Development and application of a nutrient support to age related lean tissue mass loss in an Irish population (50-70 years); Validation of methods used to investigate cross sectional observations of the intake of nutrients implicated, and a longitudinal intervention applying a nutrient support to age related lean tissue mass loss

Development and application of a nutrient support to age related lean tissue mass loss in an Irish population (50-70years);

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by

Catherine Norton

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Supervised by: Professor P Jakeman & Professor RJ Fitzgerald

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Abstract

Title: Development and application of a nutrient support to age related lean tissue mass loss in an Irish population (50-70years); Validation of methods used to investigate cross sectional observations of the intake of nutrients implicated, and a longitudinal intervention applying a nutrient support to age related lean tissue mass loss

Author: C. Norton

Prevention of the age-related loss of muscle mass and strength (sarcopaenia) is fundamental to conserving functional capability in older age and enabling independent living. To develop preventive strategies, a better understanding is needed of the lifestyle dynamics influencing age related lean tissue mass loss. Increasing evidence suggests that a modification of protein intake, which is quantitative, qualitative and optimally apportioned, is necessary to counteract progressive lean tissue loss in the elderly. Among other nutrients implicated are antioxidants. Muscle mass and strength in later life are a reflection of both the rate of muscle loss and the peak attained in early life, therefore efforts to prevent sarcopaenia also need to consider diet across the life-course and the potential effectiveness of early interventions.

This thesis aimed to assess the adequacy of protein intake (quantity, quality and apportioning), and dietary total antioxidant capacity (TAC) in a convenience population of healthy, 'young' elderly Irish adults. This necessitated modifications to traditional, and the development of novel approaches to dietary data collection and nutrient intakes analysis methods. Two innovative nutrient intake analysis methods are proposed to estimate dietary total antioxidant capacity and protein quality (as essential amino acid content) in dietary intakes. Progressing from this assessment a subsequent aim was to investigate whether supplementing dietary intake with a milk protein rich in essential amino acids at both smaller meals in the day over a 24 week intervention, would arrest the progression of sarcopaenia in this population.

The intervention study consisted of a randomised, single blind, control trial of healthy adults aged 50-70y, in which participants consumed a milk-protein based nutrient support (with >50% essential amino acid content, fortified with milk minerals and vitamin D) to age related lean tissue mass loss, or a non-nitrogenous, iso-energetic control, twice daily with smaller meals. The change in body composition, specifically whole body lean tissue mass (LTM) was the outcome assessed.

At 24 weeks the mean LTM increased by 0.45 (1.06) kg in the formulation group compared to a mean decrease of 0.16 (0.88) kg in the control. Expressed as a percentage, the increase in LTM at 24 weeks was +0.91 (2.4) % in the formulation group compared to a decrease of -0.3 (2.1) % in the control group (ANOVA; $F_{3,59}$= 2.616, P=0.06, 1-ß=0.861, η_p^2=0.12). The 1.2% mean difference in response between the treatment and control group was statistically significant (P=0.011).

Dietary modification presents a real opportunity to prevent or delay age related losses in whole body lean tissue mass. Refinement of current methods and procedures of dietary data collection and analysis are required to comprehensively investigate correlations between nutrient intakes and sarcopaenia. Further investigation is required to assess the efficacy of single nutrient interventions as opposed to the comprehensive nutrient formulation proposed here.

Authors Declaration

This work is presented in fulfilment of the requirement for the degree of Doctor of Philosophy. I hereby declare that the work contained within this thesis is my own work, and was completed without collaboration or assistance from others apart from the counsel received from my supervisors. This work has also not been submitted to any other university of higher education institution, or for any other academic award within this university. Where the use has been made of the work of other people it has been fully acknowledged and referenced (References).

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Catherine Norton

May 2014

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Sincere thanks for support from funders (Food for Health Ireland); supervisors; departmental academics, colleagues, and support staff; study participants; support staff at WISP©, Tinuviel UK; and also for love from friends and family.

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To my family – especially my parents, for their unwavering support, love and encouragement in all aspects of life; my children (Cormac, Caoimhe and Bróna) for putting up with an absentee Mum over much of the last year; most especially to Donogh, for allowing me the indulgence of a PhD 'at this stage of life', and ensuring my safe passage from the beginning to completion! I really couldn't have done it without you.

The duration of this doctoral work has seen both the best and worst of times from welcome additions to painful bereavements in my family; and from discovering new found friends and skills to realising some of my own great inadequacies. If Theodore Roosevelt was correct in saying that "Nothing in the world is worth having or worth doing unless it means effort, pain and difficulty", it must make a doctoral thesis a very worthwhile thing!

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List of Abbreviations

∑EAAs	Sum of all essential amino acids
♀	Female
♂	Male
μmol/L	micromoles per litre
25OHD	25-hydroxyvitamin D
AA	amino acid
ALTM	Appendicular lean tissue mass
AMDR	Acceptable Macronutrient Distribution Ranges
AMPK	adenosine monophosphate protein kinase
AO	Antioxidant
AR	Acceptable reporter
ASN	American Society for Nutrition
BCAA	Branched chain amino acid
BF%	Body fat percentage
BFM	Body fat mass
BIA	Bioelectrical Impedance Analysis
BM	Body mass
BMD	Bone mineral density
BMI	Body mass index
BMR	Basal metabolic rate
BV	Biological value
C	Calcitriol
CAT	Catalase
CCVP	Codex Committee on Vegetable Proteins
CHO	Carbohydrate
CI	Confidence interval
CLIP	Chronic low grade inflammatory process
COMA	Committee on Medical Aspects of Food Policy
CON	Control supplement

CRP	C-reactive protein
CT	Computed tomography
CV	Coefficient of variation
CVD	Cardiovascular disease
CYP27B1	25-Hydroxyvitamin D_3 1- α -hydroxylase
CYP2R1	vitamin D-25-hydroxylase
Cys	Cystine
DIAA	Digestible indispensable amino acid
DIAAS	Digestible indispensable amino acid score
DH %	Degree of hydrolysis
DRI	Dietary reference intake
DRV	Dietary reference value
DXA	Dual-energy x-ray absorptiometry
EAA	Essential amino acid
EAR	Estimated average requirement
EE	energy expenditure
eFIR	estimated food intake record
EFSA	European Food Safety Authority
EHSREC	Faculty of Education and Health Sciences Research Ethics Committee
EI	Energy intake
eIF4E-BP1	Eukaryotic initiation factor 4E binding protein 1
eIF4G	Eukaryotic initiation factor 4G
ER	Energy requirement
EUGMS	European Union Geriatric Medicine Society
EWGSOP	European Working Group on Sarcopaenia in Older People
FAA	Functional amino acid
FAO/UNU	Food and Agriculture Organization of the United Nations
FDA	Food & Drug Administration
FHI	Food for Health Ireland
FIR	Food intake record
FM	Fat mass

FORM	Formulation supplement
FRAP	Ferric reducing/antioxidant power
FSAI	Food Safety Authority of Ireland
FSMP	Food for special medical purposes
g	grams
g/l	grams per litre
$g.kg^{-1}.d^{-1}$	grams per kilo gram body mass per day
GAE	Gallic equivalents
GI	Gastro intestinal
GPX	Glutathione peroxidase
HDL	high-density lipoprotein
His	Histidine
H-ORAC	hydrophilic oxygen radical absorbance capacity
IAA	Indispensable amino acid
IGF^{-1}	Insulin-like growth factor 1
IL	Interleukin
Ile	Isoleucine
INCAP	Institute of Nutrition of Central America and Panama
IOM	Institute of Medicine
IQR	Interquartile range
ISCCWG	International Sarcopaenia Consensus Conference Working Group
IU	International units
IUNA	Irish Universities Nutrition Alliance
JECFA	Joint Expert Committee on Food Additives
LCPUFA	Long chain poly-unsaturated fatty acids
LDL	low-density lipoprotein
Leu	Leucine
L-ORAC	lipophilic oxygen radical absorbance capacity
LTM	Lean tissue mass
Lys	Lysine
MA	Metabolic Availability

MAFF	Ministry of Agriculture, Fisheries and Food
MDI	mean daily intake
Met	Methionine
mg	milligrams
MLA	Meal level analysis
mmol/L	millimols per litre
MPB	Muscle protein breakdown
MPC	Milk protein concentrate
MPS	Muscle protein synthesis
MPB	Muscle protein breakdown
mTOR	mammalian target of rapamysin
NANS	National Adult Nutrition Survey
NCD	non-communicable diseases
ND	normally distributed
NDB	nutrient databank
NDL	Nutrient Data Laboratory
NEAA	Non-essential amino acid
NHANES	National Health and Nutrition Examination Survey
NND	non- normally distributed
NPB	Net protein balance
NSIFCS	North / South Ireland Food Consumption Survey
ONS	Oral nutrition supplement
OPRA	Osteoporosis Prospective Risk Assessment Study
ORAC	oxygen radical absorbance capacity
p70S6K	ribosomal protein S6 kinase
PAL	Physical activity level
PS	protein synthesis
PB	protein breakdown
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PER	Protein Efficiency Ratio
Phe	Phenylalanine

PKB/Akt	protein kinase B
PPU	Postprandial Protein Utilisation
PRI	Population reference intake
PTH	Parathyroid hormone
RCT	Randomized Controlled Trials
RDA	Recommended dietary allowance
RNI	Reference nutrient intake
ROS	Reactive oxygen species
RXR	retinoid X receptor
SACN	Scientific Advisory Committee on Nutrition
SD	Standard deviation
SEE	Standard error of estimation
SOD	Super oxide dismutase
SOP	Standard operating procedure
SWA	SenseWear® armband
TAC	Total antioxidant capacity
TAG	Triacylglycerol
TE	Trolox equivalents
TEAC	Trolox equivalent antioxidant capacity
TEE	total energy expenditure
TEI	total energy intake
Thr	Threonine
TILDA	The Irish Longitudinal Study on Ageing
TNF-α	Tumour necrosis factor-alpha
T-ORAC	total oxygen radical absorbance capacity
TRAP	Total radical trapping antioxidant power
Trp	Tryptophan
Tyr	Tyrosine
UCC	University College Cork
ULBC	University of Limerick Body Composition Study
UR	under-reporting

USDA	United States Department of Agriculture
UVB	ultraviolet B radiation
Val	Valine
VDR	Vitamin D receptor
VDRE	vitamin D-responsive element
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist-to-hip ratio
WISP©	Weighed Intake analysis Software Package
WMP	Whole Milk Powder

Chapter 1

Introduction

1.1 Background

The Irish Longitudinal Study on Ageing (TILDA) estimates that by 2046 approximately 21% of the Irish population will be aged 65 years or older, and approximately 7% will be aged 80 years or older (TILDA: The Irish Longitudinal Study on Ageing 2014). This phenomenon is not unique to Ireland; demographics of the world's population show that the number of individuals aged 60 years and older will increase from 600 million in the year 2000 to more than 2 billion by 2050 (WHO 2012). Good health is essential for older persons to remain independent and to continue to actively participate in family and community life. One of the factors which play a pivotal role in the ability to maintain a healthy active lifestyle in old age is the preservation of lean tissue mass (Chumlea *et al.* 2011). However after the fourth decade of life, a progressive loss of lean tissue mass is observed with increasing age (Rosenberg 2011).

Because of its safety and ease of application, nutrition is best placed to play a key role in the challenge of 'keeping healthy people healthy' by facilitating the maintenance or accrual of lean tissue mass in old age. However enhancing nutrition for the preservation of health in old age, rather than solely as a treatment for ill health, has largely been overlooked.

In July 2013, a report from a working group assigned by the American Society for Nutrition (ASN) was published entitled 'Nutrition Research to Affect Food and a Healthy Life Span'. The authors (Ohlurst *et al*. 2013) identified 'the nutrition research needs whose advancement would have the greatest impact on the future health and well-being of global populations'. Among the high priority areas identified in the article was nutrition in health maintenance in later life. The authors proposed tools considered critical to the progression of these research needs and among the tools identified were expansion of nutrient databases. They also emphasised the importance of controlled intervention studies. At the time, the draft aims section of this thesis dissertation audaciously shared many of the sentiments expressed by these authors!

This thesis primarily investigates nutrition supports to healthy ageing; specifically interventions to offset age related whole body lean tissue mass loss. It describes the modifications to traditional methods of dietary data collection and expansion of nutritional analysis software which were necessary to elucidate associations between dietary habits and healthy ageing. This dissertation also investigates intervention strategies testing the hypothesis that optimising nutrient intakes could attenuate the age related decline in lean tissue mass, and in doing so promote healthy ageing.

Considering our ageing population, comprehensive investigation of the complex relationships between nutrition, ageing and non-communicable diseases (NCD) must become a priority for policy makers aiming to support expanding healthy ageing populations. As the accuracy of methods to assess associations between nutrition and health improves, the transfer of this evidence-based knowledge to clinicians and the public is a vital step in the promotion of healthy lifestyle choices, encouragement of self-management and improvement of health outcomes.

The context of this thesis material is within a broader programme of work funded by Food for Health Ireland (FHI). Supported by Enterprise Ireland, FHI links the scientific expertise of researchers at third level institutions with the marketing power of industry partners Irish Dairy Board, Carbery Group, Dairygold Food Ingredients Ltd., Glanbia plc. and Kerry Group plc.,. The goal of FHI is “to improve health, wellness and quality of life through world class innovation in food”. Through an intelligent milk mining programme and an extensive bioassay screening platform, FHI searches for ingredients that promote infant development, help to control weight and related health issues, boost immunity or support healthy ageing. Age-related changes in skeletal muscle, influenced by reduced levels of physical activity and malnutrition, can lead to frailty, disability and loss of independence in older adults. From a public health perspective, this has far-reaching implications and FHI is working towards a nutritional solution for healthy ageing. The FHI Healthy Ageing work package involved extensive assessment of body composition, physical activity and functionality and *in vivo* testing of bioactive compounds. The material included in this thesis constitutes some of the nutritional aspect of the FHI Healthy Ageing work programme.

1.2 Thesis aims

The primary aim of this thesis is to investigate the efficacy of a 24 week nutrient supplementation (milk protein based, twice daily with both smaller meals) in attenuating the loss of whole body lean tissue mass associated with ageing. This research was carried out in a convenience sample of healthy, community dwelling Irish adults (50 – 70 years) in a single blind, randomised, placebo controlled trial.

A reliable estimate of the intake of nutrients implicated in the aetiology of sarcopaenia is pivotal to assessing the efficacy of the supplement intervention. Consequently, it was necessary to:

1. Examine the effect of choice of screening tools used in the assessment of mis-reporting in dietary records on both sample size and estimated nutrient intake.
2. Propose novel WISP©- based methods to estimate intakes of both amino acids and dietary total anti-oxidant capacity (TAC) in the research population.

1.3 Thesis structure

This thesis comprises an introduction (Chapter 1) providing a brief preface to the research topic and the aims of the work. Chapter 2 presents a review of the literature on age related lean tissue mass loss and its associations with diet and nutrition. There are four data chapters (Chapters 3-6) and a summary / conclusion chapter (Chapter 7).

Chapter 3 addresses the phenomenon of dietary under-reporting in self-reported dietary intakes. The nutrition research community is aware that erroneous conclusions derived from self-reported energy intakes may adversely affect policy decisions involving nutrition and health , however there are no clear guidelines on how to screen for dietary under-reporters, and whether to use, or how to interpret data obtained from under-reported dietary intakes. Both Goldberg (1991) and Black (2000) proposed a method to identify under-reporters and a comparison of the rates of under-reporting, and the interpretation of nutrient data using both methods is presented. Comparisons are also made with the methods used to screen for under-reporting by the Irish Universities Nutrition Alliance (IUNA) in the National Adults Nutrition Survey (NANS).

Chapters 4 and 5 outline proposed methods to overcome the limitations of nutrient analysis software (WISP©) to estimate intakes of some of the nutrients implicated in the aetiology of age related lean tissue mass loss. Methods used in the expansions to the existing nutrient databanks that allow the estimation of intakes of both dietary total antioxidant capacity (TAC) and essential amino acids are presented, with worked examples of their application.

Chapter 6 is the main data chapter, and describes a nutrient intervention to prevent age related declines in muscle mass by supplementing protein intakes, while addressing the key issues of the quality and apportioning of the daily quantity consumed, over 24 weeks.

The final chapter (7) serves as a summary of the work presented throughout this thesis. The public health implications of these results are discussed, and potential areas of future research are highlighted.

Chapter 2

Literature Review

A version of this chapter was published as Norton, C. and Jakeman, P. (2013) 'The underappreciated role of dairy protein in the preservation of lean tissue mass in the elderly', International Journal of Dairy Technology, 66(3), 317-320.

2.1 Preamble

At the end of Homer's Odyssey, after 20 years of adventurous traveling, the goddess Athena brings Ulysses back to Ithaca disguised as an old man. With little hope for Ulysses' return, his faithful wife Penelope has reluctantly agreed to marry whoever wins a contest using Ulysses' bow and arrow. In spite of his infirmed appearance, Ulysses is the only contestant strong enough to string the bow and shoot an arrow through 12 axe-handles. The image of a hero as strong, solid and indestructible was created and has been embedded in civilization since before the beginning of written history! From time immemorial, people have equated strong muscles with youth and good health. Thousands of years later, we are beginning to understand exactly how health, nutrition and age affect muscle, and we are on the cusp of translating this knowledge into routine practice.

2.2 Introduction

This chapter will introduce the concepts of change to dietary habits, physical activity levels and body composition which occur with age. With a specific emphasis on age related whole body lean tissue mass loss (sarcopaenia), this review will explore the evidence for interactions between diet, nutrition and muscle mass, and the optimal methods of distributing the daily nutrient recommendations to offset age related whole body lean tissue mass loss.

2.3 Ageing

Ageing is the accumulation of changes in a person over time. In humans it refers to a multidimensional process of physical, psychological, and social change. However the term "ageing" is somewhat ambiguous. Ageing is subject to the constrictions by which society makes sense of old age; a person's birthday is often an important event since age is generally measured chronologically. However in contrast to the chronological milestones which mark life stages in the developed world, old age in many developing countries is seen to begin at the point when active contribution is no longer possible due to disability or physical impairment (Gorman 2000). Normal ageing affects all physiological processes. Subtle irreversible changes in the function of most organs can be shown to occur by the third or fourth decades of life, with progressive deterioration with age. The images below highlight the stark changes in lean tissue mass that occur with age, and this is the focus of this dissertation.

