
Adjusted model	15.02±0.1	15.00±0.1	14.97±0.1	15.03±0.1	15.10±0.1	0.42
Tryptophan (%E) *Unadjusted*	14.83±0.1	14.96±0.1	14.93±0.1	15.14±0.1	15.26±0.1	<0.001
Adjusted model	14.93±0.1	15.06±0.1	14.92±0.1	15.12±0.1	15.09±0.1	0.11
Valine (%E)						
Unadjusted	14.85±0.1	14.93±0.1	15.01±0.1	15.15±0.1	15.17±0.1	0.001
Adjusted model	14.96±0.1	15.00±0.1	15.00±0.1	15.12±0.1	15.04±0.1	0.26

[1] Values are means and SEM for fat free mass index by quintiles of nutrient intake.

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking history (never, former, current), and total body mass (kg).

Table 4.6 The associations between protein, essential amino acid intakes and hand grip strength (kg) in 949 women from the TwinsUK cohort aged 34-83 years[1]

Quintiles of nutrient intake	Hand grip strength (kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Energy intake (kcal/d)						
Unadjusted	28.7±0.4	28.7±0.5	28.2±0.4	29.1±0.5	29.3±0.4	0.26
Adjusted model[2]	28.7±0.3	28.9±0.4	28.3±0.4	28.9±0.4	29.1±0.4	0.61
Protein (%E)						
Unadjusted	29.2±0.4	28.2±0.5	29.4±0.4	28.6±0.4	28.5±0.5	0.42
Adjusted model	28.8±0.2	28.8±0.2	28.8±0.2	28.7±0.2	28.8±0.2	0.88
Arginine (%E)						
Unadjusted	28.68±0.4	29.13±0.4	28.95±0.4	28.74±0.5	28.41±0.5	0.51
Adjusted model	28.77±0.4	28.86±0.4	28.85±0.3	28.75±0.4	28.68±0.4	0.81
Glutamine (%E)						
Unadjusted	28.96±0.4	28.58±0.5	28.66±0.4	29.07±0.4	28.64±0.5	0.91
Adjusted model	28.55±0.3	28.76±0.4	28.84±0.4	28.95±0.3	28.82±0.4	0.55
Histidine (%E)						
Unadjusted	29.16±0.4	28.72±0.4	28.76±0.4	28.76±0.4	28.51±0.5	0.38
Adjusted model	28.47±0.4	29.22±0.4	28.39±0.4	28.69±0.3	29.15±0.4	0.64
Isoleucine (%E)						
Unadjusted	29.24±0.4	28.29±0.5	29.16±0.4	28.65±0.4	28.58±0.5	0.50
Adjusted model	28.91±0.4	28.01±0.4	29.36±0.4	28.62±0.4	29.00±0.4	0.51
Leucine (%E)						
Unadjusted	29.30±0.4	27.90±0.5	29.47±0.4	28.56±0.4	28.68±0.5	0.69
Adjusted model	29.00±0.3	27.78±0.4	29.69±0.3	28.35±0.4	29.09±0.4	0.54
Lysine (%E)						
Unadjusted	28.97±0.4	29.04±0.5	28.64±0.4	28.56±0.5	28.70±0.5	0.47
Adjusted model	28.63±0.4	28.86±0.4	28.96±0.4	28.35±0.4	29.11±0.4	0.71
Methionine (%E)						
Unadjusted	29.38±0.4	28.27±0.5	28.88±0.4	29.14±0.5	28.25±0.5	0.35
Adjusted model	28.85±0.4	28.38±0.3	29.02±0.4	28.98±0.4	28.69±0.4	0.82
Phenylalanine (%E)						
Unadjusted	29.06±0.4	28.38±0.4	29.39±0.5	28.71±0.4	28.38±0.5	0.48
Adjusted model	28.91±0.3	28.09±0.4	29.49±0.4	28.55±0.4	28.87±0.4	0.76
Threonine (%E)						
Unadjusted	29.42±0.4	28.43±0.4	29.00±0.5	28.57±0.4	28.49±0.5	0.24
Adjusted model	28.93±0.4	28.34±0.4	29.04±0.4	28.59±0.4	29.01±0.4	0.74
Tryptophan (%E)						
Unadjusted	29.32±0.4	28.20±0.5	29.13±0.4	29.01±0.4	28.25±0.5	0.35
Adjusted model	29.05±0.3	28.05±0.4	29.05±0.4	29.02±0.3	28.74±0.4	0.77
Valine (%E)						
Unadjusted	29.21±0.4	28.26±0.5	29.58±0.4	28.28±0.5	28.59±0.5	0.40
Adjusted model	28.87±0.3	28.05±0.4	29.60±0.3	28.26±0.4	29.14±0.4	0.53

[1] Values are means and SEM for hand grip strength (kg) by quintiles of nutrient intake.

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), current smoking (yes/no), height (m), menopausal status (premenopausal/postmenopausal) and for HRT use (yes/no).

Table 4.7 The associations between protein, essential amino acid intakes and muscle quality (kg/kg) in 949 women from the TwinsUK cohort aged 34-83 years[1]

Quintiles of nutrient intake	Muscle quality (kg/kg)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Energy intake (kcal/d)						
Unadjusted	0.69±0.01	0.69±0.01	0.68±0.01	0.69±0.01	0.70±0.01	0.93
Adjusted model[2]	0.69±0.01	0.69±0.01	0.68±0.01	0.69±0.01	0.70±0.01	0.93
Protein (%E)						
Unadjusted	0.70±0.01	0.69±0.01	0.71±0.01	0.69±0.01	0.67±0.01	0.14
Adjusted model	0.69±0.01	0.69±0.01	0.70±0.01	0.69±0.01	0.68±0.01	0.18
Arginine (%E)						
Unadjusted	0.69±0.01	0.70±0.01	0.70±0.01	0.69±0.01	0.67±0.01	0.31
Adjusted model	0.69±0.01	0.70±0.01	0.70±0.01	0.69±0.01	0.68±0.01	0.24
Glutamine (%E)						
Unadjusted	0.69±0.01	0.70±0.01	0.69±0.01	0.70±0.01	0.67±0.01	0.37
Adjusted model	0.69±0.01	0.70±0.01	0.69±0.01	0.70±0.01	0.67±0.01	0.28
Histidine (%E)						
Unadjusted	0.69±0.01	0.70±0.01	0.70±0.01	0.68±0.01	0.68±0.01	0.12
Adjusted model	0.69±0.01	0.70±0.01	0.70±0.01	0.68±0.01	0.68±0.01	0.22
Isoleucine (%E)						
Unadjusted	0.69±0.01	0.69±0.01	0.71±0.01	0.69±0.01	0.67±0.01	0.20
Adjusted model	0.69±0.01	0.69±0.01	0.71±0.01	0.69±0.01	0.68±0.01	0.37
Leucine (%E)						
Unadjusted	0.69±0.01	0.69±0.01	0.71±0.01	0.68±0.01	0.68±0.01	0.21
Adjusted model	0.70±0.01	0.68±0.01	0.71±0.01	0.68±0.01	0.68±0.01	0.28
Lysine (%E)						
Unadjusted	0.69±0.01	0.70±0.01	0.70±0.01	0.68±0.01	0.68±0.01	0.16
Adjusted model	0.69±0.01	0.70±0.01	0.70±0.01	0.68±0.01	0.68±0.01	0.23
Methionine (%E)						
Unadjusted	0.70±0.01	0.69±0.01	0.71±0.01	0.69±0.01	0.67±0.01	0.09
Adjusted model	0.69±0.01	0.69±0.01	0.71±0.01	0.69±0.01	0.67±0.01	0.20
Phenylalanine (%E)						
Unadjusted	0.69±0.01	0.69±0.01	0.71±0.01	0.69±0.01	0.67±0.01	0.20
Adjusted model	0.69±0.01	0.69±0.01	0.71±0.01	0.69±0.01	0.67±0.01	0.27
Threonine (%E)						
Unadjusted	0.70±0.01	0.70±0.01	0.70±0.01	0.68±0.01	0.67±0.01	0.05
Adjusted model	0.69±0.01	0.69±0.01	0.70±0.01	0.68±0.01	0.68±0.01	0.19
Tryptophan (%E)						
Unadjusted	0.70±0.01	0.68±0.01	0.70±0.01	0.70±0.01	0.67±0.01	0.15
Adjusted model	0.70±0.01	0.68±0.01	0.70±0.01	0.70±0.01	0.67±0.01	0.24
Valine (%E)						
Unadjusted	0.70±0.01	0.69±0.01	0.71±0.01	0.68±0.01	0.67±0.01	0.10
Adjusted model	0.70±0.01	0.68±0.01	0.71±0.01	0.68±0.01	0.68±0.01	0.29

[1] Values are means and SEM for muscle quality (kg/kg) by quintiles of nutrient intake.

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), current smoking (yes/no), height (m), menopausal status (premenopausal/postmenopausal) and for HRT use (yes/no).

Chapter 5

Associations between diet quality scores and indexes of muscle mass, muscle strength and muscle quality

5.0 Introduction

As an extension to previous chapters, this chapter will investigate the association of the "whole diet" assessed by diet quality scores and indexes of muscle mass, muscle strength and muscle quality. In recent years, it has been suggested that research should focus on examining associations between the "whole" diet, alongside individual foods and nutrients, and health status, as we consume a variety of foods and not simply nutrients. It is proposed that benefits in health status are more likely to be determined by the total diet Jacobs *et al.* (2009). Diet scores examining dietary patterns of a population have been used for more than a decade as an approach to study the relationship between overall diet and chronic disease risk, because they examine nutrient and food synergies and interactions instead of examining a single nutrient's effect in relation to disease risk (Hu 2002). Growing evidence has also related these diet scores to overall health (Maynard M *et al.* 2004). Diet scores are either based on government based recommendations for a healthy diet or designed to allow comparisons with other eating patterns such as the Mediterranean diet or the Dietary Approach to Stop Hypertension diet (Fung *et al.* 2008, Huijbregts *et al.* 1997a, Patterson *et al.* 1994, Trichopoulou *et al.* 1995). Diet scores are used to establish adherence of an individual or a group's diet to a particular dietary pattern (i.e. Mediterranean, Western-style diet, prudent diet, etc) and to evaluate the intake of a group of foods rather than the intake of a single nutrient or dietary component.

The role of the "whole" diet, in terms of healthy eating patterns, on sarcopenia has not yet been researched. To date, 20 distinct indexes have been constructed to assess overall diet quality (Waijers *et al.* 2007). The current analysis, investigating associations between indices of muscle mass and diet will focus on five predefined diet scores, the Mediterranean Diet score (MDS), the Healthy Diet Indicator (HDI), the Diet Quality Index (DQI), the Alternate Healthy Eating Index (AHEI), and the DASH-style score (based on the Dietary Approaches to Stop Hypertension diet), which have been used widely in relation to health outcomes previously (de Koning *et al.* 2011, Kourlaba and Panagiotakos 2009). Overall, better diet quality as assessed previously by these five most common scores has been associated with reduced mortality and decreased cardiovascular disease risk (Akbaraly *et al.* 2011, Fung *et al.* 2008, Huijbregts *et al.* 1997a, McCullough *et al.* 2002, Patterson *et al.* 1994, Seymour *et al.* 2003, Trichopoulou *et al.* 1995).

Diet quality, as assessed by the five predefined diet scores, may also be important for the conservation of muscle mass because they consist of food components high in nutrients which are known to be involved in the biology of human muscle (Frassetto *et al.* 1998, Song *et al.* 2007, Song *et al.* 2005), reduce oxidative stress and inflammation (Ferrucci *et al.* 2005, Helmersson *et al.* 2005, Young *et al.* 2004) or have been shown to be beneficial in supplementation studies (Børsheim *et al.* 2008, Dawson-Hughes 2008, Scognamiglio *et al.* 2004). In addition, higher adherence to the selected diet scores has also been associated with lower levels of plasma inflammation

markers (Chrysohoou *et al.* 2004, Dai *et al.* 2008, Fung *et al.* 2008, Fung *et al.* 2005), which further adds to the rationale for their selection.

Currently the most popular approach to evaluate the overall diet is the Mediterranean dietary pattern. The Mediterranean diet, as assessed by the Mediterranean Diet Score (MDS) has been associated with low mortality risk (Serra-Majem *et al.* 2004, Trichopoulou *et al.* 2003, Trichopoulou *et al.* 1995, Trichopoulou *et al.* 2005). The MDS has been defined as a pattern high in plant foods (fruits, vegetables, cereals, beans, nuts and seeds), minimally processed, seasonally fresh foods, olive oil (as the main source of dietary lipids) low to moderate consumption of dairy products (mainly cheese and yogurt), low frequency and amounts of red meat, and low to moderate consumption of wine (Trichopoulou *et al.* 1995).

Higher adherence to the MDS has also been inversely associated with oxidative stress as measured by the ratio of reduced to oxidised glutathione (GSH/GSSG) (Dai *et al.* 2008) and with lower plasma levels of inflammation markers (such as Interleukin-6, C-reactive protein, homocysteine, fibrinogen and lower white blood cell counts) (Chrysohoou *et al.* 2004, Dai *et al.* 2008, Fung *et al.* 2005). Therefore, this type of diet consisting of components, such as fruit and vegetables (which represent the greatest source of vitamins C and E, magnesium, potassium and carotenoids) may be beneficial for muscle mass by potentially preventing the damage to muscle tissue from reactive oxygen species and further preventing the expression of pro-inflammatory cytokines in muscle (Block

et al. 2009, Frassetto *et al.* 1997, Meng and Yu 2010, Sebastian *et al.* 1994, Walston *et al.* 2006).

The HDI was developed in the Netherlands according to the World Health Organization's guidelines for the prevention of chronic disease (Huijbregts *et al.* 1997) and has been reported to be inversely associated with all-cause mortality in men from 3 European countries, including the Netherlands, and in Dutch elderly men but not in women (Huijbregts *et al.* 1997a, Huijbregts *et al.* 1997b).

The DQI was developed based on U.S. recommendations and has been positively related to lower risk of all-cause mortality and CVD mortality, but not cancer mortality and also to markers of inflammation and endothelial dysfunction (Fung *et al.* 2005, Patterson *et al.* 1994, Seymour *et al.* 2003, Waijers *et al.* 2007). However, both the HDI and DQI scores account for high intakes in fruit and vegetables, nuts, and whole grains. It has been well established that higher intakes of nuts, whole grains and fruit and vegetables, as well as dietary patterns high in these food components are associated with lower concentrations of inflammation markers (Chrysohoou *et al.* 2004, Esmaillzadeh *et al.* 2006, Esposito *et al.* 2004, Fung *et al.* 2001, Lopez-Garcia *et al.* 2004, Lutsey *et al.* 2007, Nettleton *et al.* 2006). Also, these two scores account for low intakes of saturated fatty acids. Saturated fatty acid intakes, and dietary patterns high in saturated fatty acids have been significantly and positively associated with markers of inflammation (Esmaillzadeh *et al.* 2007, Fung *et al.* 2001, Lopez-Garcia *et*

al. 2004, Nettleton *et al.* 2006). Therefore, greater adherence to both these scores, which assign positive scoring at high intakes of fruits and vegetables, nuts and whole grains, as well as negative scoring at high intakes of saturated fatty acids may positively contribute to a better inflammatory status and potentially benefit muscle mass.

The AHEI was devised as an improved version of the original Healthy Eating Index (HEI). The HEI was originally developed in 1995 from the U.S. Department of Agriculture to measure adherence to the Dietary Guidelines for Americans and the food guide pyramid (My Pyramid), and was later revised in 2005 (HEI-2005), according to the updated Dietary guidelines for Americans (U.S. Health and Human Services and U.S. Department of Agriculture, 2005 (Kennedy *et al.* 1995). The AHEI has been inversely associated with major biomarkers of chronic disease risk, essentially CVD risk, including lower concentrations of markers of inflammation and endothelial dysfunction (Fung *et al.* 2005). Additionally, the AHEI assigns a high score to items such as fruits and vegetables, nuts and protein, particularly from fish and poultry and dietary fiber that are high in nutrients that have been previously shown to be associated with muscle mass, such as vitamins C and E, potassium, magnesium, carotenoids, protein and essential amino acids (see Chapters 3, section 3.0 and 4, section 4.0).

The DASH-style score is based on the Dietary Approaches to Stop Hypertension diet, and monitors adherence to the DASH style pattern. The DASH diet has been shown to be related to lower blood pressure and low-

density lipoprotein cholesterol in intervention trials (Appel *et al.* 1997, Obarzanek *et al.* 2001, Sacks *et al.* 2001). Also, greater adherence to the DASH-style score has been associated with lower risk of coronary heart disease, stroke and lower plasma levels of inflammation markers CRP and IL-6 (Fung *et al.* 2008). In addition, the dietary components of the DASH-style diet, such as fruits, nuts, vegetables, and whole grains are major sources of vitamins C and E, potassium, magnesium and carotenoids that have been previously shown to be associated with muscle mass (see Chapters 3, section 3.0). Also, lean meats may provide amounts of dietary protein and essential amino acids important for muscle mass (Aubertin-Leheudre and Adlercreutz 2009). Therefore, adherence to this score may be important for the conservation of muscle mass.

In summary, there are limited studies on associations between habitual diet (mainly based on nutrient intake) and muscle mass, muscle strength and no studies on muscle quality (Robinson SM *et al.* 2008, Scott *et al.* 2010). In addition, three of the measures described above, the MDS, the HDI and the DQI have also been negatively associated with body composition (mainly BMI) and with the risk of obesity in adults (Boynton *et al.* 2008, Mendez *et al.* 2006, Panagiotakos *et al.* 2006, Quatromoni *et al.* 2006, Schröder *et al.* 2004). To date, there is only one previous longitudinal study among 690 men and women aged 65 years and over which has examined associations between the Mediterranean diet score and muscle strength, as a component of frailty over 6 years follow-up, although no associations were observed (Talegawkar *et al.* 2012). However, there are currently no studies examining

the relative importance of the overall diet quality on body composition, specifically muscle mass indexes, hand grip strength and muscle quality. It is likely that this would provide a useful insight into the potential role of the overall diet to maintain muscle mass, muscle strength and muscle quality.

5.1 Aims of the study

The aim of this study was to derive five predefined diet scores, the MDS, HDI, DQI, AHEI, and DASH-style score, and examine the hypothesis that greater adherence to these five scores is associated with greater muscle mass as assessed by three indexes, fat free mass (FFM, kg), percentage fat free mass (FFM%) and fat free mass index (FFMI, kg/m^2), among women aged 18-79 years from the TwinsUK cohort (Subset 1). An additional aim of this study was to examine the hypothesis that greater adherence to the five derived diet scores was associated with greater muscle strength assessed by hand grip strength (kg) and muscle quality (muscle strength divided by muscle mass) (kg/kg) in women aged 34-83 years from the TwinsUK cohort (Subset 2).

5.2 Covariate plan and statistical analysis

All analyses were performed using STATA statistical software (version 11.0; STATA Corp, USA). The analyses were performed treating twins as individuals as previous studies have shown that participants from the TwinsUK registry were similar to age-matched singleton women in the distribution of common traits and outcomes (Andrew *et al.* 2001). Muscle quality was calculated as the ratio of hand grip strength (kg) to lean mass

(kg). Robust cluster regression was applied for continuous variables (such as age, energy intake, and total body mass) and Chi-square test for categorical variables (such as physical activity and smoking history), to test for differences in participant baseline characteristics across quartiles of the derived diet scores (**Table 5.2**). One-way ANOVA was applied to test for differences in nutrient intakes across quartiles of the five derived diet scores (**Table 5.3)**. The percentage difference in intake of different food groups between extreme quartiles of the five derived diet scores was calculated as: (quartile 1 - quartile 4 / quartile 1)* 100 for Subset 1 (**Figure 5.1)** and Subset 2 **(Figure 5.2)**.

Prior to statistical analysis an analysis plan with justification for all confounding factors in the multivariate model was developed as described in Chapter 2, section 2.2.4, p.. Multivariate regression analysis was used to assess associations between the five derived diet scores and indexes of muscle mass, hand grip strength and muscle quality using the robust cluster regression option in STATA. These models take into account clustering of individuals when calculating standard errors of the mean (Richards *et al.* 2007) to ensure familial aggregation within twin pairs was accounted for.

As age has a great influence on muscle mass, strength, and muscle quality all analyses were adjusted for age (in years) (Cruz-Jentoft *et al.* 2010, Lynch *et al.* 1999). Models were then also adjusted for physical activity and smoking status, as regular physical activity has been associated with increased muscle mass and strength (Frankel *et al.* 2006, Heath and Stuart

2002, Kuh *et al.* 2002, Roubenoff 2007, van Kan *et al.* 2008, Wolfe 2006), and smoking has been shown to increase the risk of sarcopenia (Morse *et al.* 2007, Petersen *et al.* 2007, Wüst *et al.* 2008). Energy intake (kcal/d) was also included in the multivariate models for the MDS (Trichopoulou *et al.* 1995), AHEI (McCullough *et al.* 2002) and the DASH-style score (Fung *et al.* 2008) as these scores do not take into account energy intake, in contrast to the HDI and DQI (Huijbregts *et al.* 1997, Patterson *et al.* 1994). Total body mass (kg) was included as a covariate in the evaluation of associations between diet and FFM and FFMI as described in Chapter 2, section 2.2.5, p. 57 (Subset 1) (**Tables 5.4, 5.5, 5.6**). Additional adjustments were made for hand grip strength and muscle quality including height, menopausal status (premenopausal or postmenopausal), and hormone replacement therapy use (HRT) (no or yes), as these factors have been previously shown to affect muscle strength and muscle quality (Subset 2) (**Tables 5.8, 5.9**) (Carville *et al.* 2006, Cooper *et al.* 2008, Greeves *et al.* 1999, Greising *et al.* 2009, Jerome *et al.* 1991, Kurina *et al.* 2004, Neder *et al.* 1999, Sipila *et al.* 2001).

In order to examine the relative importance of diet quality and other lifestyle characteristics on indexes of muscle mass, muscle strength and muscle quality regression analysis was performed with all independent variables standardised to represent a similar scale. For Subset 1 (2570 women, aged 18-79 years), diet quality indices were measured in quartiles, age in 10 year categories, smoking habit in two categories (current v never and former v never), physical activity in three categories (inactive, moderate, and active), energy intake per 1000 kcal, and total body mass in kg (**Table 5.7**). For

Subset 2 (949 women, aged 34-83 years), diet quality indices were measured in quartiles, age in 10 year categories, current smoking (yes/no), physical activity (inactive, moderate, and active), height in cm, energy intake per 1000 kcal, menopausal status (postmenopausal v premenopausal), and hormone replacement therapy (no/yes) (**Table 5.10**). In the subset of 2570 women, age was inversely correlated with FFM (r = -0.2, P < 0.001) and percentage FFM (r = -0.4, P < 0.001), but not with FFMI (r = -0.006, P = 0.76). Therefore, for FFMI further analysis was not stratified by age. However, since age was inversely correlated with hand grip strength (r = -0.5, P < 0.001) and muscle quality (r = -0.4, P < 0.001) analysis in the subset of 949 women was further stratified by age using two categories, less than 50 years and more than 50 years.

5.3 Results

Descriptive characteristics of the two subsets of the TwinsUK cohort are shown in **Table 5.1**. For the first subset (n = 2570 women) the mean (±SD) age of participants was 48.3 ± 12.7 years and mean (±SD) BMI was 24.9 ± 4.14 kg/m^2. Mean FFM (±SD) was 39.6 ± 5.30 kg, percentage FFM 61.1 ± 6.49 %, and FFMI 15 ± 1.71 kg/m^2. More than one-half of the participants were moderately active (54 %), and 18.2 % were current smokers. The mean (±SD) MDS was 4.48 ± 1.79 points, HDI score was 3.44 ± 1.34 points, DQI was 11.7 ± 2.03 points, AHEI was 51.5 ± 11.2 points, and the DASH-style score was 23.4 ± 5.67 points. Mean (±SD) energy intake was 1979 ± 524 kcal/d (**Table 5.1**).

For the second subset (*n* = 960 women) the mean (±SD) age of participants was 59.1 ± 9.30 years and the mean (±SD) BMI was 26.5 ± 4.73 kg/m^2. Mean hand grip strength was 28.8 ± 5.95 kg, and muscle quality was 0.69 ± 0.14 kg/kg. Mean (±SD) percentage FM was 35.7 ± 6.28 %, and percentage FFM was 61.4 ± 6.18 %. In relation to physical activity, 34.2 % of participants were moderately active and few were current smokers (9.8 %). The mean (±SD) MDS was 4.42 ± 1.47 points, HDI score was 3.38 ± 1.25 points, DQI was 11.6 ± 1.96 points, AHEI was 53.3 ± 11.0 points, and the DASH-style score was 23.5 ± 5.74 points. Mean (±SD) energy intake was 1917 ± 637 kcal/d. Notable differences in body composition were observed between the two subsets of this cohort. Participants in the second subset were heavier, had a higher BMI, fat mass, percentage fat mass and estimates of fat free mass compared to the first subset of the cohort, which is likely to be because body composition measurements in the second subset were taken 10years later. In terms of fat free mass, differences might be because total body weight was greater in the second subset and increases in fat free mass are associated with increases in total body weight (Wells *et al.* 2002, Wolfe 2006) (**Table 5.1**).

The main baseline characteristics of the two subsets across quartiles of the five derived diet scores are presented as unadjusted means ± standard errors (SEM) in **Table 5.2**. Levels of statistical significance are reported as P for trend. For the first subset age was significantly and positively associated with the DQI (P <0.001) and AHEI (P = 0.001), and inversely associated with the HDI (P = 0.051). Total body mass was significantly and positively

associated with the DQI (P = 0.042) and DASH-style score (P = 0.013), and negatively associated with the HDI (P = 0.001). All scores were associated moderate physical activity (MDS, P = 0.034; DQI, P = 0.019; AHEI, P < 0.001; DASH-style score, P = 0.002) with the exception of HDI (P = 0.124); and current smoking (MDS, P < 0.001; HDI, P = 0.002; DQI, P < 0.001; AHEI, P < 0.001; DASH-style score, P < 0.001).

For the second subset, age was inversely associated with the MDS and HDI (P < 0.001 for both). Women who scored high for the HDI, DQI and AHEI were less likely to be current smokers (P for trend = 0.011, < 0.001 and 0.001, respectively). Women who scored high for the MDS and AHEI were more likely to be postmenopausal (P for trend < 0.001 and 0.024, respectively), and those with higher AHEI were more likely to be physically active (P for trend < 0.001) (**Table 5.2**).

A comparison of nutrient intakes between the highest and the lowest quartile of the derived diet scores are presented in **Table 5.3**. For subset 1, protein intake (as % Energy) was positively associated with the DQI, AHEI and DASH-style scores (P < 0.05 for all). Higher intakes of vitamin C, magnesium and total carotene were positively associated with higher adherence to all scores (P < 0.001 for vitamin C and magnesium, and P < 0.05 for total carotene). Higher vitamin D intake was positively associated with higher MDS, AHEI and DASH-style scores (P < 0.001 for all). Higher vitamin E, potassium and selenium intakes were positively associated with

higher adherence to all scores ($P < 0.001$ for vitamin E and selenium, and $P > 0.05$ for potassium) except for the DQI.

For subset 2, higher intakes of vitamin C, magnesium, potassium and total carotene were positively associated with higher adherence to all scores ($P < 0.05$ for all) apart from total carotene and the DQI ($P > 0.05$). Also, higher vitamin D, E and selenium intakes were positively associated with higher MDS, AHEI and DASH-style scores ($P < 0.001$ for all) (**Table 5.3**).

The percentage difference in different food groups of the two subsets between extreme quartiles of the five derived diet scores is shown in **Figures 5.1** and **5.2**, respectively. For subset 1, percentage difference in intake of fruit and vegetables, whole grains, nuts and legumes was positively and significantly associated with all scores ($P < 0.001$ for all), apart from nut and legume intake and the DQI (**Figure 5.1**). For subset 2, percentage difference in intake of fruit and vegetables, whole grains, nuts and legumes was positively and significantly associated with all scores ($P < 0.001$ for all), apart from nut intake and the HDI and legume intake and the DQI (**Figure 5.2**). Percentage difference in intake of dairy products and meat was positively associated with the DASH-style score ($P < 0.001$) for both subsets 1 and 2.

Subset 1 (2570 women, 18-79 years)

Fat free mass (FFM)

In multivariate analyses, when comparing extreme quartiles of the five derived diet scores, FFM was positively and significantly associated with the MDS by 1.97 %, HDI by 1.67%, DQI by 2.28 %, AHEI by 2.46 %, and

DASH-style score by 2.13 %, compared to the lowest quartile of each score (P for difference < 0.05 for all) (**Table 5.4**).

Percentage fat free mass (FFM %)

In the multivariate model, mean percentage FFM was positively and significantly associated with a 4 point difference in the MDS (difference in FFM% 1.15 %), a 3 point difference in the HDI (difference 2.07 %), and a 13 points difference in the AHEI (1.64 %) (P < 0.05 for all). The associations were not significant for the DQI and the DASH-style scores (**Table 5.5**).

Fat free mass index (FFMI)

After multivariate adjustment, mean FFMI that was positively and significantly associated with a 4 point difference in the MDS, by 1.41 %, a 3 point difference in the HDI, by 1.34 %, a 5 point difference in the DQI by 2.30 %, 13 points difference in the AHEI by 1.28 %, and 27 points difference in the DASH-style score by 2.62 % (P < 0.05 for all) (**Table 5.6**).

Relative comparison of associations between diet quality scores, lifestyle characteristics and fat free mass indexes in subset 1

Higher fat free mass (kg) was significantly associated with greater adherence to all five diet scores. The greatest magnitude of association was observed between FFM and the AHEI score, with an increase of 0.31 kg per quartile of the AHEI score (P = 0.001). Compared with the diet scores, FFM (kg) was inversely associated with age per 10 yrs for all five diet scores and

this association was 4-5 times the scale of the association with the diet scores (P for all < 0.001). Both current and former smoking was positively associated with FFM and this was of a similar scale to age (P < 0.001). Also, a one category increase in physical activity was positively associated with FFM, with the greatest association for the HDI and the DQI (with an increase of 0.74 kg per category of physical activity) (P < 0.001) for both scores, although this association was smaller than age and smoking (**Table 5.7**).

Higher percentage FFM was significantly associated with greater adherence to all diet scores with the exception of DQI and the DASH-style scores for which the association was not significant. The relative associations with age per 10 years (P for all < 0.001) and current smoking (P for all < 0.05) and physical activity (P for all < 0.001) were of a similar scale to FFM for all diet scores (**Table 5.7**).

Higher fat free mass index (kg/m^2) was significantly associated with greater adherence to all five diet scores (P for all < 0.05). Again the relative associations for age, current smoking and physical activity were of a similar scale to those of FFM for all scores (**Table 5.7**).

Overall, the greatest magnitude of association was an almost 2 % decrease in percentage FFM for an increase in age per 10 years for all diet scores (P for all < 0.05); and an increase of almost 1.4 % in percentage FFM for every category in physical activity for all diet scores (P for all < 0.05) (**Table 5.7**).

Subset 2 (949 women, 34-83 years)

Hand grip strength and muscle quality

In multivariate models, no associations were observed between the five diet scores and hand grip strength (**Table 5.8**) and muscle quality (**Table 5.9**). In further analyses, where we stratified by age no significant associations were observed.

Relative comparison of associations between diet quality scores, lifestyle characteristics and hand grip strength and muscle quality in subset 2

When examining the relative associations between diet quality scores, lifestyle factors and hand grip strength and muscle quality, the greatest magnitude of association was observed between age per 10 years and physical activity and hand grip strength, and age per 10 years and muscle quality **(Table 5.10)**. Hand grip strength (kg) was inversely associated with age per 10 yrs by 2.70 kg per quartile of all diet scores ($P < 0.001$ for all). Also, a 1 category increase in physical activity was positively associated with hand grip strength by 0.50 kg on average per category of physical activity ($P < 0.05$ for all). Muscle quality (kg/kg) was also inversely associated with age per 10 years by 0.06 kg/kg on average for all five diet scores ($P < 0.001$ for all) (**Table 5.10**).

5.4 Discussion

This study is one of the first cross-sectional studies to show that there was a positive association between diet quality as assessed by the MDS, HDI, DQI, AHEI and DASH-style scores and fat free mass (FFM, kg); the MDS,

HDI and AHEI scores and percentage fat free mass (FFM %); and the MDS, HDI, DQI, AHEI and DASH-style scores and fat free mass index (FFMI, kg/m^2) in a cohort of women across a wide age range. However, associations between diet quality and muscle strength and muscle quality were not observed in this population. Only one previous longitudinal study among 690 men and women aged 65 years and over has examined associations between the Mediterranean diet score and muscle strength, although no associations were observed over 6 years of follow-up (Talegawkar *et al.* 2012). The current study highlighted the influence of diet quality assessed by five of the most commonly used diet quality scores, previously inversely associated with the risk of mortality, cardiovascular disease risk factors, and with the prevention of chronic disease risk (Akbaraly *et al.* 2011, Fung *et al.* 2008, Huijbregts *et al.* 1997a, McCullough *et al.* 2002, Patterson *et al.* 1994, Seymour *et al.* 2003, Trichopoulou *et al.* 1995).

Diet quality scores and indexes of muscle mass

The current study observed significantly higher FFM (kg) with higher adherence to all five diet quality scores. Differences in FFM between higher and lower adherence to the diet scores were 0.77 kg for the MDS (P = 0.001), 0.66 kg for the HDI (P = 0.023), 0.89 kg for the DQI (P = 0.002), 0.96 kg for the AHEI (P = 0.001), and 0.84 kg for the DASH-style score (P = 0.011). These differences equated to a relative mean difference of between 2% and 3% for FFM (**Table 5.4**).

Also, the results indicated that higher percentage FFM was significantly associated with and higher adherence to the MDS, HDI and AHEI. Differences in percentage FFM between higher and lower adherence to the diet scores ranged from 0.7-1.3 % for three of the scores (MDS, AHEI and HDI, respectively). These differences equated to differences that were between 2% and 3% of the mean percentage FFM for the population. No associations were found between percentage FFM and the DQI and DASH-style scores in the current study. This is most likely because those who scored higher for the DQI and DASH-style score had a higher total body mass and for the DASH-style score were also older compared to those who scored higher for the MDS, HDI and AHEI (**Tables 5.2 and 5.5**).

Significant positive associations were also observed between the FFMI and higher adherence to all five diet quality scores. Differences in FFMI between higher and lower adherence to the diet scores were ranged between 0.2-0.4 % for the MDS, HDI, DQI, AHEI (P = 0.028) and DASH-style score. These differences equated to differences that were between 1.3% and 3% of the mean FFMI for the population (**Table 5.6**).

These associations remained significant after adjustments for age, physical activity, smoking status, energy intake (for MDS, AHEI and DASH-style), and total body mass and equated to a scale of effect that was between 37% and 63% of the scale of the observed association with 10 years of age for FFMI (**Table 5.7**).

There are currently no studies examining the influence of a better diet quality on indexes of muscle mass. However, looking to our data, interestingly, the magnitude of the findings in this analysis was similar of that observed for the association between vitamin C and magnesium intakes and indexes of muscle mass described in Chapter 3, section 3.4, p. 87, 89. These findings may be explained either because higher diet scores were associated to higher intakes of vitamin C and magnesium (**Table 5.3**) or because plant-based nutrients individually are more important than the "whole diet". These findings also point towards the need to identify whether public health recommendations for the maintenance of healthy muscles in relation to diet should focus on eating patterns or individual dietary components.

The derived diet scores had distinct similarities and differences (see **APPENDICES II**, p. 329 and **III**, p. 332). Three of the scores consisted of 9 components (including the MDS, HDI, and AHEI); and the other two scores (DQI and DASH-style) consisted of 8 components. Key difference between the scores were that the MDS, AHEI and DASH-style were focused mainly on intake of significant food groups (characterised by higher intakes in fruits and vegetables-either grouped or separately, nuts and legumes, whole grains, and low intakes in red processed meat). In contrast, the HDI and DQI scores were mainly focused on intake of nutrients (characterised by higher intakes in polyunsaturated fatty acids, average intakes in protein, and low intake in saturated fatty acids and cholesterol). Yet, the MDS, AHEI, and DASH-style were based on overall dietary

patterns, and the HDI and DQI were based on healthy eating guidelines. No specific statistical weighting for individual components was used in any of the five scores with all scores assuming that all food groups and nutrients contributed equally to the diet quality.

There were also differences in the methodologies used to construct the scores. The MDS used the median intake of dietary components as the cut-off between healthy and unhealthy intakes, whereas the DASH-style score used quintiles to classify intakes of dietary components, with a "healthy" point assigned only if the quantity consumed was in the highest quintile. In contrast, the HDI, DQI, and the AHEI were cut-point specific scores based on healthy eating guidelines. Overall, these differences suggest that the DASH-style score gives more weight to high adherence to a better quality diet compared with the other four scores. However, it is likely that the use of the median as a cut-off value will not reflect a healthy level of intake *per se*, and will be different among different populations, but an advantage is that half of the sample will score positively and half negatively for each component, which ensures that each component distinguishes well between individuals (Waijers *et al.* 2007). On the other hand there are drawbacks with the use of cut-off specific values as it is likely that the intake of a specific component may be below the cut-off level for most of the subjects, limiting the discriminating power of a component (Kourlaba and Panagiotakos 2009, Waijers *et al.* 2007).

This analysis indicated that the five derived diet scores measured diet quality adequately as they were highly associated with intake of different

macro- and micronutrients in this population (**Table 5.3**). This finding was supported by other studies in different populations (Kennedy *et al.* 1995, Patterson *et al.* 1994). Interestingly, when translating the differences in nutrient intakes between extreme quartiles of the five diet scores into comparable amounts of food, the differences in vitamin C intake, for example, between extreme quartiles of the diet scores equated to 1.6 portions of oranges for the MDS, 1.5 portions for the HDI, 1 portion for the DQI, 2.3 portions for the AHEI and 2 portions for the DASH-style score (assuming one portion of oranges to be 120 g for adults).

Most of the scores captured variability of dietary constituents that have been previously associated with muscle mass (as it was outlined in Chapter 1, section 1.3, p. 21). However, there were a few differences in nutrient intake across quartiles of the five diet scores which may offer some explanation as to why associations between the percentage FFM and the DQI and DASH-style scores were not seen in this study. When different dietary constituents across quartiles of the five diet quality scores were analysed, it was observed that nutrients that have been previously associated with muscle mass, such as vitamins C and E, selenium, magnesium, potassium and total carotene were significantly and positively associated with higher adherence to the MDS, HDI and AHEI. Although higher adherence to the DQI score was also positively associated with protein, vitamin C and potassium, it was however negatively and significantly associated with vitamins D and E, and selenium, important dietary components related to muscle mass (as outlined in Chapter 1, section 1.3, p. 21, 22). The observed negative associations

between dietary components important for muscle mass and the DQI may offer some explanation as to why this score was not associated with the percentage FFM in this cohort.

Additionally, even though the DASH-style score consisted of components high in nutrients important for muscle mass, such as protein, vitamins C, E and D, magnesium, potassium, total carotene, and selenium; and also higher adherence was significantly and positively associated with intakes of these nutrients, a higher adherence to this score was not associated with percentage FFM. This finding suggests that the way the indexes of muscle mass were calculated may have lead to the differences observed. It is well documented that in order to evaluate FFM in relation to body size and nutritional status, FFM should be considered in relation to height, because FFM expressed in absolute weight or as a percentage of body weight is not usually adequate because FFM increases with height. FFMI (weight/height^2) which eliminates differences associated with height is an important marker of health and disease status (Coin *et al.* 2008, Heymsfield *et al.* 2011, Kyle *et al.* 2003). An example, of the relationship between height and FFM is that FFM might be similar between a healthy, well-nourished man and a similar aged but taller subject with protein - energy malnutrition (VanItallie *et al.* 1990).

To further evaluate whether the findings were due to the potential protective effect of a better quality diet or due to an overall healthy lifestyle, analyses was calculated for age per 10 years, smoking habit, physical activity, energy

intake per 1000 calories, and total body fat to enable comparison. Findings suggested that there was a strong effect of age on all three indexes of muscle mass with the greatest magnitude of association equivalent to 2 % decrease in percentage FFM for an increase in age per 10 years (P for all < 0.05). This finding reflects data from the literature that shows that loss of muscle mass increases with age (Fielding *et al.* 2011, Morley *et al.* 2001). Also, physical activity was significantly positively associated with higher indexes of muscle mass, with the greatest magnitude of association equivalent to 1.4 % higher percentage FFM per category of physical activity. This finding was of a similar magnitude to those from an intervention study among older men and women (aged 65.5 ± 3.7 years) that found positive effects of physical activity combined with antioxidant supplementation on muscle mass; a 1.1 % increase in percentage FFM after 6 months of resistance training and antioxidant supplementation (Bobeuf *et al.* 2010).

However, although lifestyle factors had an effect on indexes of muscle mass, the association of the diet quality scores with indexes of muscle mass remained significant for all scores (with the exception of the percentage FFM and the DQI and DASH-style) even after accounting for all relevant lifestyle factors.

Diet quality scores and hand grip strength and muscle quality

In the current study no associations were observed between diet quality and hand grip strength and muscle quality. Age and physical activity showed the greatest influence on these two measurements. When examining the relative

associations between diet quality scores, lifestyle factors and hand grip strength, the greatest magnitude of association was observed between age per 10years and hand grip strength. Hand grip strength (kg) was negatively associated with age per 10 years by 2.70 kg on average for all five diet scores ($P < 0.001$ for all). Only one previous longitudinal study among 690 men and women aged (≥ 65 years) has examined associations between the Mediterranean diet score and muscle strength, and although no associations were observed (Talegawkar *et al.* 2012) the authors did not offer an explanation for this finding neither did they report the mean value for grip strength.

In a number of clinical and epidemiological studies, hand grip strength has been suggested as an important measurement for overall health and nutritional status (Norman *et al.* 2011). Low hand grip strength has been associated with an increased risk of disability, morbidity and mortality in both men and women (≥ 45 years) and with a decline in quality of life in older individuals, and has been suggested to be a useful marker of muscle function and sarcopenia (Cruz-Jentoft *et al.* 2010, Gale *et al.* 2007, Laukkanen *et al.* 1995, Rantanen 2003, Rantanen *et al.* 1999, Rantanen *et al.* 2003, Sayer *et al.* 2006, Syddall *et al.* 2003). Mean (SD) hand grip strength in women from the TwinsUK cohort was 28.8 (5.9) kg, higher compared to the mean (SD) hand grip strength [26.5 (5.7) kg] in women from a previous UK based cohort (Robinson *et al.* 2008). However, the TwinsUK cohort was younger (34-83 years) and this may explain this observed difference, since age is a strong independent factor of grip strength

(Norman *et al.* 2011). Therefore, it is likely that the lack of association observed in the current study between diet quality and hand grip strength might be due to the fact that the cohort included younger adults as well as older individuals, however stratification for age did not alter our findings.

In relation to muscle quality (kg/kg) there was a negative association with age per 10 years by 0.06 kg/kg on average for all five diet scores ($P < 0.001$). Muscle quality is defined as the ratio of muscle strength per unit of muscle mass, and has been suggested as an approach to measure the quality of muscles (Goodpaster *et al.* 2006, Lynch *et al.* 1999). It has been positively associated with age and functional incapacities (Goodpaster *et al.* 2006, Lynch *et al.* 1999). However, in the current study no associations were observed with diet quality, even after stratification for age. Muscle quality does integrate muscle strength in its calculation, however, as muscle strength was not associated with diet quality in our analyses this may be the reason why muscle quality was not also associated with diet quality. In addition, analyses in the current study showed that after multivariate adjustment for relevant confounders, the influence of age was six (for the AHEI) to 200 (for the MDS) times greater of that of diet quality scores on muscle quality (Newman *et al.* 2003). Moreover, the effect of physical activity was one (for the AHEI) to 27 times greater (for the MDS) of that of diet quality scores on muscle quality. Notably, intervention programmes have shown that after 9 weeks strength training for three days per week, muscle quality (assessed as the ratio of strength divided by the muscle

volume of the trained muscle group) was increased in older women aged 65-73 years by 16 % from baseline (Tracy *et al.* 1999).

Muscle quality has not been previously used in nutritional studies, as to date there is no standardised protocol to assess muscle quality, although research is on-going in this field (Barbat-Artigas *et al.* 2012). However, further studies using more validated methods are needed in order to integrate this important measurement in future dietary intervention studies on sarcopenia.

The mechanisms involved in the relationship between diet quality (assessed by diet quality indices) and indexes of muscle mass cannot be specifically identified due to the nature of our study which assessed associations. Nevertheless, findings from epidemiological studies that have shown that dietary patterns characterised by increased consumption of fruits and vegetables, whole grains, dietary fiber and low intakes of saturated and trans fatty acids were associated with lower levels of markers of inflammation, oxidative stress, and endothelial dysfunction, offer support to these findings (Baer *et al.* 2004, Dai *et al.* 2008, Esmaillzadeh *et al.* 2007, Esposito *et al.* 2004, Fung *et al.* 2001, Fung *et al.* 2001, Lopez-Garcia *et al.* 2004, Lopez-Garcia *et al.* 2005, Mozaffarian *et al.* 2004, Nettleton *et al.* 2006). Indeed, adherence to the Mediterranean diet, which is characterised by a high intake in fruits, vegetables, and whole grains, and lower consumption of red meat and saturated fats has been associated with lower circulating levels of the inflammatory marker, IL-6 (Chrysohoou *et al.* 2004). It has also been shown that the alternate Mediterranean score (aMED) and the Alternate Healthy Eating Index (AHEI) are significantly associated with lower plasma both IL-6 and CRP ($P < 0.05$ for both), independent of obesity, and after

multivariate adjustment (Fung *et al.* 2005). Furthermore, in another cross-sectional investigation among women who were selected for a previous nested case-control study of diabetes from the Nurse's Health cohort Study, 62% of whom were overweight, it was observed that higher adherence to the Alternate Healthy Eating Index (AHEI) score was associated with a significantly 41% lower plasma CRP, 10% lower TNF-a receptor II levels (*sTNF-aRII*) and 22% lower IL-6 in the higher compared with lower adherence to the score (Fargnoli *et al.* 2008). In addition, a randomised, crossover design trial among type II diabetic patients who consumed the DASH-style diet for 8 weeks showed a mean decrease of 27 % in plasma CRP after the DASH-style diet period (Azadbakht *et al.* 2011). Chronic inflammation is an inevitable consequence of the ageing process and may further cause loss of myocytes and reduced function of skeletal muscle mass (Gianni *et al.* 2004, Mecocci *et al.* 1999, Morley *et al.* 2001, Pansarasa *et al.* 1999). In addition, it has been suggested that the development of age-associated declines in muscle mass, muscle strength, physical function, mobility and frailty have been attributed partly to the inflammation that occurs with the ageing process (Chung *et al.* 2009, Howard *et al.* 2007). Consequently, the greater associations we found between muscle mass and the healthy diet scores may be partly explained due to the anti-inflammatory effects of an overall healthier diet containing food components high in the nutrients previously found to be related to muscle mass (as outlined in Chapter 1, section 1.3, p. 21, 22, and Chapter 3, section 3.0, p. 82).

Minor differences in absolute values for different scores were observed between the two subsets due to particular differences in the way that the scores were constructed (which were outlined in Chapter 2, section 2.2.4.1, p. 56). Mean values in the current study were similar to mean values in UK, European and US populations, with a mean MDS of 4.5 points for the EPIC-PANACEA cohort (Romaguera *et al.* 2010), mean DASH-style score of 23 points among US women from the Nurses' Health Study (Fung *et al.* 2008), and a mean DQI score 8.6 points among adults from the American Nationwide Food Consumption Survey (NFCS) (Patterson *et al.* 1994). However, the HDI score differed between different populations, with a mean HDI of 2.5 points for Finland, 4 points for Italy, and 3.5 points for a Swedish sample population, although in the Swedish cohort the HDI was modified from the traditional score to include fish intake (based on the Nordic Nutrition recommendations from 2004) (Sjögren *et al.* 2010). The HDI score for the TwinsUK cohort was between the scores for other European populations which is likely to be reflective of differences in dietary intake with other studies. Also, the mean DQI score was higher in the TwinsUK cohort and the NFCS most likely because in the current cohort the scoring of the original score was reversed so that a higher score indicated higher diet quality, to enable comparison with the other four scores. The mean AHEI score for the current cohort was higher compared with that from the US women from the Nurses' Health Study, which was 38.4 points. However, for the development of AHEI for the TwinsUK cohort the criterion for the maximum dietary fiber intake was based on the Daily Reference Value (DRV) for non-starch polysaccharides (NSP) for the

UK population, which is 18 g/d, compared with 15 g/d which is the maximum daily intake for cereal fiber for the US population studied from the Harvard cohorts (McCullough *et al.* 2002). Overall, the mean of the scores for the current cohort was comparable with that of other populations.

5.5 Strengths and limitations

The strengths of this study include the large sample size and the wide age range of participants, as most previous studies on sarcopenia focused only on older individuals (Robinson *et al.* 2008, Scott *et al.* 2010). Moreover, this was the first study that directly compared five diet quality scores based on adherence to dietary patterns or to dietary recommendations with indexes of muscle mass, muscle strength and muscle quality in a large cohort. Also, many nutrients and food groups were significantly related to the derived diet quality scores, suggesting that the diet quality scores developed for the TwinsUK cohort captured aspects of diet quality. An additional strength was that two of the scores have been previously validated. Indeed, two modified versions of the Mediterranean diet score measured by an FFQ were recently shown to produce similar findings to conducting 10 to 12 24 h dietary recalls (Benítez-Arciniega *et al.* 2011). Yet, it has been previously shown that the Diet Quality Index (DQI) has been validated against plasma biomarkers of vitamin C, a-tocopherol, β-cryptoxanthin and phospholipid fatty acids (Neuhouser *et al.* 2003). Body composition was objectively assessed by DEXA scans and an objective measurement was used to assess muscle strength, which allowed calculation of muscle quality.