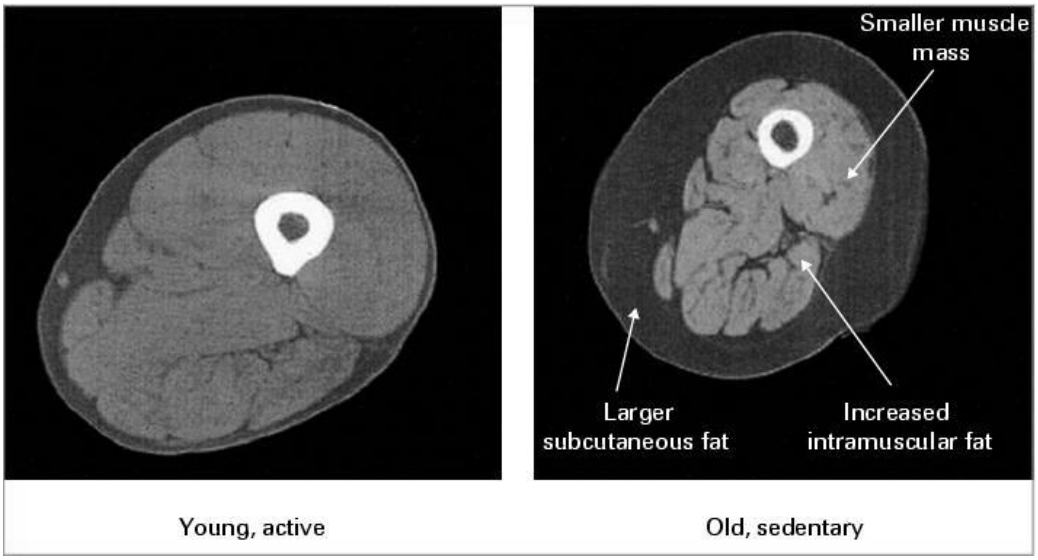


Figure 1 Muscular changes in lower limbs associated with ageing in CT scan transversal section

2.4 Nutrition & ageing

"I am grateful to old age because it has increased my desire for good conversation and decreased my desire for good food." —Cicero, DeSenectute, 43 B.C.

The concept of a physiological decrease in food intake with ageing is certainly not a new one; dietary habits change with age. It is reported that food intakes fall by 25% between 40 and 70 years of age (Hallfrisch *et al*. 1990). This condition has been referred to as 'anorexia of aging' (Morley 2001). Protein-energy malnutrition is a frequent condition in the elderly, the pathogenesis of which is likely to be multifactorial. In the Irish National Adult Nutrition Survey (NANS Summary Report 2011) total energy intake decreased with increasing age for both men and women. A low-calorie intake represents one of the main risk factors for compromised nutrition due to an increased risk of inadequate intake of essential nutrients (Morley 2001; Bartali *et al*. 2003). Many factors can influence food intakes in ageing; loneliness, poor dentition, illness or decreased appetite (Volpi 2013). Reduced nutrient intake affects both cognitive and physiological functions. Singly or concomitantly (Figure 2), these changes can lead to physical dysfunction and disability.

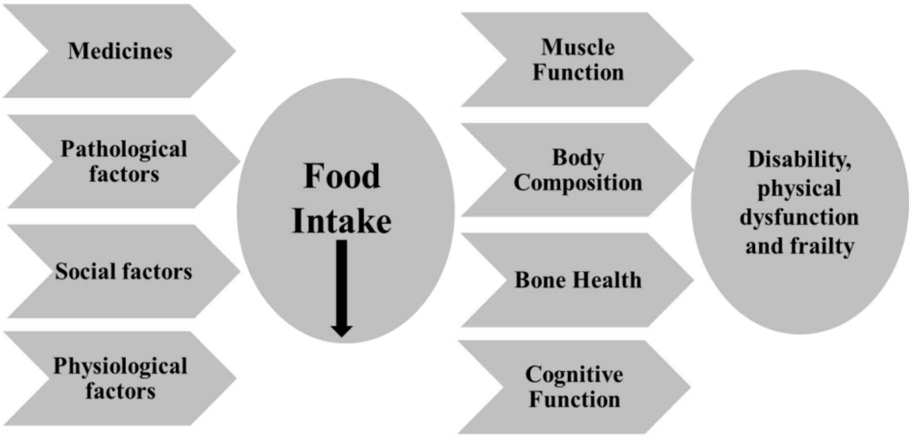


Figure 2 Possible pathways linking nutrition and diet with frailty and disability syndromes

2.5 Physical activity and ageing

Engaging in regular physical activity preserves functional ability, reduces the risk of cardiovascular disease and benefits psychological health in older people (Wagner *et al*. 1992), yet people who have physical, psychological or mental health disorders are the group most likely to report inactivity (SLÁN Report 2007). The proportion of Irish adults reporting participation in physical activity decreases with age, with 51.6% of over 75s reporting low levels of physical activity (TILDA: The Irish Longitudinal Study on Ageing 2014). This phenomenon is reported in US populations also by Caspersen (2000). Overall, two thirds of older Irish adults report moderate (33%) or high (34%) levels of physical activity (**Table 1**). For both men and women, the highest levels of physical activity are among adults aged 50 to 64 years, the lowest levels are reported in adults aged 75 and over. In both community-dwelling older people as well as those in residential care, exercise has been shown to attenuate the impact of age on mortality (Hubbard *et al.* 2009). The reasons most commonly cited as barriers to participation are listed in Figure 3 (Moschny *et al.* 2011).

Table 1 Participation in physical activity by age from The Irish Longitudinal Study on Ageing (TILDA) 2014

	Low exercise		**Moderate exercise**		**High exercise**		**Total**	**Number in sample**
	%	**95% CI**	**%**	**95% CI**	**%**	**95% CI**		
Total								
50 - 64	28	(26-29)	33	(31-34)	40	(38-42)	100	4615
64 - 74	32	(30-35)	36	(34-39)	31	(29-34)	100	2152
≥ 75	52	(49-55)	30	(28-33)	18	(15-20)	100	1334
Total	33	(32-35)	33	(32-34)	34	(32-36)	100	8101

CI, confidence interval; Missing observations = 0.94%

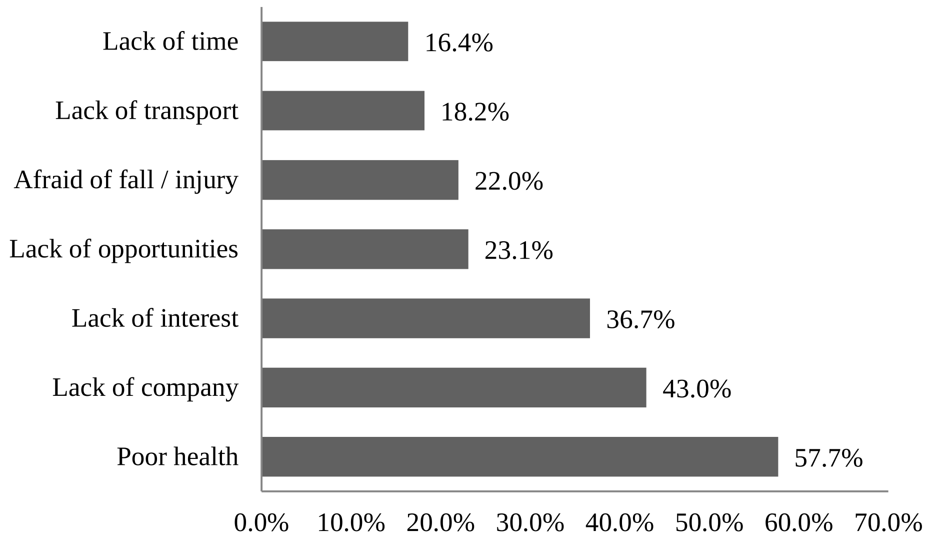


Figure 3 Barriers to physical activity in subjectively insufficiently active older adults

These age-related alterations in both dietary intake and physical activity participation are undoubtedly involved in the declines in whole body lean tissue mass observed in the elderly. These issues will be addressed in greater detail following an overview of muscle protein turnover in both young and older populations.

2.6 Muscle protein turnover

Analogous with bone tissue, muscle is in a state of constant flux. Skeletal muscle protein turnover (illustrated in **Figure** 4) is a relatively slow metabolic process that is altered by various physiological stimuli. As shown in **Figure** 5, feeding, fasting, and exercise can all influence protein turnover (Paddon-Jones *et al*. 2008). Being in a fed state temporarily shifts the balance of muscle protein turnover to anabolism lasting 2 – 3 hours (Symons 2009). One of the determinants of the magnitude of the effect of feeding on muscle protein turnover is the composition of the meal (Wolfe 2012). The effects of resistance exercise alone are powerful and longer-lasting than a feeding stimulus (Phillips *et al*. 1997) and the combined effect of resistance exercise and feeding is perhaps optimal in terms of stimulating muscle protein synthesis (MPS) (Biolo *et al*. 1997; Breen and Phillips 2012; Churchward-Venne *et al*. 2012) (**Figure** 6). In healthy young adults, muscle mass is maintained constant by the fine balance between the catabolic periods (i.e. fasting, stress) and the anabolic stimuli (i.e. feeding, muscle contraction). But at some point in the fourth decade of life adults begin to lose muscle mass and function.

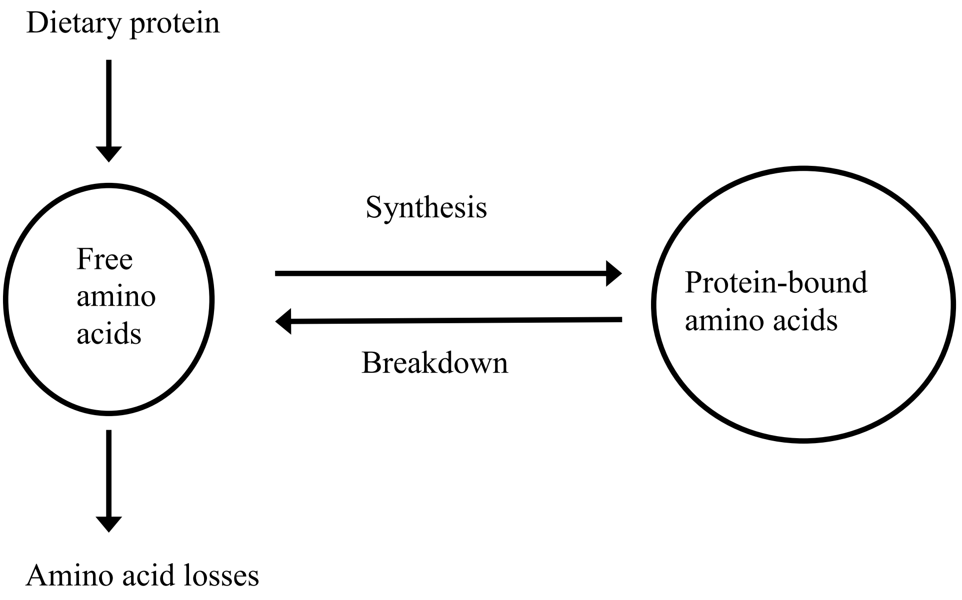


Figure 4 General model of whole-body protein turnover.

The consumption of dietary protein is instrumental to replace amino acid loses and maintain protein balance

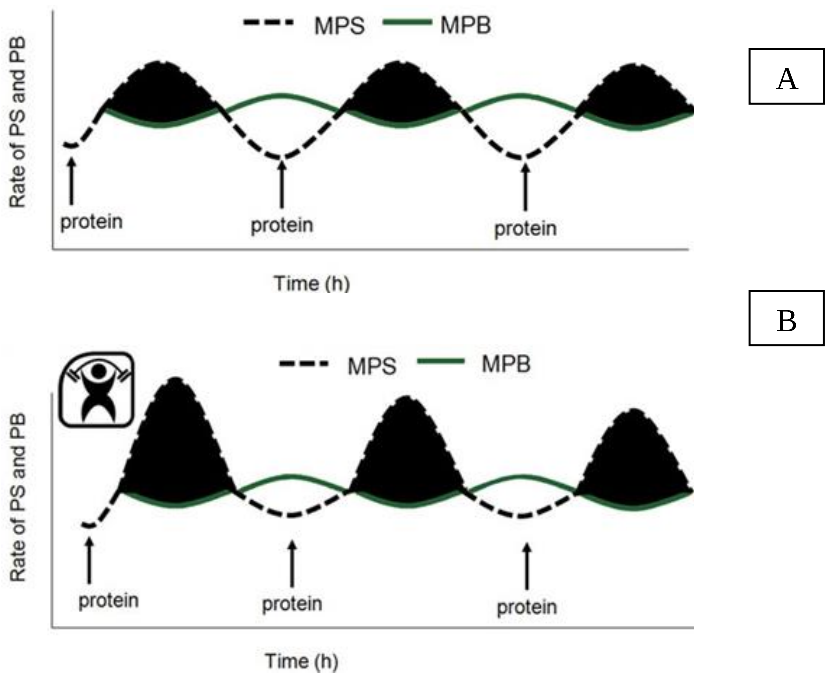


Figure 5 Changes in rates of muscle protein synthesis and degradation determine net protein balance in response to feeding (A) or feeding plus resistance training (B)

MPS muscle protein synthesis, MPB muscle protein breakdown, PS protein synthesis, PB protein breakdown

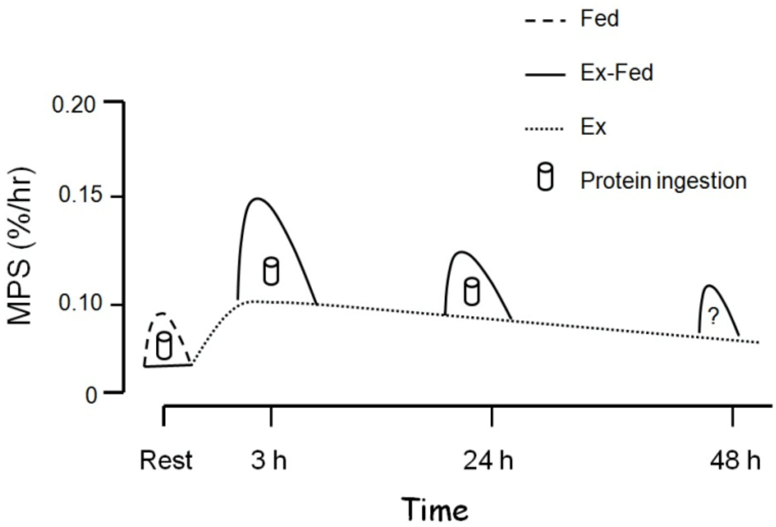


Figure 6 The synergistic effect of resistance training and protein feeding on rates of muscle protein synthesis (Burd et al. 2012) see section 2.6

2.6.1 *Age related decline in lean tissue mass*

Having reached a peak in early adult years, functional capacity gradually declines with age (Figure 7). This physical functional capacity is largely influenced by body composition, where declines in mass of bone and skeletal muscle and increases in adiposity are observed (Morley 2008, Coin et al. 2008). In humans, individuals tend to build muscle mass over the first two decades of life, begin to lose muscle mass and strength between the third and fourth decade, and the decline accelerates during the sixth decade (Roubenoff and Hughes 2000). This age trajectory of muscle mass and function is universal, but the variability in rates of decline is so striking that some individuals reach extreme age with little functional consequences while others become weak and disabled decades earlier. The loss of muscle mass with age was termed ‘sarcopaenia’ by Rosenberg in 1989 (from Greek meaning ‘poverty of flesh’), but it is also referred to as age-related muscle wasting. Sarcopaenia can be thought of as a muscular analogue of osteoporosis. Where osteoporosis is associated with an imbalance between bone resorption and formation; sarcopaenia results from a disproportionate decrease in synthesis and / or increase in breakdown of skeletal muscle proteins.

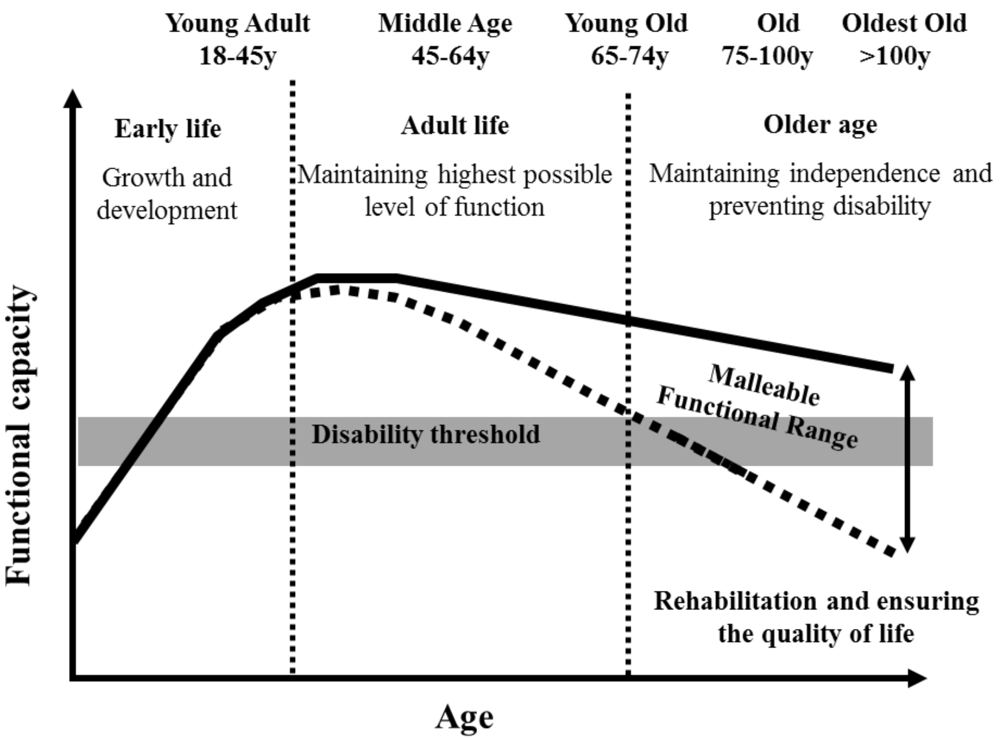


Figure 7 Changes in functional capacity across the lifespan (adapted from Kalache and Kickbusch, World Health Organisation Active Ageing Programme 1997)

Although the term sarcopaenia was first coined in 1989, and several definitions have been proposed, none has received universal agreement nor undergone any clinical validation. Baumgartner *et al.* (1998) defined sarcopaenia as "a deficiency in lean tissue mass", the European Working Group on Sarcopaenia in Older People (EWGSOP) define it as "a syndrome characterised by progressive and generalised loss of lean tissue mass and strength with a risk of adverse outcomes", and the International Sarcopaenia Consensus Conference Working Group (ISCCWG) as "age associated loss of lean tissue mass and function". A growing unanimity among both the research and clinical communities is that sarcopaenia should not be defined solely on the basis of lean tissue mass but also on aspects of functional capability.

The rate of muscle loss in sarcopaenia is estimated to be 1 – 2% annually after the age of 50 years (Sehl and Yates 2001). For the standard, reference elderly female, with an approximate lean tissue mass of 42kg (University of Limerick Body Composition Study-ULBC), a decline in lean tissue mass of 1% per year would equate to 0.4kg. This mass loss is accompanied by a decline in strength of 1.5% annually, accelerating to 3% by the age of sixty (Roubenoff 2000;

Vandervoort 2002). Compounding this lean tissue mass loss is the phenomenon whereby muscle is replaced by adipose tissue. Forbes *et al*. (1999) estimate an increase in fat mass of 0.45kg annually between the ages of 30 and 60 years. This shift in body composition is frequently undetected because of a stable total body mass. This body composition phenotype is known as sarcopaenic obesity (Hughes *et al*. 2002). Janssen (2010) suggests that as many as 15% of those with sarcopaenia are also obese.

The prevalence of sarcopaenia increases with age, however it is difficult to identify reliable incidences while a worldwide definition of sarcopaenia remains elusive. This absence of agreement leads to variation in reported prevalence, with ranges from 5 to 13% in persons aged 60 to 70 years and of 11 to 50% in persons over 80 years of age (Baumgartner *et al*. 1998; Morley *et al*. 2001; Janssen 2002; Castillo *et al*. 2003).

The cost of the physiological change and functional decline arising from sarcopaenia occur at both the individual and societal level. Advanced sarcopaenia is synonymous with physical frailty and associated with increased risk of falls and impairment in the ability to perform common-place activities (Bautmans *et al.* 2009). Poor outcomes such as loss of strength, mobility disorders, disability, and poor quality of life have been associated with sarcopaenia (Cruz-Jentoft 2013). Left unmanaged, sarcopaenia frequently results in loss of independent living and in the current environment of global ageing, the future health costs associated with sarcopaenia are worrying. Janssen *et al*. (2004) reported the estimated direct healthcare cost attributable to sarcopaenia in the United States in 2000 at $18.5 billion. They proposed that a 10% reduction in sarcopaenia prevalence would result in savings of $1.1 billion per annum in U.S. healthcare costs.

There are many purported causes of sarcopaenia including decreased physical activity levels (Baumgartner *et al.* 1999; Chodzko-Zajko *et al*. 2009), altered endocrine function (Sakuma and Yamaguchi 2012), chronic diseases (Janssen 2010), inflammation (Fulle *et al.*2004; Cerullo *et al*. 2012), insulin resistance (Batsis and Buscemi 2011), and nutritional inadequacies (Millward 2012) (**Figure** 8). Chief among these, and those most frequently researched are the modifiable lifestyle factors of physical activity and nutrition.

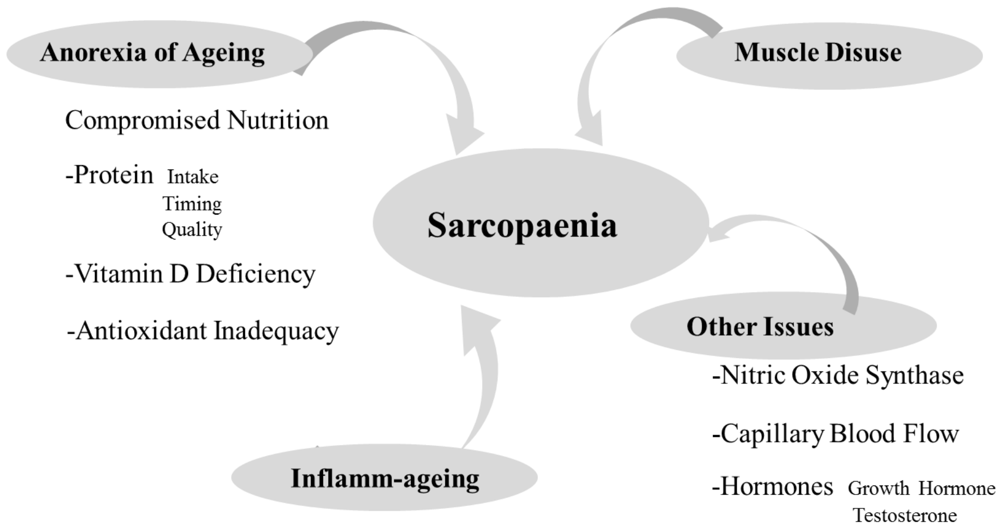


Figure 8 Causes of sarcopaenia

2.7 Physical activity and age related lean tissue mass loss

The importance of physical activity in the aetiology of age related lean tissue mass loss cannot be under estimated. Even the most ardent of nutrition supporters would not suggest that nutrition could displace physical activity as a treatment modality in the prevention of sarcopaenia. Physical inactivity or a decreased physical activity level is a part of the underlying mechanisms of sarcopaenia and, therefore, physical activity is a pivotal factor to reverse or modify the development of this condition (Montero-Fernández 2013). Both accumulation of physical activity and participation in structured exercise have been shown to result in improvements in physical fitness and the ability to perform work (Montero-Fernández 2013). The difficulty arises where there is an age related decline in purposeful activity. The proportion of Irish adults reporting low levels of physical activity increases with age, with 51.6% of over 75s reporting low levels of physical activity (TILDA: The Irish Longitudinal Study on Ageing 2014). This phenomenon is reported in US populations also by Caspersen (2000). Combatting the decline in participation rates in physical activity with age should be addressed to prevent age related lean tissue mass loss. There are four types of exercises recommended for older adults; aerobic, progressive resistance, flexibility and balance training. A systematic review of 951 articles published on exercise and sarcopaenia concluded that high intensity resistance training with sufficient periods, frequency, repetitions and sets is most effective to counteract the loss of muscle mass associated with advancing age (Miyachi 2011). An excellent review article by Roth *et al*. (2000) provides extensive information on the exercise recommendations for the prevention and treatment of age related lean tissue mass loss. This review will now focus on the nutritional components implicated in the aetiology and treatment of sarcopaenia.

2.8 Nutrition and age related lean tissue mass loss

Although the importance of adequate nutrition has been recognised for a long time, its contribution to muscle mass has not been studied extensively and much of the research is relatively new. Nutritional interventions are an appealing potential means for the prevention and treatment of sarcopaenia of the elderly due to their easy applicability and safety. A number of interventions have been studied, ranging from the provision of dietary motivation and support (Ha *et al*. 2010) to supplementation with specific nutrients. The nutrients that have been most consistently linked to muscle mass loss in older adults are energy, protein and amino acids (in particular leucine), antioxidant nutrients, and vitamin D.

2.8.1 *Energy*

The amount of energy consumed by the ageing is crucial to maintain body weight or to prevent weight loss and the concomitant loss of lean tissue mass (Morley, 1997; Bartali *et al*. 2003). 'Anorexia of aging' (Morley, 2001) is a common phenomenon among the elderly with an observed decrease of 25% in food intakes between 40 and 70 years of age (Hallfrisch *et al*. 1990). There are two consequences of declining food intakes in older age that could be important in the conservation of muscle mass. Firstly, the decrease in food intake observed is only partly balanced by reductions in energy expenditure; consequently this population loses body weight. With the ensuing weight loss, there is disproportionate loss of lean tissue mass (Candow *et al*. 2012) . Even if weight is regained, there is a net loss of lean tissue mass, predisposing to sarcopaenia, frailty and physical dysfunction. However, increasing total energy, without targeted control of specific macro- and micro-nutrients is unlikely to yield any benefits in the prevention or treatment of sarcopaenia (Bartali *et al*. 2003). This introduces the second consequence of reduced energy intake in the ageing; as older people consume smaller amounts of food it becomes increasingly challenging for them to meet their nutrient requirements. Energy deficits do not generally occur in isolation, frequently accompanied by inadequacies in macro- and micro-nutrients that compound the issue.

Addressing dietary quality rather than energy adequacy alone is necessary in preventing age related losses in lean tissue mass (Bartali *et al*. 2003).

2.8.2 *Protein*

Protein is considered a key nutrient in older age. Muscle protein synthesis occurs in response to a complex interplay among stimuli such as physical activity and hormonal signalling (Figure 5). However in all circumstances the prerequisite for muscle protein synthesis is dietary protein (Wolfe 2012). Dietary intake of protein is vital to support muscle protein synthesis, as proteins act not only as a substrate for synthesis, by also as regulators (Phillips 2005). As previously outlined the effect of a feeding provides a stimulus for muscle protein synthesis however the quantity, quality and apportioning of daily protein intakes are all important considerations in this regard (Paddon-Jones and Rasmussen 2009, Paddon-Jones et al. 2008).

2.8.3 *Protein: Quantity*

Current estimates of the nutritional requirements for protein as reported by the WHO/FAO/UNU (2007) are defined as "the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintains the body protein mass (assumed to be at a desirable level), in persons at energy balance with modest levels of physical activity and any special needs for growth, reproduction and lactation". This definition, however, does not address the optimal intake for health. Instances where individual benefits may be derived from protein intakes in excess of the currently recommended 0.8 $g.kg^{-1}.d^{-1}$ may include older individuals who may gain advantage in terms of muscle mass, strength and functionality. These benefits may in turn be reflected in improved health outcomes (Wolfe 2012).

Currently there is no consensus on whether ageing requires an increase in protein dietary requirements. The current recommendation of 0.8 $g.kg^{-1}.d^{-1}$, established by the Institute of Medicine (IOM), was derived from short term nitrogen balance studies in young adults (Trumbo *et al*. 2002 and Rand *et al*. 2003), rather than on the maintenance of lean tissue mass in the elderly. While many studies support retaining the current recommended daily allowance (RDA) (Millward *et al.* 1997; Millward 1999), among the authors suggesting a moderate increase in protein intake to 1.0 – 1.3 $g.kg^{-1}.d^{-1}$ are Bunker *et al.* (1987), Evans *et al*. (2004), Campbell *et al*. (2001), Morais *et al*. (2006), Paddon-Jones *et al*. (2008) and Breen and Phillips (2011). This increase is thought to be necessary to offset the lower total energy intake observed in ageing (Hallfrisch *et al*. 1990), and decreased protein synthetic efficiency (Mosoni *et al*.1995; Arnal *et al*. 1999). Recently, the European Union Geriatric Medicine Society (EUGMS) established the PROT-AGE Study Group with a goal of developing updated evidence based recommendations for optimal protein intake by older people. International expert participants in this working group concluded that older people do require more dietary protein than younger people, and should consume in the range of 1.0 – 1.2 $g.kg^{-1}.d^{-1}$ (Bauer *et al*. 2013).

A combined analysis of several nitrogen balance studies in aging populations (56 – 80 years) has indicated greater needs of protein for the elderly (1.14 $g.kg^{-1}.d^{-1}$) relative to the young (0.8$g.kg^{-1}.d^{-1}$) (Campbell 1994). This research provided critical information in terms of adequate protein intake as a possible mechanism underlying sarcopaenia, particularly as protein intake has been reported as being inversely proportional to age (Rousset S 2003).

Observational evidence on protein intakes suggests that the majority of adults in the developed world meet or exceed the current RDA. The National Health and Nutrition Examination Survey (NHANES) data indicate that the mean protein intake of Americans, including the elderly, meet or exceed the RDA (Fulgoni *et al*. 2008). In Ireland the National Adult Nutrition Survey (NANS) (IUNA 2011) corroborates this, as does the predecessor to this national study the North / South Ireland Food Consumption Survey (NSIFCS) whose summary document states that the vast majority of people surveyed met or exceeded dietary protein recommendations (IUNA 2001). If research indicates that protein is required for muscle protein synthesis, and these observational studies indicate that protein requirements are by and large met, why is the worldwide incidence of sarcopaenia increasing?

2.8.4 *Anabolic blunting to protein feeding in the elderly*

The increase in MPS observed in the fed versus fasted state in the young does not seem to translate to an elderly population (**Figure** 9). An apparent defect in the stimulation of muscle protein synthesis has been shown in old rats (Mosoni *et al*.1995) and elderly humans (Arnal *et al*. 1999) after the ingestion of a normal protein meal. The reason for this defect is unclear because MPS responded normally if large amounts of amino acids were continuously infused in old rats (Mosoni *et al*. 1993) or given orally in aged volunteers (Volpi *et al*. 1999). Nevertheless, the studies agreed in that aged muscle is less sensitive to the stimulatory effects of protein and amino acids at physiological concentrations but is still able to respond if the increase of aminoacidaemia is large enough (Arnal *et al*. 1999; Volpi *et al*. 1999; Paddon-Jones *et al*. 2004). The same conclusion was drawn with essential amino acids (EAAs) by Katsanos *et al*. (2005) who showed in the elderly, that a small bolus of EAAs (~7 g) was unable to stimulate muscle protein synthesis whereas an increase in protein synthesis occurred normally when EAA intake was doubled.

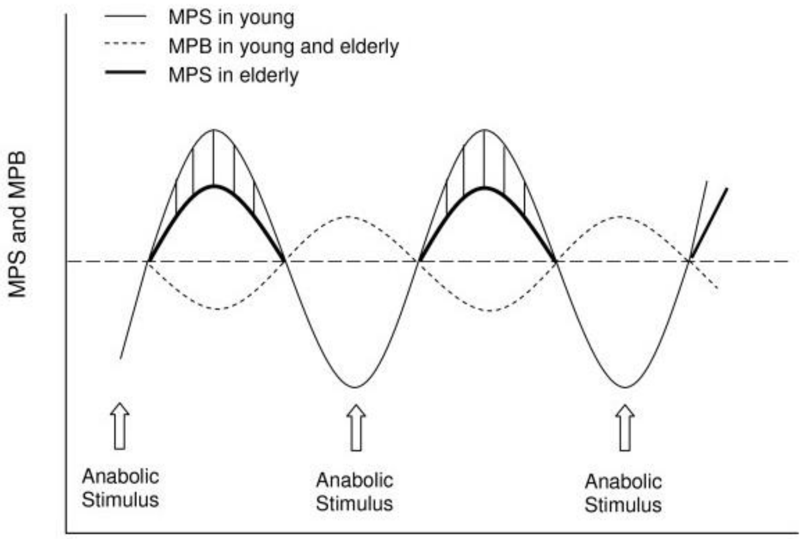


Figure 9 Schematic representation of muscle protein metabolism in response to anabolic stimuli (exercise and/or amino acid ingestion) in young and elderly.

Shaded section indicates the difference in MPS between elderly and young in response to anabolic stimuli.

In the context of anabolic blunting it is essential to consider that amino acids are physiologically introduced in the body through the gut, usually in the form of proteins. Digestion and absorption may be important limiting factors. This will be discussed further in the section on protein quality but studies have shown that the type of protein ingested may affect the protein anabolic response at the whole-body level.

Another obstacle that dietary amino acids need to overcome to reach the systemic circulation is the splanchnic tissues. Dietary amino acids must pass through the splanchnic tissues before they are made available for the peripheral tissues via the systemic circulation. It is well known that in young individuals the splanchnic tissues use a significant proportion of the dietary amino acids for their own metabolism (Mathews *et al*. 1993), limiting the amount of the ingested amino acids available for the peripheral tissues. There is some evidence that the importance of the splanchnic tissue in the regulation of whole-body protein turnover increases with age (Young 1990). If the splanchnic tissues utilise more amino acids in the elderly, the flow and availability of dietary amino acids for the peripheral tissues, including muscle, would be reduced. This hypothesis was corroborated by the observation of Boirie *et al*. (1997) that the first-pass splanchnic extraction of leucine increases with age; however, Volpi *et al*. (1999) reported that despite a higher first-pass splanchnic extraction of the amino acid phenylalanine in the elderly, the delivery to the leg increased to the same extent both in the young and in the elderly.

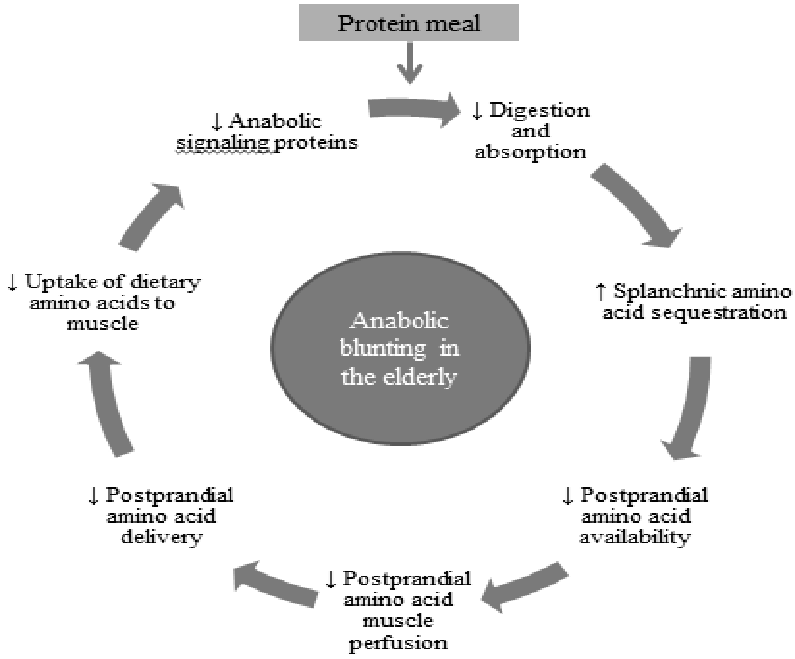


Figure 10 Factors influencing anabolic blunting to protein feeding in the elderly.