Our study also has limitations. The cross-sectional nature of the study limits causal inference, but enables us to generate hypotheses regarding associations between diet quality and indexes of muscle mass, strength and muscle quality based on plausible mechanisms of action. Another limitation is that no specific statistical weighting for each component has been used regarding the construction of each score, assuming that all food groups and nutrients contributed equally to the diet quality. Higher adherence to the diet scores may be an indicator of a healthy lifestyle, and even though all analyses were adjusted for lifestyle factors, residual confounding could not be ruled out because of the observational nature of the study. In addition, as in all observational studies, measurement error in self-reported dietary intakes is inevitable. It is also widely acknowledged that the use of FFQs for measuring dietary intake has its own limitations related to memory errors, they provide relative than absolute intake, they introduce bias due to over- or under-reporting, and they may introduce systematic errors as preparation methods are inadequately considered (McNeill *et al.* 2009). Also, FFQs measure food intake over a period of one year and reflect the habitual diet over this period of time but not lifetime dietary habits (Teucher *et al.* 2007). Nevertheless, the FFQ used in the current study was previously compared and validated against a 7-day weighed record in the EPIC Norfolk study, and although the two approaches to measure dietary intake were different, both methods identified similar intakes of macronutrients when these expressed as percentage of total energy intake (Bingham *et al.* 2001). Also, this FFQ was previously validated against urinary and plasma biomarkers of intake, such as urinary nitrogen for protein intake, urinary potassium and

sodium, plasma ascorbic acid and plasma n-3 PUFAs (McKeown *et al.* 2001, Welch *et al.* 2006). In addition, it has been shown previously that serum β-cryptoxanthin and zeaxanthin concentrations were moderately correlated with dietary carotenoids intakes measured by an FFQ (Tucker *et al.* 1999). In order to examine associations between dietary intake and health outcomes participants need to be ranked according to their usual dietary intake and FFQs have been shown to rank individuals well (Molag *et al.* 2007). The observed findings relate to women and further work is needed to investigate if these findings are replicated in a male population of the same country or in populations from different ethnic backgrounds.

5.6 Concluding remarks

This is one of the first studies to our knowledge to show that diet quality assessed by five predefined diet quality scores is associated with muscle mass in women of different ages. This is also one of the first studies to examine cross-sectional associations between diet quality and muscle strength and muscle quality. Findings from the current study extend the results from previous cross-sectional studies by focusing on the overall diet quality instead of a food group or a nutrient in association with muscle mass and muscle strength. Additionally, our findings extended the existing literature by evaluating the importance of diet quality and muscle quality to further understand the association between muscle strength and muscle quality.

In conclusion, the greatest associations were observed between the AHEI and FFM, the HDI and percentage FFM, and the DASH-style score and the FFMI and were equivalent to 2.5 %, 2 % and 2.6 % of the population mean for FFM, percentage FFM and FFMI, respectively. Although the strength of the associations were modest, this was observed after analyses was accounted for age, smoking, physical activity, body fat and energy intake, factors that are known to affect muscle mass. Nevertheless, associations were not observed between diet quality and muscle strength and muscle quality possibly due to the fact that other factors, such as age and physical activity may be more important than diet quality in improving or maintaining muscle strength and quality. Yet, it is also possible that a longitudinal study design might be a more suitable approach to detect associations between diet quality and muscle strength and muscle quality.

Despite their differences, the derived diet scores reflected a common pattern characterised by high intakes in fruit and vegetables, nuts, legumes, dietary fiber/and or whole grains, and low intake of red and processed meat, and saturated fat, suggesting that plant derived sources of vitamin C, magnesium, potassium and carotenoids may be important for muscle mass. In addition, examining dietary patterns and health outcomes may lead to recommendations which are more likely to be used for public health purposes (Tucker 2010). Although further work is needed to examine associations between other diet quality scores or dietary patterns and muscle mass, muscle strength and muscle quality, results from the current analysis suggest that it is important for adult women to consume a variety of foods to ensure greater muscle mass. From a public health perspective, these findings

will help to improve the knowledge and understanding of the effects of dietary and lifestyle factors on muscle mass and strength and provide useful information in the developing and planning dietary intervention trials to improve conservation of muscle mass and strength in adult life.

TABLES

Table 5.1 Baseline characteristics of female subjects from the TwinsUK cohort[1]

Characteristic	Subset 1 *n*=2570, 18-79 yrs	Subset 2 *n*=949, 34-83 yrs
Age (y)	48.3±12.7	59.1±9.3
Weight (kg)	65.6±11.2	69.2±12.5
Height (cm)	162±6.1	162±5.9
BMI (kg/m^2)	24.9±4.1	26.5±4.7
Fat mass (kg)	22.7±7.9	25.2±8.3
Fat mass %	33.9±7.2	35.7±6.3
Fat free mass (kg)	39.6±5.3	42.0±5.5
Fat free mass %	61.1±6.5	61.4±6.2
Fat free mass index (kg/m^2)	15.0±1.7	16.0±1.8
Hand grip strength (kg)		28.8±5.9
Muscle quality (kg/kg)		0.69±0.14
Physical activity %		
Inactive	21.9	39.6
Moderate	53.9	34.2
Active	24.2	26.2
Smoking history %		
Never	50.2	
Current	18.2	
Former	31.6	
Current smoking %		
Yes		9.79
No		90.21
Menopausal status %		
Premenopausal		10.3
Postmenopausal		89.7
Hormone replacement therapy %		
Yes		9.38
No		90.63
Derived diet scores (points)		
Mediterranean Diet Score (MDS)	4.48±1.79	4.42±1.47
Healthy Diet Indicator (HDI)	3.44±1.34	3.38±1.25
Diet Quality Index (DQI)	11.7±2.0	11.6±2.0
Alternate Healthy Eating Index (AHEI)	51.5±11.2	53.3±11.0
Dietary Approach to Stop Hypertension score (DASH-style)	23.4±5.7	23.5±5.7
Dietary components		
Energy intake (kcal/d)	1979±524	1917±637
MDS		
Fruit & nuts group (g/d)	252±195	283±209
Vegetables group (g/d)	280±157	325±178
Ratio of mono- and poly- to saturated fat	1.49±0.37	1.60±0.39
Milk & dairy group (g/d)	421±197	390±205
Meat group (g/d)	86.8±48.6	88.1±48.3
Fish group (g/d)	35.6±27.5	44.8±43.7
Legumes intake (g/d)	23.3±26.3	23.5±26.0
Cereals intake (g/d)	209±103	208±125
Alcohol intake (g/d)	9.98±13.5	9.45±11.7
HDI		
Fruit & vegetables group (g/d)	584±315	662±336
Complex carbohydrates group (% Energy/d)	47.6±18.5	49.3±20.4
Dietary fiber group (g/d)	20.3±7.6	21.0±8.5
Pulses & nuts group (g/d)	26.3±27.7	26.8±26.8

Mono- & disaccharides group (% Energy/d)	23.8±5.8	24.7±5.7
Protein intake (% Energy/d)	16.6±2.6	17.7±2.8
Saturated fat intake (%Energy/d)	11.7±2.9	11.5±2.7
Polyunsaturated fat intake (% Energy/d)	6.43±1.59	6.91±1.87
Cholesterol intake (mg/d)	224±88	238±128
DQI		
Fruit & vegetables group (servings/d)	7.29±3.92	7.97±3.97
Total fat (% Energy/d)	31.3±5.6	31.7±5.2
Complex carbohydrates (servings/d)	3.85±2.0	3.44±1.9
Saturated fat (% Energy/d)	11.7±3.0	11.5±2.7
Cholesterol (mg/d)	224±88	238±128
Protein (% RDA)	147±25.3	155±28.1
Sodium intake (mg/d)	2259±758	2329±963
Calcium intake (mg/d)	1134±374	1066±404
AHEI		
Vegetables (servings/d)	3.48±1.95	4.68±2.62
Fruit (servings/d)	3.10±2.42	2.77±2.03
Nuts and soy protein (servings/d)	0.10±0.25	0.62±0.52
Ratio of white to red meat	2.63±5.63	2.50±7.68
Dietary fiber (g/d)	20.3±7.6	21.0±8.5
Trans fat (% energy)	1.10±0.38	1.09±0.35
Polyunsaturated to saturated fat ratio	0.59±0.23	0.64±0.25
Vitamin supplement use % (yes/no)	53.5 / 46.5	41.0 / 59.0
Alcohol (servings/d)	0.77±1.02	0.68±0.86
DASH-style score		
Fruits intake (servings/d)	4.02±2.82	3.25±2.17
Vegetables intake (servings/ d)	3.26±1.91	4.68±2.62
Nuts & legumes intake (servings/d)	0.54±0.46	0.62±0.52
Whole grains intake (servings/d)	1.85±1.65	1.89±1.46
Low-fat dairy intake (servings/d)	0.93±0.66	0.93±0.64
Sodium (mg/d)	2259±758	2329±963
Red & processed meat intake (servings/d)	1.00±0.58	1.08±0.62
Sweetened beverages intake (servings/d)	0.39±0.81	0.43±0.82

[1] Values are means ± standard deviations unless indicated

Table 5.2 Baseline characteristics of female subjects from the TwinsUK cohort across the derived diet scores quartiles[1]

	MDS			HDI			DQI			AHEI			DASH-style		
Subset 1	**Q1**	**Q4**	***P***	**Q1**	**Q4**	***P***	**Q1**	**Q4**	***P***	**Q1**	**Q4**	***P***	**Q1**	**Q4**	***P***
N	**776**	**774**		**650**	**528**		**709**	**557**		**653**	**572**		**677**	**641**	
Age (y)	48.3±0.6	48.8±0.5	0.357	49.2±0.5	47.6±0.6	0.051	46.6±0.6	49.3±0.6	<0.001	46.5±0.6	49.2±0.6	0.001	47.1±0.5	48.7±0.6	0.114
Total body mass(kg)	22.6±0.3	22.4±0.3	0.642	23.2±0.3	21.7±0.3	0.001	22.2±0.3	22.9±0.3	0.042	22.2±0.3	21.8±0.3	0.620	22.2±0.3	23.5±0.4	0.013
Total energy (kcal/d)	1879±18.9	2112±19.6	<0.001	1959±22.1	2108±23.2	<0.001	2186±23.3	1816±18.0	<0.001	1868±20.8	2079±22.9	<0.001	1547±15.4	2440±18.3	<0.001
Physical activity %			0.034			0.124			0.019			<0.001			0.002
Inactive	25.9	19.8		24.0	18.6		26.6	18.5		25.8	14.2		25.7	19.0	
Moderate	51.3	54.5		50.6	54.4		50.4	55.6		54.7	57.5		55.7	54.5	
Active	22.8	25.7		25.4	27.0		23.0	25.9		19.5	28.3		18.6	26.5	
Smoking history %			<0.001			0.003			<0.001			<0.001			<0.001
Never	51.9	50.5		47.4	52.5		46.0	50.3		45.0	51.8		44.1	58.7	
Current	22.9	14.1		23.1	13.3		26.0	12.9		27.1	12.9		24.7	12.9	
Former	25.2	35.4		29.5	34.2		28.0	36.8		27.9	35.3		31.2	28.4	
Subset 2	**Q1**	**Q4**	***P***	**Q1**	**Q4**	***P***	**Q1**	**Q4**	***P***	**Q1**	**Q4**	***P***	**Q1**	**Q4**	***P***
N	**262**	**232**		**231**	**185**		**246**	**167**		**250**	**220**		**241**	**201**	
Age (y)	60.8±0.6	57.3±0.7	<0.001	60.6±0.7	57.2±0.7	<0.001	59.6±0.8	59.0±0.8	0.316	59.2±0.7	58.2±0.7	0.247	59.7±0.7	59.4±0.7	0.542
Height (cm)	161±0.4	162±0.4	0.094	162±0.4	161±0.5	0.663	162±0.5	162±0.5	0.932	161±0.4	162±0.5	0.186	161±0.4	162±0.4	0.191
Total energy (kcal/d)	1698±32.5	2121±42.2	<0.001	1879±42.4	2020±43.5	0.009	2283±55.9	1712±29.4	<0.001	1736±37.4	1981±43.9	<0.001	1399±24.0	2560±54.7	<0.001
Physical activity %			0.184			0.236			0.553			<0.001			0.117
Inactive	43.1	32.8		41.1	35.1		41.9	35.3		48.8	31.8		44.0	30.9	
Moderate	32.8	35.3		33.8	31.4		33.7	38.9		33.6	33.6		33.2	37.8	
Active	24.1	31.9		25.1	33.5		24.4	27.8		17.6	34.6		22.8	31.3	
Current smoking %			0.377			0.011			<0.001			0.001			0.162
No	87.8	92.24		86.2	95.68		82.1	94.0		84.4	95.0		87.1	90.0	
Yes	12.2	7.76		13.8	4.32		17.9	6.00		15.6	5.00		12.9	10.0	
Menopausal status %			0.004			0.095			0.885			0.024			0.603
Premenopausal	6.50	16.0		8.20	13.5		9.76	9.00		12.4	14.1		10.0	11.4	
Postmenopausal	93.5	84.0		91.7	86.5		90.24	91.0		87.6	85.9		90.0	88.6	
HRT %			0.245			0.712			0.603			0.967			0.099
No	90.0	93.5		88.8	90.0		89.4	91.0		90.4	91.36		92.0	92.5	
Yes	10.0	6.50		11.2	10.0		10.6	9.00		9.60	8.64		8.00	7.50	

[1] Values are means and robust standard errors (SEM), unless indicated

Table 5.3 Nutrient intakes across the derived diet scores quartiles[1]

	MDS		HDI		DQI		AHEI		DASH-style	
Subset 1	**Q1 (2.3±0.8)[1]**	**Q4 (6.6±0.8)**	**Q1 (1.7±0.5)**	**Q4 (5.4±0.6)**	**Q1 (9.1±1.1)**	**Q4 (14.3±0.5)**	**Q1 (37.1±14.3)**	**Q4 (66.5±5.1)**	**Q1 (16.3±2.4)**	**Q4 (30.7±2.5)**
N	**776**	**774**	**650**	**528**	**709**	**557**	**653**	**572**	**677**	**641**
Total energy (kcal/d)	1879±496	2112±516	1959±534	2108±515	2186±592	1816±412	1868±523	2079±518	1547±388	2440±444
Protein (% E)	16.8±2.8	16.6±2.5[b]	17.2±2.3	16.1±2.7	16.5±2.6	17.0±2.7	16.3±2.7	16.8±2.6[a]	16.1±2.9	16.9±2.50
Carbohydrate (% E)	50.1±6.7	53.1±6.3	44.5±5.3	52.5±6.2	47.2±5.4	56.8±6.4	45.3±6.6	51.6±5.5	46.6±7.1	50.1±5.54
Fat (% E)	32.9±5.6	29.7±5.3	35.4±4.4	27.2±5.0	36.4±4.0	25.0±3.6	33.9±5.5	28.5±5.4	32.2±6.1	30.4±5.08
Vitamin C (mg/d)	114±55.1	203±88.5	123±59.3	202±85.7	129±72.3	187±87.2	98.6±42.2	224±95.1	100±51.1	214±81.4
Vitamin D (µg/d)	2.32±1.2	3.00±1.52	2.88±1.40	2.50±1.23	3.00±1.77	2.30±1.11	2.34±1.28	2.87±1.65	1.88±0.99	3.28±1.53
Vitamin E (mg/d)	9.59±3.9	13.5±4.8	11.3±4.6	12.4±4.7	12.3±5.1	10.4±3.8	9.36±4.14	13.6±4.7	8.08±3.25	14.9±4.43
Magnesium (mg/d)	307±76.0	391±95.2	315±79.8	403±93.7	338±95.1	359±85.4	290±72.6	404±94.5	257±55.9	438±76.9
Potassium (mg/d)	3630±841	4426±1028	3750±913	4486±923	3988±1084	4055±920[b]	3417±799	4575±1021	3031±638	4980±870
Selenium (µg/d)	44.1±13.1	60.7±17.7	51.4±16.2	55.0±17.5	53.4±20.0	51.2±15.0	43.2±14.1	61.3±20.9	38.1±11.4	65.3±18.1
Carotene (µg/d)	2678±1523	4343±2197	3018±1499	4150±2214	3074±1652	3846±2221	2418±1343	4730±2431	2382±1380	4587±2029
Subset 2	**Q1 (2.6±0.6)[1]**	**Q4 (6.3±0.6)**	**Q1 (2.2±0.7)**	**Q4 (5.2±0.8)**	**Q1 (9.0±1.2)**	**Q4 (14.3±0.5)**	**Q1 (39.6±5.9)**	**Q4 (67.5±5.3)**	**Q1 (16.2±2.3)**	**Q4 (31.5±2.3)**
N	**262**	**232**	**231**	**185**	**246**	**167**	**250**	**220**	**241**	**201**
Total energy (kcal/d)	1698±501	2121±597	1879±610	2020±590[a]	2283±843	1712±379	1736±564	1981±607	1399±350	2560±740
Protein (% E)	17.9±3.0	17.3±2.5[a]	18.3±2.8	16.8±2.8	17.9±2.9	17.7±2.7[b]	17.5±2.8	17.7±2.8[b]	17.6±3.1	17.7±2.6[b]
Carbohydrate (% E)	45.6±7.2	48.3±5.6	43.3±5.9	51.8±6.0	42.9±6.0	51.4±5.9	45.6±6.8	48.9±5.9	45.7±6.7	48.3±5.6[a]
Fat (% E)	32.8±5.6	31.1±4.9[a]	35.8±4.5	27.6±4.6	36.8±4.6	26.0±3.5	33.0±5.6	29.9±5.1	32.2±5.6	31.4±5.0[b]
Vitamin C (mg/d)	137±65.9	214±89.4	143±68.7	218±97.2	168±100	197±86.4[a]	122±56.4	231±91.2	118±51.9	248±107
Vitamin D (µg/d)	2.17±1.04	3.00±1.40	2.72±1.43	2.51±1.25[b]	3.36±2.54	2.23±1.04	2.18±1.11	3.01±2.43	1.79±0.84	3.65±2.60
Vitamin E (mg/d)	9.65±4.27	13.7±5.4	11.2±5.1	12.4±5.2[b]	13.4±7.5	10.1±3.4	8.67±3.97	14.2±6.2	7.52±2.81	15.8±6.8
Magnesium (mg/d)	300±88.2	406±105	317±98.1	404±106	374±140	352±73.8[a]	286±80.4	408±109	250±57.5	472±116
Potassium (mg/d)	3523±968	4467±1090	3687±1052	4446±1159	4324±1520	3918±772	3394±895	4448±1119	2944±626	5302±1264
Selenium (µg/d)	41.3±12.7	61.9±21.4	49.4±16.6	54.6±20.4[b]	59.6±36.3	49.6±16.1	40.2±14.7	65.5±37.2	36.8±12.7	70.5±36.6
Carotene (µg/d)	3371±2021	5139±2933	3677±2389	4686±2665[a]	4316±3088	4109±2154[b]	2933±1620	5401±2660	2909±1657	5759±4658

[1] Values are means and standard deviations (SDs); P for trend < 0.001, except for those annotated as: [a)] P for trend between 0.0001 and 0.04, and [b)] P for trend > 0.05

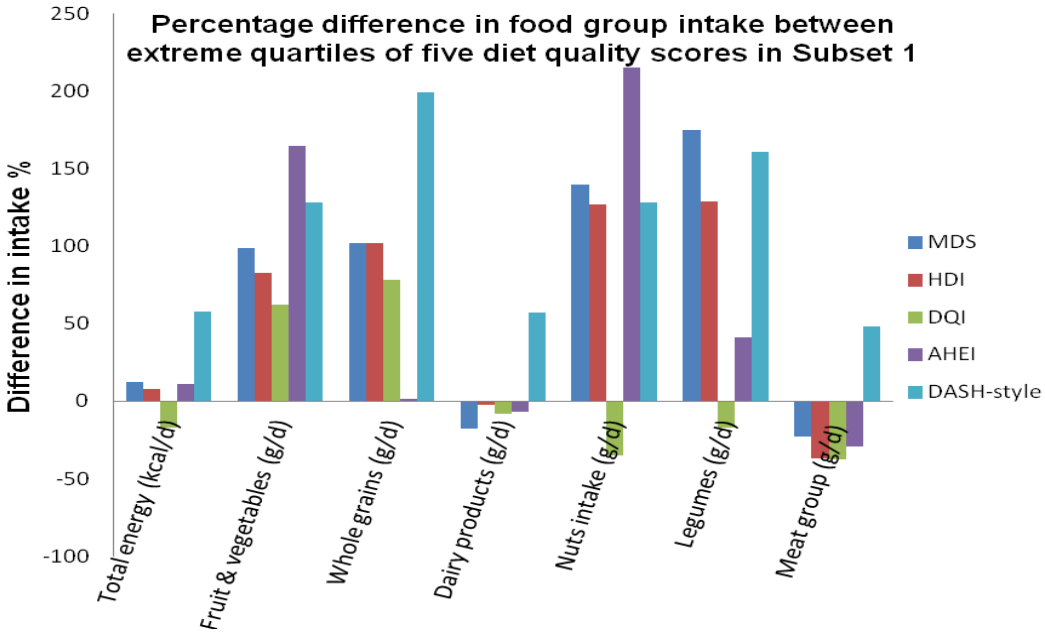


Figure 5.1: Percentage difference in intake of different food groups between extreme quartiles of the five derived diet scores in Subset 1 (*n*=2570 women) from the TwinsUK cohort. Values are differences in means (unadjusted) from quartile 4 to 1. Quartiles 1 and 4 differed significantly (P <0.05, ANOVA) for all variables except dairy products (HDI).

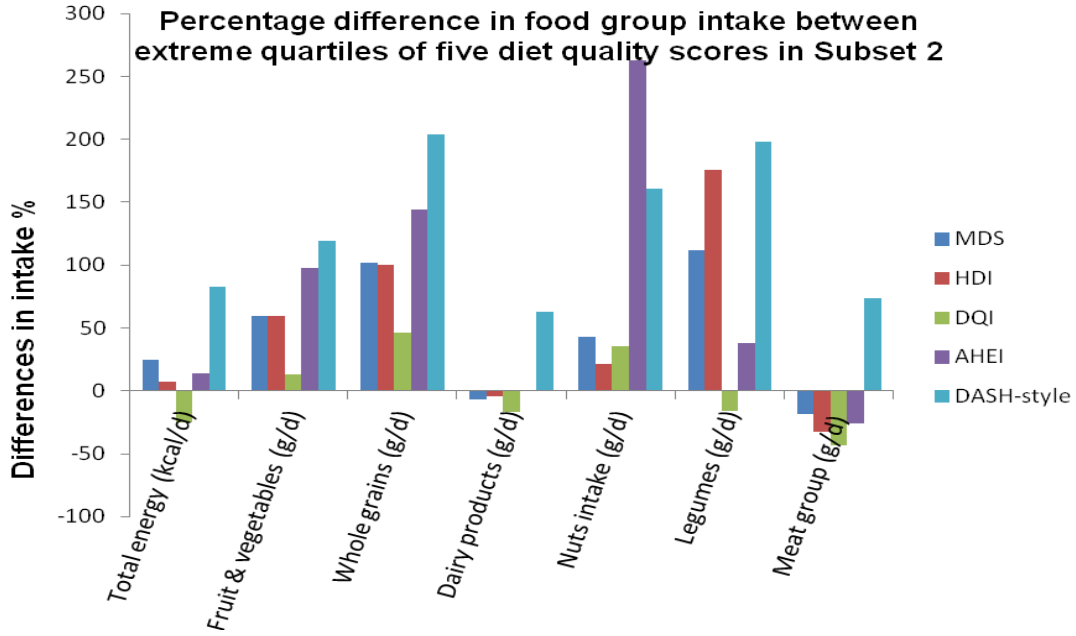


Figure 5.2: Percentage difference in intake of different food groups between extreme quartiles of the five derived diet scores in Subset 2 (*n*=949 women) from the TwinsUK cohort. Values are differences in means (unadjusted) from quartile 4 to 1. Quartiles 1 and 4 differed significantly (P <0.05, ANOVA) for all variables except dairy products (MDS, HDI, AHEI) and nuts (HDI).

Table 5.4 The associations between fat free mass (kg) and the derived diet scores quartiles in 2570 women from the TwinsUK cohort aged 18-79 years[1]

	Fat free mass (kg)				
Quartiles	**Q1**	**Q2**	**Q3**	**Q4**	**P for trend**
MDS (points)	2[3]	4	5	6	
Unadjusted	38.99±0.21	39.86±0.24	40.15±0.27	39.83±0.20	0.002
Adjusted model[2]	39.15±0.19	39.64±0.21	40.00±0.22	39.92±0.18	0.001
HDI (points)	2	3	4	5	
Unadjusted	39.53±0.24	39.61±0.21	39.62±0.22	39.88±0.24	0.313
Adjusted model[2]	39.45±0.21	39.49±0.18	39.65±0.19	40.11±0.22	0.023
DQI (points)	9	12	13	14	
Unadjusted	39.05±0.24	39.82±0.19	40.04±0.29	39.85±0.24	0.012
Adjusted model[2]	39.00±0.20	39.84±0.17	40.01±0.25	39.89±0.21	0.002
AHEI (points)	17	21	25	30	
Unadjusted	38.96±0.23	39.93±0.23	40.03±0.22	39.66±0.23	0.023
Adjusted model[2]	38.97±0.21	39.76±0.20	39.96±0.19	39.93±0.21	0.001
DASH-style (points)	36.5	49.5	54.5	63.5	
Unadjusted	39.18±0.23	39.25±0.23	39.66±0.22	40.50±0.23	<0.001
Adjusted model[2]	39.35±0.23	39.35±0.20	39.70±0.20	40.19±0.22	0.011

[1] Values are means and SEM for fat free mass (kg) by quartiles of the derived diet scores
[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking status (never, current, former), energy intake (kcal/d) (only for MDS, AHEI, and DASH-style), and total body mass (kg)
[3] Values are medians

Table 5.5 The associations between percentage fat free mass and the derived diet scores quartiles in 2570 women from the TwinsUK cohort aged 18-79 years[1]

	Percentage fat free mass				
Quartiles	**Q1**	**Q2**	**Q3**	**Q4**	**P for trend**
MDS (points)					
Unadjusted	60.90±0.27	60.74±0.31	61.11±0.29	61.52±0.26	0.057
Adjusted model[2]	60.92±0.25	60.59±0.28	61.07±0.26	61.62±0.24	0.018
HDI (points)					
Unadjusted	60.54±0.27	60.84±0.25	61.13±0.28	62.09±0.30	<0.001
Adjusted model[2]	60.67±0.26	60.83±0.22	61.14±0.25	61.93±0.28	0.001
DQI (points)					
Unadjusted	61.26±0.27	61.29±0.25	60.66±0.34	60.88±0.28	0.153
Adjusted model[2]	60.96±0.25	61.26±0.23	61.01±0.32	61.05±0.25	0.985
AHEI (points)					
Unadjusted	61.20±0.28	60.69±0.29	60.78±0.27	61.83±0.28	0.142
Adjusted model[2]	60.87±0.26	60.74±0.26	61.00±0.24	61.87±0.30	0.007
DASH-style (points)					
Unadjusted	61.28±0.28	60.86±0.28	61.32±0.26	60.91±0.30	0.580
Adjusted model[2]	61.17±0.29	61.05±0.27	61.25±0.24	60.89±0.29	0.679

[1] Values are means and SEM for percentage fat free mass by quartiles of the derived diet scores
[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking status (never, current, former), and energy intake (kcal/d) (only for MDS, AHEI, and DASH-style)

Table 5.6 The associations between fat free mass index (kg/m^2) and the derived diet scores quartiles in 2570 women from the TwinsUK cohort aged 18-79 years[1]

	Fat free mass index (kg/m^2)				
Quartiles	**Q1**	**Q2**	**Q3**	**Q4**	**P for trend**
MDS (points)					
Unadjusted	14.86±0.07	15.09±0.08	15.15±0.09	15.05±0.06	0.039
Adjusted model[2]	14.88±0.06	15.04±0.07	15.11±0.07	15.09±0.06	0.012
HDI (points)					
Unadjusted	15.00±0.08	14.99±0.07	15.06±0.07	15.06±0.07	0.451
Adjusted model[2]	14.95±0.07	14.95±0.06	15.07±0.06	15.15±0.07	0.015
DQI (points)					
Unadjusted	14.79±0.08	15.02±0.06	15.29±0.10	15.12±0.07	<0.001
Adjusted model[2]	14.79±0.07	15.03±0.05	15.26±0.09	15.13±0.07	<0.001
AHEI (points)					
Unadjusted	14.83±0.08	15.13±0.07	15.17±0.07	15.00±0.07	0.190
Adjusted model[2]	14.85±0.07	15.07±0.07	15.14±0.06	15.04±0.07	0.028
DASH-style (points)					
Unadjusted	14.87±0.07	14.95±0.07	15.00±0.08	15.27±0.07	<0.001
Adjusted model[2]	14.86±0.07	14.94±0.07	15.04±0.07	15.25±0.07	<0.001

[1] Values are means and SEM for fat free mass index by quartiles of the derived diet scores

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking status (never, current, former), energy intake (kcal/d) (only for MDS, AHEI, and DASH-style), and total body mass (kg)

Table 5.7 Relative associations between diet quality scores, lifestyle characteristics and fat free mass indices in 2570 women aged 18-79 years

	MDS			HDI			DQI			AHEI			DASH-style		
Fat free mass (kg)	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**
Diet score per quartile	0.27	0.08	0.001	0.22	0.09	0.017	0.28	0.09	0.002	0.31	0.09	0.001	0.28	0.11	0.012
Age (per 10 y)	-1.16	0.09	<0.001	-1.16	0.09	<0.001	-1.19	0.09	<0.001	-1.17	0.09	<0.001	-1.17	0.09	<0.001
Smoking habit															
Current v never	1.19	0.26	<0.001	1.09	0.26	<0.001	1.15	0.26	<0.001	1.21	0.26	<0.001	1.17	0.26	<0.001
Former v never	0.80	0.23	<0.001	0.79	0.23	0.001	0.78	0.23	0.001	0.83	0.23	<0.001	0.86	0.23	<0.001
Physical activity	0.71	0.15	<0.001	0.74	0.15	<0.001	0.74	0.15	<0.001	0.67	0.15	<0.001	0.70	0.15	<0.001
Energy intake (per 1000 cal)	0.59	0.19	0.002	*Not adjusted for energy intake*						0.66	0.19	0.001	0.37	0.24	0.114
Total body mass (kg)	0.32	0.02	<0.001	0.33	0.02	<0.001	0.32	0.02	<0.001	0.32	0.02	<0.001	0.32	0.02	<0.001
Percentage fat free mass															
Diet score per quartile	0.26	0.11	0.016	0.41	0.11	<0.001	0.009	0.11	0.934	0.32	0.12	0.007	-0.07	0.15	0.637
Age (per 10 y)	-1.85	0.12	<0.001	-1.84	0.11	<0.001	-1.85	0.12	<0.001	-1.88	0.12	<0.001	-1.86	0.12	<0.001
Smoking habit															
Current v never	1.11	0.34	0.001	1.11	0.34	0.001	1.06	0.34	0.002	1.12	0.34	0.001	1.00	0.34	0.004
Former v never	-0.08	0.29	0.786	-0.05	0.29	0.855	-0.03	0.29	0.909	-0.05	0.29	0.863	-0.04	0.29	0.895
Physical activity	1.38	0.19	<0.001	1.37	0.19	<0.001	1.40	0.19	<0.001	1.34	0.19	<0.001	1.39	0.19	<0.001
Energy intake (per 1000 cal)	-0.06	0.25	0.801	*Not adjusted for energy intake*						0.02	0.25	0.928	0.20	0.32	0.526
Fat free mass index (kg/m^2)															
Diet score per quartile	0.07	0.03	0.012	0.07	0.03	0.013	0.11	0.03	<0.001	0.07	0.03	0.028	0.12	0.04	0.001
Age (per 10 y)	-0.18	0.03	<0.001	-0.19	0.03	<0.001	-0.19	0.03	<0.001	-0.19	0.03	<0.001	-0.19	0.03	<0.001
Smoking habit															
Current v never	0.41	0.08	<0.001	0.40	0.08	<0.001	0.42	0.08	<0.001	0.41	0.08	<0.001	0.42	0.08	<0.001
Former v never	0.18	0.08	0.018	0.19	0.08	0.015	0.18	0.08	0.018	0.19	0.08	0.012	0.20	0.08	0.009
Physical activity	0.18	0.05	0.001	0.18	0.05	<0.001	0.18	0.05	0.001	0.17	0.05	0.001	0.17	0.05	0.001
Energy intake (per 1000 cal)	0.06	0.06	0.387	*Not adjusted for energy intake*						0.07	0.07	0.276	-0.08	0.08	0.340
Total body mass (kg)	0.10	0.006	<0.001	0.11	0.006	<0.001	0.10	0.006	<0.001	0.11	0.006	<0.001	0.10	0.006	<0.001

Table 5.8 The associations between hand grip strength (kg) and the derived diet scores quartiles in 949 women from the TwinsUK cohort aged 34-83 years[1]

	Hand grip strength (kg)				
Quartiles	**Q1**	**Q2**	**Q3**	**Q4**	**P for trend**
MDS (points)					
Unadjusted	28.49±0.40	28.37±0.40	28.27±0.44	29.93±0.41	0.023
Adjusted model[2]	29.03±0.33	28.46±0.33	28.24±0.37	29.26±0.34	0.824
HDI (points)					
Unadjusted	28.60±0.41	28.51±0.38	29.30±0.41	28.72±0.41	0.441
Adjusted model[2]	29.00±0.33	28.65±0.30	29.07±0.36	28.24±0.37	0.292
DQI (points)					
Unadjusted	28.73±0.43	28.53±0.32	29.54±0.56	28.59±0.47	0.723
Adjusted model[2]	28.88±0.34	28.64±0.25	29.12±0.50	28.52±0.41	0.758
AHEI (points)					
Unadjusted	28.36±0.39	29.22±0.41	28.36±0.41	29.10±0.45	0.458
Adjusted model[2]	28.62±0.33	29.32±0.34	28.45±0.35	28.60±0.38	0.557
DASH-style (points)					
Unadjusted	28.62±0.42	28.47±0.41	29.43±0.41	28.36±0.39	0.796
Adjusted model[2]	29.01±0.39	28.63±0.36	29.08±0.33	28.18±0.42	0.442

[1] Values are means and SEM for hand grip strength (kg) by quartiles of the derived diet scores

[2] Adjusted for age (years), physical activity (inactive, moderate active, active), current smoking (yes/no), energy intake (kcal/d) (only for MDS, AHEI and DASH-style), menopausal status (premenopausal/postmenopausal) and HRT use (yes/no)

Table 5.9 The associations between muscle quality (kg/kg) and the derived diet scores quartiles in 949 women from the TwinsUK cohort aged 34-83 years[1]

	Muscle quality (kg/kg)				
Quartiles	**Q1**	**Q2**	**Q3**	**Q4**	**P for trend**
MDS (points)					
Unadjusted	0.69±0.01	0.68±0.01	0.67±0.01	0.71±0.01	0.202
Adjusted model[2]	0.70±0.01	0.68±0.01	0.67±0.01	0.70±0.01	0.937
HDI (points)					
Unadjusted	0.68±0.01	0.69±0.01	0.70±0.01	0.69±0.01	0.457
Adjusted model[2]	0.69±0.01	0.69±0.01	0.70±0.01	0.67±0.01	0.337
DQI (points)					
Unadjusted	0.69±0.01	0.69±0.01	0.70±0.01	0.68±0.01	0.933
Adjusted model[2]	0.69±0.01	0.69±0.01	0.70±0.01	0.68±0.01	0.516
AHEI (points)					
Unadjusted	0.68±0.01	0.69±0.01	0.68±0.01	0.70±0.01	0.384
Adjusted model[2]	0.68±0.01	0.70±0.01	0.68±0.01	0.69±0.01	0.765
DASH-style (points)					
Unadjusted	0.70±0.01	0.69±0.01	0.70±0.01	0.67±0.01	0.150
Adjusted model[2]	0.70±0.01	0.69±0.01	0.70±0.01	0.66±0.01	0.034

[1] Values are means and SEM for hand grip strength (kg) by quartiles of the derived diet scores

[2] Adjusted for age (years), physical activity (inactive, moderate active, active), current smoking (yes/no), energy intake (kcal/d) (only for MDS, AHEI and DASH-style), menopausal status (premenopausal/postmenopausal) and HRT use (yes/no)

Table 5.10 Relative associations between diet quality scores, lifestyle characteristics and muscle strength and muscle quality in 949 women aged 34-83 years

	MDS			HDI			DQI			AHEI			DASH-style		
	ß	**SEM**	**P**	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**
Hand grip strength (kg)															
Diet score per quartile	0.03	0.15	0.826	-0.16	0.16	0.294	-0.05	0.17	0.749	-0.09	0.16	0.558	-0.16	0.21	0.440
Age (per 10 y)	-2.70	0.22	<0.001	-2.70	0.22	<0.001	-2.70	0.22	<0.001	-2.70	0.22	<0.001	-2.70	0.22	<0.001
Current smoking															
yes v no	-0.25	0.57	0.665	-0.33	0.57	0.565	-0.28	0.57	0.624	-0.30	0.57	0.600	-0.29	0.57	0.616
Physical activity	0.49	0.21	0.020	0.49	0.21	0.018	0.49	0.21	0.018	0.51	0.21	0.018	0.51	0.21	0.016
Height (cm)	0.27	0.03	<0.001	0.27	0.03	<0.001	0.27	0.03	<0.001	0.27	0.03	<0.001	0.27	0.03	<0.001
Energy intake (per 1000 cal)	-0.03	0.27	0.912	*Not adjusted for energy intake*						0.01	0.27	0.971	0.16	0.35	0.647
Menopausal status															
postmenopausal v premenopausal	0.75	0.70	0.281	0.75	0.69	0.278	0.76	0.70	0.277	0.76	0.70	0.278	0.75	0.70	0.283
HRT															
yes v no	-0.29	0.60	0.630	-0.31	0.60	0.603	-0.29	0.59	0.623	-0.29	0.59	0.621	-0.27	0.59	0.647
Muscle quality (kg/kg)															
Diet score per quartile	0.0003	0.004	0.934	-0.004	0.004	0.339	-0.003	0.004	0.507	0.001	0.004	0.763	-0.01	0.005	0.034
Age (per 10 y)	-0.06	0.006	<0.001	-0.06	0.006	<0.001	-0.06	0.006	<0.001	-0.06	0.006	<0.001	-0.06	0.006	<0.001
Current smoking															
yes v no	0.00001	0.01	0.999	-0.002	0.01	0.909	-0.001	0.01	0.928	0.0007	0.01	0.962	-0.002	0.01	0.887
Physical activity	0.008	0.005	0.159	0.008	0.005	0.156	0.008	0.005	0.154	0.007	0.005	0.186	0.009	0.005	0.102
Height (cm)	-0.0006	0.0009	0.465	-0.0007	0.0009	0.434	-0.0006	0.0009	0.455	-0.0006	0.0009	0.459	-0.0006	0.0009	0.482
Energy intake (per 1000 cal)	-0.003	0.007	0.668	*Not adjusted for energy intake*						-0.003	0.007	0.622	0.009	0.009	0.348
Menopausal status															
postmenopausal v premenopausal	0.02	0.02	0.264	0.02	0.02	0.255	0.02	0.02	0.249	0.02	0.02	0.268	0.02	0.02	0.259

HRT														
yes v no	-0.002	0.01	0.887	-0.002	0.01	0.866	-0.002	0.01	0.884	-0.002	0.01	0.881	-0.001	0.01	0.958

Chapter 6

Associations between micronutrients, protein, essential amino acid intakes and C-reactive protein levels

6.0 Introduction

Inflammation and inflammatory cytokines are associated with muscle loss, as described in Chapter 1 (section 1.4, p. 32) and C-reactive protein (CRP) is the most commonly studied marker of inflammation (Heinrich *et al.* 1990). Diet is one factor that plays a major role in the regulation of chronic inflammation as dietary constituents have the potential to exert anti-inflammatory as well as pro-inflammatory effects (Cavicchia *et al.* 2009). Diverse dietary components, such as vitamins C, E and D, magnesium, potassium and selenium, and carotenoids exert anti-inflammatory effects via a range of mechanisms including decreasing inflammatory mediator production through effects on cell signalling and gene expression and by reducing production of oxidants (Calder *et al.* 2009). Therefore, the relationship between a range of dietary factors and C-reactive protein was investigated in the TwinsUK cohort.

Nutrition may contribute to the regulation of the uncontrolled inflammatory processes, help with the maintenance and control of homeostasis and potentially reduce the risk of disease (Calder *et al.* 2009). Vitamin C, in the form of ascorbic acid, which is its functional form, is involved in the inflammatory process and immune mechanisms possibly through its ability to scavenge a variety of reactive oxygen and nitrogen species (Carr and Frei 1999). Vitamin E acts mainly in the lipid phase by interrupting lipid and especially unsaturated fatty acids' peroxidation (Kamal-Eldin and Appelqvist 1996). The constituents of vitamin E are α- and γ-tocopherol, which have different functions. Specifically, α-tocopherol acts as an

inhibitor of lipid peroxidation and protects LDL particles from oxidation (Winklhofer-Roob *et al.* 2003). However, γ-tocopherol can decrease markers of oxidative stress, such as LDL oxidation and superoxides and is potentially more effective than α-tocopherol (Liu *et al.* 1999, Saldeen *et al.* 1999). In relation to vitamin D, there is evidence that 1-alpha, 25-Dihydroxyvitamin D(3) [1,25-(OH)(2)D(3)], the active metabolite of vitamin D(3) acts as an inhibitor of the inflammatory response (Nagpal *et al.* 2005). Moreover, low vitamin D concentrations have been associated with an increased risk of developing rheumatoid arthritis (Hillman *et al.* 1994), multiple sclerosis (Munger *et al.* 2004) and type I diabetes (Mathieu and Badenhoop 2005).

Although there is currently limited evidence for an anti-inflammatory effect of potassium intake in humans, there is evidence of the anti-inflammatory effect of three potassium salts of N,N-disubstituted 4-aminoazobenzenesulfonic acids in rats with adjuvant arthritis (Kazemekaite *et al.* 2008). Magnesium is an essential micronutrient which acts as a cofactor in multiple enzymatic reactions in the human body. Notably, there are a number of studies indicating an inverse association between dietary magnesium intake and metabolic and inflammatory conditions, such as hypertension (Song *et al.* 2006), metabolic syndrome (He *et al.* 2006), insulin resistance (Paolisso and Barbagallo 1997), type 2 diabetes (Song *et al.* 2004), dyslipidemia (Song *et al.* 2005), and cardiovascular disease (Al-Delaimy *et al.* 2004). These findings are supported by experimental data from animal studies indicating that dietary magnesium deficiency may

induce an inflammatory response (Malpuech-Brugere *et al.* 2000). Although there are findings from observational and animal studies, the underlying biological mechanisms for such metabolic effects of magnesium are not yet entirely clear. Also, selenium is a cofactor of peroxidases and detoxifies lipid peroxides, instead of scavenging free radicals or interrupting peroxidation chain reactions (Paolisso *et al.* 1999) as other nutrients with potential anti-inflammatory action do. This may have a different impact on blood CRP concentrations in response to dietary intake of selenium, which remains to be elucidated in *in vitro* and *in vivo* studies. Moreover, selenium may also inhibit the activation of the nuclear factor kappa-B (NF-*k*B), which is a signaling pathway that has been associated with increased inflammatory response and its activation has been associated with the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF- α) (Duntas 2009).

Dietary carotenoids may also scavenge free radicals, which are produced intracellularly during the oxidative processes and particularly α- and β-carotene are part of cell membranes constituted of two lipid layers (Olson 1999). Consequently, lower levels of these carotenoids can result to increased lipid oxidation and potentially to modulation of inflammation (Blackwell and Christman 1997, Kontush *et al.* 2000).

In addition, it has been recently suggested that the pro-inflammatory status of muscle cells, observed in ageing but also in pathological conditions (such as cancer and sepsis) could be modulated by the branched-chain amino acids

(BCAAs) particularly leucine, which has been suggested to enhance protein translation initiation and thus attenuates proteolysis, contributing to negative protein turnover (Nicastro *et al.* 2012). Moreover, under inflammatory conditions, leucine may indirectly attenuate inflammation through transamination to glutamate and further activation of glutamine synthesis (a conditionally essential amino acid) (Nicastro *et al.* 2012). This may be explained by the fact that glutamine may play a major role on the NF-*k*B signaling pathway, attenuating local inflammation (Fillmann *et al.* 2007, Hubert-Buron *et al.* 2006). Glutamine is a substrate that is used by inflammatory cells, such as macrophages, under pathological conditions (Pithon-Curi *et al.* 2004). However, this process usually occurs only in situations of increased need of glutamine synthesis. Arginine plays an important role in multiple metabolic processes, including modulation of immune function (Wu and Morris 1998). Arginine is also considered as a conditionally essential amino acid as the endogenous synthesis of arginine may not be sufficient to meet the metabolic needs of the organism, particularly in infants and children during growth (Wu *et al.* 2004), and in acute catabolic conditions, including sepsis (Argaman *et al.* 2003) and burns (Yu *et al.* 1996). It is noteworthy that dietary protein and essential amino acid intakes play a major role in various chronic (such as cancer and chronic obstructive pulmonary disease) and acute disease (such as sepsis and burn injury) states, where inflammation is present and protein catabolism is enhanced (Jonker *et al.* 2012). These diseases are related to low protein synthesis, loss of body protein pool and enhanced skeletal muscle wasting. Therefore, it has been argued that modifying the protein and essential amino

acid content of the diet may positively affect the balance of muscle protein synthesis and breakdown, and may activate muscle protein anabolism in chronic and acute disease states (Eley *et al.* 2007, Jonker *et al.* 2012, Peters *et al.* 2011).

The most common studied marker of inflammation is C-reactive protein (*CRP*), which is an acute phase protein and which increases dramatically during an acute phase response in man (Heinrich *et al.* 1990). CRP is synthesised in the liver, and may be mediated by a number of cytokines, but mainly IL-6 (Shoelson *et al.* 2007). In addition to liver derived CRP, data have shown that adipose tissue itself may contribute to obesity associated increases in CRP levels (Berg and Scherer 2005). It has also been reported that CRP levels are higher in obese individuals who are insulin resistant and decrease with weight loss and improvement in insulin sensitivity (Kopp *et al.* 2003, McLaughlin *et al.* 2002). Conversely, IL-6 production by human adipose tissue increases with obesity and may induce hepatic CRP synthesis and promote the onset of insulin resistance (Bastard *et al.* 2006, de Luca and Olefsky 2008). The association between elevated levels of circulating CRP and increased cardiovascular risk from observational data is quite robust with more than 20 prospective studies, to date, providing evidence that high-sensitivity CRP is an independent predictor of a number of CVD events, such as myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death, even in apparently healthy subjects (Calabrò *et al.* 2009).

Although several observational studies have examined associations between plasma/serum nutrient levels (Boekholdt *et al.* 2006, Erlinger TP *et al.* 2001, Ford *et al.* 2003, Kritchevsky *et al.* 2000, van Herpen-Broekmans *et al.* 2004, Walston *et al.* 2006, Wang *et al.* 2008, Wannamethee *et al.* 2006), intake of supplements with potential antioxidant properties (Chacko *et al.* 2011, Scheurig *et al.* 2008) and different inflammatory markers, including CRP, there are few studies to date which have examined associations between habitual dietary intake and the inflammatory marker CRP (Chacko *et al.* 2010, de Oliveira Otto *et al.* 2011, Floegel *et al.* 2011, Helmersson *et al.* 2009, Song *et al.* 2007). It is thought that nutrient biomarkers may provide a stronger association with disease outcomes than those based on dietary assessments due to measurement errors occurring with dietary questionnaires (Giovannucci 2013). However, it is also argued that blood concentrations of nutrients may reflect other exposures (such as smoking and alcohol consumption) (Dietrich *et al.* 2003), and genetic , physiological, metabolic (Lee *et al.* 2012) and pathophysiological processes (Giovannucci 2013, Zhang *et al.* 2012). Therefore, in order to investigate the beneficial health effects of nutrients with potential anti-inflammatory properties it is also important that epidemiological studies examine associations between the habitual intake of specific nutrients and markers of disease outcomes, such as CRP.

One previous prospective study among 704 men from the Uppsala Longitudinal Study of Adult Men (ULSAM) aged 70 years at baseline found that dietary intake of ascorbic acid and α-tocopherol were inversely

associated with plasma high-sensitive CRP (hsCRP) at baseline and after 7 years of follow-up. However, higher β-carotene intake was not associated with hsCRP at baseline and the authors do not provide any data of associations at follow-up (Helmersson *et al.* 2009). Four cross-sectional studies have examined associations between the habitual dietary intake of different nutrients including vitamins C and E, total carotene, β-carotene, selenium and magnesium and blood CRP concentrations (Chacko *et al.* 2010, de Oliveira Otto *et al.* 2011, Floegel *et al.* 2011, Song *et al.* 2007). The Multiethnic-Ethnic Study of Atherosclerosis (MESA) among 5181 men and women aged 45 to 84 years found no significant associations between vitamins C and E, magnesium and β-carotene intakes and the inflammatory marker CRP (de Oliveira Otto *et al.* 2011). However, in the National Health and Nutrition Examination Survey (NHANES) among 8335 US adults aged ≥ 19 years old, it was observed that higher vitamin C, E, and total carotene intakes were inversely associated with the odds of increased serum CRP concentrations, after adjustment for age, gender, ethnicity, total energy intake and BMI (Floegel *et al.* 2011). Also, higher habitual magnesium intake has been inversely associated with plasma CRP concentrations in women from both the Nurse's Health Study (NHS) cohort (Song *et al.* 2007) and the Women's Health Initiative Observational Study (WHI-OS) cohort (Chacko *et al.* 2010) who were aged 43 to 69 years and 50 to 79 years, respectively. However, to our knowledge, there are currently no studies examining associations between a more complete range of nutrients and protein and essential amino acids with potential anti-inflammatory properties and the global inflammatory marker of CRP.