Ingestion of a protein meal should stimulate MPS, however many secondary factors may impact the magnitude of this response in aging, resulting in anabolic blunting

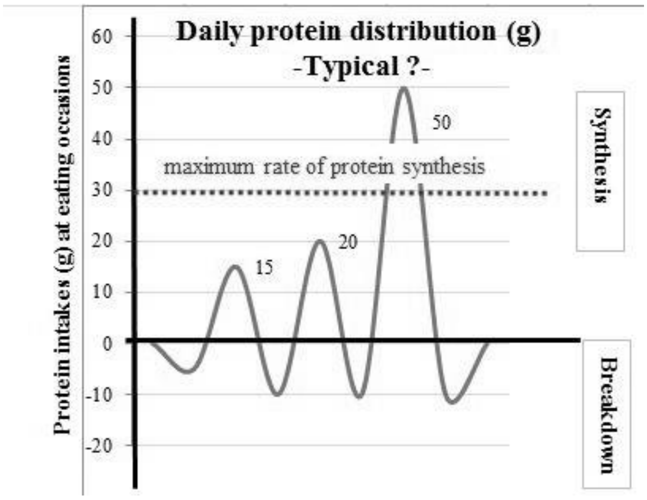
Breen and Phillips (2013) proposes that chronic rates of muscle protein synthesis in healthy older adults may not be much different from those of the young, and suggests that periods of disuse (oftentimes concomitant with disease), which occur with greater frequency during aging, are acute events during which anabolic resistance occurs. He concludes that it seems unlikely that in the healthy non- diseased aged persons there is a permanent state of anabolic resistance but more likely that acute periods of disuse are the times when the elderly may become, even transiently, anabolically resistant. During such times a period of accelerated muscle loss occurs from which it is hard for older persons to recover compared with the young (Breen and Phillips 2011).

2.8.5 *Protein: Apportioning*

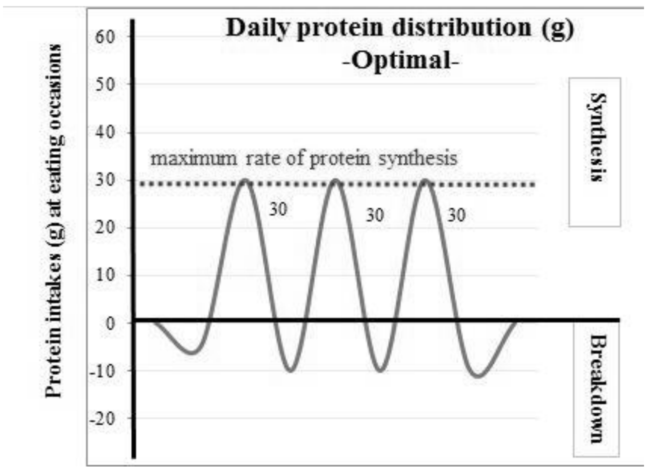
The distribution of protein intake throughout the day may influence muscle protein synthesis. The ingested daily allowance of protein should be spread equally across breakfast, lunch and dinner to optimally stimulate muscle protein synthesis (Breen and Phillips 2011). The influence of being fasted or fed on MPS has been widely reported on where feeding stimulates MPS to an extent where net protein balance (NPB) becomes positive, for a transient time. Symons *et al* (2009) indicate that it is more important to ingest a sufficient amount of high-quality protein (25–30 g) with each meal rather than 1 large bolus, because greater than 30 g in a single meal may not further stimulate muscle protein synthesis (‘muscle–full’ hypothesis) and Pennings *et al*. (2012) support this opinion. Muscle protein synthesis in both the young and older populations is increased by 50% when approximately 30g of essential amino acids (113 g lean beef) is ingested (Symons 2009). Importantly in older people, when protein is co-ingested with carbohydrate and when the protein quantity is less than 20 g per meal, muscle protein synthesis is blunted. A 20 g serving of most animal or plant based proteins should contain 5-8 g of essential amino acids (Symons 2009).

Observational studies on meal level intakes of nutrients are scarce relative to reported mean daily intakes (MDI). Rhodes *et al*. (2010) reported protein intakes on the NHANES III survey and Volpi (2013) extrapolated both day and meal level protein intakes based on distribution patterns and reported body mass. She comes to the conclusion that the average older person in the NHANES survey would only consume adequate protein to stimulate muscle protein synthesis at one meal in the day (dinner) with protein consumption estimated to be 31g. No other eating occasion would contain adequate protein to stimulate muscle protein synthesis. Tieland *et al*., (2012) report a typical feeding regimen in which the elderly typically ingest smaller amounts of protein with breakfast (~8 g) and lunch (~12 g) and the majority of dietary protein with dinner (~40 g). Lanham-New (2011) estimate typical protein feeding patterns at breakfast, lunch and dinner to be 10, 16 and 49 g respectively. These reports suggest that few achieve the optimal protein apportioning to maximise MPS.

Many studies have investigated the effects of different feeding patterns on muscle protein synthesis. In elderly people, higher protein turnover and better nitrogen retention were observed with three meals per day each providing an equal amount of protein (spread feeding) rather that pulse feeding where 80% of total protein intake was provided at a midday meal (Arnal *et al*. 1999). However, very large amounts of protein at one meal do not seem to be more effective than more moderate servings (Paddon-Jones and Rasmussen 2009). They report that a large single dose of 34 g of lean beef (containing 90 g protein) did not elicit a greater anabolic response than a serving one-third the size. They conclude that ingesting more than 30 g protein at a single meal may be an inefficient means of stimulating muscle protein synthesis. This is in agreement with a study by Borsheim *et al*. 2008, that showed no benefit of very large amounts of protein for 10 days (3.0 vs. 1.5 $g.kg^{-1}.d^{-1}$) in terms of muscle protein synthesis.



A



B

Figure 11 Changes in muscle protein synthesis with different apportioning of daily protein intake

In **Figure** 11 graph A depicts what is thought to represent a 'typical' distribution of daily protein intake where graph B depicts the optimal distribution required to maximise rates of muscle protein synthesis (Paddon-Jones and Rasmussen 2009, Symons 2009). Where these figures depict changes in muscle protein synthesis expected in just one day, the extrapolation of this theory to habitual patterns of protein intake could have very significant benefits for the preservation of lean tissue mass in the elderly. Over a six-month period with optimal protein apportioning there would be 546 (3 meals daily x 7 days weekly x 26 weeks) opportunities to maximally stimulate muscle protein synthesis. If the recommendation for protein dose at meal level is not achieved at one eating occasion daily this decreases the number of opportunities to 364, with a further decrease to 182 occasions on which muscle protein synthesis is optimally stimulated if the recommendations are met at only one meal daily. Redistributing protein foods throughout the day to optimise protein apportioning in the context of MPS seems a logical approach to preventing age related lean tissue mass loss. This strategy could increase the opportunities to stimulate MPS by 33-66%.

2.8.6 *Protein: Quality*

In addition to the quantity and apportioning of protein ingested, there appears to be subtle differences in the ability of different protein sources to promote muscle protein synthesis. Differences have been observed in the effect on muscle protein synthesis of casein, whey or soy (Wilkinson *et al*. 2007). Milk protein and its main isolated constituents, whey and casein, offer anabolic advantages over soy protein (Wilkinson *et al*. 2007; Tang *et al*. 2009). Furthermore, whey protein seems to induce a greater muscle protein synthetic response when compared with casein (Tang *et al*. 2009). The differences in the muscle protein synthetic response to the ingestion of various protein sources can be attributed to 1) differences in protein digestion and absorption kinetics (Tipton *et al*. 2004; Koopman *et al*. 2009) as well as 2) amino acid composition (Tang *et al*. 2009; Pennings *et al*. 2011).

2.8.6.1 Protein digestion and absorption kinetics

Following protein ingestion, the kinetics of protein digestion and absorption determine at which rate the dietary protein-derived amino acids become available in the circulation (Boirie 1997). This rate has been proposed to represent a key-factor regulating postprandial muscle protein synthesis (Dangin 2001). However, protein digestion and amino acid absorption kinetics seem to affect postprandial muscle protein retention differently at a more advanced age (Dangin 2001). Whereas young adults show a more positive whole-body protein balance following ingestion of a slow digestible protein (casein), older adults show a more positive whole-body protein balance following ingestion of a faster dietary protein (whey) (Koopman *et al*. 2009).

The findings on fast versus slow digestible protein indicate that the threshold of plasma amino acid concentration that needs to be reached to allow an adequate muscle protein synthetic response is elevated in the elderly. In an habitual diet, we rarely consume only ‘fast’ or ‘slow’ protein sources in isolation. In fact, most meals consist of different protein sources and combine protein with other macronutrients (Tieland 2012). Therefore, meal composition might be an important factor that affects digestion and absorption kinetics of dietary protein and its subsequent effect on muscle protein synthesis. For example, co-ingestion of other macronutrients has been reported to affect gastric emptying and modulate splanchnic extraction of dietary protein (Fouillet 2001). Besides the composition of a meal, the matrix of the food and its mastication are likely important factors that drive protein digestion and absorption kinetics. Normally, chewing is the first transformation process of consumed food on its way to the gut. Aging has been associated with decreased chewing efficiency, leading to a lower disruption of swallowed food (Mioche 2004).

Besides choosing specific fast digestible protein sources and/or changing the composition of the macronutrients in the diet, also the structure of a (slow) protein can be modified to resemble a fast digestible protein. By enzymatic hydrolysation of slow digestible intact micellar casein, a casein hydrolysate can be obtained consisting of >90% di- and tripeptides, while retaining its original amino acid composition. Koopman *et al*. (2009) performed a study in which elderly men consumed a single bolus of intact casein and casein hydrolysate in a cross-over design. The results showed that the casein hydrolysate resembled a faster digestible protein, which resulted in a greater availability of dietary amino acids in the circulation during the 6 h postprandial period and tended to further increase muscle protein synthesis rates when compared with the slower digestible intact casein.

In summary, modulating protein digestion and absorption kinetics represents a powerful tool to promote postprandial muscle protein synthesis rates in older adults. While some proteins have the appropriate chemical properties, others could be made more anabolic with some structural changes without affecting their unique amino acid composition.

2.8.6.2 Protein amino acid composition

Traditionally, amino acids have been classified by their essentiality based on growth or nitrogen balance. It was assumed that humans could synthesize sufficient amounts of all non-essential amino acids (NEAA) and did therefore not need them provided in dietary intakes for optimal nutrition and health. However, growing evidence suggests that some of the traditionally classified NEAA (glutamine, glutamate and arginine) play significant roles in signalling pathways involved in the regulation of gene expression, intracellular protein turnover, nutrient metabolism and oxidative defence (Wu 2010). As a result of this, in an insightful review (Wu 2013) it is proposed that the long-standing classification of AAs as essential or non-essential has limitations in protein nutrition. He proposes the use of the term functional amino acids (FAA), defined as those AAs that have a role to play in the regulation of metabolic pathways to improve health, survival, growth, development, lactation and reproduction of organisms. However, the Institute of Medicine does not recommend a daily intake for NEAAs, choosing to publish recommendations on EAAs only. In light of the many non-nutritive roles of both essential and nonessential amino acids, there would seem to be ample justification for Wu's proposal for a re-classification of traditional amino acid nomenclature to reflect functionality.

In the context of MPS, many researchers have conducted studies on amino acids to determine which may have the more potent effects. Volpi *et al* have conducted experiments investigating muscle anabolism and catabolism, and amino acids in both young and elderly subjects (Volpi *et al*. 2001, Volpi *et al.* 2003). In 2003, they assessed whether nonessential amino acids are required in a nutritional supplement to stimulate muscle protein anabolism in the elderly and reported that it is the essential amino acids (EAA) that are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Other research supports the theory that the EAA are primarily responsible for stimulating MPS (Tipton *et al*. 1999), whereas non-essential amino acids appear ineffective in this regard (Smith 1998).

Table 2 Traditional classification of amino acids in human nutrition Source : Laidlaw & Kopple, 1987

Essential•	**Non-essential^**	**Conditional essential***	**Precursors of Conditional essential**
Histidine	Alanine	Arginine	Glutamine / glutamate, aspartate
Isoleucine	Asparagine	Cysteine	Methionine, serine
Leucine	Aspartic acid	Glutamine	Glutamic acid / ammonia
Lysine	Glutamic acid	Glycine	Serine, choline
Methionine	Serine	Proline	Glutamate
Phenylalanine		Tyrosine	Phenylalanine
Threonine			
Tryptophan			
Valine			

•cannot be made by the body, and must be supplied by food; not essential, except in times of illness and stress; ^are made by the body from essential amino acids or in the normal breakdown of proteins*

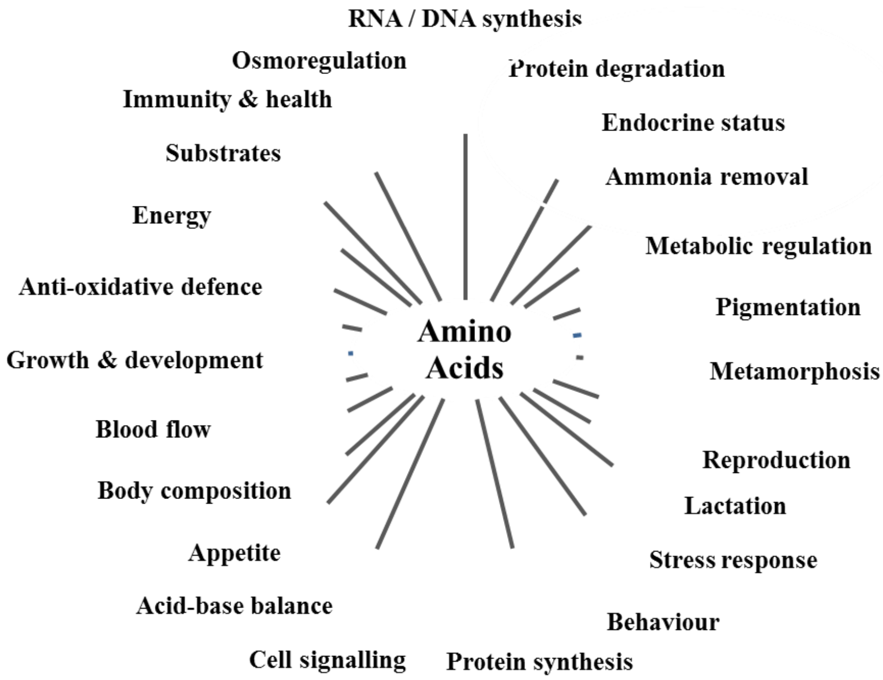


Figure 12 Roles of amino acids in homeostasis and nutrition (adapted from Wu 2013)

The same anabolic blunting described previously applies to feeding amino acids as it does to whole proteins. This age-related decline in the ability to utilise exogenous amino acids for protein synthesis could be due to alterations in the whole-body amino acid turnover that reduce the availability of exogenous amino acids for the muscle tissue limiting the availability of substrate, or to alterations in the response of muscle to the hormonal stimuli that normally follow a meal (Fujita and Volpi 2004).

Among amino acids, branched chain amino acids and especially leucine, are the most efficient for protein synthesis stimulation (Proud 2002). It has been demonstrated that leucine alone is able to stimulate muscle protein synthesis to the same extent as all amino acids (Dardevet *et al*. 2000; Anthony *et al*. 2002a; Anthony *et al*. 2002b; Lynch *et al*. 2002; Crozier *et al*. 2005). Amino acid supplements without adequate leucine reportedly do not stimulate protein synthesis (Katsanos, 2005; Rieu *et al*. 2007; Hayes 2008).

2.8.7 *Leucine*

Ingestion of an amino acid mixture containing extra leucine has the potential to affect muscle protein metabolism in several ways. The ability of leucine to influence MPS can be part explained by the fact that leucine has a dual role in lean tissue mass anabolism, both as a substrate and a regulator through activation of the mammalian target of rapamycin (mTOR) pathway (Anthony 2000; Crozier 2005; Atherton *et al*. 2010). mTOR is a protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription (Proud 2002).

The stimulatory effect of leucine on MPS (Figure 13) is mediated by up-regulation of the initiation of mRNA translation. A number of mechanisms including phosphorylation of ribosomal protein S6 kinase (p70S6K), eukaryotic initiation factor (eIF)4E binding protein1 (eIF4E-BP1), and eukaryotic initiation factor 4G (eIF4G) contribute to the effect of leucine on translation initiation. A key component in the signalling pathway controlling these phosphorylation induced mechanisms is the protein kinase, mTOR. The activity of mTOR toward downstream targets is controlled in part through its interaction with the regulatory-associated protein of mTOR (known as raptor) and the G protein β-subunit-like protein. Some studies propose that leucine activates mTOR in part through the inhibition of the adenosine monophosphate protein kinase (AMPK), i.e. the AMPK activity is reduced with intracellular presence of leucine and when AMPK is activated, the mTOR signalling is impaired (Du *et al*. 2007). While insulin alone is known not to have stimulatory effects on MPS, it seems that concomitant ingestion with the BCAA leucine acts in a permissive manner to allow signalling through mTOR.

Ingestion of extra leucine may be particularly important for the stimulation of skeletal muscle protein synthesis in the elderly because evidence from animal studies indicates that skeletal muscle protein synthesis becomes less responsive to the stimulatory effects of leucine with aging (Dardevet *et al*. 2000). Additional evidence indicates that meals supplemented with leucine improve the postprandial muscle protein synthesis in old rats (Rieu *et al*. 2003). In human subjects, investigators have reported a 'leucine threshold' (Breen & Philips 2011) which must be surpassed post protein ingestion to stimulate muscle protein synthesis. In an elderly population this threshold appears to be higher to elicit the same anabolic response (Katsanos 2006; Rieu *et al.* 2006) (**Figure** 14).

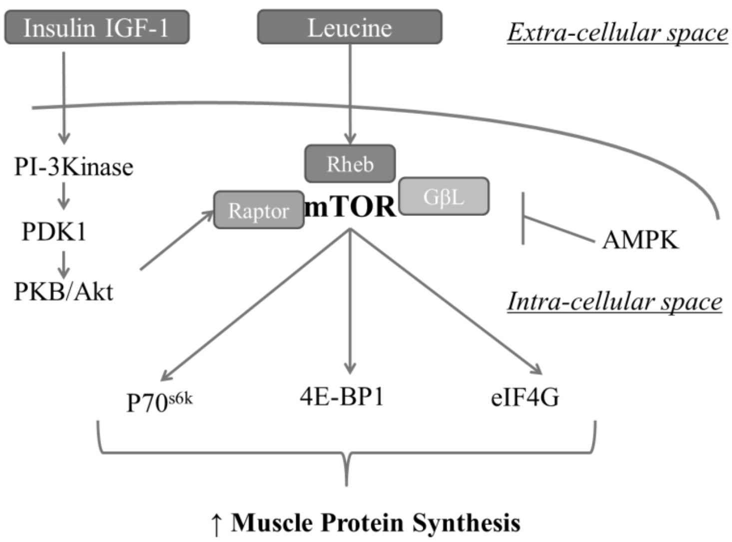


Figure 13 Signalling from leucine and IGF1/ insulin through mTOR to increase protein synthesis

(IGF^{-1}: insulin like growth factor, PKB/Akt: protein kinase B, AMPK: adenosine monophosphate protein kinase, mTOR: mammalian target of rapamycin, p70S6K: ribosomal protein S6 kinase, eIF4E-BP1: eukaryotic initiation factor 4E binding protein 1, eIF4G: eukaryotic initiation factor 4G)

The estimated threshold dose of leucine for stimulation of muscle protein synthesis in older adults has not been agreed upon by researchers in this area. Paddon-Jones & Rasmussen (2009) report the threshold to be approximately 3g, corresponding to approximately 25-30g of a high quality protein. Breen & Phillips (2011) report a slightly lower leucine threshold at approximately 1.5 – 2.0g of leucine contained in 15 – 20g whey protein. This is considerably higher than the estimated leucine threshold in a young population (<1g leucine in 2.5g EAAs). Katsanos *et al*. (2006) recommend the same dose to stimulate MPS in both young and elderly; 6.7 g of EAA (typically found in 15 g of whey protein) enriched with leucine (2.8 g).

There are few published reports on amino acid intakes in any population, making it difficult to determine whether recommended intakes for EAAs are met, either at day or meal level. Rhodes *et al*. (2010) reported protein intakes on the NHANES III survey and Volpi (2013) extrapolated both day and meal level protein intakes based on distribution patterns and reported body mass. She comes to the conclusion that the average older person in the NHANES survey would only consume adequate amino acids to stimulate muscle protein synthesis at one meal in the day (dinner). No other eating occasion would contain adequate specific amino acids to stimulate muscle protein synthesis. Similarly to the argument put forward in discussing protein apportioning, redistribution of leucine intakes could be a countermeasure to age related lean tissue mass loss.

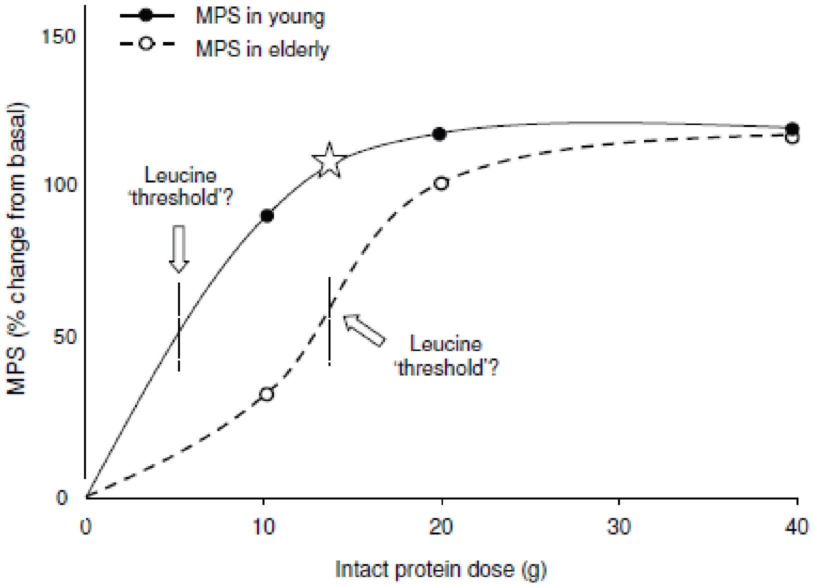


Figure 14 Leucine threshold in young and elderly (with permission from Breen and Philips 2011)

The star marks the dose reported by Katsanos et al. to stimulate MPS in both young and elderly; 6.7 g of EAA (typically found in 15 g of whey protein) enriched with leucine (2.8 g).

Overall, these data indicate a requirement both for amino acids, in particular leucine, and an energy source for activation of this key step in translation initiation. This clearly makes sense – amino acids are the precursor for protein synthesis, leucine being an essential amino acid, and protein synthesis consumes a large proportion of total cellular energy. While the headline result from the leucine studies mentioned above are encouraging, an important emerging caveat is that leucine supplementation is not unconditionally effective. Specifically, ingestion of supplemental leucine appears to be of little benefit to younger adults (Glynn *et al*. 2010) and/or, individuals who habitually consume a protein/leucine-sufficient diet (Verhoeven 2009).

2.8.8 *Anti-oxidant nutrients*

There is increasing interest in the role of oxidative stress in the aetiology of age-related muscle mass loss; markers of oxidative damage have been shown to predict impairments in physical function in older adults (Semba 2007). Damage to biomolecules such as DNA, lipid and proteins may occur when reactive oxygen species (ROS) are present in excess in cells (Meng and Yu 2010). The actions of ROS are normally counterbalanced by antioxidant defence mechanisms that include the enzymes superoxide dismutase and glutathione peroxidase, as well as exogenous antioxidants derived from the diet, such as selenium, carotenoids, tocopherols, flavonoids and other plant polyphenols (Semba 2007, Jeong-Su Kim et al. 2010). In older age, an accumulation of ROS caused by chronic low-grade inflammatory processes (CLIP) (Beyer 2012), altered dietary habits or deranged cellular responses to ROS may lead to oxidative damage and contribute to losses of both muscle mass and strength (Kim *et al*. 2010).

Skeletal muscle depletion is characterized by a negative nitrogen balance, which results from disruption of this equilibrium due to reduced synthesis, increased breakdown, or both. There has been increasing interest in experimental data considering hypercatabolism as the major cause of muscle protein depletion. Several signalling pathways that contribute to muscle atrophy have been identified (Meng and Yu 2010) (**Figure** 15) and there is increasing evidence that oxidative stress, due to ROS production overwhelming the intracellular antioxidant systems, plays a role in causing muscle depletion both during aging and in chronic pathological states. In particular, oxidative stress has been proposed to enhance protein breakdown, directly or by interacting with other factors.

The first indication that oxidative stress (when the balance between oxidants and antioxidants is perturbed) occurs in the skeletal muscle derives from the observation that physical exercise is associated with increased production of ROS (Dillard 1978). Since the concept was introduced many reports in the literature have described the occurrence of oxidative stress, due to enhanced ROS production, impaired antioxidant systems, or both, in physiological as well as pathological states associated with muscle atrophy. ROS production has been shown to increase in the skeletal muscle during aging (Vasilaki 2006, Shigenaga 1994), and oxidative stress has been claimed to be relevant to age-related cell damage (Cadenas 2000).

Meng and Yu (2010) report extensively on the interactions between oxidative stress and the progression of sarcopaenia. A schematic summary of their findings is outlined below (**Figure**

15). Some major signalling pathways are activated or inactivated during the oxidative stress and chronic inflammation observed in aged skeletal muscle. The pathways are related to an imbalance of protein synthesis and breakdown, mitochondrial dysfunction, and apoptosis, leading to muscle fibre atrophy and loss, and eventually to sarcopaenia.

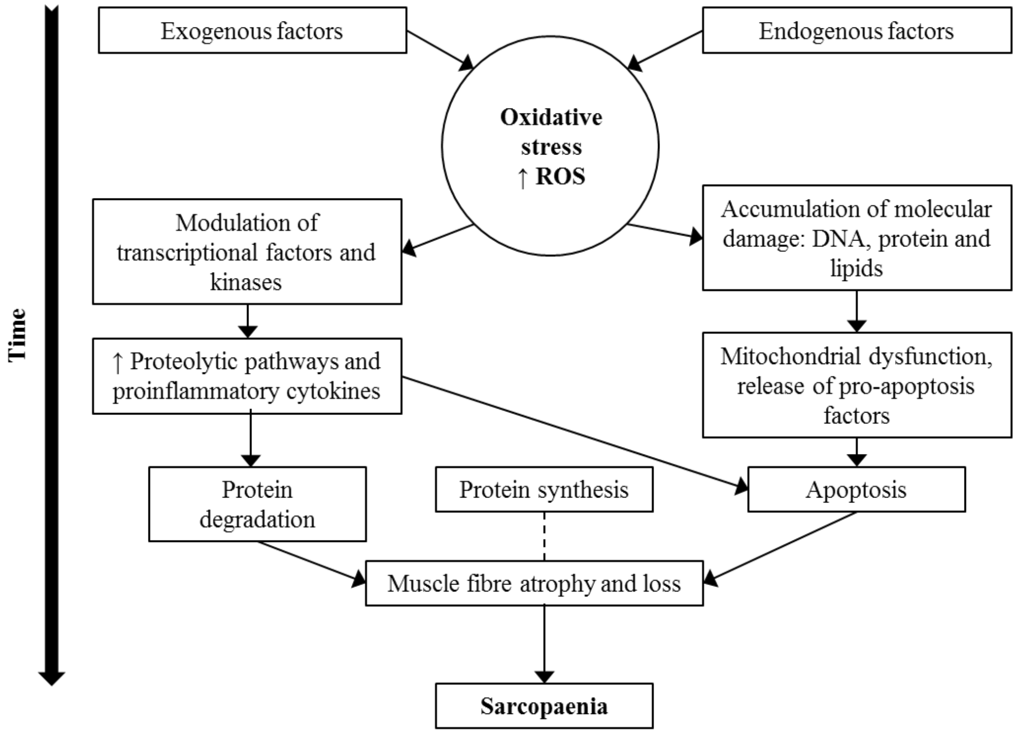


Figure 15 Schematic outlining the proposed mechanisms by which oxidative stress and chronic inflammation could contribute to sarcopaenia (adapted from Meng and Ju 2010)

In addition to derangements directly occurring at the skeletal muscle level, sarcopaenia in aged individuals is also associated with reduced antioxidant dietary intake (Chaput *et al*. 2007). Numerous studies have described low dietary intakes of antioxidant nutrients and relationships with poor physical capabilities. Inverse associations have been described for intake of vitamin C, E and carotenoids (Cesari and Pahor 2008, Semba et al. 2007) and physical function.

A number of observational studies have shown associations between antioxidant status and measures of physical function (Kaiser 2010, Semba 2007, Cesari 2004, Cesari et al. 2005). These observations are seen in both cross-sectional and longitudinal studies, where poor antioxidant status is predictive of decline in physical function and vice versa. Longitudinally, a decline in physical performance and an increased risk in becoming frail in 3 – 6 years was observed in elderly persons with low serum antioxidant levels (Semba 2007). Poor muscle strength and low physical performance have been associated with low levels of circulating carotenoids (Cesari 2004, Semba et al. 2007). In the InCHIANTI study (Cesari 2004) higher plasma carotenoid concentrations were associated with a lower risk of developing a severe walking disability over a follow up period of 6 years. Poor serum selenium status has also been associated with risk of impaired physical function (Kaiser 2010).

Although a lot of studies have investigated the possibility of delaying the aging process by enhancing, endogenously or exogenously, the organism's antioxidant capacity very little is known about the effectiveness of such strategies on the development of sarcopaenia. Some studies have shown that the treatment of aged individuals with high-dose β-carotene or vitamin E does not seem to replace the effects of diets high in fruits, vegetables, and whole grains and low in saturated fats (reviewed in Semba 2007; Harikumar *et al*. 2008). One trial shows that zinc supplementation does not affect oxidative stress status in elderly people (Andriollo-Sanchez *et al*. 2008). In addition, several reports have shown deleterious effects of antioxidant treatment on muscle function and redox state. Indeed, supplementation with vitamin E or ubiquinone has been shown to unfavourably affect endurance performance in training subjects (Sharman *et al*. 1971; Malm *et al*. 1996).

In summary, the above studies suggest that a diet supplemented with a combination of antioxidants may possibly increase antioxidant defences, lower muscle oxidative damage, and improve muscle protein balance during senescence. However, the effect of antioxidant supplementation on muscle performance is still largely controversial. Interestingly, a recent statement from the society on sarcopaenia, cachexia, and wasting disease does not even mention antioxidant supplementation as a possible tool to manage sarcopaenia in older persons (Morley *et al*. 2011). The hypothesis that derangements in the production, distribution or interactions of ROS can contribute to the aetiology of age related muscle mass loss is supported by experimental and clinical evidence. Similarly to other nutrient supports proposed in the prevention of muscle mass loss, there are both positive and null results. Caution should be used in recommending high dose antioxidants in the treatment of muscle atrophy and further work is required to substantiate routine use in wasting conditions.

A relatively new approach to assessing dietary antioxidant intakes is to examine the dietary total antioxidant capacity (TAC). It is thought that this approach may reveal the potential added benefits of concomitant ingestion of many antioxidant nutrients. The theory is that the synergistic action of these nutrients may be greater than their individual effects.

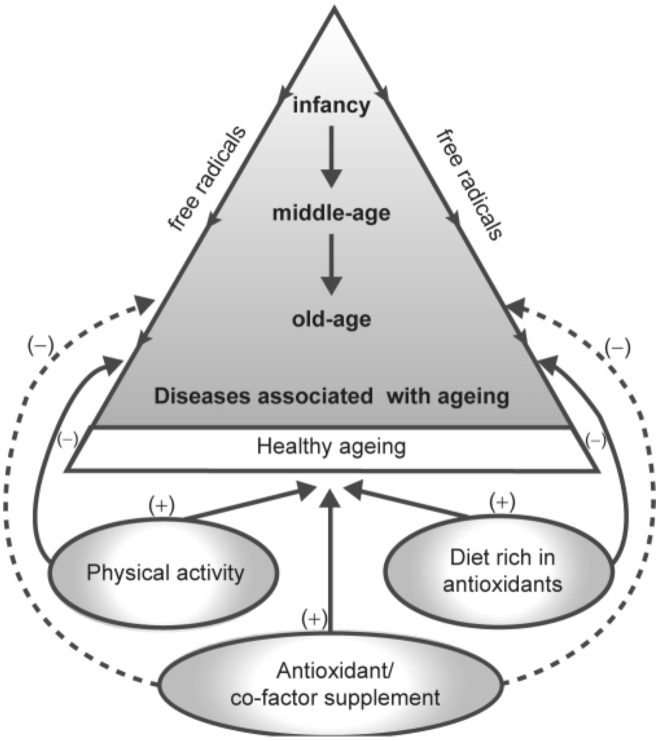


Figure 16 Summary of anti-oxidant factors involved in healthy ageing

(+) Promotes healthy aging; (–) Reduces free radical induced diseases.

Dietary TAC, based on the cumulative antioxidant activities of all the antioxidants present in food, has been shown to be inversely associated with risks of chronic diseases (Wang 2012), both in young and older populations (Valtueña 2008, Pellegrini et al. 2006, Del Rio 2011). Several methods were developed in the late 90s for measuring the TAC of food and beverages. The oxygen radical absorbance capacity (ORAC) assay is the assay of choice in the food and pharmaceutical industries and is often quoted in product literature. There is no WISP ©-based method established to evaluate dietary TAC, but this analysis has been carried out by the USDA database. This is discussed in greater detail in Chapter 4.

2.8.9 *Leucine and antioxidant nutrients concomitantly*

As discussed in previous sections muscle atrophy may be partly explained by a decreased ability of older muscle to respond to anabolic stimuli, such as food intake (Arnal *et al*. 1999).

This anabolic blunting is thought to mainly result from a decreased response and / or sensitivity of protein synthesis and degradation to amino acids in older muscle (Dardevet *et al*. 2000; Cuthbertson *et al*. 2005; Katsanos *et al*. 2005). Among amino acids, leucine can stimulate muscle protein synthesis to the same extent as all amino acids (Anthony *et al*. 2000a; Anthony *et al*. 2000b; Dardevet *et al*. 2000; Lynch *et al*. 2002; Crozier *et al*. 2005). These studies suggest that increasing leucine availability may be a viable nutritional strategy in the preservation of lean tissue mass loss in the elderly.

An alternative approach to the hypothesis of anabolic blunting in explaining age related lean tissue mass loss, outlined above, is the argument that damage caused by oxidative stress increases in the ageing. Because oxygen derived radicals are responsible for damage at the cellular and tissue level, they could be involved in the derangement of the anabolic stimulus of leucine observed in the elderly. While observational studies show an association between poor antioxidant status or intakes and muscle strength and performance (Semba 2007, Cesari 2004, Kaiser 2010), the impact of oxidative stress on muscle protein synthesis is still unknown.

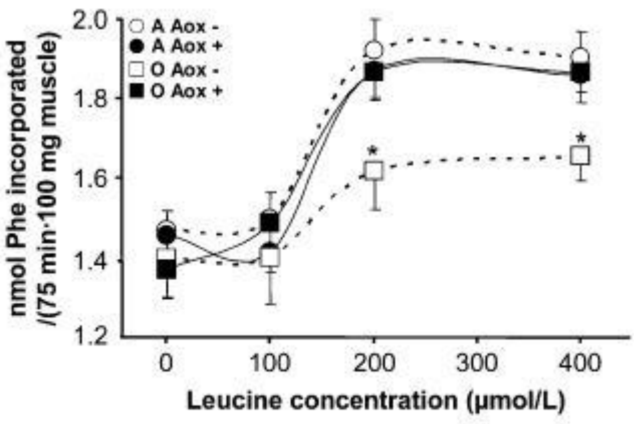


Figure 17 Dose response curve for leucine stimulated muscle protein synthesis in adult (A) and older (O) rats, with and without antioxidant supplementation (+/- ox) over 7 weeks (Marzani et al. 2008)

Marzani *et al*. (2008) aimed to address this question in a paper investigating the effect of antioxidant supplementation on muscle protein metabolism regulation by leucine in adult and older rats. The rats were fed either a control diet or the same food supplemented with antioxidant nutrients (rutin (a quercetin derivative with antioxidant and anti-inflammatory properties (Crespy 1999)), vitamin E, vitamin A, zinc and selenium) for 7 weeks. The results confirmed previous studies where the ability of leucine to stimulate muscle protein synthesis was reduced in older compared with adult rats. However, antioxidant supplementation improved the response of protein synthesis to leucine in older rats, and also improved the leucine- induced inhibition of protein degradation (Figure 17). This study suggests that the defect in leucine-stimulated protein synthesis may be reversed with antioxidant supplementation. The authors propose that this effect may be mediated by a reduction in inflammatory state or the oxidative stress, but the precise mechanisms involved remain to be determined.