6.1 Aims

The aims were to test the hypothesis that habitual dietary intake of a range of micronutrients with the potential to act against oxidative stress and with potential anti-inflammatory properties (shown in **Table 6.1**) are inversely associated with C-reactive protein (*CRP*) levels, among women aged 18-79 years from the TwinsUK cohort. Additionally, to examine the hypothesis that habitual dietary intake of protein and essential amino acids, which have been previously shown to influence inflammation, are inversely associated with C-reactive protein (*CRP*) in the same subset of the TwinsUK cohort. In addition to the nine essential amino acids (histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) two conditionally essential amino acids were included (arginine and glutamine) as shown in **Table 6.1**, as they were previously shown to modulate inflammation (section 6.0, p. 198).

The nutrients included are presented in (**Table 6.1**).

Table 6.1 Nutrients included in the analysis

Vitamins	***Minerals***	***Carotenoids***	***Macronutrients***	***Amino acids***
Vitamin C (mg/d)	Magnesium (mg/d)	α-carotene (µg/d)	Protein (% En/d)	Arginine (% En/d)
Vitamin E (mg/d)	Potassium (mg/d)	ß-carotene (µg/d)		Glutamine (% En/d)
Vitamin D (µg/d)	Selenium (µg/d)	Total carotene (µg/d)		Histidine (% En/d)
		ß-cryptoxanthin (µg/d)		Isoleucine (% En/d)
		Lycopene (µg/d)		Leucine (% En/d)
		Lutein (µg/d)		Lysine (% En/d)
		Zeaxanthin (µg/d)		Methionine (% En/d)
				Phenylalanine (% En/d)
				Threonine (% En/d)
				Tryptophan (% En/d)
				Valine (% En/d)

6.2 Covariate plan and statistical analysis

All analyses were performed using STATA statistical software (version 11.0; STATA Corp, USA). Means and standard deviations were calculated (**Table 6.2**) and quintiles of each micronutrient, protein and essential amino acid intakes were derived. The analyses were performed treating twins as individuals as previous studies have shown that participants from the TwinsUK registry were similar to age-matched singleton women in the distribution of common traits and outcomes (Andrew *et al.* 2001). Data on dietary intake (FFQ), body composition parameters, and other covariates were available for n = 1658 women, aged 18 to 79 years representing 582 monozygotic (MZ) and 1076 dizygotic (DZ) females who had completed a food frequency questionnaire (FFQ) and had fasting blood samples taken to measure C-reactive protein (*CRP*) between 1996 and 2000 (Moayyeri *et al.* 2012, Teucher *et al.* 2007).

Prior to conducting statistical analysis, an analysis plan with justification for each confounding factor in the multivariate model was developed as described in Chapter 2, section 2.2.5, p. 57. Multivariate regression analysis was used to assess associations between micronutrient, protein and essential amino acid intakes and plasma CRP concentrations using the robust cluster regression option in STATA. These models take into account clustering of individuals when calculating standard errors of the mean (Richards *et al.* 2007) to ensure familial aggregation within twin pairs was accounted for. The plasma concentration of CRP was transformed and analyzed on the natural logarithmic scale in order to meet the criterion of normality for the

linear models. Values were subsequently back transformed into geometric means and 95% confidence intervals (CI) for presentation. Amino acid intakes were expressed as percentage of energy intake rather than total intakes to enable comparisons between people with different total food and energy intakes.

As ageing has been strongly associated with an increased low-grade inflammatory status all analyses were adjusted for age (years) (Chung *et al.* 2009). Models were also adjusted for physical activity and smoking status, as physical activity has been suggested to exert anti-inflammatory properties (Gleeson *et al.* 2011, Petersen and Pedersen 2005) and smoking has been suggested to increase inflammation (Lee *et al.* 2012). Energy intake (kcal/d) was also included in the multivariate models. Additional adjustments were made for body mass index (kg/m^2), hormone replacement therapy use (HRT) and anti-inflammatory medication (including statins, non-steroid anti-inflammatory drugs and aspirin) (no or yes, respectively) as these factors have been previously shown to affect inflammatory status and may mediate the relationship between diet quality and CRP (Chapter 2, section 2.2.5, p. 64, 66, 68) (**Tables 6.3 and 6.4**).

6.3 Results

Descriptive characteristics of this subset (*n* = 1658 women, aged 18-79 years) of the TwinsUK participants are presented in **Table 6.2**. The mean age (±SD) of participants was 49.7 ± 12.5 years and mean (±SD) BMI was 24.9 ± 4.0 kg/m^2. Mean (±SD) plasma CRP concentrations were 2.49 ± 2.30

mg/L. More than one-half of the participants were moderately active (54.8 %), and 16.7 % were current smokers. Relatively few participants were taking anti-inflammatory medication (6.2 %) or hormone replacement therapy (6.3 %). Mean (±SD) energy intake was 1976 ± 512 kcal/d (**Table 6.2**).

Micronutrient intake and plasma CRP concentrations (mg/L)

In multivariate analyses, a higher magnesium intake was associated with lower plasma CRP concentrations with a difference in CRP of 0.46 mg/L between the highest [mean (SD): 480 (61) mg/d] and lowest [mean (SD): 232 (30) mg/d] quintiles of intake (P for difference < 0.051). No other statistically significant associations were observed for the other nutrients although there were trends towards lower CRP with higher nutrient intake with vitamin C, vitamin E and potassium (**Table 6.3**).

Protein, amino acid intake and plasma CRP concentrations (mg/L)

In the multivariate model, a higher intake of arginine, glutamine, histidine, isoleucine, threonine and tryptophan (as % energy) was associated with higher plasma CRP concentrations with a difference of 0.3 mg/L between extreme quintiles of intake (P for difference < 0.05 for arginine, glutamine, threonine and tryptophan; and P = 0.05 for histidine and isoleucine). There were also trends towards significance for leucine, lysine, methionine and valine in the same direction as the other amino acids. No associations were observed for protein and phenylalanine although the trends were in the same direction as the other amino acids (**Table 6.4**).

6.4 Discussion

In this study using a large cross-sectional population-based sample of women aged 18-79 years we evaluated the association between a range of micronutrients, protein and essential amino acids and the inflammatory marker CRP. This is one of the first cross-sectional studies to examine a range of micronutrients and CRP. It is also the first population-based study to examine associations between habitual dietary intake of protein and essential amino acids in association with a potential predictor of sarcopenia risk in a cohort of women across a wide age range. This study observed that higher consumption of magnesium was associated with lower plasma levels of CRP. It was also observed that higher intakes of arginine, glutamine, histidine, isoleucine, threonine and tryptophan were positively associated with mean plasma levels of CRP. A few previous studies have evaluated the associations between the habitual dietary intake of nutrients including vitamins C and E, total carotene, β-carotene (de Oliveira Otto *et al.* 2011, Floegel *et al.* 2011, Helmersson *et al.* 2009), and magnesium (Chacko *et al.* 2010, Song *et al.* 2007) and the inflammatory marker CRP, but they did not include a full range of micronutrients and carotenoids with potential anti-inflammatory properties. In addition, there are currently no studies evaluating the influence of protein and essential amino acid intakes on inflammation.

Micronutrient intake and plasma CRP concentrations (mg/L)

The current study observed a significant inverse association between higher intake of magnesium and the inflammatory marker CRP. The magnitude of

the association was a difference of 0.46 mg/L or 25 % in plasma geometric mean CRP (P = 0.051) between the highest [mean (SD): 480 (61) mg/d] and the lowest [mean (SD): 232 (30) mg/d] quintiles of intake. This association was observed after adjustments for age, physical activity, smoking, energy intake, anti-inflammatory medication (including aspirin, statins, and NSAID) and HRT use and was independent of adiposity (assessed by BMI).

Findings were of a similar magnitude to a previous cross-sectional study reporting that higher habitual intake of magnesium was inversely associated with plasma concentrations of CRP by 24 % (P = 0.03) among women from the Nurse's Health Study (NHS) cohort aged 43 to 69 years (Song *et al.* 2007). Findings from the current analysis were also of a similar trend, although of a lower magnitude, to another previous cross-sectional study among a multiethnic sample of postmenopausal women from the Women's Health Initiative Observational Study (WHI-OS), aged 50 to 79 years (Chacko *et al.* 2010). The study by Chacko *et al*. observed an inverse association between dietary magnesium intake and plasma concentrations of high-sensitive CRP (hsCRP) with a difference of 30 % between highest and lowest quintiles of intake (P = 0.005). The difference between the TwinsUK study and that of Chacko *et al*. may be explained by the fact that the women were in a narrower age range with more people in the older age groups compared to the current cohort (18-79 years) and low-grade chronic inflammation is known to increase with age (Chung *et al.* 2009, Khansari *et al.* 2009, Woods *et al.* 2012). Moreover, women from the WHI-OS cohort were generally more overweight compared to women from the current

analysis. Interestingly, when stratified by race/ethnicity, mean BMI in the WHI-OS cohort was 29 kg/m^2 in white women, 31 kg/m^2 in African American, and 29 kg/m^2 in Hispanic/Latino women (Chacko *et al.* 2010) compared to the current analysis where mean BMI (24.9 kg/m^2) was within the normal range. It is well established that the adipose tissue secretes a number of pro-inflammatory cytokines, such as interleukin-6 (IL-6), which further triggers the hepatic synthesis of CRP (Santos *et al.* 2005, Tilg and Moschen 2006, You *et al.* 2008). Also, it has been shown *in vitro* that mature adipocytes isolated from human adipose tissue were directly involved in the release of CRP (Calabro *et al.* 2005). In addition, mean CRP in the WHI-OS cohort was almost two times higher (5.01 mg/L in white women, 5.91 mg/L in African American and 4.66 mg/L in Hispanic/Latino women) compared to mean CRP in women from the current analysis (2.49 mg/L). These data suggest that higher magnesium intake may be more beneficial among populations with higher plasma CRP concentrations who are predisposed to systemic inflammation.

In the current study we did not observe any association between habitual intakes of vitamins C and E and plasma CRP concentrations, although there are only two previous epidemiological studies (Floegel *et al.* 2011, Helmersson *et al.* 2009) that observed an inverse association. One of these studies was a prospective study among 704 men which observed that dietary intakes of ascorbic acid and α-tocopherol were inversely associated with plasma CRP concentrations over 7 years follow-up (β coefficient = - 0.14 and – 0.15, $P < 0.001$, respectively) after adjustments for BMI, diabetes,

hyperlipideamia, hypertension and smoking (Helmersson *et al.* 2009). However, the authors do not provide any information about the mean age of participants or about the magnitude of association when plasma CRP concentrations were analysed across quartiles of ascorbic acid and α-tocopherol at follow-up. Also, the analysis was conducted among men and it therefore does not allow a direct comparison with our female study. Of interest, the study from Helmersson *et al*. used a 7-day pre-coded food record to assess dietary intake when participants were 70 years old and data on plasma CRP were taken 7 years later. Therefore, dietary assessment and inflammatory status assessment were conducted at different time points, which may introduce error.

The second study was a cross-sectional examination among 8335 women (50.6 %) and men aged ≥ 19 years from the National Health and Nutrition Examination Survey (NHANES) 1999-2002 (Floegel *et al.* 2011). Results from this study showed that intakes of vitamins C and E were inversely associated with the probability of having serum CRP concentrations more than 3 mg/L in multivariate logistic regression models adjusted for age, gender, ethnicity, total energy intake and BMI (OR = 0.74, 95% CI 0.62, 0.88, P < 0.001). Moreover, intakes of vitamins C and E were assessed by one 24h dietary recall in comparison to the current study that used an FFQ, which reflects the habitual dietary intake over a period of time and hence there were differences in the mean intakes of vitamin C and E between the two studies. Of interest the median vitamin C intake in the first quintile of intake was 11.7 mg/d in the Floegel *et al*. study (Floegel *et al.* 2011)

compared to 72.3 mg/d in the current analysis. Although our cohort adjusted for a number of covariates that may mediate the association between intakes of vitamins C and E and the inflammatory marker CRP, the findings did not reach statistical significance. It should be acknowledged that women from the NHANES cohort had almost two-fold higher mean serum CRP concentrations (4.88 mg/L) compared to our cohort (2.49 mg/L) were also more overweight (mean BMI = 27.9 kg/m^2) compared to our analysis (mean BMI = 24.9 kg/m^2).

The current analysis did not also observe any associations between habitual intake of selenium and plasma CRP concentrations. These findings were supported by one previous cross-sectional analysis in the NHANES cohort among men and women aged 19 years and over, in which selenium intake was not associated with CRP concentrations (Floegel *et al.* 2011). Interestingly, selenium is a cofactor of peroxidases and detoxifies lipid peroxides, instead of scavenging free radicals or interrupting peroxidation chain reactions (Paolisso *et al.* 1999) as other nutrients with potential anti-inflammatory action do. This may have a different impact on blood CRP concentrations in response to dietary intake of selenium, which remains to be elucidated in *in vitro* and *in vivo* studies. However, it has been suggested that selenium supplementation in chronic inflammatory conditions may restore the depleted hepatic and serum selenium levels by increasing the biosynthesis of selenoproteins leading to suppression of CRP production and attenuating the inflammatory process (Duntas 2009). The lack of associations in the current study may be because the selenium content of

foods varies depending on the soil content and other environmental conditions (especially the quantity and species of selenium to which the animal/plant is exposed) (Fairweather-Tait *et al*. 2010). This affects the form of selenium present in plant and animal sources and may impact on the absorption and use of selenium and consequently influence selenium's protective role in inflammation (Rederstorff *et al.* 2006). Also, food composition data may not reflect true selenium composition.

In relation to carotenoids, findings from the current analysis showed an inverse association between higher intake of a range of carotenoids and plasma CRP concentrations although the association was not statistically significant. Results from previous epidemiological studies are also contradictory. One previous prospective study among 704 men from the Uppsala Longitudinal Study of Adult Men (ULSAM) did not find an association between β-carotene and the inflammatory marker CRP both at baseline and after 7 years of follow-up (Helmersson *et al.* 2009). However, in a cross-sectional analysis among men and women aged 19 years and over from the NHANES cohort, total carotene intake was inversely associated with the odds of having elevated serum CRP concentrations after adjustments for age, gender, ethnicity, total energy intake and BMI (OR = 0.70, 95% CI 0.56, 0.87, $P < 0.05$) (Floegel *et al.* 2011).

The authors from the ULSAM study do not offer any information about the mean CRP concentrations and the study is only in men, thus comparisons with our study or the NHANES study are not possible. Findings from the current analysis were different from the study of Floegel *et al*. and this

might be due to the fact that intake of total carotene was assessed by a 24h dietary recall, not an FFQ, and hence there were differences in the mean intakes of total carotene between the two studies. Of interest the median total carotene intake in the first quintile of intake was 29.6 μg as Retinol equivalents/d in the Floegel *et al.* study (Floegel *et al.* 2011) compared to 1470 μg/d in the current analysis. Although our cohort adjusted for a number of covariates that may mediate the association between intake of total carotene and the inflammatory marker CRP, the findings did not reach statistical significance.

Notably, findings from our cross-sectional study were supported by those from the Multiethnic-Ethnic Study of Atherosclerosis (MESA) among 5181 men and women aged 45 to 84 years showing no associations between vitamins C and E, and β-carotene intakes and the inflammatory marker CRP (de Oliveira Otto *et al.* 2011). Participants from the MESA study were older (45 to 84 years) and more overweight (mean BMI = 27.9 kg/m^2) compared to the TwinsUK cohort aged 18 to 79 years (mean BMI = 24.9 kg/m^2).

In the current study mean CRP concentrations (2.49 mg/L) were three-fold lower compared with the NHANES 1999-2002 study (4.88 mg/L) (Floegel *et al.* 2011) and two-fold higher compared with the MESA cohort (1.79 mg/L). However, in both the current analysis and the MESA study plasma CRP concentrations were lower than 3 mg/L, which represents a high risk for cut point for cardiovascular disease according to the American Heart

Association Consensus panel and the Centers for Disease Control and Prevention (CDC/AHA) (de Ferranti and Rifai 2007) suggesting that associations with diet may only be observed when population ranges of CRP are higher.

In relation to vitamin D, the current study did not observe any associations with CRP, although it is evident that 1-alpha, 25-Dihydroxyvitamin D(3) [1,25-(OH)(2)D(3)], the active metabolite of vitamin D(3), acts as an inhibitor of the inflammatory response (Nagpal *et al.* 2005). Moreover, vitamin D concentrations have been inversely associated with the inflammatory marker CRP in patients with rheumatoid arthritis (Oelzner *et al.* 1998) and frailty (Abdulrab and Heun 2008). However, there are no population studies examining associations between the habitual dietary intake of vitamin D neither in healthy individuals nor in disease to enable comparisons with our study.

Interestingly, the current analysis observed an inverse association between higher potassium intake and plasma CRP concentrations although the association was not statistically significant. There are currently no studies in the literature examining associations between habitual potassium intake and plasma CRP, however, there is evidence of the anti-inflammatory effects of three potassium salts of N,N-disubstituted 4-aminoazobenzenesulfonic acids in rats with adjuvant arthritis (Kazemekaite *et al.* 2008). Also, findings from epidemiological studies have shown that dietary patterns characterised by increased consumption of fruits and vegetables (the main sources of

potassium) were associated with lower levels of markers of inflammation including CRP and may offer support to results from the current study (Esmaillzadeh *et al.* 2006, Lopez-Garcia *et al.* 2004, Nettleton *et al.* 2006).

The lack of significant associations observed in the current study might be due to the fact that other lifestyle factors than diet have a greater effect on plasma CRP concentrations. Of interest, in this cohort, BMI and physical activity had a greater effect on CRP for all nutrients. For example, after multivariate adjustment for relevant confounders, the effect of BMI [β coefficient (95 % CI): 0.09 (0.08, 0.09), P < 0.001] was 15 times greater of that of vitamin C intake [β coefficient (95 % CI): -0.006 (-0.04, 0.03), P = 0.771] on CRP. This finding reflects data from previous epidemiological (Choi *et al.* 2013, Festa *et al.* 2001, Hodge *et al.* 2010, Khoo *et al.* 2011) and experimental studies (Calabro *et al.* 2005) showing that BMI and the adipose tissue are associated with higher CRP concentrations. The observed effect of physical activity [β coefficient (95 % CI): -0.13 (-0.27, 0.01), P = 0.07] was 22 times greater of that of vitamin C intake [β coefficient (95 % CI): -0.006 (-0.04, 0.03), P = 0.771] on CRP. The effects of physical activity on inflammation are conflicting. In a recent review which evaluated the relationship between the inflammatory marker CRP and exercise, it was reported that interventions that were based just on increasing the physical activity had no effect on the inflammatory marker CRP (Michigan *et al.* 2011). However, intervention programmes that were based on lifestyle changes including both exercise and healthy diet reported decreases on CRP, probably related to weight reduction. Also, findings from a

randomised controlled trial among obese postmenopausal women aged 50-75 years, mean CRP was reduced by 10 % in exercisers and increased by 12 % in the control group from baseline after an exercise intervention programme of one year (Campbell *et al.* 2009).

Overall, this study showed that higher magnesium intake was significantly inversely associated with plasma CRP concentrations in women across a wide age range, after adjustments for age, physical activity, smoking, energy intake, anti-inflammatory medication (including aspirin, statins, and NSAID) and HRT use and BMI. The mechanisms involved in the relationship between magnesium intake and the inflammatory marker CRP cannot be specifically identified due to the nature of the study which assessed cross-sectional associations. Magnesium is an essential micronutrient which acts as a cofactor in multiple enzymatic reactions in the human body. Notably, there are a number of studies indicating an inverse association between dietary magnesium intake and metabolic and inflammatory conditions, such as hypertension (Song *et al.* 2006), metabolic syndrome (He *et al.* 2006), insulin resistance (Paolisso and Barbagallo 1997), type 2 diabetes (Song *et al.* 2004), dyslipidemia (Song *et al.* 2005), and cardiovascular disease (Al-Delaimy *et al.* 2004). These findings are supported by experimental data from animal studies indicating that dietary magnesium deficiency may induce an inflammatory response (Malpuech-Brugere *et al.* 2000). Although there are findings from observational and animal studies, the underlying biological mechanisms for such metabolic effects of magnesium are not yet entirely clear. However, there is one recent

randomised, crossover, pilot trial in 14 overall healthy, overweight individuals (BMI $\geq$ 25 kg/m^2) who were randomly assigned to receive magnesium citrate (500 mg/d) or placebo for four weeks with one month wash-out period. This study found an up-regulation of 24 genes and down-regulation of 36 genes including genes that were related to metabolic and inflammatory pathways such as C1q and tumor necrosis factor-related protein 9 (*C1QTNF9*) and pro-platelet basic protein (*PPBP*) (Chacko *et al.* 2011). In this study gene expression profiling revealed an effect on inflammatory metabolic pathways and it also showed a significant increase in the inflammatory marker IL-6 in response to magnesium supplementation (Chacko *et al.* 2011). Findings from this small study provide further evidence of the importance of magnesium in metabolic and inflammatory pathways although future studies in large populations would need to be conducted to investigate these preliminary findings further.

Findings were not significant for associations between the other micronutrients and plasma CRP concentrations possibly because other lifestyle factors, such as BMI and physical activity had a greater effect in this cohort.

Protein, amino acid intake and plasma CRP concentrations (mg/L)

A novel finding of the current analysis was the significant positive association between higher intakes of arginine, glutamine, histidine, isoleucine, threonine and tryptophan (as % energy) and the inflammatory marker CRP. The magnitude of association was a difference of 0.3 mg/L in

plasma geometric mean CRP between extreme quintiles of intake (P for difference < 0.05 for arginine, glutamine, threonine and tryptophan; and P = 0.05 for histidine and isoleucine). There were also trends towards significance for leucine, lysine, methionine and valine. No associations were observed for protein and phenylalanine although the trends were in the same direction as the other amino acids. These associations were observed after adjustments for age, physical activity, smoking, anti-inflammatory medication (including aspirin, statins, and NSAID) and HRT use and was independent of adiposity (assessed by BMI).

There are currently no population studies examining associations between the habitual dietary intake of essential amino acids and inflammatory markers. However, two previous supplementation studies providing oral administration of protein enriched with the branched-chain amino acids (BCAAs) leucine, isoleucine and valine in malnourished elderly with catabolic status (Bonnefoy *et al.* 2010) and in patients with liver cirrhosis (Ohno *et al.* 2008) have shown a small decrease in the inflammatory marker CRP. However, an example of a relationship between amino acids and inflammation from other chronic disease relationships exists with cardiovascular disease. A recent cross-sectional analysis from the Rotterdam Study among 3086 subjects aged 55 years and over showed that higher intakes of glutamic acid, arginine, lysine, and cysteine intakes (as % of protein intake) were not associated with blood pressure or risk of hypertension (Altorf-van der Kuil *et al.* 2013). The same study also observed that a higher intake of tyrosine (0.3 % of protein) was inversely

and significantly associated to systolic blood pressure (P for trend = 0.05) by 2.4 mmHg but not to diastolic blood pressure (P for trend = 0.35) (Altorf-van der Kuil *et al.* 2013).

In relation to protein there are also currently no population studies examining association between the habitual protein intake and inflammatory markers. To our knowledge, there is only one previous randomised controlled dietary intervention among overweight and obese men and women (BMI 27-34 kg/m^2) aged 18-56 years, although this study was designed to investigate weight loss on different diets (Due *et al.* 2005). In this study participants were randomly assigned to an *ad libitum* fat reduced diet (30% of energy), either high in protein (25% of energy) and low in carbohydrate (45% of energy) (HPLC) or high in carbohydrate (58% energy) and low in protein (12% of energy) (HCLP) during six months. After six months of intervention, both the HPLC and the HCLP groups showed a non-significant decrease in serum CRP concentrations by 0.5 mg/L (21%, P = 0.06) and 0.8 mg/L (28%, P = 0.09), respectively. Moreover, the difference between the two groups was not significant, although the authors do not provide the exact p value of difference. In addition, in multiple regression analyses there was no influence of protein intake (as % energy) on CRP after six months of intervention; however data for these findings were not shown (Due *et al.* 2005). Although these findings are not directly comparable with ours (due to the weight loss occurring during that study) they indicate that the action of proteins is not neutral, as might have previously been expected. Also the study design was

different from our current study and it is noteworthy that in the current analysis protein intake in the lower quintile was 13.2 % of energy and in the higher quintile 20.5 % of energy, almost 5 % lower compared to the HPLC diet in the study of Due *et al*. (Due *et al.* 2005).

The mechanisms involved in the relationship between protein, essential amino acids and the inflammatory marker CRP cannot specifically identified due to the nature of this study which assessed cross-sectional associations. Nevertheless, it is noteworthy that dietary protein and essential amino acid intakes play a major role in various chronic (such as cancer and chronic obstructive pulmonary disease) and acute disease (such as sepsis and burn injury) states, where inflammation is present and protein catabolism is enhanced (Jonker *et al.* 2012). Given that protein breakdown and muscle wasting is increased in these conditions, it has been argued that modifying the protein and essential amino acid content of the diet may positively affect the balance of muscle protein synthesis and breakdown, and may activate muscle protein anabolism in chronic and acute disease states (Eley *et al.* 2007, Jonker *et al.* 2012, Peters *et al.* 2011). However, it needs to be considered that the protein intake that is associated with the lowest rate of catabolism in conditions with high systemic inflammation is greater than the minimum protein intake that is required to achieve a neutral protein balance in healthy individuals (Guadagni and Biolo 2009). Although the protein requirement in healthy individuals is defined as the lowest protein intake sufficient to achieve neutral protein balance, this definition cannot be applied in situations characterized by increased catabolism

(Guadagni and Biolo 2009), as in these situations a higher protein intake is required to modulate metabolic and immune functions ranging from 1.3 to 1.5 g protein/kg/d (Biolo 2013). In the TwinsUK cohort the average protein intake per kg body weight per day was 1.3 g protein/kg/d higher compared to the average recommended nutrient intake (0.8 g protein/kg/d) for the UK population in accordance with the UK report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. However, neutral protein balance and optimal protein/amino acid intake may not always have an effect on inflammation for populations that have a normal weight, BMI and CRP concentrations and the biological mechanisms underlying such a relationship are largely unknown. Nevertheless, given these are the first observations that we know about, they would appear to be novel findings.

6.5 Strengths and limitations

The strengths of this study include the large sample size and the fact that this was the first population study that directly compared a full range of micronutrients and carotenoids with potential anti-inflammatory properties in association with plasma CRP concentrations. In addition, this was the first study that evaluated the influence of protein and essential amino acid intakes on the inflammatory marker CRP. Also, the FFQ used in the current study was previously compared and validated against a 7-day weighed record in the EPIC Norfolk study, and although the two approaches to measure dietary intake were different, both methods identified similar intakes of macronutrients when these were expressed as percentage of total

energy intake (Bingham *et al.* 2001). This FFQ was also validated against urinary and plasma biomarkers of intake, such as urinary nitrogen for protein intake, urinary potassium and sodium, plasma ascorbic acid and plasma n-3 PUFAs (McKeown *et al.* 2001, Welch *et al.* 2006). In addition, it has been shown previously that serum β-cryptoxanthin and zeaxanthin concentrations were moderately correlated with dietary carotenoids intakes measured by an FFQ (Tucker *et al.* 1999). Furthermore, in order to examine associations between dietary intake and health outcomes participants need to be ranked according to their usual dietary intake and FFQs have been shown to rank individuals well (Molag *et al.* 2007).

In the current analyses multiple corrections were not performed as the hypothesis of the study was well defined *a priori*, and the mechanisms linking the associations between different nutrients and the inflammatory marker CRP were independent. Multiple testing is an important consideration in exploratory studies (Benjamini 2010). However, since our hypotheses were well defined *a priori*, and the mechanisms linking the different exposures and the outcome were independent, then correction for multiple testing is not strictly necessary (Bender and Lange 2001).

This study also has a number of limitations. The cross-sectional nature of the study limits causal inference, but enables us to generate hypotheses regarding associations between the habitual dietary intake of a range of micronutrients, protein and essential amino acids and the inflammatory marker CRP based on plausible mechanisms. Although all analyses were

adjusted for a number of confounding factors, residual confounding may still be present because of the observational nature of the study. In addition, as in all observational studies, measurement error in self-reported dietary intakes is inevitable. It is also widely acknowledged that the use of FFQs for measuring dietary intake has its own limitations related to memory errors, they provide relative than absolute intake, they introduce bias due to over- or under-reporting, and they may introduce systematic errors as preparation methods are inadequately considered (McNeill *et al.* 2009). Also, FFQs measure food intake over a period of one year and reflect the habitual diet over this period of time but not lifetime dietary habits (Teucher *et al.* 2007). This study was limited in measuring only one biomarker of inflammation, which is the widely used CRP, and maybe more markers needed to be measured to reflect, in total, the complexity of such associations. The observed findings relate to women and further work is needed to investigate if these findings are replicated in a male population of the same country or in populations from different ethnic backgrounds.

6.6 Concluding remarks

In conclusion, we found in multivariate analyses that higher magnesium intakes were associated with lower plasma CRP concentrations by 25 % comparing extreme quintiles of intake. This study also observed that higher intakes of arginine, glutamine, histidine, isoleucine, threonine and tryptophan were positively associated with CRP levels. Findings from the current study extend the results from the few previous cross-sectional studies by examining simultaneously associations between a range of

micronutrients, protein and essential amino acids with potential anti-inflammatory properties in women of a wide age range. Although in the current analysis we expected an inverse association between dietary protein intake and the inflammatory marker CRP, surprisingly we did not observe an association between protein and CRP. Also associations between essential amino acid intakes and CRP were in the opposite direction to what we had expected. To our knowledge to date no other population studies have examined the associations between protein and essential amino acid intakes and CRP or other inflammatory markers.

The fact that not all amino acids (including leucine, lysine, methionine and valine) showed a significant association with CRP may be because these amino acids are involved in different pathways of the metabolic chain and may differentially influence CRP, although the FFQ included a range of foods that contribute to essential amino acid intake, such as soy products (tofu, soy milk), meat and fish, dairy, eggs, and legumes. Also, associations were not observed for all micronutrients, possibly due to the fact that other factors, such as physical activity and body mass index may be more important than diet on CRP. Yet, it is also possible that a longitudinal study design might be more suitable approach to detect associations between nutrient intakes and the inflammatory marker CRP, as it might be more likely to observe a difference in the inflammatory status over time due to a healthier diet. Additionally, maybe long-term interventions are needed to observe an effect and a dose-response relationship in order to define the

anti-inflammatory or pro-inflammatory effects of specific nutritional factors that can further modulate disease risk.

Results from this study suggest that is important for adult women to consume a variety of food sources high in micronutrients, such as magnesium to ensure lower plasma concentrations of the inflammatory marker CRP. Given the debate on protein and amino acid requirements in healthy individuals, and from a public health perspective, the novel findings from the current analysis may offer some insight in order to better understand whether the habitual dietary protein and amino acid intakes may influence inflammation in adult women with normal weight, BMI and CRP concentrations.

The next chapter explores the associations between overall dietary quality and the inflammatory marker CRP.

Table 6.2 Characteristics of female subjects from the TwinsUK cohort[1]

Characteristic	*N*=1658, 18-79 yrs
Age (y)	49.7±12.5
Weight (kg)	65.5±10.6
Height (cm)	162±5.96
BMI (kg/m^2)	24.9±4.0
Inflammation marker	
C-reactive protein (*CRP*) (mg/L)	2.49±2.30
Physical activity %	
Inactive	20.5
Moderate	54.8
Active	24.7
Smoking history %	
Never	50.2
Current	16.7
Former	33.1
Hormone replacement therapy %	
No	93.7
Yes	6.30
Anti-inflammatory medication %[2]	
No	93.8
Yes	6.20
Dietary components	
Total energy intake (kcal/d)	1976±512
Protein intake (g/d)	81.4±21.3
Protein intake (% Energy)	16.7±2.60
Vitamins	
Vitamin C intake (mg/d)	157±78.8
Vitamin E intake (mg/d)	11.4±4.4
Vitamin D intake (µg/d)	2.62±1.39
Minerals	
Magnesium intake (mg/d)	345±90
Potassium intake (mg/d)	3999±990
Selenium intake (µg/d)	44.9±16.0
Carotenoids	
α-carotene intake (µg/d)	570±419
ß-carotene intake (µg/d)	3135±1761
Total carotene intake (µg/d)	3498±1952
Lycopene intake (µg/d)	1322±896
Lutein intake (µg/d)	2224±1463
Zeaxanthin intake (µg/d)	84.4±70.8
ß-cryptoxanthin intake (µg/d)	203±191
Amino acids	
Arginine (g/d)	4.45±1.24
Arginine (% Energy/d)	0.92±0.20
Glutamine (g/d)	16.1±4.4
Glutamine (% Energy/d)	3.31±0.59
Histidine (g/d)	2.29±0.63
Histidine (% Energy/d)	0.47±0.09
Isoleucine (g/d)	3.84±1.04
Isoleucine (% Energy/d)	0.79±0.16
Leucine (g/d)	6.51±1.75
Leucine (% Energy/d)	1.34±0.26
Lysine (g/d)	5.54±1.60
Lysine (% Energy/d)	1.14±0.27
Methionine (g/d)	1.87±0.52

Methionine (% Energy/d)	0.39±0.08
Phenylalanine (g/d)	3.83±1.02
Phenylalanine (% Energy/d)	0.79±0.15
Threonine (g/d)	3.35±0.92
Threonine (% Energy/d)	0.69±0.14
Tryptophan (g/d)	1.06±0.28
Tryptophan (% Energy/d)	0.22±0.04
Valine (g/d)	4.54±1.22
Valine (% Energy/d)	0.93±0.18

[1] Values are presented as mean ± SEM or else indicated

[2] Aspirin, statins, non-steroidal anti-inflammatory drugs (NSAID)

Table 6.3 Associations between nutrient intakes and C-reactive protein (mg/L) in 1658 women from the TwinsUK cohort[1]

Quintiles of nutrient intake	C-reactive protein (mg/L)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Vitamin C (mg/d)						
Unadjusted	1.57(1.39,1.78)	1.56(1.39,1.75)	1.70(1.52,1.90)	1.60(1.43,1.78)	1.56(1.39,1.75)	0.99
Adjusted model[2]	1.60(1.42,1.80)	1.54(1.39,1.71)	1.72(1.55,1.90)	1.61(1.45,1.78)	1.53(1.37,1.70)	0.77
Vitamin D (µg/d)						
Unadjusted	1.60(1.42,1.80)	1.60(1.43,1.79)	1.49(1.33,1.66)	1.60(1.43,1.79)	1.72(1.54,1.92)	0.41
Adjusted model	1.63(1.46,1.82)	1.59(1.43,1.77)	1.52(1.37,1.68)	1.61(1.45,1.80)	1.64(1.47,1.84)	0.85
Vitamin E (mg/d)						
Unadjusted	1.72(1.53,1.93)	1.57(1.41,1.75)	1.58(1.42,1.77)	1.64(1.47,1.83)	1.49(1.33,1.67)	0.19
Adjusted model	1.75(1.53,1.99)	1.55(1.39,1.72)	1.62(1.46,1.80)	1.64(1.48,1.81)	1.45(1.28,1.65)	0.26
Magnesium (mg/d)						
Unadjusted	1.73(1.54,1.94)	1.54(1.38,1.72)	1.55(1.38,1.73)	1.66(1.48,1.87)	1.51(1.36,1.69)	0.30
Adjusted model	1.87(1.63,2.13)	1.55(1.39,1.73)	1.58(1.42,1.75)	1.62(1.46,1.81)	1.41(1.24,1.59)	0.051
Selenium (µg/d)						
Unadjusted	1.61(1.44,1.80)	1.42(1.26,1.60)	1.68(1.50,1.87)	1.63(1.45,1.83)	1.67(1.49,1.86)	0.23
Adjusted model	1.60(1.44,1.78)	1.40(1.26,1.60)	1.66(1.50,1.84)	1.68(1.50,1.87)	1.66(1.49,1.86)	0.18
Potassium (mg/d)						
Unadjusted	1.69(1.51,1.89)	1.45(1.29,1.62)	1.60(1.42,1.79)	1.61(1.42,1.82)	1.65(1.49,1.84)	0.74
Adjusted model	1.77(1.56,2.00)	1.48(1.32,1.65)	1.62(1.46,1.80)	1.59(1.42,1.78)	1.54(1.36,1.74)	0.45
Total carotene (µg/d)						
Unadjusted	1.59(1.43,1.77)	1.46(1.30,1.64)	1.65(1.48,1.85)	1.70(1.53,1.89)	1.59(1.42,1.79)	0.39
Adjusted model	1.63(1.47,1.81)	1.54(1.38,1.72)	1.62(1.45,1.80)	1.64(1.49,1.81)	1.56(1.41,1.74)	0.91
α-carotene (µg/d)						
Unadjusted	1.60(1.43,1.79)	1.47(1.30,1.65)	1.70(1.51,1.90)	1.55(1.39,1.73)	1.68(1.51,1.88)	0.40
Adjusted model	1.66(1.50,1.85)	1.53(1.37,1.71)	1.60(1.43,1.78)	1.57(1.42,1.74)	1.63(1.47,1.80)	0.92
ß-carotene (µg/d)						
Unadjusted	1.61(1.45,1.80)	1.50(1.33,1.69)	1.58(1.41,1.78)	1.70(1.52,1.89)	1.60(1.43,1.79)	0.55
Adjusted model	1.66(1.49,1.84)	1.57(1.41,1.76)	1.57(1.41,1.75)	1.63(1.47,1.80)	1.56(1.41,1.74)	0.64

Lutein (µg/d)						
Unadjusted	1.63(1.45,1.83)	1.59(1.42,1.77)	1.60(1.44,1.77)	1.66(1.48,1.86)	1.52(1.35,1.70)	0.58
Adjusted model	1.65(1.48,1.84)	1.60(1.44,1.77)	1.57(1.43,1.74)	1.63(1.47,1.80)	1.54(1.38,1.71)	0.48
Zeaxanthin (µg/d)						
Unadjusted	1.73(1.57,1.92)	1.56(1.40,1.73)	1.71(1.54,1.89)	1.41(1.27,1.57)	1.60(1.43,1.78)	0.16
Adjusted model	1.68(1.51,1.86)	1.56(1.40,1.73)	1.69(1.52,1.87)	1.43(1.29,1.60)	1.65(1.47,1.84)	0.49
ß-cryptoxanthin (µg/d)						
Unadjusted	1.64(1.47,1.83)	1.61(1.44,1.80)	1.65(1.47,1.85)	1.57(1.40,1.76)	1.52(1.36,1.70)	0.33
Adjusted model	1.62(1.46,1.80)	1.63(1.47,1.80)	1.63(1.47,1.82)	1.61(1.45,1.79)	1.50(1.34,1.67)	0.34
Lycopene (µg/d)						
Unadjusted	1.52(1.36,1.70)	1.59(1.41,1.79)	1.65(1.48,1.83)	1.62(1.46,1.80)	1.61(1.43,1.81)	0.46
Adjusted model	1.51(1.36,1.67)	1.55(1.39,1.73)	1.69(1.53,1.85)	1.63(1.47,1.81)	1.62(1.45,1.81)	0.28

[1] All values are means and SEM for CRP (mg/L) by quintiles of different micronutrient intakes

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking status (never, current, former), body mass index (kg/m^2), energy intake (kcal/d), anti-inflammatory medication (including aspirin, statins, and NSAID) (no/yes) and HRT use (no/yes)

Table 6.4 Associations between protein and essential amino acid intakes and C-reactive protein (mg/L) in 1658 women from the TwinsUK cohort[1]

Quintiles of nutrient intake	C-reactive protein (mg/L)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Energy intake (kcal/d)						
Unadjusted	1.63(1.46,1.82)	1.57(1.40,1.76)	1.62(1.44,1.81)	1.63(1.46,1.82)	1.55(1.38,1.74)	0.72
Adjusted model[2]	1.59(1.51,1.68)	1.59(1.51,1.68)	1.60(1.52,1.69)	1.60(1.52,1.68)	1.60(1.52,1.69)	0.73
Protein (%E)						
Unadjusted	1.37(1.22,1.54)	1.58(1.42,1.75)	1.62(1.45,1.82)	1.71(1.52,1.92)	1.73(1.56,1.93)	0.002
Adjusted model	1.46(1.32,1.62)	1.60(1.45,1.77)	1.68(1.50,1.87)	1.67(1.50,1.86)	1.59(1.43,1.75)	0.22
Arginine (%E)						
Unadjusted	1.35(1.20,1.52)	1.60(1.44,1.78)	1.58(1.41,1.75)	1.59(1.42,1.78)	1.93(1.73,2.15)	<0.001
Adjusted model	1.42(1.28,1.58)	1.65(1.50,1.82)	1.61(1.46,1.77)	1.57(1.42,1.75)	1.75(1.58,1.94)	0.03
Glutamine (%E)						
Unadjusted	1.39(1.24,1.55)	1.57(1.41,1.75)	1.52(1.35,1.71)	1.68(1.50,1.88)	1.86(1.68,2.07)	<0.001
Adjusted model	1.44(1.30,1.60)	1.64(1.49,1.81)	1.57(1.40,1.76)	1.65(1.49,1.84)	1.70(1.55,1.87)	0.03
Histidine (%E)						
Unadjusted	1.33(1.18,1.49)	1.62(1.46,1.81)	1.59(1.42,1.78)	1.59(1.42,1.78)	1.91(1.72,2.13)	<0.001
Adjusted model	1.42(1.28,1.58)	1.67(1.52,1.84)	1.62(1.46,1.81)	1.56(1.40,1.73)	1.73(1.57,1.91)	0.050
Isoleucine (%E)						
Unadjusted	1.33(1.18,1.50)	1.55(1.39,1.73)	1.68(1.50,1.88)	1.62(1.44,1.81)	1.86(1.67,2.07)	<0.001
Adjusted model	1.42(1.28,1.58)	1.62(1.47,1.79)	1.67(1.50,1.85)	1.61(1.44,1.80)	1.68(1.52,1.85)	0.050
Leucine (%E)						
Unadjusted	1.36(1.21,1.52)	1.59(1.43,1.77)	1.63(1.46,1.82)	1.60(1.43,1.80)	1.85(1.66,2.05)	0.001
Adjusted model	1.45(1.30,1.61)	1.63(1.48,1.80)	1.65(1.49,1.83)	1.59(1.42,1.77)	1.68(1.53,1.86)	0.09
Lysine (%E)						
Unadjusted	1.32(1.17,1.48)	1.67(1.49,1.86)	1.57(1.40,1.75)	1.62(1.45,1.81)	1.87(1.68,2.08)	<0.001
Adjusted model	1.41(1.27,1.56)	1.72(1.56,1.91)	1.58(1.42,1.75)	1.60(1.44,1.77)	1.71(1.55,1.89)	0.07
Methionine (%E)						
Unadjusted	1.40(1.24,1.57)	1.48(1.33,1.65)	1.70(1.52,1.90)	1.60(1.43,1.79)	1.86(1.67,2.06)	<0.001
Adjusted model	1.49(1.34,1.65)	1.55(1.40,1.72)	1.68(1.51,1.87)	1.59(1.42,1.76)	1.69(1.54,1.87)	0.09

Phenylalanine (%E)						
Unadjusted	1.39(1.23,1.56)	1.55(1.39,1.73)	1.63(1.46,1.81)	1.62(1.44,1.82)	1.83(1.65,2.04)	0.001
Adjusted model	1.45(1.30,1.61)	1.62(1.46,1.79)	1.68(1.52,1.87)	1.58(1.42,1.77)	1.66(1.51,1.83)	0.12
Threonine (%E)						
Unadjusted	1.33(1.18,1.49)	1.53(1.37,1.70)	1.65(1.47,1.84)	1.63(1.46,1.83)	1.91(1.71,2.12)	<0.001
Adjusted model	1.43(1.28,1.59)	1.60(1.45,1.76)	1.65(1.48,1.83)	1.62(1.45,1.80)	1.72(1.55,1.90)	0.02
Tryptophan (%E)						
Unadjusted	1.35(1.20,1.51)	1.49(1.34,1.65)	1.66(1.48,1.86)	1.67(1.49,1.87)	1.87(1.69,2.08)	<0.001
Adjusted model	1.44(1.29,1.59)	1.55(1.41,1.71)	1.69(1.51,1.88)	1.63(1.47,1.81)	1.70(1.54,1.87)	0.02
Valine (%E)						
Unadjusted	1.35(1.20,1.51)	1.56(1.40,1.74)	1.68(1.51,1.88)	1.58(1.41,1.78)	1.86(1.67,2.06)	<0.001
Adjusted model	1.43(1.28,1.58)	1.63(1.48,1.80)	1.70(1.53,1.89)	1.55(1.39,1.72)	1.70(1.54,1.87)	0.08

[1] Values are means and SEM for by quintiles of nutrient intake

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking status (never, current, former), body mass index (kg/m^2), anti-inflammatory medication (including aspirin, statins, and NSAID) (no/yes) and HRT use (no/yes)

Chapter 7

Associations between diet quality scores and C-reactive protein levels

7.0 Introduction

There is growing evidence suggesting that chronic inflammation is an important underlying aspect of sarcopenia (Cavicchia *et al.* 2009, Chung *et al.* 2009). The importance of assessing the "whole diet" in relation to disease outcomes has been discussed previously (Chapter 5, section 5.0, p. 153). The associations between the "whole diet", assessed using diet quality scores and inflammatory markers will therefore be investigated in this chapter. As an extension to Chapter 6, this chapter investigates the association of diet quality scores and the inflammatory marker C-reactive protein (*CRP*).

To date, very few epidemiological studies have examined associations between diet quality and inflammation markers. Results from the ATTICA cohort study (men and women, aged 18 to 89 years) showed that a higher adherence to a traditional Mediterranean diet, assessed using the Mediterranean diet score, was associated with 20% lower CRP values ($P = 0.015$) compared to lower adherence in an unadjusted model. After multivariate adjustment, including age, gender, smoking, physical activity, education status, presence of hypertension, diabetes, hypercholesterolemia, family history of coronary heart disease, and body mass index the authors reported a decrease of 0.21 mg/L in plasma CRP per 10 unit increase in the MDS ($P < 0.05$) (Chrysohoou *et al.* 2004). Furthermore, in the Nurse's Health Study it was found that higher adherence to the Alternate Healthy Eating Index (AHEI) and the alternate Mediterranean score (aMED) was negatively associated with plasma CRP concentrations by 30% and 24% (P

< 0.05) respectively, independent of obesity (Fung *et al.* 2005). In another Nurse's Health Study (women aged 34 to 59 years) it was reported that higher adherence to the DASH-style score was negatively associated with plasma concentrations of CRP by 20.4 % (P = 0.008) compared to lower adherence to the score (Fung *et al.* 2008). Data from this cohort have also shown that among women (aged 38 to 69 years) , 62% of whom were overweight and free from CVD, cancer and diabetes at baseline, that higher adherence to the Alternate Healthy Eating Index (AHEI) score was negatively associated with plasma CRP by 41% (P < 0.05), compared to lower adherence to the score (Fargnoli *et al.* 2008).

Although to date a number of population studies have examined the relative influence of diet quality scores on inflammation, to our knowledge, there are currently no population studies that have examined the full range of diet quality scores in association with inflammatory markers. Therefore, to further provide insight into the potential role of diet quality on inflammation as assessed by the established inflammatory marker CRP, a predictor for an increased risk of sarcopenia (Cesari *et al.* 2004, Schaap *et al.* 2006) the potential influence of diet quality on inflammation related sarcopenia was examined.

7.1 Aims

The aim of this study was to derive five predefined diet scores including the MDS, HDI, DQI, AHEI, and DASH-style score, and examine the hypothesis that greater adherence to these five scores is negatively associated with

plasma levels of CRP (mg/L), among women aged 18-79 years from the TwinsUK cohort.

7.2 Covariate plan and statistical analysis

All analyses were performed using STATA statistical software (version 11.0; STATA Corp, USA). Means and standard deviations were calculated (**Table 7.1**) and quintiles of CRP and quartiles of the five diet scores were also derived. Robust cluster regression was applied for continuous variables (such as age, energy intake, and BMI) and Chi-square test for categorical variables (such as physical activity and smoking history), to test for differences in participant baseline characteristics across quintiles of CRP (**Table 7.2**).

The analyses were performed treating twins as individuals as previous studies have shown that participants from the TwinsUK registry are similar to age-matched singleton women in the distribution of common traits and outcomes (Andrew *et al.* 2001). Data on dietary intake (food frequency questionnaire, FFQ), body composition parameters, and other covariates were available for 1658 women, aged 18 to 79 years representing 582 monozygotic (MZ) and 1076 dizygotic (DZ) females who had completed a FFQ and had fasting blood samples taken to measure C-reactive protein (CRP) between 1996 and 2000 (Moayyeri *et al.* 2012, Teucher *et al.* 2007).

Prior to conducting statistical analysis an analysis plan with justification for all confounding factors in the multivariate model was developed as

described in Chapter 2, section 2.2.5, p. 57. Multivariate regression analysis was used to assess associations between the five derived diet scores and plasma CRP using the robust cluster regression option in STATA. These models take into account clustering when calculating standard errors of the mean and the dependency of twin pairs as related individuals (Richards *et al.* 2007) to ensure familial aggregation was controlled for. The plasma concentration of CRP was transformed and analyzed on the natural logarithmic scale in order to meet the criterion of normality for the linear models. Values were subsequently back transformed into geometric means and 95% confidence intervals (CI) for presentation.