2.8.10 *Vitamin D*

Vitamin D is produced endogenously when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis via a photolytic process (Byrdwell *et al*. 2008). It is biologically inert and must be metabolised to 25-hydroxyvitaminD3 in the liver and then 1α,25-dihydroxyvitaminD3 in the kidney before having physiological effects. The compound 25-hydroxyvitaminD3 (25(OH)D3) (referred as serum vitamin D hereafter) is monitored in serum to indicate vitamin D status.

Hypovitaminosis D, encompassing both vitamin D insufficiency and deficiency (deficiency is generally defined as a serum vitamin D concentration of < 30 nmol/L, but the definition of the milder state of vitamin D insufficiency is controversial (30-50 nmol/L) (Institute of Medicine 2010)), is highly prevalent in the general population and has potentially deleterious musculoskeletal effects. Hypovitaminosis D results from several factors, including reduced cutaneous vitamin D synthesis as a consequence of sunscreen use, increased skin pigmentation, inadequate sun exposure, poor nutrition, comorbid diseases, and the use of certain medications (Hill 2006).

It has been well-established that vitamin D plays an essential role in the regulation of calcium and phosphate homeostasis and in bone development and maintenance (DeLuca 2004). There is emerging evidence that vitamin D has an influential role in many other tissues including skeletal muscle. Early clinical descriptions of a reversible muscle weakness associated with vitamin D deficiency and/or chronic renal failure recognised a potential association between vitamin D and muscle (Boland 1986). The identification of the vitamin D receptor (VDR) on muscle cells (Zanello *et al*. 1997; Bischoff-Ferrari *et al*. 2004b) provided further support for a direct effect of vitamin D on muscle tissue. Although our understanding of the relationship between vitamin D and muscle function has advanced (**Figure** 18), a comprehensive understanding of the action of vitamin D on muscle tissue and how this translates to preventative measures against sarcopaenia are yet to be clarified.

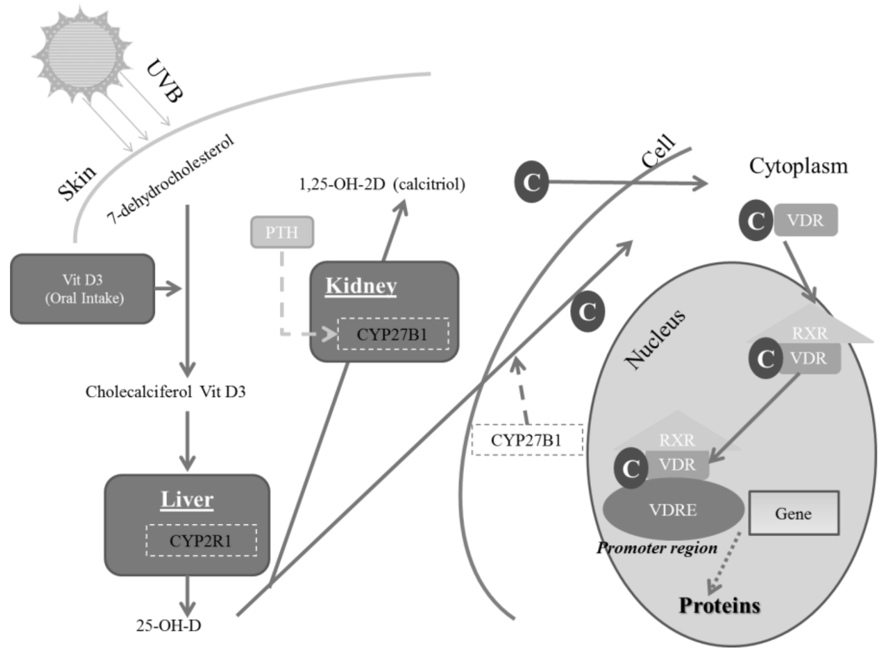


Figure 18 Schematic representation of vitamin D metabolism

Vitamin D in its activated form, 1,25-dihydroxyvitamin D, acts on muscle by binding to classical nuclear vitamin D receptors (VDR). This binding induces the heterodimerisation of active VDR and a steroid receptor, the retinoic x receptor, forming the VDR/retinoic x receptor/cofactor complex. This complex then binds to vitamin D response elements to regulate gene expression of mRNA, and subsequently de novo protein synthesis (adapted from Pierrot-Deseilligny 2013)

C: calcitriol, CYP2R1:vitamin D-25-hydroxylase, CYP27B1: 25-Hydroxyvitamin D3 1- α – hydroxylase, RXR : retinoid X receptor, UVB : ultraviolet B radiation, VDR: vitamin D receptor, VDRE: vitamin D-responsive element

In addition to the proposed role of vitamin D in increasing de novo protein synthesis, as outlined above, an additional potential benefit of vitamin D to ageing muscle may be its anti-inflammatory properties (suppression of C-reactive protein, TNF-α and IL-6 (Van den Berghe 2003, Schleithoff 2006).

The relationship between vitamin D and physical performance was investigated in NHANES (2004) and reported by Bischoff-Ferrari *et al*. (2004a). Serum vitamin D showed a significant relationship with time to perform both a walk and chair rise test. These were performed faster when serum vitamin D levels were higher. In the Longitudinal Ageing Study Amsterdam (LASA) (Visser *et al*. 2003) physical performance increased significantly (on a standard battery of tests) with increasing serum vitamin D, even after adjustment for potential confounders. In a recent randomized controlled trial in elderly institutionalized women with documented vitamin D insufficiency (documented as vitamin D levels of less than 50 nmol/L), the effects of different doses of vitamin D were tested on muscle strength, muscle mass, and bone density (Verschueren *et al*. 2011). Vitamin D supplementation at the conventional dose of 800 IU daily resulted in a level of circulating vitamin D > 50 nmol/l in all women at 6 months. During supplementation, muscle mass (assessed in the upper leg by CT scanning) did not change. However, supplements of vitamin D were associated with an improvement in dynamic muscle strength and hip bone density. The Osteoporosis Prospective Risk Assessment (OPRA) Study in Malmö in 986 women aged 25 years or older showed positive correlations between serum vitamin D levels and functional tests (Gerdhem 2007). An Australian study in Chinese adolescent girls showed a positive association between vitamin D status and handgrip strength (Foo 2009). In contrast, a German study conducted in 2001 did not find a significant association between muscle strength and serum vitamin D in postmenopausal women with osteoporosis (Pfeifer 2010).

There are no published results suggesting associations between vitamin D intakes, serum levels and increasing muscle mass. However since the growing unanimity among both the research and clinical communities is that sarcopaenia should not be defined solely on the basis of lean tissue mass but also on aspects of functional capability; there may be a role for vitamin D in treatment algorithms. The observed increases in muscle strength and function have been ascribed to binding of active vitamin D to specific vitamin D receptors found in human skeletal muscle (Figure 18) that promote protein synthesis and cellular growth (Bischoff-Ferrari *et al*. 2004; Pfeifer *et al*. 2009), although this has been recently challenged (Wang & De Luca 2011) where these authors claim that there are in fact no VDRs present in skeletal muscle, as they failed to locate them using the same methodology as Pfeifer *et al.*

The studies on vitamin D and muscle mass and function have a mixture of positive and null findings, which is unsurprising given the differences in study populations, initial degrees of insufficiency, and doses of vitamin D administered. However, collectively, they indicate a role for vitamin D in the development and preservation of skeletal muscle mass and function. In general, the associations between serum vitamin D and muscle strength or performance are significant in the lower range and may not apply in the "normal" or higher range. Perhaps correcting the increasingly prevalent hypovitaminosis D rather than supplementing those with sufficiency may be a logical approach.

The IOM advisory board on vitamin D stated (Institute of Medicine 2010) that 50 nmol/L is the serum vitamin D level that covers the needs of 97.5% of the population. Vitamin D is virtually absent from the food chain in amounts required to maintain serum levels at an adequate level or to promote health (DeLuca 2004). Due to its scarcity in food in its naturally occurring form, vitamin D is frequently added to foods in the United States and Canada (fortified milk and cereals), and frequently recommended as a dietary supplement. In Europe, vitamin D fortification of foods is largely absent and little vitamin D is made in the skin of individuals in the northern and southern regions during the winter months. The Food Safety Authority of Ireland (FSAI) suggests a daily vitamin D intake of 0 – 10 µg for adults aged 50-70 years (dependent on sunlight exposure and endogenous production). In Ireland 89% of 51-64 year olds do not achieve the 10 µg per day recommendation, with mean daily intakes (MDI) of vitamin D from food sources being 4.01 µg in men and 3.39 µg in women (Hill *et al*. 2004). Results from other studies (Hill *et al*. 2002; Andersen et al. 2003; Hill et al. 2003) observed low vitamin D status in 50-75 year old Irish women between late winter and early spring. These findings indicate that dietary intake of vitamin D is not sufficient in Ireland to reach recommendations for the elderly population and vitamin D supplementation should be promoted among this population group. Morley *et al*. (2010) recommend that those who are diagnosed sarcopaenic should receive vitamin D supplementation if serum vitamin D levels are below 100 nmol/L. Despite the fact that unanimity in the scientific community is currently lacking regarding vitamin D supplementation specifically in the prevention of age related lean tissue mass loss, the general consensus is that the recommendations for vitamin D intakes and circulating serum levels in frequently not achieved and that supplementation is wise, particularly in at risk populations.

2.8.11 *Other nutrients*

Nutritional interventions are an appealing potential means for the prevention and treatment of sarcopaenia of the elderly due to their easy applicability and safety. Many additional nutrients are under scrutiny for their potential roles in preventing age related lean tissue mass loss. Summary information on those nutrients with more tenuous links is below.

2.8.11.1 *Omega 3 fatty acids LCPUFAs*

Sarcopaenia is increasingly recognised as an inflammatory condition driven by oxidative stress and cytokines (Jensen 2008). Since eicosanoids are among the mediators and modulators of inflammation they may have a potential role in the prevention of age related lean tissue mass loss. There is some observational evidence to support an effect of n-3 long chain poly-unsaturated fatty acids (LCPUFA) on muscle function (Smith 2011), and a further RCT study suggesting an enhanced anabolic response to amino acid and insulin infusion with n-3 LCPUFA supplementation in older adults (Smith *et al*. 2011). However further evidence is needed to establish the therapeutic potential of n-3 LCPUFAs in sarcopaenia.

2.8.11.2 *Homocysteine, B_{12} and folic acid*

Higher homocysteine levels were associated with lower muscle strength, gait speed and increased disability in older persons in the NHANES study (Kuo 2007) and in the Osteoporosis Prospective Risk Assessment (OPRA) study a relationship between hyperhomocysteinaemia and poor physical function was reported (McDermott 2007). Since intakes of vitamin B_{12} and folic acid are able to correct high levels of homocysteine, it is suggested that supplementation of these nutrients may have a role to play in the preservation of lean tissue mass in the elderly (Mithal *et al*. 2012), but this has yet to be demonstrated in RCTs.

2.8.11.3 *Acid producing diets*

In light of the recommendation that the consumption of animal sources of protein should be increased to facilitate ingestion of essential amino acids throughout the day, some concerns have been raised that this may increase the acidity of dietary intakes (Dawson-Hughes 2008). It has been long known that acidosis has marked catabolic influences on muscle (May 1987), and it has been reported that alkaline diets favour lean tissue mass in older adults (Dawson-Hughes). The authors concluded that in their investigation of protein intakes and potassium excretion (as a marker of fruit and vegetable intakes) that increasing the intake of fruit and vegetables to the recommended level, may prevent some of the loss of lean tissue mass that occurs with ageing, but no further recommendation on supplementary potassium, or reducing the intakes of acid producing foods are made (Dawson-Hughes *et al*. 2008).

2.8.12 *Food and dietary patterns*

One problem with the existing evidence base for nutrition and preservation of lean tissue mass is that dietary components are often highly correlated with one another. This may explain in part why the effects of single nutrient supplementation may produce results less than that predicted by observational evidence. It also means that from observational data it may be difficult to understand the relative importance of individual nutrients on the preservation of lean tissue mass. Where β-carotene, for example, may be causally related to declines in physical function, it may also act as a marker of other components of fruit and vegetables. In turn, since diets are patterned, high fruit and vegetable consumption may be an indicator of other dietary differences which could have relevance in muscle function, such as greater consumption of oily fish. Semba *et al*. (2006) described the cumulative effects of nutrient deficiencies, where he estimated that each additional nutrient deficiency raised the risk of frailty in older women by almost 10%. This reinforces the importance of the quality as well as quantity of diets of older adults in the prevention of age related lean tissue mass loss.

2.8.13 *Lifelong nutrition habits*

An important consideration in the prevention of sarcopaenia is that muscle mass and function achieved in later life are not only determined by the rate of muscle decline, but also the peak mass accrued in early life. Factors that influence growth and development in early life, such as variations in nutrition, may contribute to muscle mass and strength in old age. A key consideration in this regard is that low weight at birth is a predictor of lower muscle mass and strength in adult life (Sayer 2010). To date few studies have investigated the role of diet in early childhood in the acquisition of muscle mass and effects on later function, despite there being some evidence that it could be important. The risk of frailty has been shown to be greater in older adults who grew up in impoverished circumstances, and who experienced hunger in childhood (Alvarado 2008). Further work is needed to understand how early life nutrition influences the acquisition of peak muscle mass, and the role played by nutrition in the trajectory of age related losses in mass and function.

2.9 Food based recommendations to prevent age related lean tissue mass loss

There is increasing evidence confirming the relationship between nutrition and muscle health, extending beyond the well-established roles of energy intake and dietary protein. There are limitations to current knowledge meaning that concrete recommendations on optimal intakes to prevent functional decline as a result of age related muscle mass loss remain elusive. Official nutrient guidelines for the prevention of sarcopaenia are not currently available. However, based on the observations described above, several recommendations can be given to those at risk which are in line with the general concepts of a healthy diet. These may well contribute to the preservation of lean tissue mass and strength and the prevention of sarcopaenia, and are unlikely to do any harm.

Unnecessary weight loss should be avoided; adequate energy must be consumed to avoid weight loss. Where weight loss is clinically recommended, guidance on adequate protein intake and appropriate resistance exercise to maintain lean tissue mass is required. In light of the lack of consensus on optimal protein intakes for the elderly, it seems reasonable to ensure an intake of at least 0.8 – 1.2 $g.kg^{-1}.d^{-1}$, particularly for those at risk of sarcopaenia. Daily protein intake should be optimally apportioned and of adequate quality to meet recommendations for amino acids. Current recommendations are per kg body mass, regardless of whether the individual is over or under weight. It seems inappropriate to feed protein at the same levels to both frail and obese populations. Feeding protein to an obese individual may increase adiposity in the absence of adequate and appropriate physical activity. Perhaps a more appropriate prescription for protein may be on the basis of lean tissue mass rather than total body mass. An adequate supply of vitamin D to prevent deficiency seems prudent in light of the research supporting optimal physical performance and fall prevention although specific recommendations on intakes required to prevent age related muscle mass loss are as yet elusive. Appropriate dietary intake of anti-oxidant nutrients may contribute to antioxidant defence, reduce oxidative damage and reduce functional decline. Since loss of peak muscle mass occurs from the age of 40 years, strategies to preserve mass should occur throughout life.

In her review entitled ‘The role of nutrition in the prevention of sarcopaenia’, Volkert (2011) outlines a summary of nutritional recommendations for the prevention of sarcopaenia (Table 3).

The conservative approach is that until more evidence is available, a varied, well-balanced diet containing adequate amounts of the nutrients outlined may be the best way to support the prevention of age related muscle mass loss (Millward 2012). Long-term studies relating dietary intake to clinical endpoints (muscle mass, physical performance and independence in daily routines), are clearly required to elucidate the precise nature of the relationship between nutrition and sarcopaenia. Casperson *et al*. (2012) are of the opinion that dietary supplementation of nutrients implicated in age related muscle mass loss, in particular protein and leucine, above reported habitual intake is required to prevent age related whole body lean tissue mass loss.

Table 3 Nutritional recommendations for the prevention of sarcopaenia

Securing of adequate amounts of energy and essential nutrients in preventing sarcopaenia	
Energy	24-36 kcal.kg^{-1}.d^{-1}
	Maintenance of body weight
	Avoidance of weight loss
	Avoidance of extreme over as well as under weight
Protein	0.8 – 1.2 g.kg^{-1}.d^{-1}
	High quality sources at each meal
Vitamin D	Regular fish consumption
	Daily exposure to sunlight
	Supplementation where required
Antioxidants	Varied diet rich in fruits, vegetables, plant oils, nuts, wholemeal products, meat, milk, cheese, tea and cacao
Omega-3-fatty acids	Vegetable oils like walnut, linseed, rapeseed and soy oil
	Fatty sea-fish like herring, mackerel or salmon
Routine screening for malnutrition	Early recognition of nutritional problems : loss of appetite, low food intake, unfavourable dietary habits and weight loss
	Identification and elimination of underlying causes

2.10 Dietary supplementation for the prevention of age related lean tissue mass loss

An interesting report from Fiatarone *et al*. (1994) describes how older subjects in an intervention study were given dietary supplements in the absence of increases in physical activity, and explains how the participants decreased their dietary intake accordingly, so that their total daily energy intake remained unchanged. This suggests that nutritional supplements for the elderly may need to be considered as dietary substitutes in practice, an idea supported by Fujita and Volpi (2004). However, if the nutrient content of the supplement is not different from the normal diet, it is unlikely that the supplementation will be effective. A nutritional supplement for the prevention of age related lean tissue mass loss should only contain the nutrients that are absolutely necessary for the stimulation of muscle protein anabolism, in order to achieve the highest anabolic efficiency per unit energy (Malafarina 2013). From the evidence presented above it would seem that consideration must be given to amino acids, in particular leucine, with vitamin D and antioxidant components recommended also.

Nutricia Medical recently launched a food for special medical purposes (FSMP) developed for the dietary management of age related loss of muscle mass and function. The product (Fortofit) was provisionally launched in Poland and Nutricia states that it is reviewing the feedback and viability of this product. The company information on key product characteristics claims that “the whey based formulation is optimal as it is fast acting, that the 10 g of EAAs and 3 g leucine per recommended portion, and that the addition of 20 µg / 800 IU vitamin D will all help to maintain muscle strength, function” (2014). The usage guidelines recommend an initiation period, when the 40 g portion is taken twice daily, at both smaller meals, followed by a maintenance period with administration once daily. With minimal modification, scientific literature would suggest that this could be a winning formula. It seems counter intuitive to prescribe this supplement once daily, considering the extant literature supports distributing protein intake across each eating occasion. The appropriateness of this maintenance dose is questionable.

Abbott Nutrition has also released a FSMP indicated for those patients with high protein requirements. ‘Promote is a complete, balanced, very-high-protein formula for patients who need a higher proportion of calories from protein, suitable for tube or oral feeding, either for supplemental or sole-source nutrition’(Abbot 2014). The suggested serving size of 237 mls contains 1kcal/ml and 14.8 g protein. With only 95 IU of Vitamin D and no essential amino acid content it appears to fall short of the product released by Nutricia.

Casperson *et al*. (2012) propose that an effective supplement should produce a robust anabolic response, and be i) low-volume and easily incorporated into existing menu plans, ii) palatable and iii) cost effective.

2.10.1 *Intervention studies on age related lean tissue mass loss*

Most of the research groups publishing in this area are calling for long term randomised controlled trials to establish the validity of the proposed nutrient solutions. Unfortunately there are not many published results on such interventions. The concomitant prescription of resistance training and nutritional strategies also make interpretation of this data more difficult (**Table 4**). A paper by Dillon *et al*. (2009) reported an increase in lean tissue mass and function following supplementation over a three month period with EAAs (7.5 g) twice daily in a between meal fashion. Wall and van Loon (2013) published a review on strategies used to offset muscle atrophy due to disuse, and conclude that there is a role for chronic EAA supplementation in this immobile population. Tieland *et al.* (2012) report increases in muscle mass and function with chronic supplementation of amino acids and optimal apportioning of intake throughout the day. A study by Payette *et al*. (2002) involving 83 community based elderly at risk of under-nutrition intervened with a nutrient-dense protein-energy liquid supplement and weekly visits providing encouragement to improve intake from other foods, over 16 weeks. They assessed anthropometric measures and functional outcomes and concluded that although weight gain was observed in the supplemented group, no other outcome indices were improved upon, and they suggest that increased physical activity may also be required to improve health and functional status. Tieland *et al*. (2012) published positive results from a 12 week nutrient intervention which received much attention from lead researchers in this field.

2.11 Conclusion

The data currently available underscore the importance of nutrition in the development, prevention and treatment of age related lean tissue mass loss. The precise composition of meals and supplements and their administration modality appear to be critical factors governing the successful use of nutrition as a means of treatment.

There are compelling data to support the ability of dietary protein to acutely stimulate muscle protein synthesis in ageing individuals, specifically essential amino acids, and chief among these is leucine. However there is insufficient longer-term research with defined health outcomes to specify an optimal value for daily ingestion or meal level apportioning. Whether and to what extent vitamin D may influence muscle mass and prevent muscle wasting remains unknown, but there is a clear indication that insufficiency will have detrimental outcomes for muscle function. The data on antioxidant nutrients are promising, particularly from observational studies but further work on interventions is necessary before RDAs can be developed. Validation of these proposed nutrient solutions to age related whole body lean tissue mass loss is imperative to facilitate the translation of these hypotheses into routine dietetic practice.

Table 4 Summary of studies investigating nutrition interventions and sarcopaenia

Authors	Study design & origin	Study population	Inclusion criteria	Exclusion Criteria	Duration	Measure of Body Comp	Treatment groups	Quantity and Type of Intervention	Main Outcomes	Functional Outcomes
Bunou *et al*. 2001	RCT, Chile n=149	Not specified	NS	Cancer, chronic infections, severe organ failure, diabetes.	18 months	DXA	4(Exer, Exer with Prot supp, Prot supp, Control)	E: 1 hour, twice a week. Prot: soup or porridge, twice a daily Cont: no exercise, no supplement	Mass outcomes: FFM no change.	Functional outcomes: walking capacity no change in E group, ↓* Cont.
Vukovitch *et al*. 2001	RCT, USA n=31	Volunteers	No contraindications to exercise, no medications, physician approval.	Uncontrolled hypertension, cardiovascular or kidney disease, diabetes.	8 weeks	DXA	2 (HMB supp, Control)	E: 5 days per week HMB Supp: 4 capsules, after each main meal (3 g day). Cont: placebo	Mass outcomes: FFM ↑ HMB Supp vs Cont.	Functional outcomes: leg curl ↑* HMB Supp vs Cont.
Wouters-Wessling *et al*. 2003	RCT, Netherlands n=101	Residence in a home for the elderly	Caucasian, ≥65 years, BMI ≤25 kg/m2, sheltered housing.	Cancer, gastrointestinal disorders, mentally unable to answer study questions.	6 months	BIA	2 (Pro supp, Control)	Prot: 125 mL, two times a day Cont: placebo	Mass outcomes: FFM no change.	Functional outcomes: grip strength no change.
Flakoll *et al*. 2004	RCT, USA n=57	Not specified	Women	Treatment for liver or kidney disease, uncontrolled hypertension, diabetes.	12 weeks	BIA	2 (HMB supp, control)	HMB Supp: 240 mL (HMB Supp 2 gr), with breakfast Cont: placebo	Mass outcomes: FFM ↑ HMB Supp vs Cont.	Functional outcomes: handgrip strength ↑* HMB Supp vs Cont.

Authors	Study design & origin	Study population	Inclusion criteria	Exclusion Criteria	Duration	Measure of Body Comp	Treatment groups	Quantity and Type of Intervention	Main Outcomes	Functional Outcomes
Solerte *et al*. 2007	RCT, Italy n=41	Not specified	Diagnosis of sarcopaenia	NS	4+4+8 months	DXA	2 (AA supp, Control)	Amino Acid Supp: snacks, two times a day. Control: NS	Mass outcomes at $8^{-1}6$ mo: LBM ↑*.	NS
Borsheim *et al*. 2008	Cohort, USA n=12	Impaired glucose tolerance	Impaired glucose tolerance	Heart, kidney or liver disease, hyperlipidaemia.	16 weeks	DXA	1 (EAA supp)	Ess Amino Acid Supp: 11 capsules between meals (11 gr/day).	Mass outcomes: LMB ↑* T12 vs T0, ↑ T16 vs T0.	Functional outcomes: ↑* walking speed T16 vs T0.
Baier *et al*. 2009	RCT, USA n=104	Not specified	≥65 years, G&G Test >10 but <20 sec.	Treatment for liver or kidney disease, uncontrolled hypertension, diabetes, morbidly obese.	12 months	BIA and DXA	2 (HMB supp, Control)	HMB Supp: 1 packet daily. Cont: placebo.	Mass outcomes: FFM ↑* in HMB Supp vs Cont.	Functional outcomes: no change G&G Test , ↓ handgrip strength in HMB Supp and Cont.
Cornish *et al*. 2009	RCT, Canada n=60	Healthy	≥60 years	Medication that affect inflammation, inflammatory disease, consumption of flaxseed products, resistance training ≥ 2 times per week	12 weeks	DXA	2 (ALA supp, Control)	E: 3 times/wk. ALA Supp: 30 mL of flaxseed oil (14 g ALA Supp) Cont: placebo	Mass outcomes: FM and total body mass ↓* in the two groups.	Functional outcomes: no change (1RM chest press and leg press).

Authors	Study design & origin	Study population	Inclusion criteria	Exclusion Criteria	Duration	Measure of Body Comp	Treatment groups	Quantity and Type of Intervention	Main Outcomes	Functional Outcomes
Dillon *et al*. 2009	RCT, USA n=14	Volunteers women	NS	Metabolically unstable medical condition, vascular disease, hypertension, cardiac abnormality, oestrogen supplementation, exercise trained.	3 months	DXA	2 (EAA supp, Control)	Ess Amino Acid Supp: 20 capsules, twice a day. Cont: placebo	Mass outcomes: LBM ↑* in Ess Amino Acid Supp vs Cont.	Functional outcomes: 1RM no change.
Verdjik *et al.* 2009	RCT, Netherlands n=26	Healthy	NS	Cardiac or peripheral vascular disease, orthopaedics limitations.	12 weeks	DXA	2(exer with Prot supp, Control)	E: 3 times/wk Prot: 250 mL (10 gr protein) before and after exercise session. Cont: placebo	Mass outcomes: leg lean mass and whole-body lean mass ↑* in Prot and Cont.	Functional outcomes: leg extension ↑* in Prot and Cont.
Verhoeven *et al*. 2009	RCT, Netherlands n=30	Healthy	NS	Cardiac or peripheral vascular disease, orthopaedic limitations, type 2 diabetes.	12 weeks	DXA	2(Leu supp, Control)	Leu Supp: 5 capsules, after each main meal (7.5 g day). Cont: placebo	Mass outcomes: FM and FFM no change.	Functional outcomes: 1RM for leg press and leg extension no change.
Baldi *et al*. 2010	RCT, Italy n=28	COPD	Weight loss >5% of body weight over previous 6 months.	Malignancy, gastrointestinal disorders, severe endocrine disorders, recent surgery, respiratory tract infection, oedema or use of diuretics.	12 weeks	DXA	2 (EAA supp, Control)	E: 2 sessions a day of 30 minutes. Ess Amino Acid Supp: 200 mL twice a day Cont: no supplement	Mass outcomes: FFM ↑* Ess Amino Acid Supp vs Cont.	NS

Authors	Study design & origin	Study population	Inclusion criteria	Exclusion Criteria	Duration	Measure of Body Comp	Treatment groups	Quantity and Type of Intervention	Main Outcomes	Functional Outcomes
Dal Negro *et al*. 2010	RCT, Italy n=32	COPD	BMI ≤23 kg/m2, > 40 yrs , FEV1 ≤50% predicted	Asthma, interstitial pulmonary disease, neoplasm, life expectancy < 1 year, mental deterioration, acute exacerbation of COPD in the last 4 wk.	12 weeks	BIA	2(EAA supp, Control)	Ess Amino Acid Supp: 4 g, two times a day. Cont: placebo	Mass outcomes: EEA ↑* FFM, LBM T12 vs T0.	Functional outcomes: EEA ↑* steps number T12 vs T0.
Carlsson *et al*. 2011	RCT, Sweden n=177	Residential care	Aged ≥65 years Dependent in 1+ ADL, Can stand from sit,	Pacemaker	3 months, w 3 months follow up	BIS	4(Exer, Exer with Prot supp, Prot supp, Control)	E: 45 minute, 5 times every 2weeks. Prot: 200 mL drink, 5 times every 2 weeks. Cont: placebo.	Mass outcomes: at 6 mo: E+Prot and E ↓* Prot and Cont muscle mass and body water.	Functional outcomes: at 6 mo: E+Prot and E ↑ Prot and Cont Berg Balance
Neelemaat *et al*. 2011	RCT, Netherlands n=210	Not specified	Aged ≥60 years, inpatient 2days +, BMI<20 kg/m2 +/- weight loss in prev 6 mnths	Senile dementia, impaired cognition / comprehension	3 months	BIS	2(Prot supp, Control)	Prot: protein enriched supplement and VitD3, once a day. Cont: no supplement.	Mass outcomes: FFM ↑ Prot vs Cont.	Functional outcomes: Prot ↑ Cont handgrip strength.

Authors	Study design & origin	Study population	Inclusion criteria	Exclusion Criteria	Duration	Measure of Body Comp	Treatment groups	Quantity and Type of Intervention	Main Outcomes	Functional Outcomes
Kim *et al*. 2012	RCT, Japan n=155	Not specified	Women Aged ≥75 years	Impaired mobility, cognition, unstable cardiac conditions, musculoskeletal conditions.	3 months	BIA	4 (Exer , exer with AA supp, AA supp, Control)	E: 60 minute, twice per week. Amino Acid Supp: packets of powdered, 3 g, two times a day. Cont: health ed.	Mass outcomes: legs muscle mass: E+Amino Acid Supp ↑*Cont.	Functional outcomes: walking speed, m/s E and E+Amino Acid Supp ↑*Cont.
Leenders *et al*. 2012	RCT, Netherlands n=60	Diabetic	Men	Cardiac or peripheral vascular disease, orthopaedics limitations, impaired renal function.	6 months	DXA	2(Leu supp, Control)	Leu Supp: 5 capsules, after each main meal Cont: placebo	Mass outcomes: FM and LBM did not change.	Functional outcomes: ↑* leg strength in Cont and Leu Supp group.

*NA not available, NS not specified, M men, W women, PM postmenopausal, +RET coupled to resistance exercise training, L(B)M lean (body) mass, FM fat mass, Prot protein, AA amino acid, CH carbohydrate, Leu leucine, EAA essential amino acid, Cont control, E exercise, BMI body mass index, BIA bioelectric impedance analysis, FSR fractional synthesis rate, US ultrasonography, 1RM one repetition maximum, DXA dual-energy X-ray absorptiometry; *, significant improvement (P < .05)*

Chapter 3

Comparing the incidence & implications of dietary under-reporting from four day estimated food intake records using 3 different methods in an Irish population (aged 50 – 70 years)

3.1 Introduction

A recent publication claimed to demonstrate that 40 years and millions of dollars of United States nutritional surveillance data were fatally flawed (Archer 2013). Among the aims of this research was to evaluate the validity of the National Health and Nutrition Examination Survey (NHANES) by comparing subjective measures of self-reported energy intakes (EI) with more objective predictions of basal metabolic rates (BMR), to determine whether reported calorie intakes were physiological plausible. Despite the frequent use of this method in nutritional surveillance data, the authors stated that 'methodological limitations compromise the validity of nutritional surveillance data'. They concluded that across the 39-year history of NHANES, EI data on the majority of respondents were not physiologically plausible. The authors continue by questioning the ability of policy makers to estimate population trends in energy intake and to generate policy relevant to diet and health from 'flawed data'. The furore that ensued suggested that the limitation to dietary intake data collection was a new discovery. This, of course is not the case, with most researchers cognisant that while a self-reported dietary assessment tool may be practical it is not always adequate (Schoeller 2013, Bingham 1987, Hirvonen et al. 1997). The nutrition research community is aware that erroneous conclusions derived from self-reported EI may adversely affect policy decisions involving nutrition and health. However, there are no clear guidelines on methods to screen for, whether to use, or how to interpret data obtained from misreported dietary intakes, that consider the practicalities of this data collection on a large scale.

Dietary data analyses present challenges that are difficult to address with traditional data collection methodology (Bingham 1987). Chief among the limitations of commonly used self-reported methods is under-estimation of dietary intake (Poslusna *et al*. 2009). The validity of food and nutrient intakes is primarily investigated through comparing reported energy intake with energy requirements, where energy intake is a proxy measure of food intake. This approach has shown that self -reported food intakes are frequently underestimated by all dietary assessments methods (Macdiarmid 1998). This underestimation is termed 'under-reporting' (UR), an as yet unresolved issue in dietary data collection.

3.1.1 *Identifying under-reporters*

3.1.1.1 Goldberg cut-offs

The most frequently used method to identify under-reporters in self-reported dietary intakes over the past two decades has involved the use of the Goldberg cut-offs. In 1991 Goldberg *et al.* introduced the idea of expressing energy intake (EI) from dietary assessment as a multiple of basal metabolic rate (EI:BMR). They devised cut-offs for EI:BMR below which energy intake for a 'person of a given age, sex and body weight could not live a normal life'. These cut-offs were presented in two forms; cut-off 1 and cut-off 2.

3.1.1.1.1 Cut-off 1

Cut-off 1 tested whether reported energy intakes represented habitual intake and was set at 1.35 where BMR was measured rather than estimated using predictive equations. The value of 1.35 was the mean physical activity level (PAL) reported in nine whole-body calorimetry studies on 207 sedentary participants. This cut-off did not accommodate for day-to-day variation in energy intake or physical activity and subsequent publications regarding this cut-off by one of the original authors (Black 2000) deemed it to be obsolete due to the failure of the equation to account for inherent variation in the required parameters.

3.1.1.1.2 Cut-off 2

Cut-off 2 tested whether reported energy intakes were 'a plausible measure of the food consumed during the actual measurement period'. Goldberg *et al.* (1991) proposed a formula for the calculation of cut-off 2 along with two tables of calculated cut-off 2 values for different sample sizes (1-2000), different periods of dietary recording (1-28 days) and according to whether BMR was measured or predicted with Schofield equations (Schofield *et al.* 1985). The formula for calculation of cut-off 2 represents the lower 95% or 99% confidence limit of agreement between EI/BMR and a PAL of 1.55, taking into consideration the within-person variation in energy intake, the within-person variation in the estimation or prediction of BMR and the within-person variation in PAL.

The formula is

$$\mathrm{EI_{rep}{:}BMR} > \mathrm{PAL} \times \exp\left[\mathrm{s.d.}_{\min} \times \frac{(S/100)}{\sqrt{n}}\right]$$

Where

$$S = \sqrt{\frac{CV_{wEI}^2}{d} + CV_{wB}^2 + CV_{tP}^2}$$

and

PAL	physical activity level
d	number of days of dietary assessment
n	sample size
s,d,min	-2 or -3 for 95% 0r 99% lower confidence limit
CV_{wEI}	within-person co-efficient of variation* in energy intake
$CV_{w\mathbf{B}}$	within-person co-efficient of variation in predicted BMR
CV_{tP}	within-person co-efficient of variation in PAL
rep	reported

**Coefficient of variation (C_V) - the normalized measure of dispersion of a probability distribution*

C_V = Standard Deviation / Mean

The within-person co-efficient of variation of energy intake used was 23% (the mean within-individual co-efficient of variation from 17 separate studies (Bingham 1987). The within-person co-efficient of variation for measured BMR used was 2.5% as it had been previously reported by Prentice *et al.* (1989). The within-person co-efficient of variation for predicted BMR using Schofield equations was 8% (Schofield 1985). The within-person co-efficient of variation for PAL, as assumed by the FAO/WHO/UNU report (1985) on energy requirements, was 12.5%. The PAL value used in the formula was 1.55. This was proposed by the FAO/WHO/UNU report (1985) and used in the absence of other accepted PALs in populations.

The cut-off 2 value for a population of 1500 respondents with 7 days of food intake data, for example is 1.54 (95% confidence limit). This value represents the minimum mean expected value of EI/BMR in this population. When each individual in the population is considered separately, the cut-off 2 value for n=1 with 7 days of food intakes is 1.1 (95% confidence limit). Individuals in the population with an EI/BMR below this value are not considered to have reported valid energy intakes over the duration of dietary assessment and should not be included in analysis (Goldberg *et al*. 1991).