As ageing has been strongly associated with an increased low-grade inflammatory status all analyses were adjusted for age (years) (Chung *et al.* 2009). Models were also adjusted for physical activity and smoking status, as physical activity has been suggested to exert anti-inflammatory properties (Gleeson *et al.* 2011, Petersen and Pedersen 2005) and smoking has been suggested to increase inflammation (Lee *et al.* 2012). Energy intake (kcal/d) was also included in the multivariate models for the MDS (Trichopoulou *et al.* 1995), AHEI (Kennedy *et al.* 1995) and DASH-style score (Fung *et al.* 2008) as these scores do not take into account energy intake in contrast to the HDI (Huijbregts *et al.* 1997) and DQI (Patterson *et al.* 1994). Additional adjustments were made for body mass index (kg/m^2), hormone replacement therapy use (HRT) and anti-inflammatory medication (including statins, non steroid anti-inflammatory drugs and aspirin) (no or yes, respectively) as these factors have been previously shown to affect inflammatory status and

may mediate the relationship between diet quality and CRP (Chapter 2, section 2.2.5, p. 64, 66, 68) (**Table 7.3**).

In order to examine the relative importance of diet quality and other lifestyle characteristics on inflammatory marker CRP, regression analysis was performed with all independent variables standardised to similar scale of measurements. The diet quality indices were measured in quartiles, age in 10 year categories, smoking habit in two categories (current v never and former v never), physical activity in three categories (inactive, moderate, and active), body mass index in kg/m^2, energy intake per 1000 kcal, anti-inflammatory medication and hormone replacement therapy use (no/yes), respectively. The plasma concentration of CRP was not transformed in this analysis to enable presentation of the data (**Table 7.4**).

7.3 Results

Descriptive characteristics of this subset (*n* = 1658 women, aged 18-79 years) of the TwinsUK cohort are presented in **Table 7.1**. The mean age (±SD) of participants was 49.7 ± 12.5 years and mean (±SD) BMI was 24.9 ± 4.0 kg/m^2. Mean (±SD) plasma CRP concentrations were 2.49 ± 2.30 mg/L. More than half of the participants were moderately active (54.8 %), and 16.7% were current smokers. Few participants reported taking anti-inflammatory medication (6.2 %) or were using hormone replacement therapy (6.3 %). The mean (±SD) MDS was 4.54 ± 1.79 points, HDI score was 3.45 ± 1.32 points, DQI was 11.8 ± 1.9 points, AHEI was 49.6 ± 11.1

points, and the DASH-style score was 23.3 ± 5.6 points. Mean (±SD) energy intake was 1976 ± 512 kcal/d.

The main baseline characteristics and the five derived diet scores across quintiles of CRP are presented as unadjusted means ± standard errors (SEM) in **Table 7.2**. There was a significant positive association between plasma CRP and age, weight and BMI (P for trend < 0.001 for all). Women with a higher range of plasma CRP levels (4.24 – 9.86 mg/L) were less likely to be active (P < 0.001). In relation to the diet scores, the HDI and AHEI were inversely associated with plasma levels of CRP (mg/L) indicating that higher diet quality was associated with lower levels of CRP (P for trend = 0.001 and 0.009, respectively) (**Table 7.2**).

In multivariate analyses, when comparing extreme quartiles of the five derived diet scores, CRP was inversely associated with the HDI by 14 % (P for difference = 0.041), and with the AHEI by 12 % (P for difference = 0.051). The associations were not significant for the MDS, DQI and DASH-style scores (**Table 7.3**)

Relative comparison of associations between diet quality scores, lifestyle characteristics and C-reactive protein

Higher adherence to the HDI and AHEI was associated with lower plasma levels of CRP. The β-coefficients and standard errors for CRP in each of the diet quality scores and lifestyle characteristics represent non-transformed values to facilitate analyses and presentation. The magnitude of associations

observed were a decrease of 0.10 mg/L in plasma CRP levels per quartile of the HDI score (P = 0.044), and a decrease of 0.12 mg/L in plasma CRP levels per quartile of the AHEI score (P = 0.018). A one category increase in physical activity was associated with 0.2 mg/L lower plasma CRP levels, and this association was 1-6 times the scale of the association with the diet scores (P for all < 0.05). For every unit increase in BMI there was a 0.2 mg/L increase in plasma CRP levels. This association was a similar scale of that of physical activity for all five diet scores (P for all < 0.001). Comparing participants who were not taking anti-inflammatory medication, plasma CRP levels were 0.5 mg/L higher for the MDS, DQI and DASH-style scores compared to women who reported taking anti-inflammatory medication (P for all < 0.05) (**Table 7.4**).

7.4 Discussion

In this study using a large population-based sample of women aged 18-79 years we evaluated the association between diet quality, as assessed by the five most commonly used predefined diet scores, the MDS, HDI, DQI, AHEI and DASH-style score and the inflammatory marker CRP. This is one of the first cross-sectional studies to examine a range of diet scores in association with a potential predictor of sarcopenia risk in a cohort of women across a wide age range. This study observed an inverse association between higher adherence to the HDI and AHEI scores and mean plasma levels of CRP highlighting the influence of diet quality on inflammation. Previous population studies have examined the relative influence of diet quality scores on inflammatory markers, including CRP associated mainly

to outcomes related to cardiovascular disease risk (Chrysohoou *et al.* 2004, Fargnoli *et al.* 2008, Fung *et al.* 2008, Fung *et al.* 2005).

The current analysis observed a significant inverse association between higher adherence to the HDI and the inflammatory marker CRP. There are currently no studies that have directly evaluated the influence of the HDI score on CRP or other inflammatory markers, although higher adherence to the HDI has been previously inversely associated with all-cause mortality and cognitive impairment (Huijbregts *et al.* 1997, Huijbregts *et al.* 1998). In addition, a higher HDI score has been previously associated with lower BMI in both US and European cohorts (Haveman-Nies *et al.* 2001). In the current study, higher adherence to the HDI was also associated with lower BMI (P for trend = 0.005). However, the association observed between the HDI and plasma concentrations of CRP in the current analysis was independent of obesity.

The magnitude of association between higher (five points) and lower (two points) adherence to the HDI score was 0.23 mg/L or 14 % of the population geometric mean for CRP (P = 0.041). This association was observed after adjustments for age, physical activity, smoking, anti-inflammatory medication (including aspirin, statins, and NSAID) and HRT use and was independent of adiposity (assessed by BMI). Alternatively, the difference in CRP concentration per quartile of the HDI score was - 0.10 mg/L [β coefficient (SEM): - 0.10 (0.05), P = 0.044] (**Table 7.4**). As mentioned earlier (sections 7.2 and 7.3), the plasma concentration of CRP was not

transformed in analysis presented in Table 7.4 to enable presentation of the data. Therefore, p values are different in Tables 7.3 and 7.4, but of interest p values were similar when the data on CRP were transformed and analysed on the natural logarithmic scale (data not shown). Findings were of a similar trend, although of a greater magnitude, to a previous cross-sectional study reporting that the difference in CRP concentration per quintile of a diet pattern score high in whole grains, fruit, nuts and green leafy vegetables was - 0.06 mg/L [β coefficient (SEM): - 0.06 (0.02), P < 0.05], among middle-aged men and women aged 45-84 years from the MESA cohort (Nettleton *et al.* 2006). This difference may be explained by the fact that the study from Nettleton *et al*. was conducted in an ethnically diverse population and included more participants (*n* = 5089 men and women) compared with the current study (*n* = 1658 women). It also used an empirical method to derive dietary patterns, whereas the current study generated diet scores based on *a priori* knowledge. Moreover, the observed size of association was higher in the current analysis compared to Nettleton's *et al*. study, possibly because the dietary pattern derived from Nettleton *et al*. included less components compared to the HDI score that we used in this study. These components included whole grains, fruit, nuts, and green leafy vegetables which have previously been associated with CRP levels.

A significant inverse association between higher adherence to the AHEI score and inflammatory marker CRP was also observed. The magnitude of association between higher (62.5 points) and lower (37.5 points) adherence to the AHEI score was 0.20 mg/L or 13 % of the population geometric mean

for CRP (P = 0.051). This association was observed after adjustments for age, physical activity, smoking, energy intake, anti-inflammatory medication (including aspirin, statins, and NSAID) and HRT use and was independent of adiposity (assessed by BMI).

There are currently two previous cross-sectional studies that have examined associations between the AHEI score and CRP. In one of these studies, among women from the Nurse's Health Study it was observed that a higher adherence to the AHEI score was associated with a 30% decrease in plasma CRP concentrations ($P < 0.05$) independent of obesity and after multivariate adjustment (Fung *et al.* 2005). In another cross-sectional investigation in a subgroup of the same cohort, a higher adherence to the AHEI score was inversely associated with plasma CRP concentration by 41% ($P < 0.05$) compared to lower adherence to the score among women free from CVD, cancer and diabetes at baseline (Fargnoli *et al.* 2008). Findings from the current analysis were of a similar trend although of a lower magnitude to these two previous studies. These differences may be explained by the fact that in both previous cross-sectional analyses women were in a narrower age range with more people in the older age groups compared to the current cohort (18-79 years). Indeed, low-grade chronic inflammation is a common and inevitable consequence of the ageing process (Chung *et al.* 2009, Khansari *et al.* 2009, Woods *et al.* 2012). Moreover, women from the Nurse's Health Study were more overweight compared to women from the current analysis. Interestingly, BMI in the lowest and highest quintiles of the AHEI score were 27.5 kg/m^2 and 25.1 kg/m^2 in the Fung *et al*. cross-

sectional analysis (Fung *et al.* 2005), and 28.7 kg/m^2 and 26.9 kg/m^2 in the Fargnoli *et al.* analysis (Fargnoli *et al.* 2008). Whereas, in the current analysis BMI between the lowest and highest quartiles of the AHEI score was within the normal range, i.e. 24.8 kg/m^2 and 24.5 kg/m^2, respectively.

Both the HDI and AHEI scores award points to diets high in fruit and vegetables, whole grains, nuts and polyunsaturated fatty acids and low in saturated fatty acids. It has been well established that higher intakes of fruit and vegetables, whole grains, nuts and polyunsaturated fatty acids, as well as dietary patterns high in these food components may exert anti-inflammatory properties and are associated with lower concentrations of inflammatory markers including CRP (Brown and Hu 2001, Chrysohoou *et al.* 2004, Esmaillzadeh *et al.* 2006, Esposito K and et al. 2004, Fung *et al.* 2001, Lopez-Garcia *et al.* 2004, Lutsey *et al.* 2007, Nettleton *et al.* 2006). Also, saturated fatty acid intakes and dietary patterns high in saturated fatty acids may exert pro-inflammatory properties and have been positively associated with inflammatory markers including CRP (Esmaillzadeh *et al.* 2007, Fung *et al.* 2001, Lopez-Garcia *et al.* 2004, Nettleton *et al.* 2006).

In the current study we did not observe any associations between plasma CRP concentrations and the MDS, DQI and DASH-style scores. In relation to the MDS our findings were inconsistent with two previous cross-sectional studies that have evaluated the association between the MDS and CRP (Chrysohoou *et al.* 2004, Fung *et al.* 2005). One of these studies showed that in men and women (aged 18 to 89 years) from the ATTICA cohort

study adherence to the Mediterranean diet score, was negatively associated with CRP by 20% (P = 0.015) (Chrysohoou *et al.* 2004). However, this association was only observed in the unadjusted model. After multivariate adjustment the authors reported a decrease in plasma CRP by 0.21 ± 0.12 mg/L (P < 0.05) per 10 units of the MDS score however, they do not provide a percentage difference in plasma CRP levels. Also, the authors do not provide a definition for the units they used for the MDS; therefore we assumed that 1 point increase in the MDS was associated with 0.021 mg/L decrease in plasma CRP. In the current analysis we observed a decrease in plasma CRP concentration of 0.030 mg/L per quartile (per 1 point increase) in the MDS [β coefficient (SEM): - 0.030 (0.05), P = 0.580] (**Table 7.4**). This finding was of a greater magnitude to that reported in the ATTICA study, although our finding did not reach significance even after considering a number of covariates that may mediate the relationship between the MDS and plasma CRP concentrations. It is noteworthy that the objective of the ATTICA study was to examine whether the benefits of the Mediterranean diet on heart disease may be explained by its ability to modulate systemic inflammation and coagulation (Chrysohoou *et al.* 2004). Therefore, this study adjusted for a number of factors, such as presence of hypertension, diabetes, hypercholesterolemia, and family history of coronary heart disease. However, these additional factors were not included in our analysis which aimed to assess whether greater adherence to the MDS is negatively associated with plasma levels of CRP (mg/L). Also, there was a considerable difference in the range of the score generated in the Attica cohort (0-55 units, although as mentioned above the authors do not provide

a definition for the units that they used for the MDS score) compared to a range between 0 and 10 points for the TwinsUK cohort, which may be indicative of the fact that the scores were constructed in a different way. This difference in addition to the fact that the MDS score is generated mainly according to the distribution of the foods within a specific cohort may provide an explanation why we did not observe an association with CRP.

In the second study among women (aged 43 to 69 years) from the Nurses' Health Study it was shown that higher adherence to the alternate Mediterranean score (aMED) was associated with 24% lower plasma levels of CRP ($P < 0.05$) (Fung *et al.* 2005). In the current study higher adherence to the MDS was only associated with a 2.5% decrease in CRP concentrations and this association was not significant ($P = 0.841$) (**Table 7.3**). Findings were of a similar trend although of a lower magnitude to that reported in the Nurses' Health Study. This difference may be explained by the fact that Fung *et al*. used a modified version of the original Mediterranean score including only whole grain products in the cereal group. This may offer an explanation as to why we did not observe a significant association in our analysis as we developed the original Mediterranean score which includes non-whole grain cereals in addition to whole grain products. Indeed, it has been previously shown that diets high in whole grains may decrease inflammation (Nettleton *et al.* 2006) (Schulze *et al.* 2005) and diets high in refined grains are associated with increased risk of diseases associated with inflammation, such as diabetes (Schulze and

Hu 2005). It is noteworthy that in the study from Fung *et al*. mean CRP concentration in the first quintile of the aMED score was 3.4 ± 4.0 mg/L. This concentration was higher compared to the mean CRP concentration in the first quartile of the MDS in the current analysis (2.5 ± 2.3 mg/L). The median CRP concentration under physiological conditions is 0.8 mg/L and CRP levels > 3 mg/L represent a high risk for cardiovascular disease according to the American Heart Association Consensus panel and the Centres for Disease Control and Prevention (CDC/AHA) (de Ferranti and Rifai 2007). This suggests that associations with diet may only be observed when population ranges of plasma CRP are higher.

In the current analysis there was also no association between the DASH-style score and CRP, although one previous cross-sectional study in women from the Nurses' Health Study cohort suggested that the DASH-style score was associated with lower plasma levels of CRP by 20 % (Fung *et al.* 2008). However, these findings were observed in a cross-sectional analysis of a sub-group from the larger prospective study focusing mainly on the influence of the DASH-style score on the incidence of nonfatal myocardial infarction, coronary heart disease death and stroke over 24 years of follow-up. However, the authors do not provide any information about the baseline characteristics of this subgroup of women, which does not enable comparisons with our study.

No association was also observed for the DQI score. This finding is consistent with the only previous cross-sectional study among women aged

43 to 69 years from the Nurse's Health Study. This study has shown that a modified version of the DQI, the diet Quality Index Revised (DQI-R) was not associated with plasma levels of CRP or other inflammatory markers (Fung *et al.* 2005).

Although the MDS, DQI and the DASH-style scores account for diets high in food components, such as fruit and vegetables and whole grains (Brown and Hu 2001, Fung *et al.* 2001, Lopez-Garcia *et al.* 2004, Nettleton *et al.* 2006), and diets low in saturated fat and red processed meat, respectively, that have been previously associated with lower levels of CRP (Esmaillzadeh *et al.* 2007), associations in this study did not reach significance. However, the DQI score also includes calcium and sodium intakes and the DASH-style score includes only sodium intake in its criteria. To date, there is no evidence suggesting that calcium intake may be related to inflammation. Also, data on sodium intake are conflicting with one cross-sectional study suggesting that sodium intake (assessed by 24-h urinary excretion) was associated with lower systemic inflammatory markers (including CRP) (Fogarty *et al.* 2009), and one randomised double blind placebo-controlled trial reporting no effects of low sodium diet on high-sensitivity CRP (hs-CRP) (Forrester *et al.* 2010). Therefore, as sodium and calcium are included in the DQI and the DASH-style scores this may impact on their ability to detect associations with CRP.

The lack of associations observed in the current study might also be due to the fact that other lifestyle factors than diet have a greater influence on CRP.

Therefore, to further evaluate whether the findings were due to the potential protective effect of a better quality diet or due to an overall healthy lifestyle, analyses was calculated for age per 10 years, smoking habit, physical activity, body mass index (BMI, kg/m^2), energy intake per 1000 cal, anti-inflammatory medication and hormone replacement use to enable comparisons (**Table 7.4**). Interestingly, in this cohort, physical activity had a greater relationship with CRP than the MDS, DQI and DASH-style scores. After multivariate adjustment for relevant confounders the effect of physical activity and BMI was 6 times greater of that of the MDS and DASH-style scores and 3 times greater of that of the DQI score on CRP ($P < 0.05$ for all) (**Table 7.4**). Specifically, there was a strong effect of BMI on CRP and the magnitude of association was equivalent to 0.2 mg/L increase in CRP for every unit increase in BMI (P for all < 0.001). This finding reflects data from previous epidemiological studies showing that a number of obesity indices, including BMI are independently associated with higher plasma CRP concentrations in various populations (Choi *et al.* 2013, Festa *et al.* 2001, Hodge *et al.* 2010, Khoo *et al.* 2011). It is also well established that the adipose tissue secretes a number of pro-inflammatory cytokines, such as interleukin-6 (IL-6), which further triggers the hepatic synthesis of CRP (Santos *et al.* 2005, Tilg and Moschen 2006, You *et al.* 2008). In addition, it has been shown *in vitro* that mature adipocytes isolated from human adipose tissue were directly involved in the release of CRP (Calabro *et al.* 2005). Also, physical activity was significantly inversely associated with plasma levels of CRP with a magnitude of association equivalent to 0.2 mg/L lower CRP per category of physical activity in this study. Notably, in a recent

review which evaluated the relationship between the inflammatory marker CRP and exercise, it was reported that interventions that were based just on increasing the physical activity had no effect on the inflammatory marker CRP (Michigan *et al.* 2011). However, intervention programmes that were based on lifestyle changes including both exercise and healthy diet reported decreases on CRP, probably related to weight reduction. The association observed in our cohort was of a similar magnitude of that of a randomised controlled trial among obese postmenopausal women aged 50-75 years, in which mean CRP was reduced by 0.24 mg/L from baseline after an exercise intervention programme of one year (Campbell *et al.* 2009).

In the current study although lifestyle factors had an effect on plasma CRP, the association of the HDI and AHEI scores remained significant even after accounting for all relevant lifestyle factors. Of interest, after multivariate adjustment, the effect of these two scores on CRP was similar to the effect of 10 years of age. However, the observed findings may be because the dietary factors included in the scores are more likely associated with beneficial effects on CRP.

The mechanisms involved in the relationship between diet quality and the inflammatory marker CRP cannot be specifically identified due to the nature of this study which assessed cross-sectional associations. Nevertheless, findings from epidemiological studies that have shown that greater consumption of fruits and vegetables, whole grains, dietary fiber, nuts and seeds and diet patterns high in these components were associated with lower

levels of inflammatory markers including CRP offer support to these findings (Esmaillzadeh *et al.* 2006, Lutsey *et al.* 2007, Nettleton *et al.* 2006). In addition, micronutrients included in these foods, such as vitamins C (Block *et al.* 2009) and E (Helmersson *et al.* 2009), magnesium (Chacko *et al.* 2010, Chacko *et al.* 2011, Moslehi *et al.* 2012, Song *et al.* 2007), selenium (Duntas 2009, Scheurig *et al.* 2007) and carotenoids (Helmersson *et al.* 2009, Walston *et al.* 2006, Wang *et al.* 2008) have been associated with lower levels of inflammatory markers including CRP. Consequently, the inverse associations we found between CRP and healthy diet scores may be partly explained due to the anti-inflammatory effects of an overall healthier diet containing food components high in nutrients with anti-inflammatory properties.

7.5 Strengths and limitations

The strengths of this study include the large sample size and the fact that this was the first study that directly compared five of the most commonly used predefined diet quality scores based on adherence to dietary patterns or to dietary recommendations with plasma levels of CRP. An additional strength was that two of the scores have been previously validated by 10 to 12 24 h dietary recalls (Benítez-Arciniega *et al.* 2011) and against plasma biomarkers of vitamin C, a-tocopherol, β-cryptoxanthin and phospholipid fatty acids (Neuhouser *et al.* 2003). Also, the FFQ used in the current study was previously compared and validated against a 7-day weighed record in the EPIC Norfolk study, and although the two approaches to measure dietary intake were different, both methods identified similar intakes of

macronutrients when these were expressed as percentage of total energy intake (Bingham *et al.* 2001). This FFQ was also validated against urinary and plasma biomarkers of intake, such as urinary nitrogen for protein intake, urinary potassium and sodium, plasma ascorbic acid and plasma n-3 PUFAs (McKeown *et al.* 2001, Welch *et al.* 2006). In addition, it has been shown previously that serum β-cryptoxanthin and zeaxanthin concentrations were moderately correlated with dietary carotenoids intakes measured by an FFQ (Tucker *et al.* 1999). Furthermore, in order to examine associations between dietary intake and health outcomes participants need to be ranked according to their usual dietary intake and FFQs have been shown to rank individuals well (Molag *et al.* 2007).

This study also has a number of limitations. The cross-sectional nature of the study limits causal inference, but enables us to generate hypotheses regarding associations between diet quality and the inflammatory marker CRP based on plausible mechanisms. Higher adherence to the diet scores may be an indicator of a healthy lifestyle besides diet, and even though all analyses were adjusted for lifestyle factors, residual confounding cannot be ruled out because of the observational nature of the study. In addition, as in all observational studies, measurement error in self-reported dietary intakes is inevitable. It is also widely acknowledged that the use of FFQs for measuring dietary intake has its own limitations related to memory errors, they provide relative than absolute intake, they introduce bias due to over- or under-reporting, and they may introduce systematic errors as preparation methods are inadequately considered (McNeill *et al.* 2009). Also, FFQs

measure food intake over a period of one year and reflect the habitual diet over this period of time but not lifetime dietary habits (Teucher *et al.* 2007). Another limitation is that no specific statistical weighting for each component has been used regarding the construction of each score, assuming that all food groups and nutrients contributed equally to the diet quality. The observed findings relate to women and further work is needed to investigate if these findings are replicated in a male population of the same country or in populations from different ethnic backgrounds.

7.6 Concluding remarks

In conclusion, we found that a better diet quality as assessed by two predefined derived diet scores was inversely associated with CRP in women of a wide age range, the HDI by 14 % (P = 0.041) and the AHEI by 13 % (P = 0.051) of the population geometric mean for CRP. These associations were observed after accounting for age, physical activity, smoking, energy intake, BMI, anti-inflammatory medication and use of hormone replacement therapy. Findings from the current study extend the results from previous cross-sectional studies by focusing on the overall diet quality developed based on *a priori* knowledge instead of patterns derived based on empirical methods in association with the inflammatory marker CRP. Nevertheless, associations were not observed for all diet quality scores possibly due to the fact that other factors, such as physical activity and body mass index may be more important than diet on CRP. Yet, it is also possible that a longitudinal study design might be more suitable approach to detect associations between diet quality and the inflammatory marker CRP, as it might be more

likely to observe a difference in the inflammatory status over time due to a healthier diet. Additionally, maybe long-term interventions are needed to observe an effect and a dose-response relationship needs to be demonstrated in order to define the anti-inflammatory or pro-inflammatory effects of specific nutritional factors that can further modulate disease risk.

Despite their differences, the derived diet scores reflected a common pattern characterised by high intakes in fruit and vegetables, nuts, legumes, dietary fiber and/or whole grains, and low intake of red and processed meat, and saturated fat, suggesting that plant derived sources of vitamin C, magnesium and carotenoids may be important for the inflammatory status. Results from this study suggest that is important for adult women to consume a variety of foods to ensure lower plasma concentrations of the inflammatory marker CRP. From a public health perspective, these findings will help to improve the knowledge and understanding of the effects of dietary and lifestyle factors on CRP, which is predictive for the development of chronic diseases, and provide useful information in developing and planning dietary intervention trials to improve the inflammatory status in adult life.

TABLES

Table 7.1 Baseline characteristics of female subjects from the TwinsUK cohort[1]

Characteristic	*N*=1658, 18-79 yrs
Age (y)	49.7±12.5
Weight (kg)	65.5±10.6
Height (cm)	162±5.96
BMI (kg/m^2)	24.9±4.0
Inflammation marker	
C-reactive protein (*CRP*) (mg/L)	2.49±2.30
Physical activity %	
Inactive	20.5
Moderate	54.8
Active	24.7
Smoking history %	
Never	50.2
Current	16.7
Former	33.1
Hormone replacement therapy %	
No	93.7
Yes	6.30
Anti-inflammatory medication %[2]	
No	93.8
Yes	6.20
Derived diet scores (points)	
Mediterranean Diet Score (MDS)	4.54±1.79
Healthy Diet Indicator (HDI)	3.45±1.32
Diet Quality Index (DQI)	11.8±1.9
Alternate Healthy Eating Index (AHEI)	49.6±11.1
Dietary Approach to Stop Hypertension score (DASH-style)	23.3±5.6
Dietary components	
Energy intake (kcal/d)	1976±512
MDS[3]	
Fruit & nuts group (g/d)	254±192
Vegetables group (g/d)	276±156
Ratio of mono- and poly- to saturated fat	1.49±0.37
Milk & dairy group (g/d)	426±197
Meat group (g/d)	86.4±47.5
Fish group (g/d)	36.3±27.8
Legumes intake (g/d)	22.8±26.6
Cereals intake (g/d)	208±101
Alcohol intake (g/d)	10.1±13.8
HDI	
Fruit & vegetables group (g/d)	583±302
Complex carbohydrates group (% Energy/d)	47.5±18.2
Dietary fiber group (g/d)	20.4±7.5
Pulses & nuts group (g/d)	25.6±27.6
Mono- & disaccharides group (% Energy/d)	23.9±5.7
Protein intake (% Energy/d)	16.7±2.6
Saturated fat intake (%Energy/d)	11.6±2.9
Polyunsaturated fat intake (% Energy/d)	6.45±1.57
Cholesterol intake (mg/d)	224±86
DQI	
Fruit & vegetables group (servings/d)	7.39±3.83
Total fat (% Energy/d)	30.9±5.5
Complex carbohydrates (servings/d)	3.82±1.91
Saturated fat (% Energy/d)	11.6±2.93

Cholesterol (mg/d)	224±86
Protein (% RDA)	147±24
Sodium intake (mg/d)	2258±733
Calcium intake (mg/d)	1144±378
AHEI	
Vegetables (servings/d)	3.54±2.01
Fruit (servings/d)	3.14±2.39
Nuts and soy protein (servings/d)	0.10±0.21
Ratio of white to red meat	2.75±6.09
Dietary fiber (g/d)	20.4±7.5
Trans fat (% energy)	1.11±0.38
Polyunsaturated to saturated fat ratio	0.59±0.23
Vitamin supplement use % (yes/no)	54.4 / 45.6
Alcohol (servings/d)	0.78±1.05
DASH-style score	
Fruits intake (servings/d)	4.06±2.73
Vegetables intake (servings/ d)	3.32±1.98
Nuts & legumes intake (servings/d)	0.52±0.40
Whole grains intake (servings/d)	1.85±1.63
Low-fat dairy intake (servings/d)	0.95±0.66
Sodium (mg/d)	2258±733
Red & processed meat intake (servings/d)	0.99±0.56
Sweetened beverages intake (servings/d)	0.38±0.79

[1] Values are presented as mean ± SEM or else indicated
[2] Aspirin, statins, non-steroidal anti-inflammatory drugs (NSAID)
[3] Values represent the mean for the variables used in construction of the dietary scores

Table 7.2 Baseline characteristics of 1658 female subjects from the TwinsUK cohort across C-reactive protein (mg/L) quintiles[1]

	Q1 (0.16-0.62)	Q2 (0.63-1.20)	Q3 (1.21-2.21)	Q4 (2.22-4.23)	Q5 (4.24-9.86)	P for trend
N	341	325	331	331	330	
Age (y)	46.9±0.8	48.7±0.7	51.3±0.7	50.3±0.8	51.2±0.8	<0.001
Weight (kg)	60.8±0.5	63.1±0.5	65.6±0.6	67.5±0.7	70.4±0.8	<0.001
Height (cm)	162±0.4	163±0.3	162±0.4	162±0.4	161±0.4	0.006
BMI (kg/m^2)	23.1±0.2	23.8±0.2	25.0±0.2	25.6±0.2	27.2±0.3	<0.001
Physical activity %						
Inactive	17.0	20.0	18.4	16.9	30.0	<0.001
Moderate	54.8	54.5	60.1	54.4	50.3	
Active	28.2	25.5	21.5	28.7	19.7	
Smoking history %						
Never	54.8	48.6	46.8	49.5	50.9	0.416
Current	13.5	19.1	18.4	15.1	17.6	
Former	31.7	32.3	34.7	35.4	31.5	
Hormone replacement therapy %						
No	91.5	95.7	91.8	94.2	93.7	0.083
Yes	8.5	4.3	8.2	5.8	6.3	
Anti-inflammatory medication %[2]						
No	94.1	94.8	94.3	94.0	92.1	0.674
Yes	5.9	5.2	5.7	6.0	7.9	
Derived diet scores (points)						
Mediterranean Diet Score (MDS)	4.55±0.11	4.54±0.11	4.66±0.10	4.59±0.10	4.38±0.10	0.378
Healthy Diet Indicator (HDI)	3.61±0.08	3.48±0.07	3.47±0.08	3.45±0.07	3.23±0.07	0.001
Diet Quality Index (DQI)	11.7±0.12	11.8±0.11	12.0±0.11	11.8±0.11	11.5±0.11	0.295
Alternate Healthy Eating Index (AHEI)	50.8±0.7	49.1±0.6	50.8±0.6	49.5±0.6	48.0±0.6	0.009
Dietary Approach to Stop Hypertension score (DASH-style)	23.5±0.31	23.1±0.32	23.3±0.33	23.3±0.31	23.5±0.33	0.828
Energy intake (kcal/d)	1996±28.5	1972±29.1	1956±28.8	1961±27.8	1992±31.3	0.834
Inflammation markers						
CRP (mg/L)	0.41±0.01	0.91±0.01	1.67±0.02	3.11±0.03	6.42±0.09	<0.001

[1] Values are means and standard errors (SEM) by quintiles of CRP (mg/L)

Table 7.3 The associations between C-reactive protein (mg/L) and the derived diet scores quartiles in 1658 female subjects from the TwinsUK cohort aged 18-79 years[1]

	C-reactive protein (mg/L)				
Quartiles	**Q1**	**Q2**	**Q3**	**Q4**	**P for trend**
MDS (points)	3[3]	4	5	6	
Unadjusted	1.61 (1.46,1.79)	1.58 (1.41,1.77)	1.68 (1.51,1.86)	1.54 (1.41,1.69)	0.651
Adjusted model[2]	1.62 (1.48,1.78)	1.56 (1.41,1.72)	1.63 (1.47,1.80)	1.58 (1.45,1.73)	0.841
HDI (points)	2	3	4	5	
Unadjusted	1.68 (1.51,1.87)	1.72 (1.57,1.88)	1.61 (1.46,1.77)	1.34 (1.20,1.50)	0.004
Adjusted model[2]	1.65 (1.49,1.82)	1.68 (1.54,1.82)	1.62 (1.47,1.77)	1.42 (1.28,1.57)	0.041
DQI (points)	9	12	13	14	
Unadjusted	1.62 (1.46,1.80)	1.57 (1.44,1.70)	1.63 (1.43,1.86)	1.60 (1.44,1.77)	0.992
Adjusted model[2]	1.68 (1.53,1.85)	1.56 (1.45,1.68)	1.55 (1.37,1.76)	1.59 (1.44,1.74)	0.417
AHEI (points)	37.5	46.5	53.5	62.5	
Unadjusted	1.67 (1.50,1.86)	1.76 (1.59,1.95)	1.56 (1.41,1.72)	1.40 (1.26,1.56)	0.009
Adjusted model[2]	1.68 (1.52,1.85)	1.67 (1.52,1.83)	1.56 (1.42,1.71)	1.48 (1.34,1.63)	0.051
DASH-style (points)	17	21	25	30	
Unadjusted	1.61 (1.46,1.77)	1.49 (1.35,1.65)	1.65 (1.49,1.82)	1.64 (1.48,1.82)	0.486
Adjusted model[2]	1.61 (1.45,1.79)	1.52 (1.38,1.68)	1.71 (1.56,1.87)	1.54 (1.38,1.73)	0.948

[1] Values are geometric means and 95% confidence intervals (CI) of C-reactive protein (mg/L) by quartiles of the derived diet scores

[2] Adjusted for age (years), physical activity (inactive, moderately active, active), smoking status (never, current, former), body mass index (kg/m^2), energy intake (kcal/d) (only for MDS, AHEI, and DASH-style), anti-inflammatory medication (including aspirin, statins, and NSAID) (no/yes) and HRT use (no/yes)

[3] Values are medians of the diet scores

Table 7.4 Relative associations between diet quality scores, lifestyle characteristics and C-reactive protein (mg/L) in 1658 female subjects from the TwinsUK cohort aged 18-79 years

	MDS			HDI			DQI			AHEI			DASH-style		
C-reactive protein (mg/L)	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**	**ß**	**SEM**	**P**
Diet score per quartile	-0.03	0.05	0.580	-0.10	0.05	0.044	-0.06	0.05	0.202	-0.12	0.05	0.018	0.03	0.06	0.690
Age (per 10 y)	0.10	0.05	0.055	0.10	0.05	0.089	0.10	0.05	0.056	0.10	0.05	0.056	0.10	0.05	0.056
Smoking habit															
Current v never	0.18	0.16	0.278	0.15	0.17	0.352	0.16	0.16	0.332	0.15	0.16	0.355	0.19	0.16	0.248
Former v never	0.01	0.13	0.931	0.003	0.13	0.982	0.005	0.13	0.971	0.03	0.13	0.812	0.004	0.13	0.972
Physical activity	-0.18	0.08	0.033	-0.19	0.02	0.042	-0.17	0.08	0.037	-0.16	0.08	0.053	-0.18	0.08	0.028
Body mass index (kg/m^2)	0.19	0.02	<0.001	0.19	0.02	<0.001	0.19	0.02	<0.001	0.19	0.02	<0.001	0.19	0.02	<0.001
Energy intake (per 1000 cal)	0.11	0.11	0.311	*Not adjusted for energy intake*						0.14	0.11	0.204	0.07	0.14	0.638
Anti-inflammatory medication															
No v Yes	0.52	0.26	0.047	0.51	0.26	0.053	0.52	0.26	0.048	0.51	0.27	0.056	0.53	0.26	0.045
HRT															
No v Yes	-0.25	0.23	0.289	-0.25	0.23	0.279	-0.24	0.23	0.292	-0.23	0.23	0.330	-0.25	0.23	0.286

Chapter 8

Association between C-reactive protein levels and indexes of muscle mass and the potential attenuation by dietary constituents

8.0 Introduction

Ongoing research on ageing and sarcopenia suggests a link between sarcopenia and inflammation (Chung *et al.* 2009). The ageing process is associated with a gradual, chronic production and increase of pro-inflammatory cytokines (Roubenoff *et al.* 1998). Age-related cytokine production is a risk factor leading to the predisposition to sarcopenia, as the pro-inflammatory environment triggers catabolism and protein breakdown and therefore leads to imbalances in the synthesis of muscle tissue (Morley and Baumgartner 2004, Roubenoff and Hughes 2000).

Chronic low-grade inflammation is considered detrimental to skeletal muscle mass and strength in both humans and animal models (Howard *et al.* 2007, Siu *et al.* 2008). The potential underlying mechanisms have been previously described in Chapter 1 (section 1.4, p. 32) (Chung *et al.* 2009, Janssen-Heininger *et al.* 2000).

Epidemiological evidence has also observed an inverse relationship between muscle mass, strength and function and inflammatory cytokines (Barbieri *et al.* 2003, Cesari *et al.* 2005, Payette *et al.* 2003, Schaap *et al.* 2006, Schaap *et al.* 2009, Visser *et al.* 2002). In the Health, Aging and Body Composition (Health ABC) study among men and women aged 70-79 years, it was observed that per standard deviation increase in IL-6, grip strength was 1.1 to 2.4 kg lower (Visser *et al.* 2002). Also participants with high levels of IL-6 (> 1.80 pg/ml) and TNF-a (> 3.20 pg/ml) had a smaller muscle area, less appendicular muscle mass, lower knee extensor strength and grip strength.

A report from the InCHIANTI cohort found that among men and women aged 20-102 years, IL-6 was an independent predictor of hand grip strength and muscle power (Barbieri *et al.* 2003). In a cohort of older adults aged 72 to 92 years, from the Framingham Heart study, IL-6 was a significant predictor of fat free mass over two years follow-up (Payette *et al.* 2003). Another report from the InCHIANTI cohort among men and women aged more than 65 years showed that there was an independent association between IL-6 and CRP and muscle strength and poor physical performance (Cesari *et al.* 2004). In the Longitudinal Aging Study Amsterdam (LASA) among men and women with a mean age of 74.6 years, high IL-6 and CRP were associated with a two to three fold higher risk of losing greater than 40 % of muscle strength, however no associations were found with appendicular muscle mass (Schaap *et al.* 2006). Similarly, in the Health, Aging and Body Composition (Health ABC) study among men and women aged 70-79 years, it was observed that TNF-a levels and its soluble receptors were associated with a five year decline in cross-sectional muscle area and hand grip strength (Schaap *et al.* 2009).

These findings were also supported by animal studies showing that administration of IL-6 and TNF-a in rats was associated with changes in muscle metabolism, including increased skeletal muscle protein breakdown, decreased protein synthesis rate, decreased amino acid concentration of skeletal muscle, and muscle wasting (Goodman 1991, Hoshino *et al.* 1991).

A number of nutrients have been associated with muscle mass and strength. Protein (Campbell *et al.* 2001, Castaneda *et al.* 1995), amino acids (Borsheim *et al.* 2008, Scognamiglio *et al.* 2004, Solerte *et al.* 2008) and vitamin D (Dawson-Hughes 2008) have been shown to be beneficial in supplementation studies, and magnesium and potassium are known to play an important role in muscle metabolism (Frassetto *et al.* 1998, Song *et al.* 2007, Song *et al.* 2005). Furthermore, vitamins C, E and selenium and carotenoids are known to reduce oxidative stress and have anti-inflammatory properties (Ferrucci *et al.* 2005, Helmersson *et al.* 2005, Young *et al.* 2004). The results from Chapter 3 (section 3.3, p. 87) showed strong, positive associations with magnesium, potassium, vitamin C, carotene, and β-carotene.

Diet also plays a major role in the regulation of chronic inflammation as dietary constituents have the potential to exert anti-inflammatory as well as pro-inflammatory effects (Cavicchia *et al.* 2009). The potential mechanisms linking diverse dietary constituents, such as magnesium, potassium, vitamin C, carotene, β-carotene, arginine and glutamine, the "whole diet" and both muscle mass and the inflammatory marker CRP have been described in detail in previous chapters (Chapter 3, section 3.4, p. 91; Chapter 4, section 4.0, p. 124; Chapter 5, section 5.4, p. 168; Chapter 6, section 6.0, p. 198; Chapter 7, section 7.4, p .241).

Inflammation has been shown to influence loss of muscle mass in other studies and in the current study was associated (cross-sectionally) with

indexes of fat free mass. I also found that certain nutrients are associated with the inflammatory marker (CRP) and investigated the association between CRP and muscle mass and dietary factors to determine whether diet would influence the association between CRP and muscle mass. To our knowledge there are currently no studies examining if both the habitual intake of nutrients that have been previously associated with muscle mass or have shown to exert anti-inflammatory properties and the "whole diet" mediate the known association between muscle mass and inflammation. This could offer valuable data for the implementation of dietary intervention trials for the prevention of sarcopenia by targeting inflammation. I hypothesised that if diet reduced the association between CRP and muscle mass in the statistical models that this would be interpreted as an influence of diet on the association between CRP and muscle.

8.1 Aims

The aims of this chapter were firstly to test the hypothesis that muscle mass, as assessed by three indexes, fat free mass (FFM, kg), percentage fat free mass (FFM%) and fat free mass index (FFMI, kg/m^2), was inversely associated with C-reactive protein (*CRP*) levels, among women aged 18-79 years from the TwinsUK participants, and whether diet would mediate the proposed association between muscle mass and CRP levels in this cohort.

8.2 Covariate plan and statistical analysis

All analyses were performed using STATA statistical software (version 11.0; STATA Corp, USA). The selection of dietary constituents that may

mediate the association between muscle mass and CRP levels was decided *a priori*. Thus, nutrients and diet scores that showed a greater association with either indexes of muscle mass (such as magnesium, vitamin C, carotene, β-carotene, potassium, MDS, HDI, AHEI, and DASH-style scores) in Chapter 3 (section 3.4, p. 91) and Chapter 5 (section 5.4, p. 168) or CRP levels (such as magnesium, HDI, AHEI scores) in Chapter 6 (section 6.4, p. 209) and Chapter 7 (section 7.4, p. 241) were included. In relation to essential amino acids, although a significant positive association with CRP levels was observed with higher intakes of arginine, glutamine, histidine, isoleucine, threonine and tryptophan (as % energy) (Chapter 6, section 6.3, p. 207), only glutamine and arginine were included in the current analysis as these amino acids have been suggested to play an important role in inflammatory processes related to muscle wasting (Nicastro *et al.* 2012, Pithon-Curi *et al.* 2004, Wu *et al.* 2004, Wu and Morris 1998). Moreover, glutamine was the amino acid with the highest intake in this cohort.

The analyses were performed treating twins as individuals, as previous studies have shown that participants from the TwinsUK registry were similar to age-matched singleton women in the distribution of common traits and outcomes (Andrew *et al.* 2001). Data on dietary intake (FFQ), body composition parameters, and other covariates were available for n = 1658 women, aged 18 to 79 years representing 582 monozygotic (MZ) and 1076 dizygotic (DZ) females who had completed a food frequency questionnaire (FFQ) and had fasting blood samples taken to measure C-

reactive protein (*CRP*) between 1996 and 2000 (Moayyeri *et al.* 2012, Teucher *et al.* 2007).

Prior to conducting statistical analysis an analysis plan with justification for each confounding factor in the multivariate models was developed as described in Chapter 2, section 2.2.5, p. 57.

Briefly, factors that have been previously shown to affect either muscle mass or the inflammatory status or both and may mediate the relationship between muscle mass and CRP were included in the multivariate models (**Model 1** in **Tables 8.1, 8.4, and 8.7**). These factors were age (years), physical activity and smoking status, body mass index (kg/m^2), hormone replacement therapy use (HRT) and anti-inflammatory medication (including statins, non-steroid anti-inflammatory drugs and aspirin) (no or yes, respectively) (Chapter 2, section 2.2.5, p. 57, 58, 60, 64, 66, 68).

The analyses were performed in a three-step process. First, multivariate regression analysis was used to assess associations between indexes of muscle mass (FFM, FFM% and FFMI) across quintiles of plasma CRP concentrations using the robust cluster regression option in STATA (**Model 1, Tables 8.1, 8.4 and 8.7**). Model 1 was adjusted for age, physical activity, smoking, BMI, anti-inflammatory medication and HRT use. Robust cluster regression takes into account clustering of individuals when calculating standard errors of the mean (Richards *et al.* 2007) to ensure familial aggregation within twin pairs was accounted for.

Second, nutrient intakes or diet quality scores that may mediate the relationship between muscle mass and CRP were added in the model (as quintiles for nutrients and as quartiles for diet scores) to assess the change in the β-coefficient for the association between indexes of muscle mass and CRP levels, per quintile of nutrient intakes or per quartile of diet scores (**Model 2, Tables 8.1, 8.4 and 8.7**). Thirdly, this analysis was repeated with additional adjustment for energy intake (as linear term) with exception of the HDI that already takes energy intake into account (**Model 3, Tables 8.1, 8.4 and 8.7**). To understand the effect of any attenuation of the relationship between CRP and muscle mass by dietary factors after multivariate analyses, the percentage difference in β-coefficients between Model 1 and Model 3 was calculated using the following formula:

[(β-coefficient in Model 1 - β-coefficient in Model 3) / β-coefficient in Model 1] x 100.

This model was applied to all the nutrients and dietary patterns except the HDI where the comparison was between Model 1 and Model 2, since energy was not included in this analysis, as explained earlier. This percentage difference (%) in β-coefficients between Model 1 and Model 3 provides an estimate of the potential attenuation of diet on the relationship between CRP and muscle mass. A positive finding indicates that the nutrient/diet score improves the association between indexes of muscle mass and CRP levels or reduces the impact of CRP on indexes of muscle mass, and a negative finding indicates that the nutrient/diet score does not improve the association between indexes of muscle mass and CRP levels, or increases the impact of CRP on indexes of muscle mass.

Since age was inversely and significantly correlated with indexes of muscle mass, this analysis was stratified by age using two categories, less than 50 years (**Tables 8.2, 8.5 and 8.8**) and more than 50 years (**Tables 8.3, 8.6 and 8.9**).

8.3 Results

Descriptive characteristics of this subset (*n* = 1658 women, aged 18-79 years) of the TwinsUK participants have been previously described in Chapter 6 (section 6.3, p. 207 and **Table 6.2,** p. 228). Briefly, the mean age (±SD) of participants was 49.7 ± 12.5 years, mean (±SD) BMI was 24.9 ± 4.0 kg/m^2, and mean (±SD) plasma CRP concentrations were 2.49 ± 2.30 mg/L.

In the multivariate analyses, higher plasma CRP levels were associated with lower indexes of muscle mass. The magnitude of associations observed was a decrease of 0.18 kg in FFM (P =0.028), 1.22 % in percentage FFM (P < 0.001), and 0.07 kg/m^2 in FFMI (P = 0.001) per quintile of plasma CRP levels (**Model 1** in **Tables 8.1, 8.4** and **8.7** (first foot note), respectively).

After stratification for age, higher plasma CRP levels were significantly associated with lower percentage FFM (**Tables 8.5, 8.6**) and FFMI (**Tables 8.8, 8.9**), but not FFM (**Tables 8.2, 8.3**) for women aged less than 50 years and the more than 50 years, respectively. The greatest magnitude of associations observed was a decrease of 1.34 % in percentage FFM (P < 0.001) per quintile of plasma CRP levels in women aged more than 50years (**Model 1** in **Tables 8.6** (first foot note)).

After the addition of nutrients or diet quality scores (**Model 2** in **Table 8.1)** and energy intake (**Model 3** in **Table 8.1**) in the model, the associations between FFM and CRP levels were attenuated (after the addition of magnesium, vitamin C, carotene, β-carotene, and potassium intakes, and the MDS, HDI and AHEI scores). In contrast, intakes of arginine and glutamine did not attenuate the association, and the addition of the DASH-style score in the multivariate model did not alter the association (**Table 8.1**). The greatest percentage difference in β coefficients between Model 1 and Model 3 was observed with the addition of magnesium (6.67%) and the AHEI (7.78%) and HDI (5.00%) scores for the association between FFM and CRP levels (**Table 8.1**). After stratification for age and the addition of nutrients and diet quality scores (**Model 2** in **Tables 8.2 and 8.3),** and energy intake (**Model 3** in **Tables 8.2 and 8.3)** in the model, the associations between FFM and CRP levels were not attenuated in both age groups.

The association between percentage FFM and CRP levels was reduced after the addition of magnesium, arginine and glutamine intakes, and the MDS, HDI, AHEI and DASH-style scores in the model. In contrast, intake of carotene did not improve the association, and the addition of vitamin C, β-carotene, and potassium intakes did not alter the association (**Table 8.4**). The greatest percentage difference in β coefficients between Model 1 and Model 3 was observed with the addition of magnesium (0.74%), arginine (0.99%), the HDI (1.07%) and AHEI (0.99%) scores for the association between percentage FFM and CRP levels (**Table 8.4**). After stratification for age and the addition of nutrients and diet quality scores (**Model 2** in

Tables 8.5 and 8.6), and energy intake (**Model 3** in **Tables 8.5 and 8.6)** in the model, the associations between percentage FFM and CRP levels were reduced after the addition of magnesium, vitamin C, carotene, β-carotene, arginine, glutamine, and the MDS, HDI, AHEI for women aged less than 50 years. In contrast, intake of potassium and the addition of the DASH-style score in the multivariate model did not improve the association (**Table 8.5**). For women aged over 50 years, the associations between percentage FFM and CRP levels were reduced after the addition of magnesium, vitamin C, carotene, β-carotene, potassium, arginine, glutamine, and the HDI, AHEI and DASH-style scores. The addition of the MDS score in the multivariate model did not alter the association (**Table 8.6**).

The association between FFMI and CRP levels was improved after the addition of magnesium, vitamin C, carotene, β-carotene, and potassium intakes, the MDS, HDI and AHEI scores in the model. In contrast, intake of glutamine did not improve the association, and the addition of arginine and the DASH-style score in the multivariate model did not alter the association (**Table 8.7**). The greatest percentage difference in β coefficients between Model 1 and Model 3 was observed with the addition of magnesium (5.41%), potassium (2.70%) and the HDI (5.41%) and AHEI (4.05%) scores for the association between FFMI and CRP levels (**Table 8.7**). After stratification for age and the addition of nutrients and diet quality scores (**Model 2** in **Tables 8.8 and 8.9)** and energy intake (**Model 3** in **Tables 8.8 and 8.9)** in the model, the associations between FFMI and CRP levels were reduced after the addition of magnesium, vitamin C, carotene, β-carotene,

potassium, arginine, glutamine, and the MDS, HDI, AHEI and DASH-style scores for women aged less than 50 years (**Table 8.8**). For women aged over 50 years, the associations between FFMI and CRP levels were reduced after the addition of magnesium, vitamin C, carotene, β-carotene, potassium, and the HDI, AHEI and DASH-style scores. The addition of arginine, glutamine and the MDS score in the multivariate model did not reduce the association (**Table 8.9**).

8.4 Discussion

In this study using a large cross-sectional population-based sample of women aged 18-79 years we evaluated the association between indexes of muscle mass and the inflammatory marker CRP and the potential mediation by dietary components. This is the first population-based study to examine the influence of a range of nutrients and diet quality scores (that have been associated with either indexes of muscle mass or CRP levels in previous chapters of the current thesis) on the association between muscle mass and CRP levels. This study observed that higher concentrations of CRP were associated with lower indexes of muscle mass. It was also shown that magnesium, vitamin C, carotene, β-carotene, potassium intakes, and the MDS, HDI and AHEI scores reduced the impact of CRP on FFM and FFMI; and magnesium, arginine, glutamine intakes, and the MDS, HDI, AHEI and DASH-style scores improved the association between percentage FFM and CRP levels. There is currently only one recent longitudinal study showing that lower protein intake was associated with a greater decline in muscle strength in individuals with high levels of inflammatory markers including

CRP (Bartali *et al.* 2012). This study differed to the current study in that, although it was longitudinal, the study was designed to measure different objectives, it did not include a complete range of nutrients or diet quality scores that may potentially mediate the inflammation and muscle mass relationship, and examined only muscle strength.

The current study observed a significant inverse association between higher levels of CRP and all three indexes of muscle mass. The magnitude of the associations observed was a decrease of 0.18 kg in FFM (P =0.028), 1.22 % in percentage FFM (P < 0.001), and 0.07 kg/m^2 in FFMI (P = 0.001) per quintile of plasma CRP levels (**Model 1** in **Tables 8.1, 8.2** and **8.3**). These associations were observed after adjustments for age, physical activity, smoking, anti-inflammatory medication (including aspirin, statins, and NSAID) and HRT use and were independent of adiposity (assessed by BMI for FFM and FFMI). These findings were consistent with one previous cross-sectional study from the Health, Aging and Body Composition (Health ABC) cohort that observed that participants (aged 70-79 years) with high levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-a) had a smaller muscle area and less appendicular muscle mass (Visser *et al.* 2002). However, this study included both men and women of a different age range, inflammation was measured by different markers, and muscle mass was assessed by different measurements so it is difficult to draw direct comparisons with our study.

Findings from the current study were not consistent with data from the Longitudinal Aging Study Amsterdam (LASA) among men and women

with a mean age of 74.6 years (Schaap *et al.* 2006). In this study high CRP levels (>6.1 μg/mL) were not associated with appendicular muscle mass after three years of follow-up. Although the authors adjusted for a number of confounding factors including several inflammation-related chronic diseases and three years weight change, it might be possible that other subclinical diseases may have been present in the study population and may have confounded the observed associations (Schaap *et al.* 2006).

It is noteworthy that in the current study although analysis was adjusted for a number of confounding factors, the effect of CRP on indexes of muscle mass was 1.5- for FFM, 3- for FFMI and 7.2- times greater than that of age for FFM, FFMI and %FFM, respectively. Of interest the ß-coefficient (95 % CI) for FFMI was -0.074 (-0.12, -0.03), P = 0.001 compared to the ß-coefficient (95 % CI) for age which was -0.025 (-0.03, -0.02), P < 0.001.

The current analysis observed that magnesium intake mediated the association between CRP levels and indexes of muscle mass by reducing the association observed between CRP and all three indexes of muscle mass with a range of difference between 0.74 to 6.67 %. Furthermore, the 6.67 % reduction in the association between CRP and FFM (kg) reported per quintile of magnesium intake equated to a 43 mg/d mean difference in magnesium intake between the lower (315 mg/d) and the higher (358 mg/d) intake in the third quintile. We chose the range difference in the third quintile as equivalent of one quintile as a reference for the mean quintile difference. In food terms, this difference would correspond to consumption

of 1.8 portions of whole meal bread per day (64.8 g/d) from the habitual diet (assuming 1 portion of whole meal bread to be 36 g for adults).

The mechanisms involved in the impact of magnesium intake on the association between muscle mass and the inflammatory marker CRP cannot be specifically identified due to the nature of the study which was designed to assess cross-sectional associations. A number of previous studies however, have indicated an inverse association between dietary magnesium intake and metabolic and inflammatory conditions, and its importance in metabolic and inflammatory pathways has been acknowledged in Chapter 6 (section 6.4, p. 218 and 219). The important role of magnesium (an essential micronutrient which acts as a cofactor in multiple enzymatic reactions in the human body) on muscle metabolism, specifically on the function of muscle mitochondria, the control of oxidative stress, and its potential anti-inflammatory properties have also been discussed in Chapter 3 (section 3.4, p. 97 and 98) and offers support to our findings.

The current analysis also observed a greater effect of the HDI and AHEI scores on the association between CRP levels and indexes of muscle mass. The greatest percentage difference in β coefficients between Model 1 and Model 3 was observed for the AHEI score which mediated the impact of CRP on FFM (kg) by almost 8 %. In other words, for every 9 points increase in the AHEI score there was an 8 % improvement in the association between CRP and FFM. Although the strength of the association was modest, this was observed after analyses was accounted for age, physical

activity, smoking, anti-inflammatory medication (including aspirin, statins, and NSAID) and HRT use and were independent of adiposity (assessed by BMI) factors that are known to affect both muscle mass and CRP. When translating the differences in nutrient intakes between extreme quartiles of the AHEI score into comparable amounts of food, the differences in vitamin C intake, for example, between extreme quartiles of the diet scores (which was shown in Chapter 5, **Table 5.3**, p. 189) equated to 2.2 portions of oranges for the AHEI (assuming one portion of oranges to be 120 g for adults).

Again, the mechanisms involved in the influence of improved diet quality (assessed by diet quality indices) on the association between CRP levels and indexes of muscle mass cannot be specifically identified due to the cross-sectional nature of our study. Nevertheless, findings from previous epidemiological studies have shown that dietary patterns characterised by increased consumption of fruits and vegetables, whole grains, dietary fiber and low intakes of saturated and trans fatty acids were associated with lower levels of markers of inflammation, oxidative stress, and endothelial dysfunction, offer support to the current findings (Baer *et al.* 2004, Dai *et al.* 2008, Esmaillzadeh *et al.* 2007, Esposito *et al.* 2004, Fung *et al.* 2001, Fung *et al.* 2001, Lopez-Garcia *et al.* 2004, Lopez-Garcia *et al.* 2005, Mozaffarian *et al.* 2004, Nettleton *et al.* 2006). Moreover, the Alternate Healthy Eating Index (AHEI) has been significantly associated with lower plasma CRP levels in diverse populations, including women (Fargnoli *et al.* 2008, Fung *et al.* 2005). The development of age-associated declines in muscle mass,

muscle strength, physical function, mobility and frailty have been attributed, in part, to the inflammation that occurs with the ageing process (Chung *et al.* 2009, Howard *et al.* 2007). Notably, although age is known to be highly correlated with both muscle mass (Cruz-Jentoft *et al.* 2010, Lynch *et al.* 1999) and inflammation (Chung *et al.* 2009), in the current study after multivariate analysis, the effect of the AHEI score [ß coefficient (95 % CI): 0.33 (-0.14, -0.52), P = 0.001] on the association between CRP levels and FFM was almost 3 times greater of that of age [ß coefficient (95 % CI): -0.12 (-0.14, -0.10), P < 0.001]. This finding indicates that diet quality may be a beneficial factor in attenuating the negative association between inflammation and muscle mass, independent of age.

Our findings indicated similar patterns for vitamin C, carotene, β-carotene, potassium and the MDS score for both FFM and FFMI. The observed impact of healthy diet scores on the association between CRP levels and muscle mass, may be partly explained by the anti-inflammatory effects of an overall healthier diet containing food components high in nutrients, such as magnesium, vitamin C, potassium and carotenoids.

In relation to the essential amino acids, arginine and glutamine, it was observed that they did not mediate the association between FFM, FFMI and CRP levels (**Tables 8.1** and **8.2**, respectively). This finding was expected as in previous analyses in the same participants from the TwinsUK study in Chapter 6 (section 6.3, p. 207) higher intakes of arginine and glutamine were associated with higher CRP levels, although the biological mechanisms underlying such a relationship are largely unknown, and these

are the first observations that we know about in an overall healthy population.

However, these dietary constituents did not attenuate the association between CRP levels and percentage FFM in a similar pattern to FFM and FFMI. As previously discussed in Chapter 5 (section 5.4, p. 168) the way the indexes of muscle mass were calculated may have led to the differences observed. It is well documented that in order to evaluate FFM in relation to body size and nutritional status, FFM should be considered in relation to height, because FFM expressed in absolute weight or as a percentage of body weight is not adequate because FFM increases with height. Fat free mass index (weight/height^2) which eliminates differences associated with height is an important marker of health and disease status (Coin *et al.* 2008, Heymsfield *et al.* 2011, Kyle *et al.* 2003). Moreover, percentage FFM has a large variation in the current analysis (38.8-80.6 %) which might have influenced the results.

After stratification for age, the current analyses showed that results remained the same for the older group of women aged more than 50 years. Therefore, although age is a major determinant of muscle mass, it does not make a difference in the direction of mediation between indexes of muscle mass and CRP levels by the dietary constituents studied. Interestingly, the percentage attenuation of dietary intake of magnesium, vitamin C, carotene, β-carotene, and potassium, on the association between FFMI and CRP levels was higher in the older group of women compared to the whole sample. This finding may indicate that intake of these dietary constituents is

important for older ages and may improve the negative impact of inflammation as per CRP on muscle mass. In addition, the percentage attenuation of dietary intake of magnesium, vitamin C, carotene, β-carotene, potassium, arginine and glutamine, and a healthier dietary patterns such as the MDS, HDI, AHEI and DASH-style score, on the association between FFMI and CRP levels was also higher in the younger group of women compared to the whole sample. This finding may further indicate that it is important for women in this age group to consume a variety of plant-based constituents and a healthier diet higher in fruits and vegetables, whole grains, nuts, dietary fiber and low in red and processed meat and saturated fats in order to potentially prevent the negative impact of inflammation as per CRP on muscle mass.

8.5 Strengths and limitations

The strengths of this study include the large sample size and the fact that this was the first study to examine the influence of a range of nutrients and diet quality scores (that have been associated with either indexes of muscle mass or CRP levels in previous chapters of the current thesis) on the association between muscle mass and CRP levels. Also, the FFQ used in the current study was previously compared and validated against a 7-day weighed record in the EPIC Norfolk study, and although the two approaches to measure dietary intake were different, both methods identified similar intakes of macronutrients when these were expressed as percentage of total energy intake (Bingham *et al.* 2001). This FFQ was also validated against urinary and plasma biomarkers of intake, such as urinary nitrogen for

protein intake, urinary potassium and sodium, plasma ascorbic acid and plasma n-3 PUFAs (McKeown *et al.* 2001, Welch *et al.* 2006). In addition, it has been shown previously that serum β-cryptoxanthin and zeaxanthin concentrations were moderately correlated with dietary carotenoids intakes measured by an FFQ (Tucker *et al.* 1999). Furthermore, in order to examine associations between dietary intake and health outcomes participants need to be ranked according to their usual dietary intake and FFQs have been shown to rank individuals well (Molag *et al.* 2007).

This study also has a number of limitations. The cross-sectional nature of the study limits causal inference, but enables us to generate hypotheses regarding associations between indexes of muscle mass and the inflammatory marker CRP and the potential mediation by dietary constituents based on plausible mechanisms. In addition, the analysis was performed in a population of a wide age range of participants with relatively low CRP levels and this hypothesis may be more important in older people with higher CRP levels. On the other hand, this is the first study to investigate this in a cohort including older people. Ideally this hypothesis would have been better investigated in an intervention study.

In addition, as in all observational studies, measurement error in self-reported dietary intakes is inevitable. It is also widely acknowledged that the use of FFQs for measuring dietary intake has its own limitations related to memory errors, they provide relative than absolute intake, they introduce bias due to over- or under-reporting, and they may introduce systematic

errors as preparation methods are inadequately considered (McNeill *et al.* 2009). Also, FFQs measure food intake over a period of one year and reflect the habitual diet over this period of time but not lifetime dietary habits (Teucher *et al.* 2007). Another limitation is that no specific statistical weighting for each component has been used regarding the construction of each score, assuming that all food groups and nutrients contributed equally to the diet quality. The observed findings relate to women and further work is needed to investigate if these findings are replicated in a male population of the same country or in populations from different ethnic backgrounds.

8.6 Concluding remarks

In conclusion, we found in multivariate analyses that higher CRP levels were associated with lower FFM by 0.18 kg, percentage FFM by 1.22 %, and FFMI by 0.07 kg/m^2 per quintile of plasma CRP levels. Intakes of magnesium, vitamin C, carotene, β-carotene, potassium, and the MDS, HDI and AHEI scores appeared to reduce the impact of CRP on FFM and FFMI; and magnesium, arginine, glutamine intakes, and the MDS, HDI, AHEI and DASH-style scores appeared to reduce the association between percentage FFM and CRP levels. Notably, although age is known to be highly correlated with both muscle mass (Cruz-Jentoft *et al.* 2010, Lynch *et al.* 1999) and inflammation (Chung *et al.* 2009), findings from the current analysis indicated that diet quality may be a beneficial factor in attenuating the negative association between inflammation and muscle mass, independent of age. Notably, in additional stratification for age analyses, results remained the same regarding the direction of mediation of indexes

muscle mass by CRP levels by the dietary constituents studied. However, the percentage attenuation by a variety of plant-based constituents and healthier dietary patterns (higher in fruits and vegetables, whole grains, nuts, dietary fiber and low in red and processed meat and saturated fats) appeared to be higher in the women aged over 50 years, suggesting that following these dietary patterns may potentially be beneficial, reducing the negative impact of inflammation as per CRP on muscle mass in older women.

To our knowledge, to date, no other population studies have examined the influence of a number of dietary constituents on the association between muscle mass and CRP levels or other inflammatory markers.

Results from this study suggest that for adult women a healthier dietary pattern characterised by high intakes in fruit and vegetables, nuts, legumes, dietary fiber and/or whole grains, as well as consumption of food sources of vitamin C, magnesium, potassium and carotenoids, and low intakes of red and processed meat and saturated fat, may be important in reducing the negative impact of CRP levels on muscle mass seen with age. From a public health perspective, the novel findings from the current analysis may offer some insight in order to better understand whether and to what extent dietary and lifestyle factors may impact on the association between muscle mass and inflammation in adult women with normal weight, BMI and CRP concentrations. These preliminary findings may also provide useful information in developing and planning dietary intervention trials for the prevention of sarcopenia by targeting inflammation.

TABLES

Table 8.1 The association between fat free mass (FFM, kg) and quintiles of CRP and potential mediation by dietary constituents

FFM-CRP[1]	β-coefficient (Model 2)[2]	P	β-coefficient (Model 3)[3]	P	Percentage difference (%) in β-coefficients between Model 1 and Model 3[4]
Per quintile					
Magnesium	-0.166	0.041	-0.168	0.039	6.67
Vitamin C	-0.178	0.029	-0.178	0.029	1.11
Carotene	-0.178	0.030	-0.178	0.029	1.11
β-carotene	-0.176	0.031	-0.177	0.031	1.67
Potassium	-0.175	0.033	-0.177	0.031	1.67
Arginine	-0.187	0.023	-0.181	0.028	-0.56
Glutamine	-0.186	0.024	-0.181	0.027	-0.56
Per quartile					
MDS	-0.178	0.028	-0.178	0.029	1.11
HDI	-0.171	0.036			5.00
AHEI	-0.167	0.044	-0.166	0.042	7.78
DASH-style score	-0.180	0.028	-0.180	0.028	0

[1] **Model 1:** FFM was negatively associated to CRP (β-coefficient for the association between FFM and CRP = -0.180, P = 0.028). Adjusted for age, physical activity, smoking, BMI, anti-inflammatory medication, HRT use

[2] **Model 2:** Adjusted as Model 1 and additionally for nutrient intake or diet scores

[3] **Model 3:** Adjusted as Model 2 and for energy intake (apart from HDI)

[4] **Formula for calculation:**

[(β coefficient in Model 1 - β coefficient in Model 3) / β-coefficient in Model 1] x 100

A positive finding indicates that the nutrient/diet score improves the association between FFM and CRP levels or reduces the impact of CRP on FFM, and a negative finding indicates that the nutrient/diet score does not improve the association between FFM and CRP levels or increases the impact of CRP on FFM.

MDS: Mediterranean diet score; **HDI:** Healthy Diet Indicator; **AHEI:** Alternate Healthy Eating Index; **DASH-style score:** based on Dietary Approaches to Stop Hypertension diet pattern

Table 8.2 The association between fat free mass (FFM, kg) and quintiles of CRP and potential mediation by dietary constituents in women aged less than 50 years

FFM-CRP[1]	β-coefficient (Model 2)[2]	P	β-coefficient (Model 3)[3]	P	Percentage difference (%) in β-coefficients between Model 1 and Model 3[4]
Per quintile					
Magnesium	-0.138	0.219	-0.139	0.218	11.5
Vitamin C	-0.148	0.190	-0.150	0.187	4.46
Carotene	-0.151	0.180	-0.150	0.186	4.46
β-carotene	-0.151	0.182	-0.149	0.187	5.10
Potassium	-0.145	0.199	-0.149	0.189	5.10
Arginine	-0.158	0.165	-0.133	0.235	15.3
Glutamine	-0.160	0.161	-0.138	0.218	12.1
Per quartile					
MDS	-0.143	0.200	-0.140	0.209	10.8
HDI	-0.140	0.215			10.8
AHEI	-0.128	0.252	-0.128	0.256	18.5
DASH-style score	-0.153	0.177	-0.154	0.175	1.91

[1] **Model 1:** FFM was negatively associated to CRP (β-coefficient for the association between FFM and CRP = -0.157, P = 0.166). Adjusted for age, physical activity, smoking, BMI, anti-inflammatory medication, HRT use

[2] **Model 2:** Adjusted as Model 1 and additionally for nutrient intake or diet scores

[3] **Model 3:** Adjusted as Model 2 and for energy intake (apart from HDI)

[4] **Formula for calculation:**

[(β coefficient in Model 1 - β coefficient in Model 3) / β-coefficient in Model 1] x 100

A positive finding indicates that the nutrient/diet score improves the association between FFM and CRP levels or reduces the impact of CRP on FFM, and a negative finding indicates that the nutrient/diet score does not improve the association between FFM and CRP levels or increases the impact of CRP on FFM.

MDS: Mediterranean diet score; **HDI:** Healthy Diet Indicator; **AHEI:** Alternate Healthy Eating Index; **DASH-style score:** based on Dietary Approaches to Stop Hypertension diet pattern

Table 8.3 The association between fat free mass (FFM, kg) and quintiles of CRP and potential mediation by dietary constituents in women aged more than 50 years

FFM-CRP[1]	β-coefficient (Model 2)[2]	P	β-coefficient (Model 3)[3]	P	Percentage difference (%) in β-coefficients between Model 1 and Model 3[4]
Per quintile					
Magnesium	-0.145	0.199	-0.145	0.201	8.23
Vitamin C	-0.155	0.172	-0.159	0.162	-0.63
Carotene	-0.156	0.173	-0.159	0.162	-0.63
β-carotene	-0.156	0.174	-0.159	0.164	-0.63
Potassium	-0.151	0.185	-0.152	0.181	3.80
Arginine	-0.170	0.138	-0.168	0.142	-6.33
Glutamine	-0.172	0.132	-0.170	0.137	-7.59
Per quartile					
MDS	-0.166	0.143	-0.167	0.140	-5.69
HDI	-0.157	0.170			0.63
AHEI	-0.153	0.178	-0.156	0.169	1.27
DASH-style score	-0.159	0.164	-0.161	0.159	-1.90

[1] **Model 1:** FFM was negatively associated to CRP (β-coefficient for the association between FFM and CRP = -0.161, P = 0.158). Adjusted for age, physical activity, smoking, BMI, anti-inflammatory medication, HRT use

[2] **Model 2:** Adjusted as Model 1 and additionally for nutrient intake or diet scores

[3] **Model 3:** Adjusted as Model 2 and for energy intake (apart from HDI)

[4] **Formula for calculation:**

[(β coefficient in Model 1 - β coefficient in Model 3) / β-coefficient in Model 1] x 100

A positive finding indicates that the nutrient/diet score improves the association between FFM and CRP levels or reduces the impact of CRP on FFM, and a negative finding indicates that the nutrient/diet score does not improve the association between FFM and CRP levels or increases the impact of CRP on FFM.

MDS: Mediterranean diet score; **HDI:** Healthy Diet Indicator; **AHEI:** Alternate Healthy Eating Index; **DASH-style score:** based on Dietary Approaches to Stop Hypertension diet pattern

Table 8.4 The association between percentage fat free mass (FFM %) and quintiles of CRP and potential mediation by dietary constituents

FFM%-CRP[1]	β-coefficient (Model 2)[2]	P	β-coefficient (Model 3)[3]	P	Percentage difference (%) in β-coefficients between Model 1 and Model 3[4]
Per quintile					
Magnesium	-1.212	<0.001	-1.209	<0.001	0.74
Vitamin C	-1.218	<0.001	-1.218	<0.001	0
Carotene	-1.219	<0.001	-1.219	<0.001	-0.08
β-carotene	-1.218	<0.001	-1.218	<0.001	0
Potassium	-1.218	<0.001	-1.218	<0.001	0
Arginine	-1.215	<0.001	-1.206	<0.001	0.99
Glutamine	-1.219	<0.001	-1.214	<0.001	0.33
Per quartile					
MDS	-1.217	<0.001	-1.217	<0.001	0.08
HDI	-1.205	<0.001			1.07
AHEI	-1.204	<0.001	-1.206	<0.001	0.99
DASH-style score	-1.218	<0.001	-1.217	<0.001	0.08

[1] **Model 1:** FFM% was negatively associated to CRP (β-coefficient for the association between FFM% and CRP = -1.218, P < 0.001). Adjusted for age, physical activity, smoking, anti-inflammatory medication, HRT use

[2] **Model 2:** Adjusted as Model 1 and additionally adjusted for nutrient intake or diet scores

[3] **Model 3:** Adjusted as Model 2 and for energy intake (apart from HDI)

[4] **Formula for calculation:**

[(β coefficient in Model 1 - β coefficient in Model 3) / β-coefficient in Model 1] x 100

A positive finding indicates that the nutrient/diet score improves the association between FFM% and CRP levels or reduces the impact of CRP on FFM%, and a negative finding indicates that the nutrient/diet score does not improve the association between FFM% and CRP levels or increases the impact of CRP on FFM%.

MDS: Mediterranean diet score; **HDI:** Healthy Diet Indicator; **AHEI:** Alternate Healthy Eating Index; **DASH-style score:** based on Dietary Approaches to Stop Hypertension diet pattern

Table 8.5 The association between percentage fat free mass (FFM %) and quintiles of CRP and potential mediation by dietary constituents in women aged less than 50 years

FFM%-CRP[1]	β-coefficient (Model 2)[2]	P	β-coefficient (Model 3)[3]	P	Percentage difference (%) in β-coefficients between Model 1 and Model 3[4]
Per quintile					
Magnesium	-1.026	<0.001	-1.025	<0.001	0.19
Vitamin C	-1.021	<0.001	-1.021	<0.001	0.58
Carotene	-1.026	<0.001	-1.026	<0.001	0.10
β-carotene	-1.025	<0.001	-1.025	<0.001	0.19
Potassium	-1.027	<0.001	-1.028	<0.001	-0.10
Arginine	-1.018	<0.001	-1.004	<0.001	2.24
Glutamine	-1.020	<0.001	-0.999	<0.001	2.73
Per quartile					
MDS	-1.024	<0.001	-1.024	<0.001	0.29
HDI	-0.997	<0.001			2.92
AHEI	-1.007	<0.001	-1.007	<0.001	1.95
DASH-style score	-1.027	<0.001	-1.028	<0.001	-0.10

[1] **Model 1:** FFM% was negatively associated to CRP (β-coefficient for the association between FFM% and CRP = -1.027, P < 0.001). Adjusted for age, physical activity, smoking, anti-inflammatory medication, HRT use

[2] **Model 2:** Adjusted as Model 1 and additionally adjusted for nutrient intake or diet scores

[3] **Model 3:** Adjusted as Model 2 and for energy intake (apart from HDI)

[4] **Formula for calculation:**

[(β coefficient in Model 1 - β coefficient in Model 3) / β-coefficient in Model 1] x 100

A positive finding indicates that the nutrient/diet score improves the association between FFM% and CRP levels or reduces the impact of CRP on FFM%, and a negative finding indicates that the nutrient/diet score does not improve the association between FFM% and CRP levels or increases the impact of CRP on FFM%.

MDS: Mediterranean diet score; **HDI:** Healthy Diet Indicator; **AHEI:** Alternate Healthy Eating Index; **DASH-style score:** based on Dietary Approaches to Stop Hypertension diet pattern

Table 8.6 The association between percentage fat free mass (FFM %) and quintiles of CRP and potential mediation by dietary constituents in women aged more than 50 years

FFM%-CRP[1]	β-coefficient (Model 2)[2]	P	β-coefficient (Model 3)[3]	P	Percentage difference (%) in β-coefficients between Model 1 and Model 3[4]
Per quintile					
Magnesium	-1.319	<0.001	-1.312	<0.001	1.86
Vitamin C	-1.331	<0.001	-1.332	<0.001	0.37
Carotene	-1.328	<0.001	-1.330	<0.001	0.52
β-carotene	-1.328	<0.001	-1.329	<0.001	0.60
Potassium	-1.332	<0.001	-1.333	<0.001	0.30
Arginine	-1.339	<0.001	-1.328	<0.001	0.67
Glutamine	-1.342	<0.001	-1.329	<0.001	0.60
Per quartile					
MDS	-1.337	<0.001	-1.337	<0.001	0
HDI	-1.325	<0.001			0.90
AHEI	-1.326	<0.001	-1.328	<0.001	0.67
DASH-style score	-1.338	<0.001	-1.335	<0.001	0.15

[1] **Model 1:** FFM% was negatively associated to CRP (β-coefficient for the association between FFM% and CRP = -1.337, P < 0.001). Adjusted for age, physical activity, smoking, anti-inflammatory medication, HRT use

[2] **Model 2:** Adjusted as Model 1 and additionally adjusted for nutrient intake or diet scores

[3] **Model 3:** Adjusted as Model 2 and for energy intake (apart from HDI)

[4] **Formula for calculation:**

[(β coefficient in Model 1 - β coefficient in Model 3) / β-coefficient in Model 1] x 100

A positive finding indicates that the nutrient/diet score improves the association between FFM% and CRP levels or reduces the impact of CRP on FFM%, and a negative finding indicates that the nutrient/diet score does not improve the association between FFM% and CRP levels or increases the impact of CRP on FFM%.

MDS: Mediterranean diet score; **HDI:** Healthy Diet Indicator; **AHEI:** Alternate Healthy Eating Index; **DASH-style score:** based on Dietary Approaches to Stop Hypertension diet pattern

Table 8.7 The association between fat free mass index (FFMI, kg/m^2) and quintiles of CRP and potential mediation by dietary constituents

FFMI-CRP[1]	β-coefficient (Model 2)[2]	P	β-coefficient (Model 3)[3]	P	Percentage difference (%) in β-coefficients between Model 1 and Model 3[4]
Per quintile					
Magnesium	-0.071	0.001	-0.070	0.001	5.41
Vitamin C	-0.073	0.001	-0.073	0.001	1.35
Carotene	-0.073	0.001	-0.073	0.001	1.35
β-carotene	-0.073	0.001	-0.073	0.001	1.35
Potassium	-0.073	0.001	-0.072	0.001	2.70
Arginine	-0.075	0.001	-0.074	0.001	0
Glutamine	-0.075	0.001	-0.075	0.001	-1.35
Per quartile					
MDS	-0.073	0.001	-0.073	0.001	1.35
HDI	-0.070	0.002			5.41
AHEI	-0.070	0.001	-0.071	0.001	4.05
DASH-style score	-0.074	0.001	-0.074	0.001	0

[1] **Model 1:** FFMI was negatively associated to CRP (β-coefficient for the association between FFMI and CRP = -0.074, P = 0.001). Adjusted for age, physical activity, smoking, BMI, anti-inflammatory medication, HRT use

[2] **Model 2:** Adjusted as Model 1 and additionally for nutrient intake or diet scores

[3] **Model 3:** Adjusted as Model 2 and for energy intake (apart from HDI)

[4] **Formula for calculation:**

[(β coefficient in Model 1 - β coefficient in Model 3) / β-coefficient in Model 1] x 100

A positive finding indicates that the nutrient/diet score improves the association between FFMI and CRP levels or reduces the impact of CRP on FFMI, and a negative attenuation indicates that the nutrient/diet score does not improve the association between FFMI and CRP levels or increases the impact of CRP on FFMI.

MDS: Mediterranean diet score; **HDI:** Healthy Diet Indicator; **AHEI:** Alternate Healthy Eating Index; **DASH-style score:** based on Dietary Approaches to Stop Hypertension diet pattern

Table 8.8 The association between fat free mass index (FFMI, kg/m^2) and quintiles of CRP and potential mediation by dietary constituents in women aged less than 50 years

FFMI-CRP[1]	β-coefficient (Model 2)[2]	P	β-coefficient (Model 3)[3]	P	Percentage difference (%) in β-coefficients between Model 1 and Model 3[4]
Per quintile					
Magnesium	-0.064	0.024	-0.063	0.026	7.35
Vitamin C	-0.065	0.023	-0.065	0.023	4.41
Carotene	-0.065	0.021	-0.065	0.022	4.41
β-carotene	-0.065	0.022	-0.065	0.022	4.41
Potassium	-0.065	0.022	-0.065	0.023	4.41
Arginine	-0.067	0.018	-0.064	0.024	5.88
Glutamine	-0.068	0.018	-0.065	0.021	4.41
Per quartile					
MDS	-0.065	0.021	-0.065	0.022	4.41
HDI	-0.061	0.029			10.3
AHEI	-0.061	0.031	-0.061	0.031	10.3
DASH-style score	-0.067	0.019	-0.067	0.019	1.49

[1] **Model 1:** FFMI was negatively associated to CRP (β-coefficient for the association between FFMI and CRP = -0.068, P = 0.018). Adjusted for age, physical activity, smoking, BMI, anti-inflammatory medication, HRT use

[2] **Model 2:** Adjusted as Model 1 and additionally for nutrient intake or diet scores

[3] **Model 3:** Adjusted as Model 2 and for energy intake (apart from HDI)

[4] **Formula for calculation:**

[(β coefficient in Model 1 - β coefficient in Model 3) / β-coefficient in Model 1] x 100

A positive finding indicates that the nutrient/diet score improves the association between FFMI and CRP levels or reduces the impact of CRP on FFMI, and a negative attenuation indicates that the nutrient/diet score does not improve the association between FFMI and CRP levels or increases the impact of CRP on FFMI.

MDS: Mediterranean diet score; **HDI:** Healthy Diet Indicator; **AHEI:** Alternate Healthy Eating Index; **DASH-style score:** based on Dietary Approaches to Stop Hypertension diet pattern

Table 8.9 The association between fat free mass index (FFMI, kg/m^2) and quintiles of CRP and potential mediation by dietary constituents in women aged more than 50 years

FFMI-CRP[1]	β-coefficient (Model 2)[2]	P	β-coefficient (Model 3)[3]	P	Percentage difference (%) in β-coefficients between Model 1 and Model 3[4]
Per quintile					
Magnesium	-0.064	0.047	-0.063	0.054	7.35
Vitamin C	-0.066	0.042	-0.066	0.041	2.94
Carotene	-0.066	0.042	-0.066	0.041	2.94
β-carotene	-0.066	0.043	-0.066	0.042	2.94
Potassium	-0.065	0.044	-0.064	0.047	5.88
Arginine	-0.090	0.033	-0.070	0.034	-2.94
Glutamine	-0.070	0.032	-0.070	0.031	-2.94
Per quartile					
MDS	-0.069	0.034	-0.069	0.034	-1.47
HDI	-0.065	0.047			4.41
AHEI	-0.066	0.042	-0.067	0.041	1.47
DASH-style score	-0.067	0.039	-0.067	0.040	1.47

[1] **Model 1:** FFMI was negatively associated to CRP (β-coefficient for the association between FFMI and CRP = -0.068, P = 0.038). Adjusted for age, physical activity, smoking, BMI, anti-inflammatory medication, HRT use

[2] **Model 2:** Adjusted as Model 1 and additionally for nutrient intake or diet scores

[3] **Model 3:** Adjusted as Model 2 and for energy intake (apart from HDI)

[4] **Formula for calculation:**

[(β coefficient in Model 1 - β coefficient in Model 3) / β-coefficient in Model 1] x 100

A positive finding indicates that the nutrient/diet score improves the association between FFMI and CRP levels or reduces the impact of CRP on FFMI, and a negative attenuation indicates that the nutrient/diet score does not improve the association between FFMI and CRP levels or increases the impact of CRP on FFMI.

MDS: Mediterranean diet score; **HDI:** Healthy Diet Indicator; **AHEI:** Alternate Healthy Eating Index; **DASH-style score:** based on Dietary Approaches to Stop Hypertension diet pattern

Chapter 9

General discussion & future perspectives

9.0 Summary of research findings

This thesis has described the links between sarcopenia (as muscle mass and strength) and inflammation which are also both linked to the ageing process (Chung *et al.* 2009). Chronic low-grade inflammation is a risk factor leading to the predisposition of sarcopenia (Morley and Baumgartner 2004, Roubenoff and Hughes 2000), and is considered detrimental to skeletal muscle mass and strength in both humans and animal models (Howard *et al.* 2007, Siu *et al.* 2008). Diet plays a major role in the regulation of chronic inflammation as dietary constituents have the potential to exert anti-inflammatory as well as pro-inflammatory effects (Cavicchia *et al.* 2009). Moreover, a number of nutrients may be associated with muscle mass and strength. Protein (Campbell *et al.* 2001, Castaneda *et al.* 1995), amino acids (Borsheim *et al.* 2008, Scognamiglio *et al.* 2004, Solerte *et al.* 2008) and vitamin D (Dawson-Hughes 2008) have been shown to be beneficial in supplementation studies for muscle mass and/or strength, and magnesium and potassium are known to play an important role in muscle metabolism (Frassetto *et al.* 1998, Song *et al.* 2007, Song *et al.* 2005). Furthermore, vitamins C, E, selenium, and carotenoids are known to reduce oxidative stress which has been implicated in the aetiology of age-related muscle loss and have anti-inflammatory properties (Ferrucci *et al.* 2005, Helmersson *et al.* 2005, Young *et al.* 2004). It has also been well established that higher intakes of fruit and vegetables, whole grains, nuts and polyunsaturated fatty acids, as well as dietary patterns high in these food components may exert anti-inflammatory properties and are associated with lower concentrations of inflammatory markers including CRP (Brown and Hu 2001, Chrysohoou

et al. 2004, Esmaillzadeh *et al.* 2006, Esposito K and et al. 2004, Fung *et al.* 2001, Lopez-Garcia *et al.* 2004, Lutsey *et al.* 2007, Nettleton *et al.* 2006). Also, saturated fatty acid intakes and dietary patterns high in saturated fatty acids may exert pro-inflammatory properties and have been positively associated with inflammatory markers including CRP (Esmaillzadeh *et al.* 2007, Fung *et al.* 2001, Lopez-Garcia *et al.* 2004, Nettleton *et al.* 2006). [**Figure 9.1 (A)**].

However, until now little evidence has existed on the influence of habitual dietary intake (either for nutrients or dietary patterns) on muscle mass, muscle strength or muscle quality (Robinson SM *et al.* 2008, Scott *et al.* 2010). Moreover, there are currently no studies examining if habitual dietary intake of nutrients that have been previously associated with muscle mass, or have shown to exert anti-inflammatory properties and the "whole diet", mediate any potential association between muscle mass and inflammation. Understanding the ability of diet to mediate the association between inflammation and muscle mass could offer valuable data for the implementation of dietary intervention trials for the prevention of sarcopenia, by targeting inflammation which is known to be influenced by diet.

The present investigation aimed to evaluate i) associations between both habitual nutrient intake and the "whole diet" and indexes of muscle mass, muscle strength and muscle quality, ii) associations between diet and inflammation as assessed by the inflammatory marker C-reactive protein

(*CRP*), and iii) examine whether diet mediates the proposed association between muscle mass and CRP levels in adult women from the TwinsUK participants [**Figure 9.1 (A)**].

The findings of the current thesis are summarised in **Figure 9.1 (B)**.

Diet and muscle mass, strength and quality

Results from the current analyses suggested a significant positive association between intakes of vitamins C and E, magnesium, potassium and a range of carotenoids and indexes of muscle mass with the scale of associations ranging between 1.5-4.6 %, when women were considered as singletons. Among the nutrients studied, magnesium appeared to be independently predictive for all three indexes of muscle mass after accounting for intakes of vitamin C, total carotene and ß-cryptoxanthin. Indicatively, when the within-pair analysis was conducted for the association between magnesium intake and fat free mass index, associations were towards significance, suggesting that potential shared lifestyle and genetic factors other than dietary intake of magnesium were driving this apparent association. However, these findings need to be verified in other twin studies. These factors are not yet known and it would be interesting to be investigated in future genetic studies.

In addition, higher adherence to the Mediterranean Diet score (MDS), Healthy Diet Indicator (HDI), Diet Quality Index (DQI), Alternate Healthy Eating Index (AHEI), and DASH-style score (based on the Dietary Approaches to Stop Hypertension diet) was significantly associated with

measurements of muscle mass, with associations ranging between 1-3 %. However, no associations were observed for protein and essential amino acid intakes. Surprisingly, a significant inverse association between protein, essential amino acid intake and percentage FFM was observed. However, it needs to be highlighted that the range of protein intake in this analysis was higher compared to studies with older participants (Houston *et al.* 2008, Meng *et al.* 2009). This may suggest that higher protein intake may be more beneficial for older individuals with very low protein intakes, as in older persons the metabolic efficiency is decreased and they require higher protein intake for protein synthesis compared to younger persons (Rattan 2010). Nevertheless, findings from this study may help to improve the knowledge and understanding of the effects of protein and amino acids on muscle mass in a healthy population. These findings contribute towards the debate on protein and amino acid requirements in healthy non frail individuals.

Diet and CRP

Furthermore, a number of nutrients examined and all diet quality scores were inversely associated with plasma levels of the inflammatory marker C-reactive protein (*CRP*). The greatest magnitude of association was observed for magnesium intake which was associated with lower plasma CRP concentrations of 25 % comparing extreme quintiles of intake. Although in the current analysis we expected an inverse association between dietary protein intake and the inflammatory marker CRP, surprisingly we did not observe an association between protein and CRP. Also associations between

essential amino acid intakes and CRP were in the opposite direction to what we had initially hypothesised. However, neutral protein balance and optimal protein/amino acid intake may not always have an effect on inflammation for populations that have a normal weight, BMI and CRP concentrations (as opposed to populations with higher body weight, BMI and CRP levels) (Due *et al.* 2005), and the biological mechanisms underlying such a relationship are largely unknown. Nevertheless, given these are the first observations that we know about, they would appear to be novel findings. There is debate on the level of protein and amino acid requirements in healthy individuals, and from a public health perspective, the novel findings from the current analysis may offer some insight to better understand whether the habitual dietary protein and amino acid intakes may influence inflammation in apparently healthy adult women. This study also observed that higher intakes of the essential amino acids arginine, glutamine, histidine, isoleucine, threonine and tryptophan were significantly, positively associated with CRP levels. This finding was surprising. In relation to the "whole diet", higher adherence to the HDI and AHEI scores was inversely associated with CRP levels by 14 % and 13 % of the population geometric mean for CRP, respectively. Moreover, we found that higher CRP levels were associated with lower indexes of muscle mass with associations ranging from 0.1-1.2 %. Of interest, after multivariate adjustment, the association between the HDI and AHEI scores and CRP levels was equivalent to association of 10 years of age in this cohort.

This was the first study to examine the influence of a range of nutrients and diet quality scores (that have been associated with either indexes of muscle

mass or CRP levels in previous chapters of the current thesis) on the association between muscle mass and CRP levels.

CRP and muscle mass & mediation by diet

We also observed that higher CRP levels were associated with lower indexes of muscle mass in women aged 18-79 years from the TwinsUK participants and the effect of CRP on indexes of muscle mass was 1.5-7.2 times greater of that of age, after analysis was adjusted for a number of confounding factors including age, physical activity, smoking, anti-inflammatory medication (including aspirin, statins, and NSAID) and HRT use and were independent of adiposity (assessed by BMI for FFM and FFMI).

Interestingly, dietary intake of magnesium, potassium, vitamin C, carotene, β-carotene, and diet quality including the MDS, HDI, AHEI and DASH-style scores appeared to reduce the impact of CRP on indexes of muscle mass by 1-8 %. Notably, in additional stratification for age analyses, results remained the same regarding the direction of mediation of indexes muscle mass by CRP levels by the dietary constituents studied. However, the percentage attenuation by a variety of plant-based constituents and healthier dietary patterns (higher in fruits and vegetables, whole grains, nuts, dietary fiber and low in red and processed meat and saturated fats) appeared to be higher in the women aged over 50 years, suggesting that following these dietary patterns may potentially be beneficial, reducing the negative impact of inflammation as per CRP on muscle mass in older women.

Our findings extend the results of the few previous studies by examining simultaneously associations between the habitual intake of a range of micronutrients, protein and amino acids relevant for muscle mass in a cohort of women aged 18-79 years. It is evident that notable changes in skeletal muscle mass may occur earlier in adult life (between 30 and 45 years of age), and it is important to examine dietary associations with muscle mass in individuals of all ages (Cesari and Pahor 2008, Janssen *et al.* 2000). Moreover, this was the first study that directly compared five diet quality scores based on adherence to dietary patterns or to dietary recommendations with indexes of muscle mass, muscle strength and muscle quality in a large cohort. In addition, this was the first population study that directly compared a full range of micronutrients and carotenoids with potential anti-inflammatory properties, and evaluated the influence of protein and essential amino acid intakes in association with plasma CRP concentrations. Findings from the current study also extended the results from previous cross-sectional studies by focusing on the overall diet quality developed based on *a priori* knowledge, instead of patterns derived based on empirical methods, in association with the inflammatory marker CRP. This was also one of the first studies to examine cross-sectional associations between a range of micronutrients, protein and amino acids, the "whole diet" and muscle strength and muscle quality. Findings were not significant possibly because other lifestyle factors, such as physical activity and the ageing process itself, had a greater effect in this cohort.

These novel findings in this thesis infer that plant-derived dietary components and diet scores high in these constituents are achievable from the habitual diet and mediate the relationship between muscle mass and inflammation. Findings of this thesis also emphasise the importance of consumption of a variety of plant-based nutrients and of the overall diet quality for the conservation of muscle mass, and shed new light on the influence of these dietary components on sarcopenia related inflammation.

9.1 Overview of strengths and limitations

This work has a number of strengths and limitations. A strength of the current analysis was the focus on a cohort of women including younger adults compared to previous studies evaluating associations between diet and muscle mass (Robinson SM *et al.* 2008, Scott *et al.* 2010) or diet and inflammation (Chacko *et al.* 2010, Song *et al.* 2007) in older populations. The wide age range of this cohort may be considered advantageous, as declines in muscle mass may occur earlier in adult life (between 30 and 45 years of age), and it is important to examine dietary associations with muscle mass in individuals of all ages (Cesari and Pahor 2008, Janssen *et al.* 2000). However, the observed findings relate only to women and further work is needed to investigate if these findings are replicated in a male population of the same country or in populations from different ethnic backgrounds. Although men have, on average, greater amounts of muscle mass than women, they have lower survival compared to women, implying that sarcopenia may potentially become a greater public health concern

among female than male populations (Abellan Van Kan 2009, Roubenoff and Hughes 2000).

A limitation of the current study was that we did not measure skeletal muscle, but whole body fat free mass. Fat free mass is composed of non-fat lean soft tissues and bone mineral content and is an important metabolically active component of body composition (Nelson *et al.* 1992). Skeletal muscle mass represents the largest fraction of the fat free body mass (Heymsfield *et al.* 1990) and therefore, whole body fat free mass is commonly used in epidemiological studies (Jourdan *et al.* 2012). Yet, although body composition was objectively assessed by DEXA scans, this method involves its own limitations. DEXA is the most widely available method to assess body composition and muscle mass in both the clinical and research settings and it exposes the person to minimal radiation compared to other body imaging techniques, such as computed tomography (CT scan) or magnetic resonance imaging (MRI) (Cruz-Jentoft *et al.* 2010, Pahor *et al.* 2009). However, DEXA may overestimate muscle mass (Wang *et al.* 1996) as it does not differentiate between bone-free lean tissue and water and this may overestimate muscle mass in older people who may have extracellular fluid accumulation (Proctor *et al.* 1999).

A further strength was the objective measurement of assess muscle strength, which allowed calculation of muscle quality. Grip strength is an important measurement for overall health, as it has been associated with an increased risk of disability, morbidity and mortality in both men and women, with declines in quality of life in older individuals, and has been suggested as a

useful marker of muscle function and sarcopenia. Muscle quality which is defined as the ratio of muscle strength per unit of muscle mass has been used as an approach to measure the quality of muscles. Lower muscle quality has been associated with greater age (Lynch *et al.* 1999) and functional incapacities (Goodpaster *et al.* 2006). Although the preservation of muscle mass may be considerably important for the prevention of muscle strength loss during ageing, muscle quality may also be important in determining the loss of muscle strength with ageing (Barbat-Artigas *et al.* 2012). To date, muscle quality has not been widely studied in relation to diet as such and this was the first population study that examined such associations.

The cross-sectional nature of the study limits causal inference, but enables us to generate hypotheses regarding associations between diet, indexes of muscle mass and inflammation based on plausible mechanisms of action. In addition, as in all observational studies, measurement error in self-reported dietary intakes is inevitable. In this study, dietary intake was not assessed by a food diary which is considered as the gold standard but by a food frequency questionnaire (FFQ). This FFQ was compared and validated against a 7-day weighed record in the EPIC Norfolk study (Bingham *et al.* 2001). This FFQ was also validated against urinary and plasma biomarkers of intake, such as urinary nitrogen for protein intake, urinary potassium and sodium, plasma ascorbic acid and plasma n-3 PUFAs (McKeown *et al.* 2001, Welch *et al.* 2006). In addition, it has been shown previously that serum β-cryptoxanthin and zeaxanthin concentrations were moderately correlated with dietary carotenoids intakes measured by an FFQ (Tucker *et*

al. 1999). Nevertheless, it is widely acknowledged that the use of FFQs for measuring dietary intake has its own limitations related to memory errors (McNeill *et al.* 2009). They provide relative rather than absolute intake, they introduce bias due to over- or under-reporting, and they may introduce systematic errors as preparation methods are inadequately considered (McNeill *et al.* 2009). Also, FFQs measure food intake over a period of one year and reflect the habitual diet over this period of time but not lifetime dietary habits (Teucher *et al.* 2007). As mentioned previously in Chapter 3, FFQs have been shown to rank individuals well within populations and have the advantage of being capable of use for large studies.

Nevertheless, it is thought that nutrient biomarkers may provide a stronger association with disease outcomes than those based on dietary assessments due to measurement errors occurring with dietary questionnaires (Giovannucci 2013). However, it is also argued that blood concentrations of nutrients may reflect other exposures (such as smoking and alcohol consumption) (Dietrich *et al.* 2003), and genetic , physiological, metabolic (Lee *et al.* 2012) and pathophysiological processes (Giovannucci 2013, Zhang *et al.* 2012).

In our dataset we did not have nutritional status data, therefore it was not possible to examine whether muscle mass and inflammation could be expected to relate to status of the nutrient. Ideally, assuming that a causal association exists, altering the concentration of the biomarker directly through the intake of the nutrient would directly affect the disease or outcome risk (Giovannucci 2013). It needs, however, to be considered that

status is affected by various factors that may also affect disease risk. For example, cigarette smoking has been associated with lower plasma ascorbic acid concentrations (Wei *et al.* 2001). In addition, at low doses dietary vitamin C is almost completely absorbed, but over a certain range of usual dietary intake (30-180 mg/day), absorption may decrease to 75 percent because of competing factors in the food (Graumlich *et al.* 1997). Therefore, in order to investigate the beneficial health effects of nutrients with potential anti-inflammatory properties it is also important that epidemiological studies examine associations between the habitual intake of specific nutrients and nutrient status, and markers of disease outcomes, such as CRP for inflammation and indexes of muscle mass for sarcopenia. Even though status may be a useful marker of intake we need to know how much a population is consuming so that we can provide guidelines for population intakes for prevention.

In the current analyses, the proportions of women that had intakes lower than the RNI/EAR and RDA, were low for the reported intakes of vitamin C, magnesium and potassium, and high for the reported intakes of selenium, vitamins D and E (Chapter 3, page 101). It is noteworthy that there are currently no relevant recommendations in terms of different carotenoid intakes for the UK population. It is plausible that overall nutrient adequacy may have blunted the ability to detect an association between selenium, vitamins C and E and muscle mass or CRP. However, as mentioned above, the assessment of dietary intake using FFQs have their own limitations and self-reported dietary intakes are inherent limitations to estimating nutrient

intakes from a FFQ. Moreover, given the fact that for the sample population intakes of selenium, and vitamins D and E were not sufficient and the range of adequacy not great, this may have reflected on the ability of the dataset to assess effects on muscle mass or CRP concentrations of low and high intakes. Greater ranges of adequacy concerning selenium, and vitamins D and E may have resulted to a greater ability of the dataset to more informative results. Notably, vitamin C, magnesium and potassium are found mainly in healthy plant based foods, such as fruit and vegetables, and their intake may be a marker of a generally healthy lifestyle behavior. However, associations with muscle mass and CRP concentrations remained significant even after adjustment for lifestyle factors.

For this thesis, lifetime physical activity was assessed using a physical activity questionnaire, which was previously shown to be strongly correlated with more in-depth assessment of time spent in physical activity in the cohort (Cherkas *et al.* 2008). A recent review suggest that objectively measured physical activity by accelerometers, is a better assessment method (Ekelund *et al.* 2011). This review refers to childhood and youth but similar findings may be applicable to adults too. Ideally, objective measures of physical activity should complement self-report assessments.

This study was limited in measuring only one biomarker of inflammation, which is the widely used CRP, and more markers are needed to reflect, in total, the complexity of associations between diet, muscle mass and inflammation.

In this study analyses were adjusted for a number of confounding factors that are known to impact the association between diet, muscle mass and inflammation, such as age, physical activity, smoking, BMI, medication, and use of hormone replacement therapy. However, little is yet known about what other factors may affect muscle mass as much of the research in this area is relatively new. There might be other factors that may affect the association between diet, muscle mass and inflammation and therefore residual confounding cannot be ruled out. More factors are yet to be identified as this a very fertile field of research.

9.2 Results from this thesis and relationship with public health recommendations

There is a growing body of evidence suggesting that nutrition/diet may be an important modifiable factor of sarcopenia (Robinson *et al.* 2012). Currently, nutritional and public health recommendations for the management of sarcopenia are limited to protein, essential amino acids, mainly leucine and vitamin D intakes (Morley *et al.* 2010). The Society for Sarcopenia, Cachexia and Wasting Disease in their review in 2010 has recommended that older adults may ingest between 1.0 and 1.5 g/kg/d of protein for the preservation of muscle mass, however this report is limited only in older adults and does not specify the corresponding age range for the suggested protein intake (Morley *et al.* 2010). The same report recommended that older adults may consume a leucine-enriched balanced amino acid supplement for the preservation of muscle mass, although, the authors do not clarify a suggested dose. However, the results of our findings

point to no beneficial effect of either total protein or specific amino acid intakes. In fact they suggest that in this age group protein may not be related specifically to muscle mass. However, given the advantages and disadvantages of our study it would be wise for protein recommendations to remain as they are.

In addition, only very few observational studies have pointed out the potential importance of antioxidant nutrients on muscle mass and muscle strength (Robinson SM *et al.* 2008, Scott *et al.* 2010), however, there is a lack of definitive recommendations concerning intakes of antioxidant nutrients from the habitual diet. In terms of healthy dietary patterns, only one previous observational study found that a pattern characterized by higher intakes in fruit and vegetables and wholemeal cereals was associated with greater muscle strength in older adults (Robinson *et al.* 2009).

Notably, the existing recommendations refer to older individuals (although age ranges are not defined) when sarcopenia has already become established and therefore treatment is important. Given the findings observed in the current analysis that diet and muscle mass are also associated in younger individuals and importance of prevention, recommendations need to start earlier in life as the importance of life-course influences may be predictive of muscle mass and strength later in life (Robinson *et al.* 2012, Sayer *et al.* 2004). Nevertheless, there is currently a paucity of data approaching the role of nutrition on the preservation of muscle mass from a public health preventative point of view.

Given, the lack of such evidence, we expanded our research in examining the associations between other micronutrients and healthy eating patterns and muscle mass. The current analysis suggests that it is important for adult women of a wide age range (18-79 years) to consume a variety of foods high in vitamins C and E, magnesium, potassium and a range of carotenoids (nutrients found in foods of plant-based origin, such as fruit and vegetables, whole grain products, plant-based oils and nuts) to ensure greater muscle mass. Indicatively, a difference of 3.4 % in FFMI reported between extreme quintiles of magnesium intake corresponds to consumption of 1.8 portions of all bran per day (108 g/d) from the habitual diet (assuming 1 portion of all bran to be 60 g for adults). A difference of 3.11 % in FFMI also observed between extreme quintiles of vitamin C intake corresponds to consumption of two oranges (assuming one portion of oranges to be 120 g for adults) and one portion of strawberries (100 g/d), highlighting the importance of incorporating more plant-based food components into the habitual diet. Indeed, in terms of protection from muscle loss at the population level it would appear wise for individuals to follow the 5-a-day recommendations for prevention of muscle loss as well as for their other health effects.

Furthermore, the current study has confirmed that healthy eating patterns characterised by high intakes in fruit and vegetables, nuts, legumes, dietary fiber/and or whole grains, and low intake of red and processed meat, and saturated fat, are likely to be protective for muscle mass. Interestingly, when translating the differences in nutrient intakes between extreme quartiles of the five diet scores into comparable amounts of food, the differences in vitamin C intake, for example, between extreme quartiles of the diet scores

equated to 1.6 portions of oranges for the MDS, 1.5 portions for the HDI, 1 portion for the DQI, 2.3 portions for the AHEI and 2 portions for the DASH-style score (assuming one portion of oranges to be 120 g for adults).

The results from this study suggest that is important for adult women (aged 18-79 years) to consume a variety of food sources high in micronutrients, such as magnesium to ensure lower plasma concentrations of the inflammatory marker CRP. Similarly, healthy eating patterns which award points to diets high in fruit and vegetables, whole grains, dietary fiber, nuts and polyunsaturated fatty acids and low in saturated fatty acids (specifically the HDI and AHEI scores) may be important for the inflammatory status.

The most exiting finding of the current work was that dietary intake of magnesium, potassium, vitamin C, carotene, β-carotene, and diet quality including the MDS, HDI, AHEI and DASH-style scores appeared to reduce the impact of CRP on indexes of muscle mass by 1-8 %. In regard to nutrients, the greatest associations were observed for magnesium intake which appeared to reduce the association between FFM (kg) and CRP levels by almost 7% per quintile of intake which was equated to a mean difference of 43 mg/d. Although the strength of the associations was modest, this was observed after analyses was accounted for age, physical activity, smoking, anti-inflammatory medication (including aspirin, statins, and NSAID) and HRT use and were independent of adiposity (assessed by BMI) factors that are known to affect both muscle mass and CRP. In food terms, this difference would correspond to consumption of 1.8 portions of whole meal

bread per day (64.8 g/d) from the habitual diet (assuming 1 portion of whole meal bread to be 36 g for adults). In relation to the "whole diet", the greatest associations were observed for the AHEI score which improved the impact of CRP on FFM (kg) by almost 8 % for every 9 points increase in the score. When translating the differences in nutrient intakes between extreme quartiles of the AHEI score into comparable amounts of food, the differences in vitamin C intake, for example, between extreme quartiles of the diet scores (which was shown in Chapter 5, **Table 5.3**, p. 189) equated to 2.2 portions of oranges for the AHEI (assuming one portion of oranges to be 120 g for adults).

From a public health perspective, the novel findings from the current analysis may offer some insight in order to better understand the impact of dietary and lifestyle factors on the association between muscle mass and inflammation in adult women with normal weight, BMI and CRP concentrations. They indicate that eating a diet that includes sufficient protein, and is high in a variety of foods high in vitamin C, magnesium, potassium and a range of carotenoids (nutrients found in foods of plant-based origin, such as fruit and vegetables, whole grain products/dietary fiber, legumes and nuts) may prevent muscle loss in female populations across a wide age range and particularly in women aged over 50 years. These preliminary findings may also provide useful information in developing and planning dietary intervention trials for the prevention of sarcopenia by targeting inflammation.

9.3 Future perspectives

9.3.1 Observational studies

In terms of future work as far as observational studies are concerned [**Figure 9.1 (C)**], a longitudinal study design might be a more suitable approach to detect associations between diet and muscle mass, strength and quality, as it is more likely to observe changes in body composition over time due a healthier diet. Also, it might be more likely to observe a difference in the inflammatory status over time due to a healthier diet. In our study we did not apply a longitudinal analysis as very few people had dietary data in both FFQs (in subset 1, 2570 women, 18-79 years and subset 2, 949 women, 34-83 years).

Although, there is growing evidence suggesting that behavioural factors, such as diet, physical activity and smoking influence muscle mass, as yet, their combined impact is not known. Therefore, it would be interesting to derive a simple diet and lifestyle score based, for example, on fruit and vegetables intake, physical activity and smoking and examine whether a better diet and lifestyle score is predictive of preservation of muscle mass later in life.

9.3.2 Intervention trials

Interventions on sarcopenia may be challenging because of a number of methodological issues. The most important considerations are the definition of the sarcopenic group, which depends upon the method used to measure body composition, and the identification of the target population for

intervention. This is due to the definitions available for describing sub-normal values for sarcopenia in different reference populations. For example, a cut-point for sarcopenia in older adults has been defined as a skeletal muscle index two standard deviations below the mean of young male and female (aged 18-40 years) reference groups (Baumgartner *et al.* 1998). In this study muscle mass was assessed by DEXA scans and estimated as the sum of the muscle mass of the four limbs (appendicular skeletal muscle mass); skeletal muscle mass was therefore calculated as the ratio of appendicular skeletal muscle mass to height squared. In another observational multiethnic cohort of older people (aged 70-79 years), body composition was assessed by DEXA scans and participants were classified as sarcopenic using two different approaches to adjust lean mass for height and body size (Newman *et al.* 2003). The first was the ratio of appendicular muscle mass divided by height squared and the in second approach appendicular muscle mass was adjusted for height and body fat. As reference values for multiethnic young adults were not available, the gender specific 20^{th} percentile was arbitrarily used as a cut-off point for each method (Newman *et al.* 2003). These findings highlight the urgent need of reference values for different populations. This information will enable the distinction between preventive and treatment interventions on sarcopenia (Cesari and Pahor 2008).

A second consideration for interventions on sarcopenia may also be the target population. In regard to the target population for intervention trials on sarcopenia, only recently, in 2010, the European consensus on definition

and diagnosis of sarcopenia has developed a suggested algorithm based on gait speed measurement as the most reliable and easiest approach for screening subjects in clinical practice and for selection of participants for clinical trials (Cruz-Jentoft *et al.* 2010). This algorithm suggests that participants (aged > 65 years) are at risk of sarcopenia if their gait speed is >0.8 m/s. The selection of this cut-off point for gait speed was based on the fact that it has been previously associated with adverse health outcomes, such as disability, cognitive impairment, falls and/or mortality (Abellan van Kan *et al.* 2009). If gait speed is >0.8 m/s then muscle grip strength assessment should follow. If the grip strength is normal then the person is classified as non sarcopenic, if the grip strength is low (according to population specific cut-off values) then muscle mass should be assessed. If muscle mass is also low then the person is classified as sarcopenic as opposed to normal (Cruz-Jentoft *et al.* 2010). The consensus suggested that this cut-off may also be relevant for younger individuals, nevertheless, this report does not specify an age range for the younger population. Again the lack of population specific cut-off values for muscle mass and muscle strength and the fact that this screening tool has not yet been validated raises important considerations for the design of future interventions on sarcopenia.

Therefore, in the light of the existing evidence and considering methodological issues, future clinical trials for the treatment of age-related loss of muscle mass and strength could potentially focus on sarcopenic adults [**Figure 9.1 (C)**] without clinical impairments (such as arthritis,

diabetes, cancer, anemia, kidney disease, neurological diseases) as these are highly related to body composition modifications and may bias the study results (Cesari and Pahor 2008). Exclusion of participants with inflammatory diseases, hormonal abnormalities, and use of anti-inflammatory medication or hormone replacement therapy (in case of female participants) are also encouraged as these factors have been indicated that contribute to muscle decline (Cesari and Pahor 2008). Other important factors that may need to be considered for the selection population for clinical trials besides age are gender as there is evidence that muscle mass declines are steeper in men compared to women (Janssen *et al.* 2000, Lauretani *et al.* 2003), and race/ethnicity differences as it has been shown that African-American have greater muscle mass compared to Asian, Causasian or Hispanic populations (Castaneda and Janssen 2005, Wang *et al.* 2001). In relation to lifestyle factors, total body mass, smoking and physical activity need to be considered as they influence muscle mass as discussed in previous Chapter 2, section 2.2.5, p. 57. The lack of clinical interventions on sarcopenia may be due to the methodological issues mentioned earlier and to difficulties related to the selection of the sample population or maybe other issues that are yet to be researched. It is certain that future work is needed for the formulation of comprehensive assessment methods, validated screening tools, identification of outcome measures and robust biomarkers of sarcopenia.

Even though treatment is very important when sarcopenia has been diagnosed, prevention is also of great importance in our ageing society.

There is growing interest in the role of diet and lifestyle in the aetiology of sarcopenia (Sayer *et al.* 2013) and in interventions that encourage behaviour changes for the management of sarcopenia (Waters *et al.* 2010). Most of the existing evidence on the potential role of diet on muscle mass and strength is observational and limited to protein (Campbell *et al.* 2001, Castaneda *et al.* 1995), essential amino acids (Borsheim *et al.* 2008, Scognamiglio *et al.* 2004, Solerte *et al.* 2008) and vitamin D supplementation (Dawson-Hughes 2008), and low antioxidant status (Cesari *et al.* 2004, Dominguez *et al.* 2006, Lauretani *et al.* 2008, Semba *et al.* 2007) in older participants. However, given that dietary constituents are often highly correlated, the focus on healthy dietary patterns could be more efficient in terms of intervention, instead of single nutrient supplementation. Our findings on the positive association between diet quality scores and muscle mass may strengthen this argument.

On the other hand physical exercise interventions including progressive resistance exercise training have been shown to have positive effects on strength and physical function in older adults (Liu and Latham 2009). At present the potential implications of long term effects of exercise in combination with high protein/amino acid supplementation on sarcopenia are largely uncertain (Koopman 2011). Therefore, another consideration for future intervention trials on the prevention of muscle mass and strength loss may be the possible combined effects of both diet and exercise training which may be more effective than either alone. Future research in this direction could point towards effective strategies to prevent and/or treat sarcopenia.

In relation to dietary interventions, the potential influence of the "whole diet" for the management of muscle mass and strength loss would be a very interesting area of future research as it is still unexplored. Interventions designed to change dietary patterns would be expected to change the intake of a number of nutrients and thus may be more effective that single nutrient supplementations (Sayer *et al.* 2013). Findings from our population study although cross-sectional showed that dietary patterns high in fruits and vegetables, whole grains, nuts, dietary fiber that could provide a range of nutrients important for muscle mass, such as the plant-derived nutrients magnesium, potassium, vitamin C and carotenoids, and low in saturated fat and processed meat mediate the relationship between muscle mass and inflammation. These findings could provide more evidence-based data for the design of future dietary interventions targeting inflammation related sarcopenia. Although our study did not show evidence on the associations between diet and muscle strength, interventions on diet and sarcopenia should capture the bi-dimensionality of this condition by assessing both muscle mass and muscle strength.

Given the existing assessment methods, screening tools, methodological issues that were mentioned earlier for the design of clinical trials for sarcopenia which may also apply to preventive interventions; and the challenges of changing dietary and lifestyle behaviours in the elderly, the characteristics of a potential ideal intervention trial combining both nutrition and exercise could be the following:

- ***Primary objective:*** To determine the potential effect of a healthy dietary pattern high in plant based dietary components such as citrus

fruits and cruciferous and green leafy vegetables, whole grain products, nuts and dietary fiber and low in saturated fat and processed meat, combined with a progressive resistance exercise training program on skeletal muscle mass and strength among older sarcopenic women.

- ***Secondary objective:*** To measure the changes in inflammatory markers, biomarkers of compliance and other biochemical markers in response to the interventions.
- ***Study design:*** Randomized controlled human intervention trial, parallel design.
- ***Inclusion criteria:*** Older sarcopenic women, aged 65-75 years with a gait speed >0.8 m/s and skeletal muscle mass index two standard deviations below the reference group of a Caucasian population. Able to participate in the study for one year. Recruitment could be from the general population. The intervention and control groups will be matched for age, BMI and other baseline characteristics to control for confounding from these factors.
- ***Exclusion criteria:*** Recent diagnosis of disease, smoking, use of dietary supplements, any medication, use of hormone replacement therapy, known allergy to nuts. Body mass index >30 kg/m^2 to ensure exclusion of subjects with sarcopenic obesity. Extreme physical activity.
- ***Intervention:*** Half of the participants will be encouraged to simple changes to dietary habits following a healthier dietary pattern high in fruit and vegetables, whole grain products, nuts and dietary fiber and

low in saturated fat and processed meat. These participants may also be asked to follow a progressive resistance exercise training program. The other half of the participants will be asked to follow their usual dietary habits and physical activity.

- ***Compliance:*** Compliance of dietary modifications will be monitored using food diaries.
- ***Assessment of body composition and blood & urine samples:*** Body composition changes will be assessed using DEXA scans and measurements may be taken at baseline and at the end of the study. Specific measure may include body weight, BMI (kg/m^2), fat mass (%), skeletal muscle mass (kg or %). Muscle strength will be assessed by hand grip strength (kg). Fasting blood and urine samples will be obtained at baseline, six and twelve months. The effect of healthy dietary patterns on muscle mass and strength will be measured by biomarkers of intake, such as plasma vitamin C and carotenoids and urinary potassium. Physical exercise will ideally be accessed by accelerometers complemented by self-report assessments. Changes in different inflammatory markers (such as CRP, IL-6, TNF-a) will be assessed using validated immune assays.

As a further thought, it would of great interest to study the lifelong influences of nutrition on age-related changes in muscle mass and strength. Although we were able to identify some physical and lifestyle characteristics that influence muscle mass and strength further work is needed to identify more factors in order to design effective interventions.

Indeed, this is an area that has been very little examined but there is some evidence showing that factors that influence growth, such as early nutrition (Robinson *et al.* 2012) or low birth weight (Sayer *et al.* 2004) may influence muscle mass and strength later in life. The approach of lifecourse influences on muscle mass and strength has been recently suggested and is indeed an exciting area of research that will potentially give us the opportunity to understand more about the determinants of muscle mass loss, influences that are associated with the peak muscle mass and strength attained at early life and potential underlying mechanisms of such associations (Patel 2012). This approach suggests that there might be opportunities to preventively intervene at earlier stages of life and not just when sarcopenia has already been manifested.

Finally, human and animal studies will allow a more in depth understanding of the molecular and cellular mechanisms of muscle mass and strength loss. This would potentially allow the development of more robust biomarkers to test the efficacy of different interventions (Sayer *et al.* 2013). More sophisticated methods related to the integrated personal "omics" profile (iPOP), such as high-throughput analytic platforms may predict the development of various diseases, including sarcopenia (Chen *et al.* 2012, Ferguson 2012). Moreover, nutrigenomics and metobolomics can characterize the nutritional and metabolic status of an individual and may provide additional information for the management of diet at the individual level (Jones *et al.* 2012). The "omics" approaches may reveal new horizons for the design of dietary interventions tailored to individualized needs, and

in general may alter the metabolic phenotype to a healthier profile (Calvani *et al.* 2013).

In terms of the measures of fat free mass, fat free mass index (weight/height^2) seems to be the most suitable and therefore it would be useful to be utilised in future research. It is well documented that in order to evaluate fat free mass in relation to body size and nutritional status, fat free mass should be considered in relation to height, because fat free mass expressed in absolute weight or as a percentage of body weight is not adequate because it increases with height. Fat free mass index which eliminates differences associated with height is an important marker of health and disease status (Coin *et al.* 2008, Heymsfield *et al.* 2011, Kyle *et al.* 2003). Moreover, percentage fat free mass showed a large variation in the current analysis (38.8-80.6 %) which might have influenced the results.

In relation to the diet scoring indices, the MDS used the median intake of dietary components as the cut-off between healthy and unhealthy intakes, whereas the DASH-style score used quintiles to classify intakes of dietary components, with a "healthy" point assigned only if the quantity consumed was in the highest quintile. In contrast, the HDI, DQI, and the AHEI were cut-point specific scores based on healthy eating guidelines. These differences suggest that the DASH-style score gives more weight to high adherence to a better quality diet compared with the other four scores. However, it is likely that the use of the median as a cut-off value will not reflect a healthy level of intake *per se*, and will be different among different

populations, but an advantage is that half of the sample will score positively and half negatively for each component, which ensures that each component distinguishes well between individuals (Waijers *et al.* 2007). On the other hand there are drawbacks with the use of cut-off specific values as it is likely that the intake of a specific component may be below the cut-off level for most of the subjects, limiting the discriminating power of a component (Kourlaba and Panagiotakos 2009, Waijers *et al.* 2007).

Considering the advantages and disadvantages of the different diet scores, the use of the AHEI score would be recommended as the most relevant for future research in this area. The AHEI score was focused mainly on intake of significant food groups (characterised by higher intakes in fruits and vegetables, nuts and dietary fiber, and low intakes in red meat). Dietary patterns characterised by increased consumption of fruits and vegetables, whole grains, dietary fiber and low intakes of saturated and trans fatty acids have been previously associated with lower levels of markers of inflammation, oxidative stress, and endothelial dysfunction in epidemiological studies (Baer *et al.* 2004, Dai *et al.* 2008, Esmaillzadeh *et al.* 2007, Esposito *et al.* 2004, Fung *et al.* 2001, Fung *et al.* 2001, Lopez-Garcia *et al.* 2004, Lopez-Garcia *et al.* 2005, Mozaffarian *et al.* 2004, Nettleton *et al.* 2006). In addition, the associations we found between muscle mass and the AHEI dietary pattern may be partly explained due to the anti-inflammatory effects of an overall healthier diet containing food components high in the nutrients previously found to be related to muscle mass (as outlined in Chapter 1, section 1.3, p. 21, 22, and Chapter 3, section

3.0, p. 82). Therefore, this score may be more suitable for future studies investigating the role of diet on muscle mass.

The MDS and DASH-style scores, although they consists of similar components to the AHEI score; they are however population specific, as the MDS score is generated according to the distribution of foods in a given dataset, and the DASH-style scores classifies intakes of dietary components based on the quintiles.

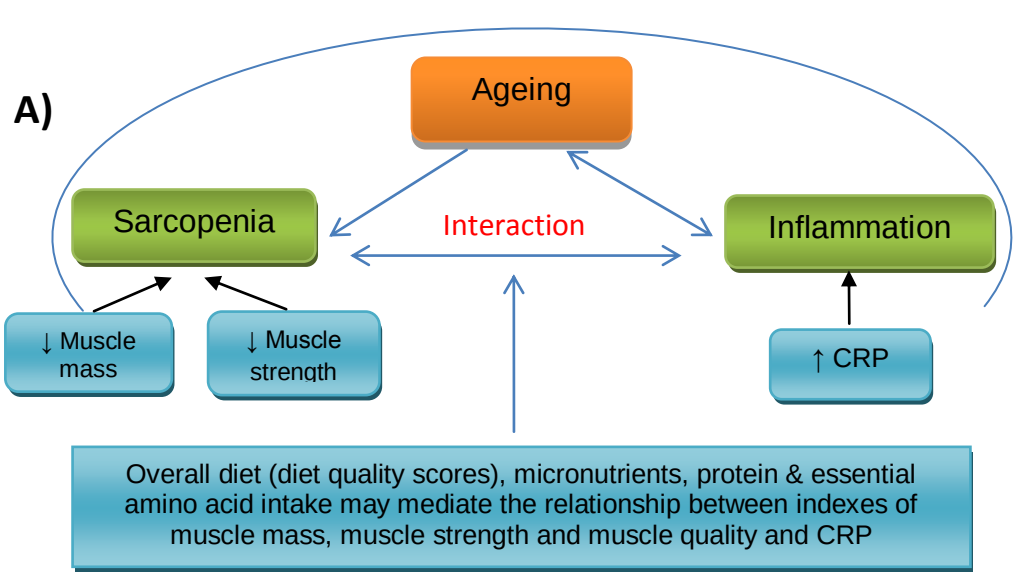
In contrast, the HDI and DQI scores were mainly focused on intake of nutrients (characterised by higher intakes in polyunsaturated fatty acids, average intakes in protein, and low intake in saturated fatty acids and cholesterol). Therefore, these two scores would not be recommended in future research as they may not be able to capture overall dietary patterns of a population.

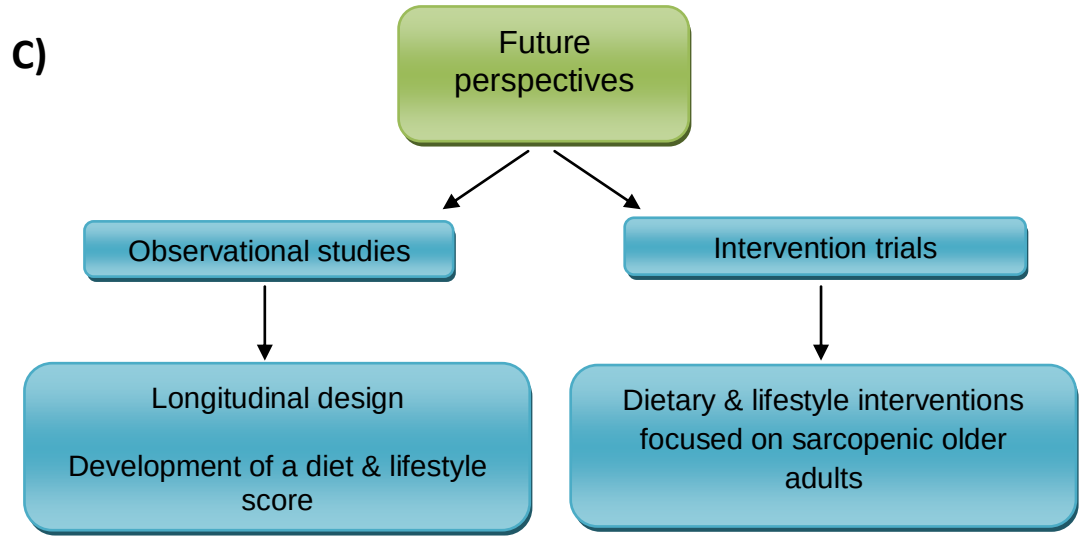
Overall, combining the knowledge from well-designed human intervention trials, epidemiological studies, animal studies, innovative technologies, comprehensive imaging and functional assessments and robust screening tools may help to uncover the factors that may be linked to sarcopenia and provide new approaches for its management, and therefore allow for robust recommendations for both prevention and treatment.

In conclusion, my research has shown that consumption of a diet that includes sufficient protein, and is high in a variety of foods high in vitamin C, magnesium, potassium and a range of carotenoids (nutrients found in foods of plant-based origin, such as fruit and vegetables, whole grain

products/dietary fiber, legumes and nuts) and a healthy dietary pattern high in these food components may prevent muscle loss in a female population across a wide age range. The novel findings from the current thesis have added to knowledge by better understanding the impact of dietary and lifestyle factors on the association between muscle mass and inflammation in adult women with normal weight, BMI and CRP concentrations.

These preliminary findings will form the basis in developing and planning dietary intervention trials for the prevention of sarcopenia by targeting inflammation.





B)

	VitaminC	VitaminE	Magnesium	Potassium	α-carotene	β-carotene	Total carotene	Lutein	β-cryptoxanthin	Lycopene	Diet scores	Protein & EAAs[1]
FFM (kg)	+		+	+		+	+	+	+		• MDS, HDI, DQI, AHEI, DASH-style **(+)**	
FFM %		+	+					+			• MDS, HDI, AHEI **(+)**	• Protein, arginine, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine **(-)**
FFMI (kg/m^2)	+		+	+	+	+	+		+	+	• MDS, HDI, DQI, AHEI, DASH-style **(+)**	
CRP (mg/L)			-								• HDI, AHEI **(-)**	• Arginine, glutamine, histidine, isoleucine, threonine, tryptophan **(+)**

- ✓ Magnesium, vitamin C, carotene, β-carotene, potassium, MDS, HDI and AHEI attenuate the association between FFM and CRP
- ✓ Magnesium, glutamine, arginine, MDS, HDI, AHEI and DASH-style attenuate the association between percentage FFM and CRP
- ✓ Magnesium, vitamin C, carotene, β-carotene, potassium, MDS, HDI and AHEI attenuate the association between FFMI and CRP

Figure 9.1: A) The interaction of sarcopenia and inflammation as consequences of the ageing process. Diet quality scores, micronutrient, protein & essential amino acid (EAA) intake may mediate the relationship between sarcopenia (assessed by indexes of muscle mass, hand grip strength and muscle quality) and inflammation (assessed by C-reactive protein – *CRP*). **B)** Results: Significant associations between diet quality scores, micronutrients, protein & EAAs and indexes of muscle mass and *CRP*; **(+)** indicates a positive association, **(-)** indicates a negative association; ([1]) protein and EAAs as % energy. **C)** Future perspectives

APPENDICES

APPENDIX I

PORTION SIZES (IN GRAMS) OF DIFFERENT FOOD COMPONENTS USED TO DERIVE THE DIET SCORES

Apples	100
Avocado	75
Baked Beans	135
Bananas	100
Bean sprouts	20
Beer	287
Beetroot	40
Biscuits choc	18
Biscuits plain	10
Block Marg	5
Boiled potato	180
Bran flakes	40
Broccoli	85
Brown Bread	36
Brown Rice	180
Brussels Sprouts	90
Bunshome	54
Bunsready	68
Butter	5
Cabbage	95
Cakeshome	90
Cakesready	60
Carrots	60
Cauliflower	90
Cereal Bars	33
Channel Island Milk	585
Cheerios	40
Chips	165
Chips Roast Pots	175.5
Chocolate bar	55
Chocolates	7
Chocs Dark	7
Chocs Milk	7
Chol Lower Spread	5
Cocoa	260
CocoPops	40
Coleslaw	45
Cornflakes	40
Cream Crackers	7
Crispbread	10

Crisps	28
Crunchynut Cornflakes	40
Dairy cheese	40
Dairy Cottage Cheese	55
Dairy Desserts	125
Dairy double	30
Dairy full fat yogurt	125
Dairy low fat cheese	30
Dairy low fat yogurt	125
Dairy single	15
Diet fizzy	160
Dried Fruit	30
Dried Lentils	70
Dried Milk	585
Eggs	50
Evaporated Milk	585
Fizzy Soft Drinks	160
French Dressing	15
Frosties	40
Fruit' n Fibre	40
Fruit pie home	110
Fruit pies ready	110
Fruit Squash	160
Full Milk	585
Garlic	5
Goats Milk	585
Grapefruit	80
Grape nuts	40
Grapes	80
Green Beans	90
Green Salad	30
Green Tea	260
High Fibre Cereal	40
Horlicks	260
Ice Cream	75
Jam	18
Just Right Type	40
Lasagne	420
Leeks	75
Liqueurs	50
Low calorie salad cream	20
Low Fat Hot Chocolate	18
Low Fat Spread	5
Marmite	9
Marrow	90

Mayo	20
Meat Soup	220
Melon	200
Bacon	25
Beef	128
Burgers	78
Corned Beef	30
Fish Fingers	50
Fishroe	45
Friedfish	180
Ham	23
Lamb	94
Liver	40
Oily fish	96
Pies	124
Pork	128
Poultry	125
Sausages	40
Shellfish	60
Whitefish	120
Milk Puddings	200
Muesli	60
Mushrooms	56
Nuts	40
Nuts Salted	25
Nuts Unsalted	16
Oat Based Cereal	60
Onions	60
Oranges	120
Other Cereal	40
Other dressing	50
Other Soft Margarine	5
Parsnips	65
Peaches	70
Peanut Butter	20
Pears	170
Peas	70
Pickles Chutney	40
Porridge	160
Potato Salad	85
Pufa Margarine	5
Pure Fruit Juice	160
Quiche	140
Red Wine	175
Rice Krispies	40

Rice Milk	585
Roast Potatoes	200
Sauces	50
Semi Skimmed Milk	585
Shredded Wheat	45
Shreddies	45
Skimmed Milk	585
Soya Milk	585
SpecialK	40
Spinach	90
Spirits	23
Sponge Pudhome	110
Sponge Pudready	110
Start	40
Strawberries	100
Sugar Added	6
Sugar Puff Type	40
Sultana Bran	40
Sweet corn	85
Sweet Peppers	80
Sweets Toffees	8
Tea	260
Tinned Fruit	100
Tofu	50
Tomatoes	85
Very Low Fat Spread	5
Watercress	20
Weetabix Type	40
Wheat Flakes	40
White Bread	36
White pasta	230
White Rice	180
White Wine	125
Wholemeal Bread	36
Wholemeal Pasta	230

APPENDIX II

Subset 1: Food groups and foods included in each of the derived diet scores

MDS	HDI	DQI	AHEI	DASH-style
Fruit & nuts Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges, nuts	**Fruit & vegetables** Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges, pure fruit juice, spinach, parsnips, leeks, onions, garlic, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, watercress, green salad, tomatoes, sweet corn, bean sprouts, coleslaw, avocado, beetroot, tofu, mushrooms, sweet peppers	**Fruit & vegetables** Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges, pure fruit juice, spinach, parsnips, leeks, onions, garlic, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, marrow, watercress, green salad, tomatoes, sweet corn, bean sprouts, coleslaw, avocado, beetroot, tofu, mushrooms, sweet peppers	**Fruit** Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges	**Fruit** Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges, pure fruit juice
Vegetables Spinach, parsnips, leeks, onions, garlic, peas, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, watercress, green salad, tomatoes, sweet corn, bean sprouts, coleslaw, avocado, beetroot, tofu, mushrooms, sweet peppers	**Complex carbohydrates** Breads, cereals, legumes, peas	**Total fat (%E)** Daily intake from FFQ	**Vegetables** Spinach, parsnips, leeks, onions, garlic, peas, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, watercress, green salad, tomatoes, sweet corn, bean sprouts, coleslaw, avocado, baked beans, beetroot, mushrooms, sweet peppers	**Vegetables** Spinach, parsnips, leeks, onions, garlic, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, watercress, green salad, tomatoes, sweet corn, bean sprouts, coleslaw, avocado, beetroot, mushrooms, sweet peppers
Legumes Baked beans, dried lentils	**Dietary fiber** NSP from FFQ (g)	**Complex carbohydrates** Breads, cereals, legumes, peas	**Nuts & soy protein** Nuts, tofu	**Nuts & legumes** Nuts, baked beans, dried lentils, peas, tofu
Fish Fried fish, fish fingers, white fish, oily fish, shell fish	**Pulses & nuts** Baked beans, nuts, and dried lentils	**Saturated fat (%E)** Daily intake from FFQ	**Ratio of white (including fish) to red meat** Fried fish, fish fingers, white fish,	**Whole grains** Bran flakes, sultana bran, fruit n fibre, all bran, muesli,

			oily fish, shell fish **+** poultry TO Beef, burgers, pork, lamb, bacon, ham, corned beef, sausages, pies, liver	shreddies, shredded wheat, weetabix type, grape nuts, wheat flakes, oat based, brown bread, wholemeal bread, crispbread (rye), porridge, wholemeal spaghetti, brown rice
Cereals Cornflakes, Crunchynut, cornflakes, bran flakes, sultana bran, fruit n fiber, rice krispies, sugar puffs, all bran, muesli, start, cheerios, just right type, shreddies, shredded wheat, weetabix type, special K, coco pops, frosties, grape nuts, wheat flakes, oat based, other cereal, porridge, white bread, brown bread, whole meal bread, cream crackers, crisp bread, lasagne, spaghetti, whole meal pasta, white pasta, brown rice, white rice	**Mono- & disaccharides** Daily intake of total sugars from FFQ	**Cholesterol** Daily intake from FFQ	**Dietary fiber** NSP from FFQ (g)	**Low fat dairy products** Semi-skimmed milk, skimmed milk, dried skimmed milk, low-fat yogurt, cottage cheese
Fat Ratio of monounsaturated & polyunsaturated to saturated fat	**Protein (%E)** Daily intake from FFQ	**Protein (%RDA)** Based on UK RNI: 1.5 g protein/kg/day	**Trans fat (%E)** Daily intake from FFQ	**Sodium** Daily intake from FFQ
Dairy products & eggs Full milk, semi-skimmed milk, skimmed milk, channel island milk, dried skimmed milk, soya milk, rice milk, goats milk, evaporated milk, cottage cheese, cream fresh single, cream fresh double, full fat yogurt, low fat yogurt, dairy desserts, dairy cheese (e.g. cheddar, brie, edam), eggs	**Saturated fat (%E)** Daily intake from FFQ	**Sodium** Daily intake from FFQ	**Polyunsaturated to saturated fat ratio** Ratio of daily intakes from FFQ	**Red & processed meat** Beef, burgers, pork, lamb, poultry, bacon, ham, corned beef, sausages, pies, liver

Meat Beef, burgers, pork, lamb, poultry, bacon, ham, corned beef, sausages, pies, liver	**Polyunsaturated fat (%E)** Daily intake from FFQ	**Calcium (%RDA)** Based on UK RDA: 525 mg/day	**Vitamin supplement use** From questionnaire (Yes/no)	**Sweetened beverages** Fizzy soft drinks (cola, lemonade), low calorie diet fizzy drinks
Alcohol Daily alcohol intake from FFQ (g)	**Cholesterol** Daily intake from FFQ		**Alcoholic drinks** Beer, spirits, wine, liqueur (servings/d)	

APPENDIX III

Subset 2: Food groups and foods included in each of the derived diet scores

MDS	HDI	DQI	AHEI	DASH-style
Fruit & nuts Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges, salted nuts, unsalted nuts	**Fruit & vegetables** Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges, pure fruit juice, spinach, parsnips, leeks, onions, garlic, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, watercress, green salad, tomatoes, sweet corn, bean sprouts, coleslaw, avocado, beetroot, tofu, mushrooms, sweet peppers	**Fruit & vegetables** Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges, pure fruit juice, spinach, parsnips, leeks, onions, garlic, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, marrow, watercress, green salad, tomatoes, sweet corn, bean sprouts, coleslaw, avocado, beetroot, tofu, mushrooms, sweet peppers	**Fruit** Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges	**Fruit** Peaches, watermelon, grapes, bananas, grapefruit, pears, apples, strawberries, tinned fruit, dried fruit, oranges, pure fruit juice
Vegetables Spinach, parsnips, leeks, onions, garlic, peas, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, watercress, green salad, tomatoes, sweet corn, bean sprouts, coleslaw, avocado, beetroot, tofu, mushrooms, sweet peppers	**Complex carbohydrates** Breads, cereals, legumes, peas	**Total fat (%E)** Daily intake from FFQ	**Vegetables** Spinach, parsnips, leeks, onions, garlic, peas, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, watercress, green salad, tomatoes, sweet corn, beans sprouts, coleslaw, avocado, baked beans, beetroot, mushrooms, sweet peppers	**Vegetables** Spinach, parsnips, leeks, onions, garlic, cabbage, broccoli, Bruccel sprouts, green beans, cauliflower, carrots, watercress, green salad, tomatoes, sweet corn, bean sprouts, coleslaw, avocado, beetroot, mushrooms, sweet peppers
Legumes Baked beans, dried lentils	**Dietary fiber** NSP from FFQ (g)	**Complex carbohydrates** Breads, cereals, legumes, peas	**Nuts & soy protein** Nuts, tofu	**Nuts & legumes** Nuts, baked beans, dried lentils, peas, tofu
Fish Fried fish, fish fingers, white fish, oily fish, shell fish	**Pulses & nuts** Baked beans, salted nuts, unsalted nuts, and dried lentils	**Saturated fat (%E)** Daily intake from FFQ	**Ratio of white (including fish) to red meat** Fried fish, fish fingers, white fish,	**Whole grains** Bran flakes, sultana bran, fruit n fibre, all bran, muesli,

			oily fish, shell fish **+** poultry TO Beef, burgers, pork, lamb, bacon, ham, corned beef, sausages, pies, liver	shreddies, shredded wheat, weetabix type, grape nuts, wheat flakes, oat based, brown bread, wholemeal bread, crispbread (rye), porridge, wholemeal spaghetti, brown rice
Cereals Breakfast cereal, high fiber cereal, cereal bars, porridge, white bread, brown bread, whole meal bread, cream crackers, crisp bread, lasagne, spaghetti, whole meal pasta, white pasta, brown rice, white rice	**Mono- & disaccharides** Daily intake of total sugars from FFQ	**Cholesterol** Daily intake from FFQ	**Dietary fiber** NSP from FFQ (g)	**Low fat dairy products** Semi-skimmed milk, skimmed milk, dried skimmed milk, low-fat yogurt, cottage cheese
Fat Ratio of monounsaturated & polyunsaturated to saturated fat	**Protein (%E)** Daily intake from FFQ	**Protein (%RDA)** Based on UK RNI: 1.5 g protein/kg/day	**Trans fat (%E)** Daily intake from FFQ	**Sodium** Daily intake from FFQ
Dairy products & eggs Full milk, semi-skimmed milk, skimmed milk, channel island milk, dried skimmed milk, soya milk, rice milk, goats milk, evaporated milk, cottage cheese, cream fresh single, cream fresh double, full fat yogurt, low fat yogurt, dairy desserts, dairy cheese (e.g. cheddar, brie, edam), eggs	**Saturated fat (%E)** Daily intake from FFQ	**Sodium** Daily intake from FFQ	**Polyunsaturated to saturated fat ratio** Ratio of daily intakes from FFQ	**Red & processed meat** Beef, burgers, pork, lamb, poultry, bacon, ham, corned beef, sausages, pies, liver
Meat Beef, burgers, pork, lamb, poultry, bacon, ham, corned beef, sausages, pies, liver	**Polyunsaturated fat (%E)** Daily intake from FFQ	**Calcium (%RDA)** Based on UK RDA: 525 mg/day	**Vitamin supplement use** From questionnaire (Yes/no)	**Sweetened beverages** Fizzy soft drinks (cola, lemonade), low calorie diet fizzy drinks
Alcohol Daily alcohol intake from FFQ (g)	**Cholesterol** Daily intake from FFQ		**Alcoholic drinks** Beer, spirits, red wine, white wine, liqueur (servings/d)	

REFERENCES

Abdulrab K and Heun R (2008) Subjective memory impairement. A review of its definitions indicates the need for a comprehensive set of standardised and validated criteria. European Phychiatry 23 (5): 321-330.

Abellan Van Kan G (2009) Epidemiology and consequences of sarcopenia. The Journal of Nutrition, Health & Aging 13 (8): 708-712.

Abellan Van Kan G, André E, Bischoff-Ferrari H, Boirie Y, Onder G, Pahor M, Ritz P, Rolland Y, Sampaio C, Studenski S, Visser M and Vellas B (2009) Carla Task Force on Sarcopenia: Propositions for clinical trials. The Journal of Nutrition, Health & Aging 13 (8): 700-707.

Abellan van Kan G, Rolland Y, Andrieu S, Bauer J, Beauchet O, Bonnefoy M, Cesari M, Donini L, Gillette-Guyonnet S, Inzitari M, Nourhashemi F, Onser G, Ritz P, Salva A, Visser M and Vellas B (2009) Gait speed at usual pace as apredictor of adverse outcomes in community-dwelling plder people. An International Academy on Nutrition and Aging (IANA) Task Force. The Journal of Nutrition, Health & Aging 13 (10): 881-889.

Akbaraly TN, Ferrie JE, Berr C, Brunner EJ, Head J, Marmot MG, Singh-Manoux A, Ritchie K, Shipley MJ and Kivimaki M (2011) Alternative Healthy Eating Index and mortality over 18 y of follow-up: results from the Whitehall II cohort. The American Journal of Clinical Nutrition 94 (1): 247-253.

Al-Delaimy W, Rimm E, Willett W, Stampfer M and Hu F (2004) Magnesium Intake and Risk of Coronary Heart Disease among Men. Journal of the American College of Nutrition 23 (1): 63-70.

Alipanah N, Ferrucci L, Sun K, Fried L, Walston J, Varadhan R, Guralnik J and Semba R (2007) Relationship of antioxidant nutrients with oxidative protein damage among older women living in the community. The FASEB Journal 21 700.713.

Altorf-van der Kuil W, Engberink M, De Neve M, van Rooij F, Hofman A, van't Veer P, Witteman J, Franco O and Geleijnse J (2013) Dietary amino acids and the risk of hypertension in a Dutch older population: the Rotterdam Study. The American Journal of Clinical Nutrition 97 (2): 403-410.

Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD and MacGregor AJ (2001) Are Twins and Singletons Comparable? A Study of Disease-related and Lifestyle Characteristics in Adult Women. Twin Research 4 (6): 464-477.

Anker SD, Ponikowski PP, Clark AL, Leyva F, Rauchhaus M, Kemp M, Teixeira MM, Hellewell PG, Hooper J, Poole-Wilson PA and Coats AJS (1999) Cytokines and neurohormones relating to body composition alterations in the wasting syndrome of chronic heart failure. European Heart Journal 20 (9): 683-693.

Annweiler C, Schott A, Berrut G, Fantino B and Beauche O (2009) Vitamin D-related changes in physical performance: A systematic review. The Journal of Nutrition, Health & Aging 1-6.

Anthony J, Reiter A, Anthony T, Crozier S, Lang C, MacLean D, Kimball S and Jefferson L (2002) Orally Administered Leucine Enhances Protein Synthesis in Skeletal Muscle of Diabetic Rats in the Absence of Increases in 4E-BP1 or S6K1 Phosphorylation. Diabetes 51 (4): 928-936.

Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin P-H, Karanja N,

Simons-Morton D, McCullough M, Swain J, Steele P, Evans MA, Miller ER and Harsha DW (1997) A Clinical Trial of the Effects of Dietary Patterns on Blood Pressure. New England Journal of Medicine 336 (16): 1117-1124.

Arden NK, Baker J, Hogg C, Baan K and Spector TD (1996) The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: A study of postmenopausal twins. Journal of Bone and Mineral Research 11 (4): 530-534.

Arden NK and Spector TD (1997) Genetic Influences on Muscle Strength, Lean Body Mass, and Bone Mineral Density: A Twin Study. Journal of Bone and Mineral Research 12 (12): 2076-2081.

Argaman Z, Young V, Noviski N, Castillo-Rosas L, Lu X, Zurakowski D, Cooper M, Davison C, Tharakan J, Ajami A and Castillo L (2003) Arginine and nitric oxide metabolism in critically ill septic pediatric patients. Critical Care Medicine 31 (2): 591-597.

Atlantis E, Martin SA, Haren MT, Taylor AW, Wittert GA and Study ftFAMA (2008) Lifestyle factors associated with age-related differences in body composition: the Florey Adelaide Male Aging Study. The American Journal of Clinical Nutrition 88 (1): 95-104.

Aubertin-Leheudre M and Adlercreutz H (2009) Relationship between animal protein intake and muscle mass index in healthy women. British Journal of Nutrition 102 (12): 1803-1810.

Aviv A, Valdes A, Gardner JP, Swaminathan R, Kimura M and Spector TD (2006) Menopause Modifies the Association of Leukocyte Telomere Length with Insulin Resistance and Inflammation. The Journal of Clinical Endocrinology & Metabolism 91 (2): 635-640.

Azadbakht L, Surkan P, Esmaillzadeh A and Willett W (2011) The Dietary Approaches to Stop Hypertension Eating Plan Affects C-Reactive Protein, Coagulation Abnormalities, and Hepatic Function Tests among Type 2 Diabetic Patients. The Journal of Nutrition 141 (6): 1083-1088.

Baer DJ, Judd JT, Clevidence BA and Tracy RP (2004) Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. The American Journal of Clinical Nutrition 79 (6): 969-973.

Barbat-Artigas S, Rolland Y, Zamboni M and Aubertin-Leheudre M (2012) How to assess functional status: A new muscle quality index. The Journal of Nutrition, Health & Aging 16 (1): 67-77.

Barbieri M, Ferrucci L, Ragno E, Corsi A, Bandinelli S, Bonafè M, Olivieri F, Giovagnetti S, Franceschi C, Guralnik JM and Paolisso G (2003) Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. American Journal of Physiology - Endocrinology And Metabolism 284 (3): E481-E487.

Bartali B, Frongillo EA, Bandinelli S, Lauretani F, Semba RD, Fried LP and Ferrucci L (2006) Low Nutrient Intake is an essential component of frailty in older persons. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 61 (6): 589-593.

Bartali B, Frongillo E, Stipanuk M, Bandinelli S, Salvini S, Palli D, Morais J, Volpato S, Guralnik J and Ferrucci L (2012) Protein Intake and Muscle Strength in Older Persons: Does Inflammation Matter? Journal of the American Geriatrics Society 60 (3): 480-484.

Bassey E (1997) Physical capabilities, exercise and aging. Reviews in Clinical Gerontology 7 (04): 289-297.

Bastard J, Mustapha M and Lagathu C (2006) Recent advances in the relationship between obesity, inflammation and insulin resistance. European Cytokine Network 17 4-12.

Basu A, Devaraj S and Jialal I (2006) Dietary Factors That Promote or Retard Inflammation. Arteriosclerosis Thrombosis & Vascular Biology 26 (5): 995-1001.

Baumgartner RN (2000) Body Composition in Healthy Aging. Annals of the New York Academy of Sciences 904 (1): 437-448.

Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ and Lindeman RD (1998) Epidemiology of Sarcopenia among the Elderly in New Mexico. American Journal of Epidemiology 147 (8): 755-763.

Baumgartner RN and Waters DL (2006). Sarcopenia and Sarcopenic-Obesity. Principles and Practice of Geriatric Medicine, John Wiley & Sons, Ltd**:** 909-933.

Baumgartner RN, Waters DL, Gallagher D, Morley JE and Garry PJ (1999) Predictors of skeletal muscle mass in elderly men and women. Mechanisms of Ageing and Development 107 (2): 123-136.

Beard JR, Biggs S, Bloom DE, Fried LP, Hogan P, Kalache A and SJ O (2011). Global Population Ageing: Peril or Promise, Geneva: World Economic Forum 2011.

Beavers KM, Brinkley TE and Nicklas BJ (2010) Effect of exercise training on chronic inflammation. Clinica Chimica Acta In Press, Corrected Proof

Becker W, Foley S, Shelley E and Gibney M (1999) Energy under-reporting in Swedish and Irish dietary surveys: implications for food-based dietary guidelines. British Journal of Nutrition 81 Suppl 2 S127-131.

Bender R and Lange S (2001) Adjusting for multiple testing-when and how? Jounal of Clinical Epidemiology 54 343-349.

Benítez-Arciniega AA, Mendez MA, Baena-Díez JM, Rovira Martori M-A, Soler C, Marrugat J, Covas M-I, Sanz H, Llopis A and Schröder H (2011) Concurrent and construct validity of Mediterranean diet scores as assessed by an FFQ. Public Health Nutrition 14 (11): 2015-2021.

Benjamini Y (2010) Simultaneous and selective inference: Current successes and future challenges. Biometrical Journal 52 (6): 708-721.

Berg AH and Scherer PE (2005) Adipose Tissue, Inflammation, and Cardiovascular Disease. Circulation Research 96 (9): 939-949.

Bingham SA, Gill C, Welch A, Cassidy A, Runswick SA, Oakes S, Lubin R, Thurnham DI, Key TJ, Roe L, Khaw KT and Day NE (1997) Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers. International Journal of Epidemiology 26 (suppl 1): S137.

Bingham SA, Welch AA, McTaggart A, Mulligan AA, Runswick SA, Luben R, Oakes S, Khaw KT, Wareham N and Day NE (2001) Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. Public Health Nutrition 4 (03): 847-858.

Biolo G (2013) Protein metabolism and requirements. World Review of Nutrition & Dietetics 105: 12-20.

Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, Wong JB, Egli A, Kiel DP and Henschkowski J (2009) Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. British Medical Journal 339

Bischoff HA, Borchers M, Gudat F, Duermueller U, Theiler R, Stahelin HB and Dick W (2001) In situ detection of 1,25-dihydroxyvitamin D3 receptor in human skeletal muscle tissue. Journal of Histochemistry 33 (1): 19-24.

Blackwell TS and Christman JW (1997) The Role of Nuclear Factor- κ B in Cytokine Gene Regulation. American Journal of Respiratory Cell and Molecular Biology 17 (1): 3-9.

Block G, Jensen CD, Dalvi TB, Norkus EP, Hudes M, Crawford PB, Holland N, Fung EB, Schumacher L and Harmatz P (2009) Vitamin C treatment reduces elevated C-reactive protein. Free Radical Biology and Medicine 46 (1): 70-77.

Bobeuf F, Labonté M, Khalil A and Dionne IJ (2010) Effects of resistance training combined with antioxidant supplementation on fat-free mass and insulin sensitivity in healthy elderly subjects. Diabetes Research and Clinical Practice 87 (1): e1-e3.

Boekholdt SM, Meuwese MC, Day NE, Luben R, Welch A, Wareham NJ and Khaw KT (2006) Plasma concentrations of ascorbic acid and C-reactive protein, and risk of future coronary artery disease, in apparently healthy men and women: the EPIC-Norfolk prospective population study. British Journal of Nutrition 96 (3): 516-522.

Bonnefoy M, Laville M, Ecochard R, Jusot J, Normand S, Maillot S, Lebreton B and Jauffret M (2010) Effects of Branched amino acids

supplementation in malnourished elderly with catabolic status. The Journal of Nutrition, Health & Aging 14 579-584.

Børsheim E, Bui Q-UT, Tissier S, Kobayashi H, Ferrando AA and Wolfe RR (2008) Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. Clinical Nutrition 27 (2): 189-195.

Borst SE (2004) Interventions for sarcopenia and muscle weakness in older people. Age Ageing 33 (6): 548-555.

Bouchard DR, Dionne IJ and Brochu M (2009) Sarcopenic/Obesity and Physical Capacity in Older Men and Women: Data From the Nutrition as a Determinant of Successful Aging (NuAge) the Quebec Longitudinal Study. Obesity 17 (11): 2082-2088.

Boynton A, Neuhouser ML, Sorensen B, McTiernan A and Ulrich CM (2008) Predictors of Diet Quality among Overweight and Obese Postmenopausal Women. Journal of the American Dietetic Association 108 (1): 125-130.

Brilla L and Haley T (1992) Effect of magnesium supplementation on strength training in humans. Journal of the American College of Nutrition 11: 326-329.

Britton A, Shipley M, Singh-Manoux A and Marmot MG (2008) Successful aging: the contribution of early-life and midlife risk factors. Journal of the American Geriatrics Society 56 (6): 1098-1105.

Brown AA and Hu FB (2001) Dietary modulation of endothelial function: implications for cardiovascular disease. The American Journal of Clinical Nutrition 73 (4): 673-686.

Burkle A, Beneke S, Brabeck C, Leake A, Meyer R, Muiras ML and Pfeiffer R (2002) Poly(ADP-ribose) polymerase-1, DNA repair and mammalian longevity. Experimental Gerontology 37 (10-11): 1203-1205.

Calabro P, Chang DW, Willerson JT and Yeh ET (2005) Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: linking obesity to vascular inflammation. Journal of the American College of Cardiology 46 (6): 1112-1113.

Calabrò P, Golia E, Maddaloni V, Malvezzi M, Casillo B, Marotta C, Calabrò R and Golino P (2009) Adipose tissue-mediated inflammation: the missing link between obesity and cardiovascular disease? Internal and Emergency Medicine 4 (1): 25-34.

Calder PC, Albers R, Antoine J-M, Blum S, Bourdet-Sicard R, Ferns GA, Folkerts G, Friedmann PS, Frost GS, Guarner F, Løvik M, Macfarlane S, Meyer PD, M'Rabet L, Serafini M, van Eden W, van Loo J, Vas Dias W, Vidry S, Winklhofer-Roob BM and Zhao J (2009) Inflammatory Disease Processes and Interactions with Nutrition. British Journal of Nutrition 101 (SupplementS1): 1-45.

Calvani R, Miccheli A, Landi F, Bossola M, Cesari M, Leeuwenburgh C, Sieber C, Bernabei R and Marzetti E (2013) Current nutritional recommendations and novel dietary strategies to manage sarcopenia. The Journal of Frailty & Aging 2 38-53.

Campbell P, Campbell K, Wener M, Wood B, Potter J, McTiernan A and Ulrich C (2009) A year-long exercise intervention decreases CRP among obese postmenopausal women. Medicine & Science in Sports & Exercise 41 (8): 1533-1539.

Campbell WW (2007) Synergistic Use of Higher-Protein Diets or Nutritional Supplements with Resistance Training to Counter Sarcopenia. Nutrition Reviews 65 (9): 416-422.

Campbell WW, Trappe TA, Wolfe RR and Evans WJ (2001) The Recommended Dietary Allowance for Protein May Not Be Adequate for Older People to Maintain Skeletal Muscle. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 56 (6): M373-M380.

Carey KA, Farnfield MM, Tarquinio SD and Cameron-Smith D (2007) Impaired Expression of Notch Signaling Genes in Aged Human Skeletal Muscle. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 62 (1): 9-17.

Carlin JB, Gurrin LC, Sterne JA, Morley R and Dwyer T (2005) Regression models for twin studies: a critical review. International Journal of Epidemiology 34 (5): 1089-1099.

Carr A and Frei B (1999) Does vitamin C act as a pro-oxidant under physiological conditions? The FASEB Journal 13 (9): 1007-1024.

Carr AC, Bozonet SM, Pullar JM, Simcock JW and Vissers MC (2013) Human skeletal muscle ascorbate is highly responsive to changes in vitamin C intake and plasma concentrations. The American Journal of Clinical Nutrition 97 (4): 800-807.

Carville S, Rutherford O and Newham D (2006) Power output, isometric strength and steadiness in the leg muscles of pre- and postmenopausal women; the effects of hormone replacement therapy. European Journal of Applied Physiology 96 (3): 292-298.

Casas JP, Shah T, Hingorani AD, Danesh J and Pepys MB (2008) C-reactive protein and coronary heart disease: a critical review. Journal of Internal Medicine 264 (4): 295-314.

Cassidy A, Skidmore P, Rimm EB, Welch A, Fairweather-Tait S, Skinner J, Burling K, Richards JB, Spector TD and MacGregor AJ (2009) Plasma Adiponectin Concentrations Are Associated with Body Composition and Plant-Based Dietary Factors in Female Twins. The Journal of Nutrition 139 (2): 353-358.

Castaneda C, Charnley J, Evans W and Crim M (1995) Elderly women accommodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. The American Journal of Clinical Nutrition 62 (1): 30-39.

Castaneda C and Janssen I (2005) Ethnic comparisons of sarcopenia and obesity in diabetes. Ethnicity & Disease 15 (4): 664-670.

Cavicchia PP, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS and Hebert JR (2009) A new dietary inflammatory index predicts interval changes in serum high-sensitivity C-reactive protein. The Journal of Nutrition 139 (12): 2365-2372.

Cesari M, Kritchevsky SB, Baumgartner RN, Atkinson HH, Penninx BW, Lenchik L, Palla SL, Ambrosius WT, Tracy RP and Pahor M (2005) Sarcopenia, obesity, and inflammation—results from the Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors study. The American Journal of Clinical Nutrition 82 (2): 428-434.

Cesari M and Pahor M (2008) Target population for clinical trials on sarcopenia. The Journal of Nutrition, Health & Aging 12 (7): 470-478.
Cesari M and Pahor M (2008) Target population for clinical trials on sarcopenia. The Journal of Nutrition, Health & Aging 12 (7): 470-478.

Cesari M, Pahor M, Bartali B, Cherubini A, Penninx BW, Williams GR, Atkinson H, Martin A, Guralnik JM and Ferrucci L (2004) Antioxidants and physical performance in elderly persons: the Invecchiare in Chianti (InCHIANTI) study. The American Journal of Clinical Nutrition 79 (2): 289-294.

Cesari M, Pahor M, Lauretani F, Zamboni V, Bandinelli S, Bernabei R, Guralnik JM and Ferrucci L (2009) Skeletal Muscle and Mortality Results From the InCHIANTI Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 64A (3): 377-384.

Cesari M, Penninx BW, Pahor M, Lauretani F, Corsi AM, Rhys Williams G, Guralnik JM and Ferrucci L (2004) Inflammatory markers and physical performance in older persons: the InCHIANTI study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 59 (3): 242-248.

Chacko SA, Song Y, Nathan L, Tinker L, de Boer IH, Tylavsky F, Wallace R and Liu S (2010) Relations of Dietary Magnesium Intake to Biomarkers of Inflammation and Endothelial Dysfunction in an Ethnically Diverse Cohort of Postmenopausal Women. Diabetes Care 33 (2): 304-310.

Chacko SA, Sul J, Song Y, Li X, LeBlanc J, You Y, Butch A and Liu S (2011) Magnesium supplementation, metabolic and inflammatory markers, and global genomic and proteomic profiling: a randomized, double-blind, controlled, crossover trial in overweight individuals. The American Journal of Clinical Nutrition 93 (2): 463-473.

Chapman IM (2007) The Anorexia of Aging. Clinics in Geriatric Medicine 23 (4): 735-756.

Chen L, Nelson D, Zhao Y, Cui Z and Johnston J (2013) Relationship between muscle mass and muscle strength, and the impact of comorbidities:

a population-based, cross-sectional study of older adults in the United States. BMC Geriatrics 13: 74.

Chen R, Mias GI, Li-Pook-Than J, Jiang L, Lam HY, Chen R, Miriami E, Karczewski KJ, Hariharan M, Dewey FE, Cheng Y, Clark MJ, Im H, Habegger L, Balasubramanian S, O'Huallachain M, Dudley JT, Hillenmeyer S, Haraksingh R, Sharon D, Euskirchen G, Lacroute P, Bettinger K, Boyle AP, Kasowski M, Grubert F, Seki S, Garcia M, Whirl-Carrillo M, Gallardo M, Blasco MA, Greenberg PL, Snyder P, Klein TE, Altman RB, Butte AJ, Ashley EA, Gerstein M, Nadeau KC, Tang H and Snyder M (2012) Personal omics profiling reveals dynamic molecular and medical phenotypes. Cell 148 (6): 1293-1307.

Cherkas LF, Hunkin JL, Kato BS and et al. (2008) The association between physical activity in leisure time and leukocyte telomere length. Archives Internal Med 168 (2): 154-158.

Choi J, Joseph L and Pilote L (2013) Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. Obesity Reviews 14 (3): 232-244.

Chrysohoou C, Panagiotakos DB, Pitsavos C, Das UN and Stefanadis C (2004) Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: The ATTICA study. Journal of the American College of Cardiology 44 (1): 152-158.

Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, Carter C, Yu BP and Leeuwenburgh C (2009) Molecular inflammation: Underpinnings of aging and age-related diseases. Ageing Research Reviews 8 (1): 18-30.

Chung HY, Lee EK, Choi YJ, Kim JM, Kim DH, Zou Y, Kim CH, Lee J, Kim HS, Kim ND, Jung JH and Yu BP (2011) Molecular Inflammation as

an Underlying Mechanism of the Aging Process and Age-related Diseases. Journal of Dental Research 90 (7): 830-840.

Ciana P, Raviscioni M, Mussi P, Vegeto E, Que I, Parker MG, Lowik C and Maggi A (2002) In vivo imaging of transcriptionally active estrogen receptors. Nature Medicine 9 (1): 82-86.

Clark BFC (2008) Healthy human ageing. New Biotechnology 25 (1): 13-15.

Coin A, Sergi G, Minicuci N, Giannini S, Barbiero E, Manzato E, Pedrazzoni M, Minisola S, Rossini M, Del Puente A, Zamboni M, Inelmen EM and Enzi G (2008) Fat-free mass and fat mass reference values by dual-energy X-ray absorptiometry (DEXA) in a 20-80 year-old Italian population. Clinical Nutrition 27 (1): 87-94.

Cooper R, Mishra G, Clennell S, Guralnik J and Kuh D (2008) Menopausal status and physical performance in midlife: findings from a British birth cohort study. Menopause 15 (6): 1079-1085.

National Research Council (2011) Dietary Reference Intakes for Calcium and Vitamin D. The National Academies Press.

Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel J-P, Rolland Y, Schneider SM, Topinkova E, Vandewoude M and Zamboni M (2010) Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing 39 (4): 412-423.

Dai J, Jones DP, Goldberg J, Ziegler TR, Bostick RM, Wilson PW, Manatunga AK, Shallenberger L, Jones L and Vaccarino V (2008) Association between adherence to the Mediterranean diet and oxidative stress. The American Journal of Clinical Nutrition 88 (5): 1364-1370.

Dardevet D, Sornet C, Balage M and Grizard J (2000) Stimulation of in vitro rat muscle protein synthesis by leucine decreases with age. Journal of Nutrition 130 (11): 2630-2635.

Davison S and Davis SR (2003) New Markers for Cardiovascular Disease Risk in Women: Impact of Endogenous Estrogen Status and Exogenous Postmenopausal Hormone Therapy. Journal of Clinical Endocrinology & Metabolism 88 (6): 2470-2478.

Dawson-Hughes B (2008) Serum 25-hydroxyvitamin D and functional outcomes in the elderly. The American Journal of Clinical Nutrition 88 (2): 537S-540S.

de Ferranti SD and Rifai N (2007) C-reactive protein: a nontraditional serum marker of cardiovascular risk. Cardiovascular Pathology 16 (1): 14-21.

de Koning L, Chiuve SE, Fung TT, Willett WC, Rimm EB and Hu FB (2011) Diet-Quality Scores and the Risk of Type 2 Diabetes in Men. Diabetes Care 34 (5): 1150-1156.

de Luca C and Olefsky JM (2008) Inflammation and insulin resistance. FEBS Letters 582 (1): 97-105.

de Oliveira Otto MCC, Alonso A, Lee D-H, Delclos GL, Jenny NS, Jiang R, Lima JA, Symanski E, Jacobs DR and Nettleton JA (2011) Dietary Micronutrient Intakes Are Associated with Markers of Inflammation but Not with Markers of Subclinical Atherosclerosis. The Journal of Nutrition 141 (8): 1508-1515.

Dickinson JM, Fry CS, Drummond MJ, Gundermann DM, Walker DK, Glynn EL, Timmerman KL, Dhanani S, Volpi E and Rasmussen BB (2011) Mammalian Target of Rapamycin Complex 1 Activation Is Required for the

Stimulation of Human Skeletal Muscle Protein Synthesis by Essential Amino Acids. The Journal of Nutrition 141 (5): 856-862.

Dietrich M, Block G, Norkus EP, Hudes M, Traber MG, Cross CE and Packer L (2003) Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase γ-tocopherol in vivo after adjustment for dietary antioxidant intakes. The American Journal of Clinical Nutrition 77 (1): 160-166.

Ding J, Kritchevsky SB, Newman AB, Taaffe DR, Nicklas BJ, Visser M, Lee JS, Nevitt M, Tylavsky FA, Rubin SM, Pahor M, Harris TB and Health ABCS (2007) Effects of birth cohort and age on body composition in a sample of community-based elderly. The American Journal of Clinical Nutrition 85 (2): 405-410.

Doherty TJ (2003) Invited review: Aging and sarcopenia. Journal of Applied Physiology 95 (4): 1717-1727.

Dominguez LJ, Barbagallo M, Lauretani F, Bandinelli S, Bos A, Corsi AM, Simonsick EM and Ferrucci L (2006) Magnesium and muscle performance in older persons: the InCHIANTI study. The American Journal of Clinical Nutrition 84 (2): 419-426.

Due A, Toubro S, Stender S, Skov AR and Astrup A (2005) The effect of diets high in protein or carbohydrate on inflammatory markers in overweight subjects. Diabetes, Obesity and Metabolism 7 (3): 223-229.

Duntas L (2009) Selenium and inflammation: underlying anti-inflammatory mechanisms. Hormone and Metabolic Research 41 (6): 443-447.

Ekelund U, Tomkinson G and Armstrong N (2011) What proportion of youth are physically active? Measurement issues, levels and recent time trends. British Journal of Sports Medicine 45 (11): 859-865.

Eley HL, Russell ST and Tisdale MJ (2007) Effect of branched-chain amino acids on muscle atrophy in cancer cachexia. Biochemical Journal 407 (1): 113-120.

Erlinger TP, Guallar E, Miller ER III, Stolzenberg-Solomon R and LJ A (2001) Relationship between systemic markers of inflammation and serum β-carotene levels. Archives of Internal Medicine 161 (15): 1903-1908.

Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB and Willett WC (2006) Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. The American Journal of Clinical Nutrition 84 (6): 1489-1497.

Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB and Willett WC (2006) Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. The American Journal of Clinical Nutrition 84 (6): 1489-1497.

Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB and Willett WC (2007) Dietary patterns, insulin resistance, and prevalence of the metabolic syndrome in women. The American Journal of Clinical Nutrition 85 (3): 910-918.

Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F and Giugliano D (2004) Effect of a Mediterranean-Style Diet on Endothelial Dysfunction and Markers of Vascular Inflammation in the Metabolic Syndrome: A Randomized Trial. The Journal of the American Medical Association 292 (12): 1440-1446.

Evans W (1997) Functional and Metabolic Consequences of Sarcopenia. The Journal of Nutrition 127 (5): 998S-1003S.

Fagot-Campagna A, Bourdel-Marchasson I and D S (2005) Burden of diabetes in an aging population: prevalence, incidence, mortality, characteristics and quality of care. Diabetes & Metabolism 31 5S35-35S52.

Fairweather-Tait SJ, Collings R and Hurst R (2010) Selenium bioavailability: current knowledge and future research requirements. The American Journal of Clinical Nutrition 91 (5): 1484S-1491S.

Fairweather-Tait SJ, Skinner J, Guile GR, Cassidy A, Spector TD and MacGregor AJ (2011) Diet and bone mineral density study in postmenopausal women from the TwinsUK registry shows a negative association with a traditional English dietary pattern and a positive association with wine. The American Journal of Clinical Nutrition 94: 1371-1375.

Fargnoli JL, Fung TT, Olenczuk DM, Chamberland JP, Hu FB and Mantzoros CS (2008) Adherence to healthy eating patterns is associated with higher circulating total and high-molecular-weight adiponectin and lower resistin concentrations in women from the Nurses' Health Study. The American Journal of Clinical Nutrition 88 (5): 1213-1224.

Ferguson JF (2012) Omics gets personal: integrative profiling of health and disease. Circulation: Cardiovascular Genetics 5 (3): 381-382.

Ferrando AA, Paddon-Jones D, Hays NP, Kortebein P, Ronsen O, Williams RH, McComb A, Symons TB, Wolfe RR and Evans W (2010) EAA supplementation to increase nitrogen intake improves muscle function during bed rest in the elderly. Clinical Nutrition 29 (1): 18-23.

Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub DD, Guralnik JM and Longo DL (2005) The origins of age-related proinflammatory state. Blood 105 (6): 2294-2299.

Festa A, D'Agostino R, Jr., Williams K, Karter AJ, Mayer-Davis EJ, Tracy RP and Haffner SM (2001) The relation of body fat mass and distribution to markers of chronic inflammation. International Journal of Obesity Related Metabolic Disorders 25 (10): 1407-1415.

Fiatarone Singh MA, Bernstein MA, Ryan AD, O'Neill EF, Clements KM and Evans WJ (2000) The effect of oral nutritional supplements on habitual dietary quality and quantity in frail elders. The Journal of Nutrition, Health & Aging 4 (1): 5-12.

Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, Abellan van Kan G, Andrieu S, Bauer J, Breuille D, Cederholm T, Chandler J, De Meynard C, Donini L, Harris T, Kannt A, Keime Guibert F, Onder G, Papanicolaou D, Rolland Y, Rooks D, Sieber C, Souhami E, Verlaan S and Zamboni M (2011) Sarcopenia: An Undiagnosed Condition in Older Adults. Current Consensus Definition: Prevalence, Etiology, and Consequences. International Working Group on Sarcopenia. Journal of the American Medical Directors Association 12 (4): 249-256.

Fillmann H, Kretzmann N, San-Miguel B, Llesuy S, Marroni N, Gonzalez-Gallego J and Tunon M (2007) Glutamine inhibits over-expression of pro-inflammatory genes and down-regulates the nuclear factor kappaB pathway in an experimental model of colitis in the rat. Toxicology 236 217-226.

Finkel T and Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408 (6809): 239-247.

Floegel A, Chung SJ, von Ruesten A, Yang M, Chung CE, Song WO, Koo SI, Pischon T and Chun OK (2011) Antioxidant intake from diet and supplements and elevated serum C-reactive protein and plasma homocysteine concentrations in US adults: a cross-sectional study. Public Health Nutrition 14 (11): 2055-2064.

Fogarty AW, Lewis SA, McKeever TM and Britton JR (2009) Is higher sodium intake associated with elevated systemic inflammation? A population-based study. The American Journal of Clinical Nutrition 89 (6): 1901-1904.

Fontana L (2009) Modulating human aging and age-associated diseases. Biochemica et Biophysica Acta (BBA) - General Subjects 1790 (10): 1133-1138.

Ford ES, Giles WH, Mokdad AH and Myers GL (2004) Distribution and Correlates of C-Reactive Protein Concentrations among Adult US Women. Clinical Chemistry 50 (3): 574-581.

Ford ES, Liu S, Mannino DM, Giles WH and Smith SJ (2003) C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. European Journal of Clinical Nutrition 57 (9): 1157-1163.

Forrester DL, Britton J, Lewis SA, Pogson Z, Antoniak M, Pacey SJ, Purcell G and Fogarty AW (2010) Impact of adopting low sodium diet on biomarkers of inflammation and coagulation: a randomised controlled trial. Journal of Nephrology 23 (1): 49-54.

Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC and Salvioli S (2007) Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. Mechanisms of Ageing and Development 128 (1): 92-105.

Frankel JE, Bean JF and Frontera WR (2006) Exercise in the Elderly: Research and Clinical Practice. Clinics in Geriatric Medicine 22 (2): 239-256.

Frankenfield D, Muth E and Rowe W (1998). The Harris-Benedict studies of human basal metabolism: History and limitations. Journal of the American Dietetic Association**:** 98: 439-445.

Frassetto L, Morris RC and Sebastian A (1997) Potassium Bicarbonate Reduces Urinary Nitrogen Excretion in Postmenopausal Women. Journal of Clinical Endocrinology & Metabolism 82 (1): 254-259.

Frassetto L, Todd K, Morris R and Sebastian A (1998) Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. The American Journal of Clinical Nutrition 68 (3): 576-583.

Fröhlich M, Mühlberger N, Hanke H, Imhof A, Döring A, Pepys MB and Koenig W (2003) Markers of inflammation in women on different hormone replacement therapies. Annals of Medicine 35 (5): 353-361.

Frontera WR, Hughes VA, Lutz KJ and Evans WJ (1991) A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. Journal of Applied Physiology 71 (2): 644-650.

Fujita S, Dreyer HC, Drummond MJ, Glynn EL, Cadenas JG, Yoshizawa F, Volpi E and Rasmussen BB (2007) Nutrient signalling in the regulation of human muscle protein synthesis. The Journal of Physiology 582 (2): 813-823.

Fujita S and Volpi E (2006) Amino Acids and Muscle Loss with Aging. The Journal of Nutrition 136 (1): 277S-280S.

Fung TT, Chiuve SE, McCullough ML, Rexrode KM, Logroscino G and Hu FB (2008) Adherence to a DASH-Style Diet and Risk of Coronary Heart Disease and Stroke in Women. Archives of Internal Medicine 168 (7): 713-720.

Fung TT, McCullough ML, Newby P, Manson JE, Meigs JB, Rifai N, Willett WC and Hu FB (2005) Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. The American Journal of Clinical Nutrition 82 (1): 163-173.

Fung TT, Rimm EB, Spiegelman D, Rifai N, Tofler GH, Willett WC and Hu FB (2001) Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. The American Journal of Clinical Nutrition 73 (1): 61-67.

Gale CR, Martyn CN, Cooper C and Sayer AA (2007) Grip strength, body composition, and mortality. International Journal of Epidemiology 36 (1): 228-235.

Gallagher D, Visser M, De Meersman RE, Sepúlveda D, Baumgartner RN, Pierson RN, Harris T and Heymsfield SB (1997) Appendicular skeletal muscle mass: effects of age, gender, and ethnicity. Journal of Applied Physiology 83 (1): 229-239.

Galland L (2010) Diet and Inflammation. Nutrition in Clinical Practice 25 (6): 634-640.

Gerdhem P, Ringsberg K, Obrant K and Akesson K (2005) Association between 25-hydroxy vitamin D levels, physical activity, muscle strength and fractures in the prospective population-based OPRA Study of Elderly Women. Osteoporosis International 16 (11): 1425-1431.

Giampaoli S, Ferrucci L, Cecchi F, Noce CL, Poce A, Dima F, Santaquilani A, Vescio MF and Menotti A (1999) Hand-grip strength predicts incident disability in non-disabled older men. Age and Ageing 28 (3): 283-288.

Gianni P, Jan KJ, Douglas MJ, Stuart PM and Tarnopolsky MA (2004) Oxidative stress and the mitochondrial theory of aging in human skeletal muscle. Experimental Gerontology 39 (9): 1391-1400.

Gibney MJ, Lanham-New SA, Cassidy A and HH V (2009). Introduction to Human Nutrition, 2nd Edition (Wiley-Blackwell), p.148.

Gil del Valle L (2011) Oxidative stress in aging: Theoretical outcomes and clinical evidences in humans. Biomedicine & Pharmacotherapy 1 (0): 1-7.

Giovannucci E (2013) Nutrient biomarkers are not always simple markers of nutrient intake. The American Journal of Clinical Nutrition 97 (3): 657-659.

Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS and Nimmo MA (2011) The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nature Reviews Immunology 11 (9): 607-615.

Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA and Prentice AM (1991) Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. European Journal of Clinical Nutrition 45 (12): 569-581.

Gomez-Cabrera MC, Borras C, Pallardo FV, Sastre J, Ji LL and Vina J (2005) Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. Journal of Physiology 567 (1): 113-120.

Gomez-Cabrera MC, Martinez A, Santangelo G, Pallardo FV, Sastre J and Vina J (2006) Oxidative stress in marathon runners: interest of antioxidant supplementation. British Journal of Nutrition 96 Suppl 1 S31-33.

Gonyea J (2005) The economic well-being of older Americans and teh persistent divide. Public Health & Aging Report 15 1-11.

Goodman MN (1991) Tumor necrosis factor induces skeletal muscle protein breakdown in rats. American Journal of Physiology - Endocrinology And Metabolism 260 (5): E727-E730.

Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, Simonsick EM, Tylavsky FA, Visser M and Newman AB (2006) The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 61 (10): 1059-1064.

Graumlich JF, Ludden TM, Conry-Cantilena C, Cantilena LR, Jr., Wang Y and Levine M (1997) Pharmacokinetic model of ascorbic acid in healthy male volunteers during depletion and repletion. Pharmaceutical Research 14 (9): 1133-1139.

Greenfield JR, Samaras K, Jenkins AB, Kelly PJ, Spector TD, Gallimore JR, Pepys MB and Campbell LV (2004) Obesity Is an Important Determinant of Baseline Serum C-Reactive Protein Concentration in Monozygotic Twins, Independent of Genetic Influences. Circulation 109 (24): 3022-3028.

Greeves JP, Cable NT, Reilly T and Kingsland C (1999) Changes in muscle strength in women following the menopause: a longitudinal assessment of the efficacy of hormone replacement therapy. Clinical Science 97 (1): 79-84.

Greising SM, Baltgalvis KA, Lowe DA and Warren GL (2009) Hormone Therapy and Skeletal Muscle Strength: A Meta-Analysis. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 64A (10): 1071-1081.

Guadagni M and Biolo G (2009) Effects of inflammation and/or inactivity on the need for dietary protein. Current Opinion in Clinical Nutrition & Metabolic Care 12 (6): 617-622.

Guo SS, Zeller C, Chumlea WC and Siervogel RM (1999) Aging, body composition, and lifestyle: the Fels Longitudinal Study. The American Journal of Clinical Nutrition 70 (3): 405-411.

Haight T, Tager I, Sternfeld B, Satariano W and van der Laan M (2005) Effects of Body Composition and Leisure-time Physical Activity on Transitions in Physical Functioning in the Elderly. American Journal of Epidemiology 162 (7): 607-617.

Halton TL, Willett WC, Liu S, Manson JE, Stampfer MJ and Hu FB (2006) Potato and french fry consumption and risk of type 2 diabetes in women. The American Journal of Clinical Nutrition 83 (2): 284-290.

Hamilton B (2010) Vitamin D and human skeletal muscle. Scandinavian Journal of Medicine, Science & Sports 20 (2): 182-190.

Harris T (1997) Muscle Mass and Strength: Relation to Function in Population Studies. The Journal of Nutrition 127 (5): 1004S-1006S.

Haveman-Nies A, Tucker KL, de Groot LC, Wilson PW and van Staveren WA (2001) Evaluation of dietary quality in relationship to nutritional and lifestyle factors in elderly people of the US Framingham Heart Study and the European SENECA study. European Journal of Clinical Nutrition 55 (10): 870-880.

Haveman-Nies A, de Groot LCPGM and van Staveren WA (2003) Dietary quality, lifestyle factors and healthy ageing in Europe: the SENECA study. Age and Ageing 32 (4): 427-434.

He K, Liu K, Daviglus ML, Morris SJ, Loria CM, Van Horn L, Jacobs DR and Savage PJ (2006) Magnesium Intake and Incidence of Metabolic Syndrome Among Young Adults. Circulation 113 (13): 1675-1682.

Heath JM and Stuart MR (2002) Prescribing exercise for frail elders. The Journal of the American Board of Family Practice 15 (3): 218-228.

Heinrich PC, Castell JV and Andus T (1990) Interleukin-6 and the acute phase response. Biochemical Journal 265 (3): 621-636.

Helmersson J, Ärnlöv J, Larsson A and Basu S (2009) Low dietary intake of β-carotene, α-tocopherol and ascorbic acid is associated with increased inflammatory and oxidative stress status in a Swedish cohort. British Journal of Nutrition 101 (12): 1775-1782.

Helmersson J, Ärnlöv J, Vessby B, Larsson A, Alfthan G and Basu S (2005) Serum selenium predicts levels of F2-isoprostanes and prostaglandin F2α in a 27 year follow-up study of Swedish men. Free Radical Research 39 (7): 763-770.

Henderson L, Gregory J, Irving K and Swan G (2004). The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 2. Energy, protein, carbohydrate, fat and alcohol intake. London: Her Majesty's Stationery Office.

Heymsfield SB, Heo M, Thomas D and Pietrobelli A (2011) Scaling of body composition to height: relevance to height-normalized indexes. The American Journal of Clinical Nutrition 93 (4): 736-740.

Heymsfield SB, Smith R, Aulet M, Bensen B, Lichtman S, Wang J and Pierson RN (1990) Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry. The American Journal of Clinical Nutrition 52 (2): 214-218.

Hillman L, Cassidy J, Johnson L, Lee D and Allen S (1994) Vitamin D metabolism and bone mineralization in children with juvenile rheumatoid arthritis. Journal of Pediatrics 124 910-916.

Hodge A, Maple-Brown L, Cunningham J, Boyle J, Dunbar T, Weeramanthri T, Shaw J and O'Dea K (2010) Abdominal obesity and other risk factors largely explain the high CRP in Indigenous Australians relative to the general population, but not gender differences: a cross-sectional study. BMC Public Health 10 (1): 700.

Holick M (1994) McCollum Award Lecture, 1994: vitamin D--new horizons for the 21st century. The American Journal of Clinical Nutrition 60 (4): 619-630.

Hoshino E, Pichard C, Greenwood CE, Kuo GC, Cameron RG, Kurian R, Kearns JP, Allard JP and Jeejeebhoy KN (1991) Body composition and metabolic rate in rat during a continuous infusion of cachectin. American Journal of Physiology - Endocrinology And Metabolism 260 (1): E27-E36.

Houston DK, Cesari M, Ferrucci L and Cherubini A (2007) Association between vitamin D status and physical performance The INCHIANTI Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 62 (4): 440-446.

Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, Lee JS, Sahyoun NR, Visser M, Kritchevsky SB and Study ftHA (2008) Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. The American Journal of Clinical Nutrition 87 (1): 150-155.

Howard C, Ferrucci L, Sun K, Fried LP, Walston J, Varadhan R, Guralnik JM and Semba RD (2007) Oxidative protein damage is associated with poor

grip strength among older women living in the community. Journal of Applied Physiology 103 (1): 17-20.

Hu FB (2002) Dietary pattern analysis: a new direction in nutritional epidemiology. Current Opinion in Lipidology 13 (1): 3-9.

Hubert-Buron A, Leblond J, Jacquot A, Ducrotté P, Déchelotte P and Coëffier M (2006) Glutamine Pretreatment Reduces IL-8 Production in Human Intestinal Epithelial Cells by Limiting IκBα Ubiquitination. Journal of Nutrition 136 (6): 1461-1465.

Hughes VA, Frontera WR, Roubenoff R, Evans WJ and Singh MAF (2002) Longitudinal changes in body composition in older men and women: role of body weight change and physical activity. The American Journal of Clinical Nutrition 76 (2): 473-481.

Huijbregts P, Feskens E, Rasanen L, Fidanza F, Nissinen A, Menotti A and Kromhout D (1997) Dietary pattern and 20 year mortality in elderly men in Finland, Italy, and The Netherlands: longitudinal cohort study. British Medical Journal 315 (7099): 13-17.

Huijbregts P, Vegt dF, Feskens E, Bowles C and Kromhout D (1997b). Dietary patterns and mortality in an elderly population in the Netherlands. A comparison between cluster analysis and the healthy diet indicator. In Dietary Patterns and Health in the Elderly, pp. 33-42 [PPCW Huijbregts, editor]. The Netherlands: Wageningen University.

Huijbregts PP, Feskens EJ, Rasanen L, Fidanza F, Alberti-Fidanza A, Nissinen A, Giampaoli S and Kromhout D (1998) Dietary patterns and cognitive function in elderly men in Finland, Italy and The Netherlands. European Journal of Clinical Nutrition 52 (11): 826-831.

Hung H-C, Joshipura KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA, Colditz GA, Rosner B, Spiegelman D and Willett WC (2004) Fruit and Vegetable Intake and Risk of Major Chronic Disease. Journal of the National Cancer Institute 96 (21): 1577-1584.

Hung J, Knuiman MW, Divitini ML, Davis T and Beilby JP (2008) Prevalence and Risk Factor Correlates of Elevated C-Reactive Protein in an Adult Australian Population. The American Journal of Cardiology 101 (2): 193-198.

Inskip HM, Godfrey KM, Martin HJ, Simmonds SJ and C C (2007) Size at birth and its relation to muscle strength in young adult women. Journal of Internal Medicine

Institute of Medicine (2002) Dietary Reference Intakes for energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press.

Institute of Medicine (2010) Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academies Press.

Jackson MJ (2009) Strategies for reducing oxidative damage in ageing skeletal muscle. Advanced Drug Delivery Reviews 61 (14): 1363-1368.

Jackson MJ, Khassaf M, Vasilaki A, McArdle F and McArdle A (2004) Vitamin E and the oxidative stress of exercise. Annals of the New York Academy of Sciences 1031 158-168.

Jacobs DR, Gross MD and Tapsell LC (2009) Food synergy: an operational concept for understanding nutrition. The American Journal of Clinical Nutrition 89 (5): 1543S-1548S.

Jakes R, Day N, Luben R, Welch A, Bingham S, Mitchell J, Hennings S, Rennie K and Wareham N (2004). Adjusting for energy intake-what measure to use in nutritional epidemiological studies? International Journal of Epidemiology**:** 33: 1382-1386.

Janssen-Heininger YMW, Poynter ME and Baeuerle PA (2000) Recent advances torwards understanding redox mechanisms in the activation of nuclear factor κb. Free Radical Biology and Medicine 28 (9): 1317-1327.

Janssen I, Heymsfield SB, Wang Z and Ross R (2000) Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. Journal of Applied Physiology 89 (1): 81-88.

Jennings A, Welch AA, Fairweather-Tait SJ, Kay C, Minihane A-M, Chowienczyk P, Jiang B, Cecelja M, Spector T, Macgregor A and Cassidy A (2012) Higher anthocyanin intake is associated with lower arterial stiffness and central blood pressure in women. The American Journal of Clinical Nutrition 96 (4): 781-788.

Jerome JA, Hunter K, Gordon P and McKay N (1991) A new robust index for measuring isokinetic trunk flexion and extension. Outcome from a regional study. Spine (Phila Pa 1976) 16 (7): 804-808.

Jones DP, Park Y and Ziegler TR (2012) Nutritional metabolomics: progress in addressing complexity in diet and health. Annual Review of Nutrition 32 183-202.

Jones TE, Stephenson KW, King JG, Knight KR, Marshall TL and Scott WB (2009) Sarcopenia - Mechanisms and Treatments. Journal of Geriatric Physical Therapy 32 (2): 39-45.

Jonker R, Engelen MP and Deutz NE (2012) Role of specific dietary amino acids in clinical conditions. British Journal of Nutrition 108 (2): S139-148.

Jourdan C, Petersen A-K, Gieger C, Döring A, Illig T, Wang-Sattler R, Meisinger C, Peters A, Adamski J, Prehn C, Suhre K, Altmaier E, Kastenmüller G, Römisch-Margl W, Theis FJ, Krumsiek J, Wichmann HE and Linseisen J (2012) Body Fat Free Mass Is Associated with the Serum Metabolite Profile in a Population-Based Study. PLoS ONE 7 (6): e40009.

Kadar L, Albertsson M, Areberg J and Landberg T (2000) The Prognostic Value of Body Protein in Patients with Lung Cancer. Annals of the New York Academy of Sciences 584-591.

Kamal-Eldin A and Appelqvist L-Å (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31 (7): 671-701.

Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A and Wolfe RR (2006) A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. American Journal of Physiology - Endocrinology And Metabolism 291 (2): E381-E387.

Kazemekaite M, Leonaviciene L, Bradunaite R, Staniulyte Z, Palaima A and Razumas V (2008) Anti-inflammatory activity of some potassium salts of N,N-disubstituted 4-aminoazobenzenesulfonic acids in rat adjuvant arthritis. Archives of Pharmacal Research 31 (6): 736-741.

Kennedy ET, Ohls J, Carlson S and Fleming K (1995) The Healthy Eating Index: Design and Applications. Journal of the American Dietetic Association 95 (10): 1103-1108.

Khansari N, Shakiba Y and Mahmoudi M (2009) Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent Patents on Inflammation & Allergy Drug Discovery 3 73-80.

Khassaf M, McArdle A, Esanu C, Vasilaki A, McArdle F, Griffiths RD, Brodie DA and Jackson MJ (2003) Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. Journal of Physiology 549 (2): 645-652.

Khaw K-T, Jakes R, Bingham S, Welch A, Luben R, Day N and Wareham N (2006) Work and leisure time physical activity assessed using a simple, pragmatic, validated questionnaire and incident cardiovascular disease and all-cause mortality in men and women: The European Prospective Investigation into Cancer in Norfolk prospective population study. International Journal of Epidemiology 35 (4): 1034-1043.

Khaw K-T, Wareham N, Bingham S, Welch A, Luben R and Day N (2008) Combined Impact of Health Behaviours and Mortality in Men and Women: The EPIC-Norfolk Prospective Population Study. PLoS Med 5 (1): e12.

Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH, Jr, Grundy SM and de Lemos JA (2005) Race and Gender Differences in C-Reactive Protein Levels. Journal of the American College of Cardiology 46 (3): 464-469.

Khoo CM, Tan M, Wu Y, Wai CH, Subramaniam T, Lee J and Tai ES (2011) Central obesity and smoking are key modifiable risk factors for elevated C-reactive protein in Asian individuals who are not eligible for statin therapy. Nutrition and Diabetes 1: e8.

Khoo CM, Tan M, Wu Y, Wai CH, Subramaniam T, Lee J and Tai ES (2011) Central obesity and smoking are key modifiable risk factors for elevated C-reactive protein in Asian individuals who are not eligible for statin therapy. Nutrition & Diabetes 1: e8.

Kim J-S, Wilson JM and Lee S-R (2010) Dietary implications on mechanisms of sarcopenia: roles of protein, amino acids and antioxidants. The Journal of Nutritional Biochemistry 21 (1): 1-13.

Kirkwood TBL (2005) Understanding the Odd Science of Aging. Cell 120 (4): 437-447.

Kirkwood TBL (2008) A systematic look at an old problem. Nature 451 (7179): 644-647.

Kontush A, Weber W and Beisiegel U (2000) Alpha- and beta-carotenes in low density lipoprotein are the preferred target for nitric oxide-induced oxidation. Atherosclerosis 148 87-93.

Koopman R (2011) Dietary protein and exercise training in ageing. Proceedings of the Nutrition Society 70 (1): 104-113.

Koopman R and van Loon LJ (2009) Aging, exercise, and muscle protein metabolism. Journal of Applied Physiology 106 (6): 2040-2048.

Kopp HP, Kopp CW, Festa A, Krzyzanowska K, Kriwanek S, Minar E, Roka R and Schernthaner G (2003) Impact of Weight Loss on Inflammatory Proteins and Their Association With the Insulin Resistance Syndrome in Morbidly Obese Patients. Arteriosclerosis, Thrombosis, and Vascular Biology 23 (6): 1042-1047.

Kourlaba G and Panagiotakos DB (2009) Dietary quality indices and human health: A review. Maturitas 62 (1): 1-8.

Kramer JH, Mak IT, Phillips TM and Weglicki WB (2003) Dietary Magnesium Intake Influences Circulating Pro-Inflammatory Neuropeptide Levels and Loss of Myocardial Tolerance to Postischemic Stress. Experimental Biology and Medicine 228 (6): 665-673.

Kritchevsky SB, Bush AJ, Pahor M and Gross MD (2000) Serum Carotenoids and Markers of Inflammation in Nonsmokers. American Journal of Epidemiology 152 (11): 1065-1071.

Kuh D, Bassey J, Hardy R, Aihie Sayer A, Wadsworth M and Cooper C (2002) Birth Weight, Childhood Size, and Muscle Strength in Adult Life: Evidence from a Birth Cohort Study. American Journal of Epidemiology 156 (7): 627-633.

Kurina LM, Gulati M, Everson-Rose SA, Chung PJ, Karavolos K, Cohen NJ, Kandula N, Lukezic R, Dugan SA, Sowers M, Powell LH and Pickett KE (2004) The Effect of Menopause on Grip and Pinch Strength: Results from the Chicago, Illinois, Site of the Study of Women's Health Across the Nation. American Journal of Epidemiology 160 (5): 484-491.

Kyle UG, Genton L, Hans D, Karsegard VL, Michel J-P, Slosman DO and Pichard C (2001) Total Body Mass, Fat Mass, Fat-Free Mass, and Skeletal Muscle in Older People: Cross-Sectional Differences in 60-Year-Old Persons. Journal of the American Geriatrics Society 49 (12): 1633-1640.

Kyle UG, Genton L, Slosman DO and Pichard C (2001) Fat-free and fat mass percentiles in 5225 healthy subjects aged 15 to 98 years. Nutrition 17 (7-8): 534-541.

Kyle UG, Melzer K, Kayser B, Picard-Kossovsky M, Gremion G and Pichard C (2006) Eight-Year Longitudinal Changes in Body Composition in Healthy Swiss Adults. Journal of the American College of Nutrition 25 (6): 493-501.

Kyle UG, Schutz Y, Dupertuis YM and Pichard C (2003) Body composition interpretation: Contributions of the fat-free mass index and the body fat mass index. Nutrition 19 (7-8): 597-604.

Lang T, Streeper T, Cawthon P, Baldwin K, Taaffe D and Harris T (2010) Sarcopenia: etiology, clinical consequences, intervention, and assessment. Osteoporosis International 21 (4): 543-559.

Laukkanen P, Heikkinen E and Kauppinen M (1995) Muscle Strength and Mobility as Predictors of Survival in 75–84 - Year - old People. Age and Ageing 24 (6): 468-473.

Lauretani F, Semba RD, Bandinelli S and al e (2007) Plasma selenium & muscle strength. The American Journal of Clinical Nutrition 86: 347-352.

Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di Iorio A, Corsi AM, Rantanen T, Guralnik JM and Ferrucci L (2003) Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. Journal of Applied Physiology 95 (5): 1851-1860.

Lauretani F, Semba RD, Bandinelli S, Dayhoff-Brannigan M, Giacomini V, Corsi AM, Guralnik JM and Ferrucci L (2008) Low Plasma Carotenoids and Skeletal Muscle Strength Decline Over 6 Years. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 63 (4): 376-383.

Laviano A, Muscaritoli M, Cascino A, Preziosa I, Inui A, Mantovani G and Rossi-Fanelli F (2005) Branched-chain amino acids: the best compromise to achieve anabolism? Current Opinion of Clinical Nutrition & Metabolic Care 8 (4): 408-414.

Lee J, Taneja V and Vassallo R (2012) Cigarette Smoking and Inflammation: Cellular and Molecular Mechanisms. Journal of Dental Research 91 (2): 142-149.

Lee JE, Wei EK, Fuchs CS, Hunter DJ, Lee IM, Selhub J, Stampfer MJ, Willett WC, Ma J and Giovannucci E (2012) Plasma folate,

methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies. Cancer Causes Control 23 (4): 537-545.

Lemoine S, Granier P, Tiffoche C, Rannou-Bekono F, Thieulant M and Delamarche P (2003) Estrogen receptor alpha mRNA in human skeletal muscles. Medicine & Science in Sports & Exercise 35 439-443.

Lennon EJ, Lemann J and Litzow JR (1966) The effects of diet and stool composition on the net external acid balance of normal subjects. The Journal of Clinical Investigation 45 (10): 1601-1607.

Lisa C (2008) Vitamin D and skeletal muscle tissue and function. Molecular Aspects of Medicine 29 (6): 407-414.

Liu CJ and Latham NK (2009) Progressive resistance strength training for improving physical function in older adults. Cochrane Database Systematic Review (3): CD002759.

Liu S, Stampfer MJ, Hu FB, Giovannucci E, Rimm E, Manson JE, Hennekens CH and Willett WC (1999) Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. The American Journal of Clinical Nutrition 70 (3): 412-419.

Livingstone MB and Black AE (2003) Markers of the validity of reported energy intake. Journal of Nutrition 133 (3): 895S-920S.

Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE and Hu FB (2004) Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. The American Journal of Clinical Nutrition 80 (4): 1029-1035.

Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, Willett WC and Hu FB (2005) Consumption of Trans Fatty Acids Is Related to Plasma Biomarkers of Inflammation and Endothelial Dysfunction. The Journal of Nutrition 135 (3): 562-566.

Lowe DA, Baltgalvis KA and Greising SM (2010) Mechanisms Behind Estrogen's Beneficial Effect on Muscle Strength in Females. Exercise and Sport Sciences Reviews 38 (2): 61-67.

Lukaski HC (2004) Vitamin and mineral status: effects on physical performance. Nutrition 20 (7-8): 632-644.

Lundman P, Boquist S, Samnegård A, Bennermo M, Held C, Ericsson C-G, Silveira A, Hamsten A and Tornvall P (2007) A high-fat meal is accompanied by increased plasma interleukin-6 concentrations. Nutrition, Metabolism and Cardiovascular Diseases 17 (3): 195-202.

Lutsey PL, Jacobs DR, Kori S, Mayer-Davis E, Shea S, Steffen LM, Szklo M and Tracy R (2007) Whole grain intake and its cross-sectional association with obesity, insulin resistance, inflammation, diabetes and subclinical CVD: The MESA Study. British Journal of Nutrition 98 (02): 397-405.

Luyer MDP, Khosla S, Owen WG and Miller VM (2001) Prospective randomized study of effects of unopposed estrogen replacement therapy on markers of coagulation and inflammation in postmenopausal women. Journal of Clinical Endocrinology and Metabolism 86 (8): 3629-3634.

Lynch NA, Metter EJ, Lindle RS, Fozard JL, Tobin JD, Roy TA, Fleg JL and Hurley BF (1999) Muscle quality. I. Age-associated differences between arm and leg muscle groups. Journal of Applied Physiology 86 (1): 188-194.

Macdonald H, Mavroeidi A, Fraser W, Darling A, Black A, Aucott L, O'Neill F, Hart K, Berry J, Lanham-New S and Reid D (2011) Sunlight and dietary contributions to the seasonal vitamin D status of cohorts of healthy postmenopausal women living at northerly latitudes: a major cause for concern? Osteoporosis International 22 (9): 2461-2472.

Mackerras D (1996) Energy adjustment: The concepts underlying the debate. Journal of Clinical Epidemiology 49 (9): 957-962.

Maguire ME and Cowan JA (2002) Magnesium chemistry and biochemistry. BioMetals 15 (3): 203-210.

Malbut KE, Dinan S and Young A (2002) Aerobic training in the 'oldest old': the effect of 24 weeks of training. Age and Ageing 31 (4): 255-260.

Malpuech-Brugere C, Nowacki W, Daveau M, Gueux E, Linard C, Rock C, Rock E, Lebreton J-P, Majur A and Rayssiguier Y (2000) Inflammatory response following acute magnesium deficiency in the rat. Biochimica et Biophysica Acta (BBA)- Molecular Basis of Disease 1501 91-98.

Manns PJ, Williams DP, Snow CM and Wander RC (2003) Physical activity, body fat, and serum c-reactive protein in postmenopausal women with and without hormone replacement. American Journal of Human Biology 15 (1): 91-100.

Martin HJ, Syddall HE, Dennison EM, Cooper C and Sayer AA (2008) Relationship between customary physical activity, muscle strength and physical performance in older men and women: findings from the Hertfordshire Cohort Study. Age and Ageing 37 (5): 589-593.

Mathers J and Ford D (2009) in Nutrients and Epigenetics [S-W Choi & S Friso, editors], CRC Press.

Mathers JC (2002) Pulses and carcinogenesis: potential for the prevention of colon, breast and other cancers. British Journal of Nutrition 88 (SupplementS3): 273-279.

Mathieu C and Badenhoop K (2005) Vitamin D and type 1 diabetes mellitus: state of the art. Trends in Endocrinology & Metabolism 16 261-266.

Matthews D (1999). Proteins and amino acids. In: Shils, ME., editor. Modern nutrition in health and disease.Williams & Wilkins; Baltimore: 1999. p. 11-48.

May R, Kelly R and Mitch W (1987) Mechanism for defects in muscle protein metabolism in rats with chronic uremia: the influence of metabolic acidosis. The Journal of Clinical Investigation 79 1099-1100.

Maynard M, Ness AR, Abraham L, Blane D, Bates D and DJ G (2004) Selecting a healthy diet score: lessons from a study of diet and health in early old age (the Boyd Orr cohort). Public Health Nutrition 8 (3): 321-326.

McCance R, Widdowson E, Holland B, Welch A and Buss D (1991). McCance and Widdowson's the composition of foods. 5th ed. Cambridge: Royal Society of Chemistry.

McCrory MA, McCrory MA, Hajduk CL and Roberts SB (2002) Procedures for screening out inaccurate reports of dietary energy intake. Public Health Nutrition 5 (6A): 873-882.

McCullough ML, Feskanich D, Stampfer MJ, Giovannucci EL, Rimm EB, Hu FB, Spiegelman D, Hunter DJ, Colditz GA and Willett WC (2002) Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. The American Journal of Clinical Nutrition 76 (6): 1261-1271.

McDermott MM, Greenland P, Ferrucci L, Criqui MH, Liu K, Sharma L, Chan C, Celic L, Priyanath A and Guralnik JM (2002) Lower Extremity Performance Is Associated with Daily Life Physical Activity in Individuals with and without Peripheral Arterial Disease. Journal of the American Geriatrics Society 50 (2): 247-255.

McDermott MM, Guralnik JM, Albay M, Bandinelli S, Miniati B and Ferrucci L (2004) Impairments of Muscles and Nerves Associated with Peripheral Arterial Disease and Their Relationship with Lower Extremity Functioning: The InCHIANTI Study. Journal of the American Geriatrics Society 52 (3): 405-410.

McDonough AA, Thompson CB and Youn JH (2002) Skeletal muscle regulates extracellular potassium. American Journal of Physiology - Renal Physiology 282 (6): F967-F974.

McKeown NM, Day NE, Welch AA, Runswick SA, Luben RN, Mulligan AA, McTaggart A and Bingham SA (2001) Use of biological markers to validate self-reported dietary intake in a random sample of the European Prospective Investigation into Cancer United Kingdom Norfolk cohort. The American Journal of Clinical Nutrition 74 (2): 188-196.

McLaughlin T, Abbasi F, Lamendola C, Liang L, Reaven G, Schaaf P and Reaven P (2002) Differentiation Between Obesity and Insulin Resistance in the Association With C-Reactive Protein. Circulation 106 (23): 2908-2912.

McNeill G, Masson L, Macdonald H, Haggarty P, Macdiarmid J, Craig L and Kyle J (2009) Food frequency questionnaires vs diet diaries. International Journal of Epidemiology 38 (3): 884-885.

Mecocci P, Fanó G, Fulle S, MacGarvey U, Shinobu L, Polidori MC, Cherubini A, Vecchiet J, Senin U and Beal MF (1999) Age-dependent increases in oxidative damage to DNA, lipids, and proteins in human skeletal muscle. Free Radical Biology and Medicine 26 (3-4): 303-308.

Mendez MA, Popkin BM, Jakszyn P, Berenguer A, Tormo MJ, Sanchéz MJ, Quirós JR, Pera G, Navarro C, Martinez C, Larrañaga N, Dorronsoro M, Chirlaque MD, Barricarte A, Ardanaz E, Amiano P, Agudo A and González CA (2006) Adherence to a Mediterranean Diet Is Associated with Reduced 3-Year Incidence of Obesity. The Journal of Nutrition 136 (11): 2934-2938.

Meng S-J and Yu L-J (2010) Oxidative Stress, Molecular Inflammation and Sarcopenia. International Journal of Molecular Sciences 11 (4): 1509-1526.

Meng SJ and Yu LJ (2010) Oxidative stress, molecular inflammation and sarcopenia. International Journal of Molecular Science 11 (4): 1509-1526.

Meng X, Zhu K, Devine A, Kerr DA, Binns CW and Prince RL (2009) A 5-year cohort study of the effects of high protein intake on lean mass and BMC in elderly postmenopausal women. Journal of Bone and Mineral Research 24 (11): 1827-1834.

Michigan A, Johnson T and Master V (2011) Review of the relationship between C-reactive protein and exercise. Molecular Diagnosis & Therary 15 265-275.

Millward DJ (2012) Nutrition and sarcopenia: evidence for an interaction. Proceedings of the Nutrition Society.

Mitchell D, Haan MN, Steinberg FM and Visser M (2003) Body composition in the elderly: the influence of nutritional factors and physical activity. The Journal of Nutrition, Health & Aging 7 (3): 130-139.

Moayyeri A, Hammond CJ, Valdes AM and Spector TD (2012) Cohort Profile: TwinsUK and Healthy Ageing Twin Study. International Journal of Epidemiology. 1-10.

Molag ML, de Vries JHM, Ocké MC, Dagnelie PC, van den Brandt PA, Jansen MCJF, van Staveren WA and van't Veer P (2007) Design

Characteristics of Food Frequency Questionnaires in Relation to Their Validity. American Journal of Epidemiology 166 (12): 1468-1478.
Morley J (2008) Sarcopenia: Diagnosis and treatment. The Journal of Nutrition, Health & Aging 12 (7): 452-456.

Morley JE, Argiles JM, Evans WJ, Bhasin S, Cella D, Deutz NE, Doehner W, Fearon KC, Ferrucci L, Hellerstein MK, Kalantar-Zadeh K, Lochs H, MacDonald N, Mulligan K, Muscaritoli M, Ponikowski P, Posthauer ME, Rossi Fanelli F, Schambelan M, Schols AM, Schuster MW, Anker SD, Society for Sarcopenia C and Wasting D (2010) Nutritional recommendations for the management of sarcopenia. Journal of the American Medical Directors Association 11 (6): 391-396.

Morley JE and Baumgartner RN (2004) Cytokine-Related Aging Process. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 59 (9): M924-M929.

Morley JE, Baumgartner RN, Roubenoff R, Mayer J and Nair KS (2001) Sarcopenia. Journal of Laboratory and Clinical Medicine 137 (4): 231-243.

Morse CI, Wüst RCI, Jones DA, De Haan A and Degens H (2007) Muscle fatigue resistance during stimulated contractions is reduced in young male smokers. Acta Physiologica 191 (2): 123-129.

Moslehi N, Vafa M, Rahimi-Foroushani A and Golestan B (2012). Effects of oral magnesium supplementation on inflammatory markers in middle-aged overweight women. Journal of Research in Medical Sciences 17 (7).

Mozaffarian D, Pischon T, Hankinson SE, Rifai N, Joshipura K, Willett WC and Rimm EB (2004) Dietary intake of trans fatty acids and systemic inflammation in women. The American Journal of Clinical Nutrition 79 (4): 606-612.

Munger K, Zhang S and O'Reilly E (2004) Vitamin D intake and incidence of multiple sclerosis. Neurology 62 60-65.

Nagpal S, Na S and Rathnachalam R (2005) Noncalcemic actions of vitamin D receptor ligands. Endocrinology Reviews 26 (5): 662-687.

Neder JA, Nery LE, Shinzato GT, Andrade MS, Peres C and Silva AC (1999) Reference values for concentric knee isokinetic strength and power in nonathletic men and women from 20 to 80 years old. Journal of Orthopaedic & Sports Physical Therapy 29 (2): 116-126.

Nelson KM, Weinsier RL, Long CL and Schutz Y (1992) Prediction of resting energy expenditure from fat-free mass and fat mass. The American Journal of Clinical Nutrition 56 (5): 848-856.

Nettleton JA, Steffen LM, Mayer-Davis EJ, Jenny NS, Jiang R, Herrington DM and Jacobs DR (2006) Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). The American Journal of Clinical Nutrition 83 (6): 1369-1379.

Neuhouser ML, Patterson RE, King IB, Horner NK and Lampe JW (2003) Selected nutritional biomarkers predict diet quality. Public Health Nutrition 6 (07): 703-709.

Newman AB, Haggerty CL, Goodpaster B, Harris T, Kritchevsky S, Nevitt M, Miles TP, Visser M, Health A and Body Composition Research G (2003) Strength and muscle quality in a well-functioning cohort of older adults: the Health, Aging and Body Composition Study. Journal of the American Geriatrics Society 51 (3): 323-330.

Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, Tylavsky FA, Rubin SM and Harris TB (2006) Strength,

But Not Muscle Mass, Is Associated With Mortality in the Health, Aging and Body Composition Study Cohort. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 61 (1): 72-77.

Nicastro H, da Luz CR, Chaves DFS, Bechara LRG, Voltarelli VA, Rogero MM and Lancha AH (2012) Does Branched-Chain Amino Acids Supplementation Modulate Skeletal Muscle Remodeling through Inflammation Modulation? Possible Mechanisms of Action. Journal of Nutrition and Metabolism 2012 10.

Nooyens AC, Visscher TL, Verschuren WM, Schuit AJ, Boshuizen HC, van Mechelen W and Seidell JC (2009) Age, period and cohort effects on body weight and body mass index in adults: The Doetinchem Cohort Study. Public Health Nutrition 12 (6): 862-870.

Norman K, Stobaus N, Gonzalez MC, Schulzke JD and Pirlich M (2011) Hand grip strength: outcome predictor and marker of nutritional status. Clinical Nutrition 30 (2): 135-142.

Obarzanek E, Sacks FM, Vollmer WM, Bray GA, Miller ER, 3rd, Lin PH, Karanja NM, Most-Windhauser MM, Moore TJ, Swain JF, Bales CW and Proschan MA (2001) Effects on blood lipids of a blood pressure-lowering diet: the Dietary Approaches to Stop Hypertension (DASH) Trial. The American Journal of Clinical Nutrition 74 (1): 80-89.

Oelzner P, Muller A, Deschner F, Huller M, Abendroth K, Hein G and Stein G (1998) Relationship between disease activity and serum levels of vitamin D metabolites and PTH in rheumatoid arthritis. Calcified Tissue International 62: 193-198.

Oeppen J and Vaupel JW (2002) Broken Limits to Life Expectancy. Science 296 (5570): 1029-1031.

Ohno T, Tanaka Y, Sugauchi F, Orito E, Hasegawa I, Nukaya H, Kato A, Matunaga S, Endo M, Tanaka Y, Sakakibara K and Mizokami M (2008) Suppressive effect of oral administration of branched-chain amino acid granules on oxidative stress and inflammation in HCV-positive patients with liver cirrhosis. Hepatology Research 38 (7): 683-688.

Olson J (1999) Carotenoids. In: Shils ME, Olson JA, Shike M, et al, eds. Modern nutrition in health and disease. Baltimore, MD: Williams & Wilkins, 1999: 525-41.

Otten J, Hellwig J and Meyers L (2006) DRI, dietary reference intakes: the essential guide to nutrient requirements. National Academies Press, Washinton, D.C.

Paddon-Jones D, Sheffield-Moore M, Zhang X-J, Volpi E, Wolf SE, Aarsland A, Ferrando AA and Wolfe RR (2004) Amino acid ingestion improves muscle protein synthesis in the young and elderly. American Journal of Physiology - Endocrinology And Metabolism 286 (3): E321-E328.

Pahor M, Manini T and Cesari M (2009) Sarcopenia: clinical evaluation, biological markers and other evaluation tools. The Journal of Nutrition, Health & Aging 13 (8): 724-728.

Palmer JR (2008) Sugar-Sweetened Beverages and Incidence of Type 2 Diabetes Mellitus in African American Women. Archives of Internal Medicine 168 (14): 1487.

Panagiotakos DB, Chrysohoou C, Pitsavos C and Stefanadis C (2006) Association between the prevalence of obesity and adherence to the Mediterranean diet: the ATTICA study. Nutrition 22 (5): 449-456.

Pannacciulli N, Cantotore F, Minenna A, Bellacicco M, Giorgino R and De Pergola G (2001) C-reactive protein is independently associated with total body fat, central fat, an dinsulin resistance in adult women. International Journal of Obesity 25: 1416-1420.

Pansarasa O, Bertorelli L, Vecchiet J, Felzani G and Marzatico F (1999) Age-dependent changes of antioxidant activities and markers of free radical damage in human skeletal muscle. Free Radical Biology and Medicine 27 (5-6): 617-622.

Paolisso G and Barbagallo M (1997) Hyoertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. American Journal of Hypertension 10: 346-355.

Paolisso G, Esposito R, D'Alessio M and al e (1999) Primary and secondary prevention of atherosclerosis: is there a role for antioxidants? Diabetes Metabolism 25: 298-306.

Paolo B (2011) Internal and external validity of cohort studies. Annals of Agricultural and Environmental Medicine 18 283-284.

Papadakis MA, Grady D, Black D, Tierney MJ, Gooding GAW, Schambelan M and Grunfeld C (1996) Growth Hormone Replacement in Healthy Older Men Improves Body Composition but Not Functional Ability. Annals of Internal Medicine 124 (8): 708-716.

Park SW, Goodpaster BH, Lee JS, Kuller LH, Boudreau R, de Rekeneire N, Harris TB, Kritchevsky S, Tylavsky FA, Nevitt M, Cho Y-w, Newman AB and Study ftHA (2009) Excessive Loss of Skeletal Muscle Mass in Older Adults with Type 2 Diabetes. Diabetes Care 32: 1993-1997.

Park SW, Goodpaster BH, Strotmeyer ES, de Rekeneire N, Harris TB, Schwartz AV, Tylavsky FA and Newman AB (2006) Decreased muscle

strength and quality in older adults with type 2 diabetes: the health, aging, and body composition study. Diabetes 55 (6): 1813-1818.

Patterson R, Haines P and Popkin B (1994) Diet Quality Index: capturing a multidimensional behavior. Journal of the American Dietetic Association 94 57-64.

Patterson RE, Haines PS and Popkin BM (1994) Diet quality index: Capturing a multidimensional behavior. Journal of the American Dietetic Association 94 (1): 57-64.

Pattison DJ, Symmons DP, Lunt M, Welch A, Bingham SA, Day NE and Silman AJ (2005) Dietary ß-cryptoxanthin and inflammatory polyarthritis: results from a population-based prospective study. The American Journal of Clinical Nutrition 82 (2): 451-455.

Payette H, Roubenoff R, Jacques PF, Charles AD, Peter WFW, Leslie WA and Tamara H (2003) Insulin-Like Growth Factor-1 and Interleukin 6 Predict Sarcopenia in Very Old Community-Living Men and Women: The Framingham Heart Study. Journal of the American Geriatrics Society 51 (9): 1237-1243.

Peel NM, McClure RJ and Bartlett HP (2005) Behavioral determinants of healthy aging1. American journal of preventive medicine 28 (3): 298-304.

Peters S, van Helvoort A, Kegler D, Argiles J, Luiking Y, Laviano A, van Bergenhenegouwen J, Deutz E, Haagsman H, Gorselink M and van Norren K (2011) Dose-dependent effects of leucine supplementation on preservation of muscle mass in cancer cachectic mice. Oncology Reports 26 247-254.

Petersen AMW, Magkos F, Atherton P, Selby A, Smith K, Rennie MJ, Pedersen BK and Mittendorfer B (2007) Smoking impairs muscle protein

synthesis and increases the expression of myostatin and MAFbx in muscle. American Journal of Physiology - Endocrinology And Metabolism 293 (3): E843-E848.

Petersen AMW and Pedersen BK (2005) The anti-inflammatory effect of exercise. Journal of Applied Physiology 98 (4): 1154-1162.

Peterson MD, Rhea MR, Sen A and Gordon PM (2010) Resistance exercise for muscular strength in older adults: a meta-analysis. Ageing Research Reviews 9 (3): 226-237.

Peterson MD, Sen A and Gordon PM (2011) Influence of resistance exercise on lean body mass in aging adults: a meta-analysis. Medicine & Science in Sports & Exercise 43 (2): 249-258.

Phillips WT, Batterham AM, Valenzuela JE and Burkett LN (2004) Reliability of maximal strength testing in older adults. Archives of Physical Medicine and Rehabilitation 85 (2): 329-334.

Pithon-Curi TC, De Melo MP and Curi R (2004) Glucose and glutamine utilization by rat lymphocytes, monocytes and neutrophils in culture: a comparative study. Cell Biochemistry and Function 22 (5): 321-326.

Poslusna K, Ruprich J, de Vries JH, Jakubikova M and van't Veer P (2009) Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. British Journal of Nutrition 101: S73-85.

Poslusna K, Ruprich J, de Vries JHM, Jakubikova M and van't Veer P (2009) Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. British Journal of Nutrition 101 (2): S73.

Proctor DN, O'Brien PC, Atkinson EJ and Nair KS (1999) Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. American Journal of Physiology 277 (3): E489-495.

Promislow DE (1994) DNA repair and the evolution of longevity: a critical analysis. Journal of Theoretical Biology 170 (3): 291-300.

Puggaard L (2003) Effects of training on functional performance in 65, 75 and 85 year-old women: Experiences deriving from community based studies in Odense, Denmark. Scandinavian Journal of Medicine & Science in Sports 13 (1): 70-76.

Quatromoni PA, Pencina M, Cobain MR, Jacques PF and D/'Agostino RB (2006) Dietary Quality Predicts Adult Weight Gain: Findings from the Framingham Offspring Study. Obesity 14 (8): 1383-1391.

Rantanen T (2003) Muscle strength, disability and mortality. Scandinavian Journal of Medicine & Science in Sports 13 (1): 3-8.

Rantanen T, Guralnik JM, Sakari-Rantala R, Leveille S, Simonsick EM, Ling S and Fried LP (1999) Disability, physical activity, and muscle strength in older women: The women's health and aging study. Archives of Physical Medicine and Rehabilitation 80 (2): 130-135.

Rantanen T, Masaki K, Foley D, Izmirlian G, White L and Guralnik JM (1998) Grip strength changes over 27 yr in Japanese-American men. Journal of Applied Physiology 85 (6): 2047-2053.

Rantanen T, Volpato S, Luigi Ferrucci MD, Eino Heikkinen MD, Fried LP and Guralnik JM (2003) Handgrip Strength and Cause-Specific and Total Mortality in Older Disabled Women: Exploring the Mechanism. Journal of the American Geriatrics Society 51 (5): 636-641.

Rattan SI (2010) Synthesis, modification and turnover of proteins during aging. Advances in Experimental Medicine and Biology 694: 1-13.

Raue U, Slivka D, Minchev K and Trappe S (2009) Improvements in whole muscle and myocellular function are limited with high-intensity resistance training in octogenarian women. Journal of Applied Physiology 106 (5): 1611-1617.

Rederstorff M, Krol A and Lescure A (2006) Understanding the importance of selenium and selenoproteins in muscle function. Cellular and Molecular Life Sciences 63 (1): 52-59.

Rederstorff M, Krol A and Lescure A (2006) Understanding the importance of selenium and selenoproteins in muscle function. Cellular and Molecular Life Sciences 63 (1): 52-59.

Reed DM, Foley DJ, White LR, Heimovitz H, Burchfiel CM and Masaki K (1998) Predictors of healthy aging in men with high life expectancies. American Journal of Public Health 88 (10): 1463-1468.

Rennie KL, Coward A and Jebb SA (2007) Estimating under-reporting of energy intake in dietary surveys using an individualised method. British Journal of Nutrition 97 (6): 1169-1176.

Richards JB, Valdes AM, Gardner JP, Paximadas D, Kimura M, Nessa A, Lu X, Surdulescu GL, Swaminathan R, Spector TD and Aviv A (2007) Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. The American Journal of Clinical Nutrition 86 (5): 1420-1425.

Rieu I, Balage M, Sornet C, Debras E, Ripes S, Rochon-Bonhomme C, Pouyet C, Grizard J and Dardevet D (2007) Increased availability of leucine

with leucine-rich whey proteins improves postprandial muscle protein synthesis in aging rats. Nutrition 23 (4): 323-331.

Rieu I, Balage M, Sornet C, Giraudet C, Pujos E, Grizard J, Mosoni L and Dardevet D (2006) Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. Journal of Physiology 575 (1): 305-315.

Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR and Bluher M (2009) Antioxidants prevent health-promoting effects of physical exercise in humans. Proceedings of the National Academy of Sciences of the USA 106 (21): 8665-8670.

Robinson S, Cooper C and AA S (2012) Nutrition and Sarcopenia: A Review of the Evidence and Implications for Preventive Strategies. Journal of Aging Research vol. 2012, Article ID 510801, 6 pages, 2012. doi:10.1155/2012/510801

Robinson S, Syddall H, Jameson K, Batelaan S, Martin H, Dennison EM, Cooper C, Sayer AA and Hertfordshire Study G (2009) Current patterns of diet in community-dwelling older men and women: results from the Hertfordshire Cohort Study. Age Ageing 38 (5): 594-599.

Robinson SM, Jameson KA, Batelaan SF, Martin HJ, Syddall HE, Dennison EM, Cooper C, Sayer AA (2008) Diet and its relationship with grip strength in community-dwelling older men and women: the Hertfordshire cohort study. Journal of the American Geriatrics Society 56 (1): 84-90.

Robinson SM, Simmonds SJ, Jameson KA, Syddall HE, Dennison EM, Cooper C, Sayer AA and Group HCS (2012) Muscle Strength in Older Community-Dwelling Men Is Related to Type of Milk Feeding in Infancy. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 67 (9): 990-996.

Rolland Y, Czerwinski S, van Kan G, Morley J, Cesari M, Onder G, Woo J, Baumgartner R, Pillard F, Boirie Y, Chumlea W and Vellas B (2008) Sarcopenia: Its assessment, etiology, pathogenesis, consequences and future perspectives. The Journal of Nutrition, Health & Aging 12 (7): 433-450.

Romaguera D, Norat T, Vergnaud AC, Mouw T, May AM, Agudo A, Buckland G, Slimani N, Rinaldi S, Couto E, Clavel-Chapelon F, Boutron-Ruault MC, Cottet V, Rohrmann S, Teucher B, Bergmann M, Boeing H, Tjonneland A, Halkjaer J, Jakobsen MU, Dahm CC, Travier N, Rodriguez L, Sanchez MJ, Amiano P, Barricarte A, Huerta JM, Luan J, Wareham N, Key TJ, Spencer EA, Orfanos P, Naska A, Trichopoulou A, Palli D, Agnoli C, Mattiello A, Tumino R, Vineis P, Bueno-de-Mesquita HB, Buchner FL, Manjer J, Wirfalt E, Johansson I, Hellstrom V, Lund E, Braaten T, Engeset D, Odysseos A, Riboli E and Peeters PH (2010) Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. The American Journal of Clinical Nutrition 92 (4): 912-921.

Rosenberg IH (1997) Sarcopenia: origins and clinical relevance. Journal of Nutrition 127 (5): 990S-991S.

Roubenoff R (2007) Physical Activity, Inflammation, and Muscle Loss. Nutrition Reviews 65: S208-S212.

Roubenoff R, Harris TB, Abad LW, Wilson PWF, Dallal GE and Dinarello CA (1998) Monocyte Cytokine Production in an Elderly Population: Effect of Age and Inflammation. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 53A (1): M20-M26.

Roubenoff R and Hughes VA (2000) Sarcopenia. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 55 (12): M716-M724.

Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, Simons-Morton DG, Karanja N, Lin P-H, Aickin M, Most-Windhauser MM, Moore TJ, Proschan MA and Cutler JA (2001) Effects on Blood Pressure of Reduced Dietary Sodium and the Dietary Approaches to Stop Hypertension (DASH) Diet. New England Journal of Medicine 344 (1): 3-10.

Saldeen T, Li D and Mehta J (1999) Differential effects of alpha- and gamma-tocopherol on low-density lipoprotein oxidation, superoxide activity, platelet aggregation and arterial thrombogenesis. Journal of the American College of Cardiology 34: 1208-1215.

Salmon A, Richardson A and Perez V (2010) Update on the oxidative stress theory of aging: Does oxidative stress play a role in aging or healthy aging? Free Radical Biology & Medicine 48: 642-655.

Santos AC, Lopes C, Guimaraes JT and Barros H (2005) Central obesity as a major determinant of increased high-sensitivity C-reactive protein in metabolic syndrome. International Journal of Obesity & Related Metabolic Disorders 29 (12): 1452-1456.

Savini I, Catani MV, Duranti G, Ceci R, Sabatini S and Avigliano L (2005) Vitamin C homeostasis in skeletal muscle cells. Free Radical Biology & Medicine 38 (7): 898-907.

Sayer AA, Robinson SM, Patel HP, Shavlakadze T, Cooper C and Grounds MD (2013) New horizons in the pathogenesis, diagnosis and management of sarcopenia. Age Ageing 42 (2): 145-150.

Sayer AA, Syddall HE, Gilbody HJ, Dennison EM and Cooper C (2004) Does Sarcopenia Originate in Early Life? Findings From the Hertfordshire Cohort Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 59 (9): M930-M934.

Sayer AA, Syddall HE, Martin HJ, Dennison EM, Roberts HC and Cooper C (2006) Is grip strength associated with health-related quality of life? Findings from the Hertfordshire Cohort Study. Age and Ageing 35 (4): 409-415.

Schaap LA, Pluijm SM, Deeg DJ and Visser M (2006) Inflammatory markers and loss of muscle mass (sarcopenia) and strength. American Journal of Medicine 119 (6): 526 e529-517.

Schaap LA, Pluijm SMF, Deeg DJH, Harris TB, Kritchevsky SB, Newman AB, Colbert LH, Pahor M, Rubin SM, Tylavsky FA, Visser M and Study ftHA (2009) Higher Inflammatory Marker Levels in Older Persons: Associations With 5-Year Change in Muscle Mass and Muscle Strength. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 64A (11): 1183-1189.

Scheurig AC, Thorand B, Fischer B, Heier M and Koenig W (2007) Association between the intake of vitamins and trace elements from supplements and C-reactive protein: results of the MONICA/KORA Augsburg study. European Journal of Clinical Nutrition 62 (1): 127-137.

Schröder H, Marrugat J, Vila J, Covas MI and Elosua R (2004) Adherence to the Traditional Mediterranean Diet Is Inversely Associated with Body Mass Index and Obesity in a Spanish Population. The Journal of Nutrition 134 (12): 3355-3361.

Schulze MB, Hoffmann K, Manson JE, Willett WC, Meigs JB, Weikert C, Heidemann C, Colditz GA and Hu FB (2005) Dietary pattern, inflammation, and incidence of type 2 diabetes in women. The American Journal of Clinical Nutrition 82 (3): 675-684

Schulze MB and Hu FB (2005) Primary prevention of diabetes: What Can Be Done and How Much Can Be Prevented? Annual Review of Public Health 26 (1): 445-467.

Schwartz RS and Weindruch R (1995) Interventions Based on the Possibility That Oxidative Stress Contributes to Sarcopenia. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 50A (Special Issue): 157-161.

Scognamiglio R, Avogaro A, Negut C, Piccolotto R, de Kreutzenberg SV and Tiengo A (2004) The effects of oral amino acid intake on ambulatory capacity in elderly subjects. Aging Clinical and Experimental Research 16 (6): 443-447.

Scott D, Blizzard L, Fell J, Ding C, Winzenberg T and Jones G (2010) A prospective study of the associations between 25-hydroxy-vitamin D, sarcopenia progression and physical activity in older adults. Clinical Endocrinology 73 (5): 581-587.

Scott D, Blizzard L, Fell J, Giles G and Jones G (2010) Associations Between Dietary Nutrient Intake and Muscle Mass and Strength in Community-Dwelling Older Adults: The Tasmanian Older Adult Cohort Study. Journal of the American Geriatrics Society 58 (11): 2129-2134.

Sebastian A, Harris ST, Ottaway JH, Todd KM and Morris RC (1994) Improved Mineral Balance and Skeletal Metabolism in Postmenopausal Women Treated with Potassium Bicarbonate. New England Journal of Medicine 330 (25): 1776-1781.

Selman C and Withers DJ (2011) Mammalian models of extended healthy lifespan. Philosophical Transactions of the Royal Society B: Biological Sciences 366 (1561): 99-107.

Semba RD, Blaum C, Guralnik JM, Moncrief DT, Ricks MO and Fried LP (2003) Carotenoid and vitamin E status are associated with indicators of sarcopenia among older women living in the community. Aging Clinical & Experimental Research 15 (6): 482-487.

Semba RD, Lauretani F and Ferrucci L (2007) Carotenoids as protection against sarcopenia in older adults. Archives of Biochemistry and Biophysics 458 (2): 141-145.

Serra-Majem L, Trichopoulou A, de la Cruz JN, Cervera P, Álvarez AG, La Vecchia C, Lemtouni A and Trichopoulos D (2004) Does the definition of the Mediterranean diet need to be updated? Public Health Nutrition 7 (07): 927-929.

Seymour JD, Calle EE, Flagg EW, Coates RJ, Ford ES and Thun MJ (2003) Diet Quality Index as a Predictor of Short-term Mortality in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. American Journal of Epidemiology 157 (11): 980-988.

Shoelson SE, Herrero L and Naaz A (2007) Obesity, Inflammation, and Insulin Resistance. Gastroenterology 132 (6): 2169-2180.

Silveira LR, Pilegaard H, Kusuhara K, Curi R and Hellsten Y (2006) The contraction induced increase in gene expression of peroxisome proliferator-activated receptor (PPAR)-gamma coactivator 1alpha (PGC-1alpha), mitochondrial uncoupling protein 3 (UCP3) and hexokinase II (HKII) in primary rat skeletal muscle cells is dependent on reactive oxygen species. Biochimica & Biophysica Acta 1763 (9): 969-976.

Singh T and Newman AB (2011) Inflammatory markers in population studies of aging. Ageing Research Reviews 10 (3): 319-329.

Sipila S, Taaffe DR, Cheng S, Puolakka J, Toivanen J and Suominen H (2001) Effects of hormone replacement therapy and high-impact physical exercise on skeletal muscle in post-menopausal women: a randomized placebo-controlled study. Clinical Science 101 (2): 147-157.

Sitnick M, Foley AM, Brown M and Spangenburg EE (2006) Ovariectomy prevents the recovery of atrophied gastrocnemius skeletal muscle mass. Journal of Applied Physiology 100 (1): 286-293.

Siu PM, Pistilli EE and Alway SE (2008) Age-dependent increase in oxidative stress in gastrocnemius muscle with unloading. Journal of Applied Physiology 105 (6): 1695-1705.

Sjögren P, Becker W, Warensjö E, Olsson E, Byberg L, Gustafsson I-B, Karlström B and Cederholm T (2010) Mediterranean and carbohydrate-restricted diets and mortality among elderly men: a cohort study in Sweden. The American Journal of Clinical Nutrition 92 (4): 967-974.

Snieder H, Hayward CS, Perks U, Kelly RP, Kelly PJ and Spector TD (2000) Heritability of Central Systolic Pressure Augmentation. Hypertension 35 (2): 574-579.

Sofi F, Cesari F, Abbate R, Gensini GF and Casini A (2008) Adherence to Mediterranean diet and health status: meta-analysis. British Medical Journal 337: a1344.

Solerte SB, Gazzaruso C, Bonacasa R, Rondanelli M, Zamboni M, Basso C, Locatelli E, Schifino N, Giustina A and Fioravanti M (2008) Nutritional Supplements with Oral Amino Acid Mixtures Increases Whole-Body Lean Mass and Insulin Sensitivity in Elderly Subjects with Sarcopenia. American Journal of Cardiology 101 (11): S69-S77.

Song Y, Li TY, van Dam RM, Manson JE and Hu FB (2007) Magnesium intake and plasma concentrations of markers of systemic inflammation and endothelial dysfunction in women. The American Journal of Clinical Nutrition 85 (4): 1068-1074.

Song Y, Manson JE, Buring JE and Liu S (2004) Dietary Magnesium Intake in Relation to Plasma Insulin Levels and Risk of Type 2 Diabetes in Women. Diabetes Care 27 (1): 59-65.

Song Y, Ridker PM, Manson JE, Cook NR, Buring JE and Liu S (2005) Magnesium Intake, C-Reactive Protein, and the Prevalence of Metabolic Syndrome in Middle-Aged and Older U.S. Women. Diabetes Care 28 (6): 1438-1444.

Song Y, Sesso HD, Manson JE, Cook NR, Buring JE and Liu S (2006) Dietary Magnesium Intake and Risk of Incident Hypertension Among Middle-Aged and Older US Women in a 10-Year Follow-Up Study. American Journal of Cardiology 98 (12): 1616-1621.

Spector T and Williams F (2006) The UK Adult Twin Registry (TwinsUK). Twin Research and Human Genetics 9 (6): 899-906.

Spector TD and MacGregor AJ (2002) The St. Thomas' UK Adult Twin Registry. Twin Research 5 (5): 440-443.

Spector TD and MacGregor AJ (2004) Risk factors for osteoarthritis: genetics. Osteoarthritis Cartilage 12 Suppl A S39-44.

Sproston K and Primatesta P (2004). Health Survey for England 2003. Volume 2. Risk factors for cardiovascular disease. London: Her Majesty's Stationery Office.

Stenholm S, Harris TB, Rantanen T, Visser M, Kritchevsky SB and Ferrucci L (2008) Sarcopenic obesity: definition, cause and consequences. Current Opinion of Clinical Nutrition & Metabolic Care 11 (6): 693-700.

Stookey J, Adair L and Popkin B (2005) Do protein and energy intakes explain long-term changes in body composition? The Journal of Nutrition, Health & Aging 9 (1): 5-17.

Strawbridge W, Cohen R, Shema S and Kaplan G (1996) Successful aging: Predictors and associated activities. American Journal of Epidemiology 144: 135-141.

Stubbs R (2009) Detecting and modelling MIS-reporting of food intake in adults. http://www.foodbase.org.uk/results.php?f_report_id=289.

Stuck A, Walthert J, Nikolaus T, Bula C and Hohmann C (1999) Risk factors for functional status decline in community-living elderly people: a systematic review. Social Science & Medicine 48 (4): 445-469.

Syddall H, Cooper C, Martin F, Briggs R and Aihie Sayer A (2003) Is grip strength a useful single marker of frailty? Age and Ageing 32 (6): 650-656.
Symons TB, Schutzler SE, Cocke TL, Chinkes DL, Wolfe RR and Paddon-Jones D (2007) Aging does not impair the anabolic response to a protein-rich meal. The American Journal of Clinical Nutrition 86 (2): 451-456.

Talegawkar SA, Bandinelli S, Bandeen-Roche K, Chen P, Milaneschi Y, Tanaka T, Semba RD, Guralnik JM and Ferrucci L (2012) A Higher Adherence to a Mediterranean-Style Diet Is Inversely Associated with the Development of Frailty in Community-Dwelling Elderly Men and Women. The Journal of Nutrition 142 (12): 2161-2166.

Teucher B, Skinner J, Skidmore PM, Cassidy A, Fairweather-Tait SJ, Hooper L, Roe MA, Foxall R, Oyston SL, Cherkas LF, Perks UC, Spector

TD and MacGregor AJ (2007) Dietary patterns and heritability of food choice in a UK female twin cohort. Twin Research and Human Genetics 10 (5): 734-748.

Thompson JL, Butterfield GE, Gylfadottir UK, Yesavage J, Marcus R, Hintz RL, Pearman A and Hoffman AR (1998) Effects of Human Growth Hormone, Insulin-Like Growth Factor I, and Diet and Exercise on Body Composition of Obese Postmenopausal Women. Journal of Clinical Endocrinology & Metabolism 83 (5): 1477-1484.

Tilg H and Moschen AR (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nature Reviews Immunology 6 (10): 772-783.

Tracy BL, Ivey FM, Hurlbut D, Martel GF, Lemmer JT, Siegel EL, Metter EJ, Fozard JL, Fleg JL and Hurley BF (1999) Muscle quality. II. Effects of strength training in 65- to 75-yr-old men and women. Journal of Applied Physiology 86 (1): 195-201.

Trichopoulou A, Bamia C and Trichopoulos D (2009) Anatomy of health effects of Mediterranean diet: Greek EPIC prospective cohort study. British Medical Journal 338: b2337.

Trichopoulou A, Costacou T, Bamia C and Trichopoulos D (2003) Adherence to a Mediterranean Diet and Survival in a Greek Population. New England Journal of Medicine 348 (26): 2599-2608.

Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Polychronopoulos E, Vassilakou T, Lipworth L and Trichopoulos D (1995) Diet and overall survival in elderly people. British Medical Journal 311 (7018): 1457-1460.

Trichopoulou A, Naska A, Orfanos P and Trichopoulos D (2005) Mediterranean diet in relation to body mass index and waist-to-hip ratio: the Greek European Prospective Investigation into Cancer and Nutrition Study. The American Journal of Clinical Nutrition 82 (5): 935-940.

Trichopoulou A, Orfanos P, Norat T, Bueno-de-Mesquita B, Ocke MC, Peeters PH, van der Schouw YT, Boeing H, Hoffmann K, Boffetta P, Nagel G, Masala G, Krogh V, Panico S, Tumino R, Vineis P, Bamia C, Naska A, Benetou V, Ferrari P, Slimani N, Pera G, Martinez-Garcia C, Navarro C, Rodriguez-Barranco M, Dorronsoro M, Spencer EA, Key TJ, Bingham S, Khaw KT, Kesse E, Clavel-Chapelon F, Boutron-Ruault MC, Berglund G, Wirfalt E, Hallmans G, Johansson I, Tjonneland A, Olsen A, Overvad K, Hundborg HH, Riboli E and Trichopoulos D (2005) Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. British Medical Journal 330 (7498): 991.

Tucker KL (2010) Dietary patterns, approaches, and multicultural perspective. Applied Physiology, Nutrition and Metabolism 35 (2): 211-218.

Tucker KL, Chen H, Vogel S, Wilson PWF, Schaefer EJ and Lammi-Keefe CJ (1999) Carotenoid Intakes, Assessed by Dietary Questionnaire, Are Associated with Plasma Carotenoid Concentrations in an Elderly Population. The Journal of Nutrition 129 (2): 438-445.

van Herpen-Broekmans WM, Klopping-Ketelaars IA, Bots ML, Kluft C, Princen H, Hendriks HF, Tijburg LB, van Poppel G and Kardinaal AF (2004) Serum carotenoids and vitamins in relation to markers of endothelial function and inflammation. European Journal of Epidemiology 19 (10): 915-921.

van Kan GA, Rolland YM, Morley JE and Vellas B (2008) Frailty: Toward a Clinical Definition. Journal of the American Medical Directors Association 9 (2): 71-72.

VanItallie T, Yang M, Heymsfield S, Funk R and Boileau R (1990) Height-normalized indices of the body's fat-free mass and fat mass: potentially useful indicators of nutritional status. The American Journal of Clinical Nutrition 52 (6): 953-959.

Vasto S, Carruba G, Lio D, Colonna-Romano G, Di Bona D, Candore G and C C (2009) Inflammation, ageing and cancer. Mechanisms of Ageing and Development 130 (1-2): 40-45.

Viña J, Borrás C and Miquel J (2007) Theories of ageing. IUBMB Life 59 (4-5): 249-254.

Visser M, Deeg DJH and Lips P (2003) Low Vitamin D and High Parathyroid Hormone Levels as Determinants of Loss of Muscle Strength and Muscle Mass (Sarcopenia): The Longitudinal Aging Study Amsterdam. Journal of Clinical Endocrinology & Metabolism 88 (12): 5766-5772.

Visser M, Pahor M, Taaffe D, Goodpaster BH, Simonsick EM, Newman AB, Nevitt M and Harris TB (2002) Relationship of Interleukin-6 and Tumor Necrosis Factor-alpha With Muscle Mass and Muscle Strength in Elderly Men and Women. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 57 (5): M326-M332.

Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B and Wolfe RR (2003) Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. The American Journal of Clinical Nutrition 78 (2): 250-258.

von Haehling S, Morley J and Anker S (2010) An overview of sarcopenia: facts and numbers on prevalence and clinical impact. Journal of Cachexia, Sarcopenia and Muscle 1 (2): 129-133.

Waijers PMCM, Feskens EJM and Ocké MC (2007) A critical review of predefined diet quality scores. British Journal of Nutrition 97 (02): 219-231.

Wald NJ, Simmonds M and Morris JK (2011) Screening for Future Cardiovascular Disease Using Age Alone Compared with Multiple Risk Factors and Age. PLoS ONE 6 (5): e18742.

Walston J, Xue Q, Semba RD, Ferrucci L, Cappola AR, Ricks M, Guralnik J and Fried LP (2006) Serum antioxidants, inflammation, and total mortality in older women. American Journal of Epidemiology 163 (1): 18-26.

Wang L, Gaziano JM, Norkus EP, Buring JE and Sesso HD (2008) Associations of plasma carotenoids with risk factors and biomarkers related to cardiovascular disease in middle-aged and older women. The American Journal of Clinical Nutrition 88 (3): 747-754.

Wang Z, Heo M, Lee RC, Kotler DP, Withers RT and Heymsfield SB (2001) Muscularity in adult humans: Proportion of adipose tissue-free body mass as skeletal muscle. American Journal of Human Biology 13 (5): 612-619.

Wang ZM, Visser M, Ma R, Baumgartner RN, Kotler D, Gallagher D and Heymsfield SB (1996) Skeletal muscle mass: evaluation of neutron activation and dual-energy X-ray absorptiometry methods. Journal of Applied Physiology 80 (3): 824-831.

Wannamethee SG, Lowe GD, Rumley A, Bruckdorfer KR and Whincup PH (2006) Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. The American Journal of Clinical Nutrition 83 (3): 567-574.

Wareham NJ, Jakes RW, Rennie KL, Mitchell J, Hennings S and Day NE (2002) Validity and repeatability of the EPIC-Norfolk Physical Activity Questionnaire. International Journal of Epidemiology 31 (1): 168-174.

Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S and Day NE (2003) Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutrition 6 (04): 407-413.

Waters DL, Baumgartner RN, Garry PJ and Vellas B (2010) Advantages of dietary, exercise-related, and therapeutic interventions to prevent and treat sarcopenia in adult patients: an update. Journal of Clinical Interventions in Aging 5: 259-270.

WCRF/AICR (2007). World Cancer Research Fund and American Institute for Cancer Research. Food, nutrition, physical activity and teh prevention of cancer: a global perspective. Washington DC: AICR, 2007.

Weber J, Gillain S and Petermans J (2010) Sarcopenia: a physical marker of frailty. Revue Medicale Liege 65 (9): 514-520.

Wei W, Kim Y and Boudreau N (2001) Association of smoking with serum and dietary levels of antioxidants in adults: NHANES III, 1988-1994. American Journal of Public Health 91 (2): 258-264.

Welch A, MacGregor A, Jennings A, Fairweather-Tait S, Spector T and Cassidy A (2012) Habitual flavonoid intakes are positively associated with bone mineral density in women. Journal of Bone and Mineral Research 27 (9): 1872-1878.

Welch AA, Bingham SA, Ive J, Friesen MD, Wareham NJ, Riboli E and Khaw K (2006) Dietary fish intake and plasma phospholipid n–3 polyunsaturated fatty acid concentrations in men and women in the European Prospective Investigation into Cancer–Norfolk United Kingdom cohort. The American Journal of Clinical Nutrition 84 (6): 1330-1339.

Welch AA, MacGregor AJ, Skinner J, Spector TD, Moayyeri A and Cassidy A (2012) A higher alkaline dietary load is associated with greater indexes of skeletal muscle mass in women. Osteoporosis International 1-10.

Welle S (1999). Human protein metabolism. Springer; New York: 1999.

Wells JC, Cole TJ and steam As (2002) Adjustment of fat-free mass and fat mass for height in children aged 8 y. Int J Obes Relat Metab Disord 26 (7): 947-952.

Wells JCK, Cole TJ and team As (2002) Adjustment of fat free mass and fat mass for height in children aged 8 y. International Journal of Obesity 26 (7): 947-952.

WHO (1987) WHO Expert Committee on Biological Standardization, 37th Report. WHO Technical Report Series 760. Geneva, Switzerland: World Health Organization. 21-22.

WHO (1998). World Health Organisation (1998). Report of a WHO consultation on obesity. Obesity: Preventing and managing the global epidemic. Geneva: World Health Organization.

Wilkins J, Gallimore JR, Moore EG and Pepys MB (1998) Rapid automated high sensitivity enzyme immunoassay of C-reactive protein. Clinical Chemistry 44: 1358-1361.

Willett W (1986). Total energy intake: implications for epidemiologic analyses. American Journal of Epidemiology**:** 124: 117-127.

Willett W, Howe G and Kushi L (1997) Adjustment for total energy intake in epidemiologic studies. The American Journal of Clinical Nutrition 65 (4): 1220S-1228S.

Williams F, Cherkas L, Spector T and MacGregor A (2004) A common genetic factor underlies hypertension and other cardiovascular disorders. BMC Cardiovascular Disorders 4 (1): 20.

Winklhofer-Roob B, Rock E, Ribalta J, Shmerling D and Roob J (2003) Effects of vitamin E and carotenoid status on oxidative stress in health and disease. Evidence obtained from human intervention studies. Molecular Aspects of Medicine 24 391-402.

Wolf FI and Cittadini A (2003) Chemistry and biochemistry of magnesium. Molecular Aspects of Medicine 24 (1-3): 3-9.

Wolfe RR (2006) The underappreciated role of muscle in health and disease. The American Journal of Clinical Nutrition 84 (3): 475-482.

Woods J, Wilund K, Martin S and Kistler B (2012) Exercise, Inflammation and Aging. Aging and Disease 3 (1): 130-140.

Woodward M, Rumley A, Lowe G and Tunstall-Pedoe H (2003) C-reactive protein associations with haematological variables, cardiovascular risk factors and prevalent cardiovascular disease. British Journal of Haematology 122: 135-141.

Wu G, Jaeger LA, Bazer FW and Rhoads JM (2004) Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. The Journal of Nutritional Biochemistry 15 (8): 442-451.

Wu G and Morris SM, Jr. (1998) Arginine metabolism: nitric oxide and beyond. Biochemical Journal 336 (1) 1-17.

Wüst R, Morse C, de Haan A, Rittweger J, Jones D and Degens H (2008) Skeletal muscle properties and fatigue resistance in relation to smoking history. European Journal of Applied Physiology 104 (1): 103-110.

You T, Nicklas BJ, Ding J, Penninx BWJH, Goodpaster BH, Bauer DC, Tylavsky FA, Harris TB, Kritchevsky SB, for the Health A and Study BC (2008) The Metabolic Syndrome Is Associated With Circulating Adipokines in Older Adults Across a Wide Range of Adiposity. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 63 (4): 414-419.

Young A, Phillip D and Lowe G (2004). in: Krinsky NI, Mayne ST, Sies H (Eds.), Carotenoids in Health and Disease, Marcel Dekker, New York, 2004, pp. 105-126.

Yu YM, Sheridan RL, Burke JF, Chapman TE, Tompkins RG and Young VR (1996) Kinetics of plasma arginine and leucine in pediatric burn patients. The American Journal of Clinical Nutrition 64 (1): 60-66.

Zaninotto M, Mion MM, Novello E, Altinier S and Plebani M (2007) New biochemical markers: From bench to bedside. Clinica Chimica Acta 381 (1): 14-20.

Zhang X, Lee JE, Ma J, Je Y, Wu K, Willett WC, Fuchs CS and Giovannucci EL (2012) Prospective cohort studies of vitamin B-6 intake and colorectal cancer incidence: modification by time? The American Journal of Clinical Nutrition 96 (4): 874-881.