3.1.1.1.2.1.1 Criticism for Goldberg cut-off 2

Black (2000), one of the original authors, has subsequently critically reviewed and re-assessed the use and application of the Goldberg cut-offs. She recommends that at the group level the Goldberg cut-off can be used to determine the probable degree of overall bias to reported energy intake in a study, stating that comparison should be made with a PAL value appropriate to the study population based on information about physical activity or lifestyle. The cut-off has low sensitivity (the proportion of those who under-report and who are identified) and poor specificity (the proportion of those who are not identified as under-reporters and who have under-reported) at the individual level (Livingstone and Black 2003). To address this shortcoming the authors recommend the use of a short questionnaire to capture data on activity levels in large studies . In small studies (n<100), Black writes that it is desirable to obtain a measure of energy expenditure. Detailed activity diaries for factorial calculation of energy expenditure, or the use of accelerometers or heart rate monitoring are all mooted as possibilities. If these measurements are obtained, energy intake may be compared directly with energy expenditure and the Goldberg cut-off is obsolete.

The FAO/UNU/WHO report now recommends a population PAL of 1.6 for adults following a meta-analysis of studies that involved a total of 411 men and women from 18 to 64 years of age. This showed a model value for PAL of 1.60 (range 1.55 to 1.65) for both men and women (Black *et al*. 1996). Median PAL values identified for adolescents and adults in this new report are 1.78 and 1.63 respectively with 1.63 representing the median PAL value of a reference adult population in which, like the UK, approximately 60% are overweight or obese (SACN 2012).

Since many authors conclude that the Goldberg approach of identifying under-reporters may be prone to misclassification due to the assumptions and formulae used to estimate total energy expenditure (TEE), (Tooze 2012, Ferrari et al. 2002, Poslusna et al. 2009, Bazelmans et al. 2007), other methods of identifying dietary misreporters have been proposed.

3.1.1.2 Other methods of identifying dietary under-reporters

Alternative and simpler cut-offs have been proposed based on the pivotal work of Goldberg (1991) and Black (2000). Predictive equations for BMR (based on sex, age, weight, height or a combination of these factors) can be used to identify persons whose self-reported energy intakes fall below some physiologically plausible cut-off (WHO 1985). This method is easy and inexpensive; however, predictive equations may not represent some population subgroups (Arciero 1993), include no measure of planned or structured activity-related energy expenditure, and identify only extremes of reporting error (Starling 1998). McCrory *et al*. (2002) propose the use of percentage difference between total energy expenditure (TEE) predicted from standard equations and reported EI, taking account of the within-individual error in these parameters. Carlsen and colleagues (2010) recommended calculating the accuracy of reported EI by expressing EI/EE, for which a value of 1.0 would mean complete agreement between EI and EE. Acceptable reporters (AR) were defined as having the EI/EE ratio in the range of 0.8 – 1.2, with under-reporters (UR) as EI/EE <0.8. These definitions were partly based on the 95% confidence limits of agreement between EI and EE measured by the double labelled water method previously reported by Black (2000).

3.1.2 *Incidence of under-reporting*

The prevalence of under-reporting in large nutritional surveys ranges from 18 to 54 % of the whole sample, but can be as high as 70 % in particular subgroups (Macdiarmid 1998, Rennie 2007). This wide variation between studies is partly due to different criteria used to identify under-reporters and also to non-uniformity of under-reporting across populations. The most consistent differences found are between men and women and between groups differing in body mass index. Women are more likely to under-report than men, and under-reporting is more common among overweight and obese individuals (Macdiarmid 1998). Other associated characteristics, for which there is less consistent evidence, include age, smoking habits, level of education, social class, physical activity and dietary restraint (Macdiarmid 1998).

Ferrari *et al*. (2002) reported on the validity of data from 24 hour recalls in 35,955 adults in ten countries from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. The prevalence of under-reporting (EI/BMR < 0.88) and over-reporting (EI/BMR > 2.72) was presented from men (8 countries) and women (all 10 countries). Under-reporting was highest in Greece at 20% of men and 33% of women. In the UK, 12% of men and 15% of women were classed as under-reporters. In all countries the prevalence for under-reporting was higher among women. It is noteworthy that the higher prevalence of dieting among women (lifetime prevalence estimated to be 75% in women and 47% in men (Jeffery et al.1991) should be considered in the context of any assessment of validity of self-reported dietary habits. A much smaller proportion of subjects over-reported energy intake (1 – 3% of men and 1 – 2% of women).

Briefel *et al.* (1997) analysed data collected with the primary dietary assessment instrument used in the US National Health & Nutrition Examination Survey (NHANES III), the 24-h recall, for 7769 non-pregnant adults to investigate under-reporting of total energy intake. Under-reporting was addressed by computing a ratio of energy intake (EI) to estimated basal metabolic rate (BMRest). EI:BMRest was 1.47 for men and 1.26 for non-pregnant women. A population level of 1.55 is expected for a sedentary population; 18% of the men and 28% of the women were classified as under-reporters. The Irish National Adult Nutrition Survey (NANS) (2011) details having had to exclude 33% of food intake records for incompleteness / inaccuracies / under-reporting where a ratio of EI:BMR of 1.1 was used as a cut-off point. This may not be the most appropriate screen to use as it is reported that a EI:BMR cut-off set at 1.1 identifies only 66% of those who under-report. (Medical Research Council (MRC) Dietary Assessment Report, 2014). As previously mentioned, Archer et al. (2013) evaluated the validity of the National Health and Nutrition Examination Survey (NHANES) by two methods. The first compared reported energy intakes (EI) with basal metabolic rates (BMR), while the second employed the use of the IOM factorial equations to estimate energy requirements and compared these to reported EI. They aimed to establish whether reported calorie intakes were physiological plausible. They concluded that across the 39-year history of NHANES, EI data on the majority of respondents were not compatible with normal life.

Given the absence of inexpensive, validated methods to readily identify under-reporting and statistical methods to correct for same, researchers are limited in their options to address this problem. Energy under-reporting can severely compromise the validity of self-reported dietary data. Goldberg’s cut-off 2 has been widely accepted and used throughout the last decade to identify dietary misreporters, but the recommendation from Black (2000) to increase both sensitivity and specificity to measure TEE is worthy of further investigation. Particularly in instances where correlations are made between dietary intakes and risks to health (age related whole body lean tissue mass loss), the validity of the reported intake is paramount.

3.2 Aims

The aim of this study was to compare the incidence of dietary under-reporting (UR) from self-reported estimated food intake records in a healthy, elderly Irish population by three established methods. Firstly, UR was identified by using Goldberg cut-off 2 with a revised physical activity level (1.6), as recommended by the (Scientific Advisory Committee on Nutrition (SACN) 2012)). The second method used a portable armband (BodyMedia™, SenseWear® Armband, Pittsburgh, PA) to measure PAL, as recommended by Black and colleagues (Black *et al.* 2001) in their review of the Goldberg cut-offs. An energy intake (EI) to energy expenditure (EE) ratio of between 0.8 and 1.2 as outlined by Carlsen *et al*. (2010) was used to define acceptable dietary reporters in this second method. Finally, the incidence of under-reporting was assessed using the IUNA NANS criteria under-reporting where a ratio of EI:BMR of 1.1 was used as a cut-off point.

An additional aim was to determine the influence on the estimated nutrient intakes of using different screening methods from self-reported food intake records in the study population.

3.3 Methods

Ethical approval was granted for the study by the Faculty of Education & Health Sciences Research Ethics Committee (EHSREC10/45), University of Limerick.

A cohort of elderly people (n=70, 53 female, 17 male) recruited from the *Healthy Ageing Study* (Chapter 6) was invited to participate in this study.

A four-day estimated food intake record (eFIR) was used to collect dietary intake data. Participants were instructed to record all food and drink consumed for four consecutive days to include one weekend day. Participants were asked to record detailed information regarding the amount (using household measure and estimates of portion sizes), and types of all foods, beverages and nutritional supplements consumed over the recording period and where applicable, the cooking methods used, brand names of the foods consumed and details of recipes. Data were also collected on the time of each eating or drinking occasion, the respondent's definition of each eating or drinking occasion (e.g. morning snack, lunch) and the location of the preparation of the meal or snack consumed (e.g. home, takeaway). Participants were weighed at the beginning and end of the four day eFIR to assess any change in body mass over the recording phase.

Food intake data were coded and subsequently analysed using WISP© (Tinuviel Software, Anglesey, UK). WISP© uses data from McCance and Widdowson's The Composition of Foods, sixth (Food Standards Agency 2002) and fifth (Holland *et al*. 1995) editions plus all nine supplemental volumes (Holland *et al*. 1988; Holland *et al*. 1989; Holland *et al*. 1991; Holland *et al*. 1992; Holland *et al*. 1993; Chan *et al*. 1994; Chan *et al*. 1995; Chan *et al*. 1996; Holland *et al*. 1996) to generate nutrient intake data. During this study, modifications were made to the food composition database to include recipes of composite dishes, nutritional supplements, generic Irish foods that were commonly consumed and new foods on the market. All previous modifications to the food composition database were also checked and updated from current manufacturers' information as necessary.

A number of quality procedures were put in place in an attempt to minimise error and ensure consistency throughout the collection and manipulation of food intake data. This was carried out by the same observer on two occasions separated by a minimum of one week and also by another independent researcher following the same data coding and entry procedures. It was stressed to respondents that they should not try to change or 'improve' their diet during the recording period. Random dual entries were performed on multiple food intake records to identify and reduce errors. Respondents were also asked if there were any items consumed during the recording period which had not yet been written down. Details on such items were recorded and subsequently entered to food intake records.

A SenseWear®™ Pro3 Armband (Bodymedia™) (SWA) was fitted and worn on the right arm for seven days (inclusive of the four day eFIR data collection phase) to monitor physical activity (Guidelines Appendix 7). The armband is a portable device worn on the upper, right arm that uses predictive equations to estimate EE based on data from accelerometers and four sensors capturing skin and near-body temperature, heat flux and galvanic skin response. The collected data, along with descriptors on age, gender, weight and height, allow calculation of EE through algorithms in the programmes software system (InnerView Professional Research Software7.1) (Bodymedia™). A log sheet was used to track activities performed when the device should not be worn such as swimming, other water based activities, bathing or showering.

Inclusion criteria necessitated at least one day of a matched valid eFIR with a day where the participant had >95% on body time for the SWA as described in a validation study by Scheers and colleagues (2012).

Three estimation methods were employed to calculate the extent of under-reporting in this population:

3.3.1 *Goldberg cut-off 2*

The first estimation method assessed under-reporting using the established Goldberg Cut-off based on EI:BMR (Goldberg *et al.* 1991). This cut-off is a population based method that takes into account the size of the sample population and the number of days of dietary intake recording. In the present study, the value using the lower 95% confidence interval was applied. This lower confidence interval represents the value below which it is statistically unlikely that the reported mean intake represents either 'habitual' intake or a low intake obtained by chance. For this sample population, a group mean EI:BMR of < 1.599 was indicative of under-reporting, with a value of < 1.594 for individuals assessed within the sample population.

$$\mathrm{EI_{rep}{:}BMR} > \mathrm{PAL} \times \exp\left[\mathrm{s.d._{min}} \times \frac{(S/100)}{\sqrt{n}}\right]$$

$$S = \sqrt{\frac{\mathrm{CV}^2_{\mathrm{wEI}}}{d} + \mathrm{CV}^2_{\mathrm{wB}} + \mathrm{CV}^2_{\mathrm{tP}}}$$

PAL	physical activity level	**1.6**
d	number of days of dietary assessment	**4**
n	sample size	**52**
s,d,min	-2 or -3 for 95% 0r 99% lower confidence limit	**-2**
CV_{wEI}	within-person co-efficient of variation in energy intake	**23%**
CV_{wB}	within-person co-efficient of variation in predicted BMR	**8%**
CV_{tP}	within-person co-efficient of variation in PAL	**12.50%**
S		**18.77%**
EIrep:BMR		**1.599**

Equation 1 Calculation of cut –off (EI:BMR) to identify dietary under-reporters based on Goldberg Cut-off 2

The second method used arose from a recommendation by Black (2003) that in populations with a sample size of less than 100, energy expenditure should be measured to assess under-reporting. Using the SWA and associated software mean EE was calculated. EE is a multiple of BMR and therefore represents the activity level of each subject as a ratio of BMR. Cut-off points identified by Carlsen and colleagues (2010) were then applied to these data to identify under-reporters.

3.3.2 *Carlsen method of identifying under-reporters*

Carlsen and colleagues (2010) recommended calculating the accuracy of reported EI by expressing EI/EE, for which a value of 1.0 would mean complete agreement between EI and EE. Acceptable reporters (AR) were defined as having the EI/EE ratio in the range of 0.8 – 1.2, with under-reporters (UR) as EI/EE <0.8. These definitions were partly based on the 95% confidence limits of agreement between EI and EE measured by the double labelled water method previously reported by Black (2000).

3.3.3 *IUNA NANS method of identifying under-reporters*

To allow comparison of nutrient intake data from the studies outlined in this dissertation (drawn from a convenience population local to the research site) to a more nationally representative sample, the methodology used to identify under-reporters used by the National Adult Nutrition Survey carried out by the Irish Universities Nutrition Alliance has also been applied. Here a ratio of EI:BMR of 1.1 was used as a cut-off point; values below this are deemed to have under-reported.

Data manipulation was conducted using SPSS© Version 20.0 for Windows ™ (SPSS Inc. Chicago, IL, USA). All data were tested for normality and homoscedasticity using Shapiro-Wilk and Levene's test respectively. Variables were described using the mean, median, standard deviation (SD) and inter-quartile range (IQR). Variables were tabulated for total population and split by gender as appropriate. A P value cut off of <0.05 was used to determine statistical significance.

3.4 Results

Twenty-two percent (n=16) of the participants were excluded as they failed to record dietary intake within ± 7 days of when the SWA was worn. A further 3% (n=2) were excluded as they failed to wear the SWA for the recommended ≤95% on body time (Scheers *et al.* 2012). Fifty-two healthy, elderly participants met the inclusion criteria and their physical activity and dietary data were further analysed.

The mean (SD) age of the population included in analysis (n=52, ♀=41, ♂=11) was 62.8(5.6) years. The mean BMI for the group was 27.1 (5.8) kg/m^2, with a prevalence of overweight of 56% (BMI ≥25 kg/m^2) of whom 17% were obese (BMI ≥ 30kg/m^2). **Table 5** (below) outlines mean energy and macronutrient intakes for the population. Data on physical activity and energy expenditure were extracted from the SWA and analysed using SenseWear® software (InnerView Professional Research Software7.1) (Bodymedia™). **Table 6** outlines summarised results of data on EE, EI and population, by gender. Applying the criteria previously outlined (Goldberg 1991; Black 2000; IUNA NANS 2011) those who under-reported dietary intakes were identified (**Table 7).** Self-reported dietary intake data with the various screens applied (Goldberg 1991; Black 2000; IUNA NANS 2011), reveal differences in both macronutrient and micronutrient intakes (Table 8).

Table 5 Reported daily energy and macronutrient intakes for population

	Mean	**SD**
	Total population n=52	
Energy (kcal)	1902	572
Protein (g)	79.8	18.6
Protein (%TEI)	17%	4%
CHO (g)	218.7	77.4
CHO (%TEI)	46%	6%
Total Fat (g)*	76.2	27.6
Fat (%TEI)	36%	6%
	Females n=41	
Energy (kcal)	1763	490
Protein (g)	74.7	15.1
Protein (%TEI)	18%	4%
CHO (g)	202.6	68.2
CHO (%TEI)	46%	6%
Total Fat (g)	72.5	25.7
Fat (%TEI)	37%	6%
	Males n=11	
Energy (kcal)	2419	580
Protein (g)	98.8	18.6
Protein (%TEI)	17%	3%
CHO (g)	279	82.9
CHO (%TEI)	46%	9%
Total Fat (g)*	90	31.2
Fat (%TEI)	33%	7%

*Data are presented as mean ± SD, (n=52, ♀=41, ♂=11);*not normally distributed; Normally distributed (Shapiro-Wilk p > 0.05). TEI, total energy intake; (the remaining 4% of TEI was contributed to by alcohol)*

Table 6 Estimated basal metabolic rate, reported energy intake, total measured energy expenditure and associated ratios, with misreporting data: kcal under-reported daily (and the degree of under-reporting in relation to reported energy intake

	All (n=52)		Female (n=41)		Male (n=11)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
BMR kcal	1520	306	1445	277	1828	221
EI_{rep} kcal/d	1902	572	1763	490	2419	580
TEE_{SWA} kcal/d	2388	419	2258	331	2921	309
EI_{rep}/BMR	1.30	0.48	1.27	0.45	1.40	0.63
TEE_{SWA}/BMR	1.59	0.22	1.59	0.24	1.61	0.14
EI_{rep}/TEE_{SWA}	0.81	0.27	0.80	0.26	0.86	0.32
EI UR by%	20%	29%	20%	26%	22%	40%
UR kcal/d	307	427	309	403	298	542

Basal metabolic rate (BMR kcal), reported energy intake (EIrep kcal/d), total measured energy expenditure (TEESWA kcal/d) kcal under-reported daily (UR kcal/d) and the degree of under-reporting in relation to reported energy intake (EI UR by%) (n=52, ♀=41, ♂=11) Values are means and SD, BMR was calculated by the Schofield equation, EI_{rep} Reported EI, TEE_{SWA} TEE measured by SenseWear® Armband, UR under-reporting

Table 7 Comparison on the incidence of dietary under-reporting using Goldberg, Carlsen and IUNA NANS methods

		Goldberg Method		Black Method		IUNA NANS Method	
		n	***%***	***n***	***%***	***n***	***%***
	All	42	81%	30	58%	22	42%
Under-reporters	**Female**	33	80%	23	56%	18	44%
	Male	9	82%	7	64%	4	36%

(Goldberg (recommended PAL and cut-off 2), Carlsen (measured PAL with an EI:EE ratio of >0.8 and <1.2), NANS methods (EI:BMR<1.1)(data are presented as mean ± SD, n=52, ♀=41, ♂=11)

The Goldberg method uses EI:BMR to assess under-reporting with a calculated cut-off based on assumed PAL for this sample population of 1.59. The alternative method proposed by Black is to use EI:EE where EE is measured using, in this instance, SWA. The acceptable EI:EE ratio range is 0.8 – 1.2, suggested by Carlsen *et al.* (2010) In the NAN Survey (IUNA 2011) a cut off of <1.1 EI:BMR was used to identify dietary under-reporters.

Goldberg cut-off 2 identified 81% of the population (n=42) as dietary under-reporters, where the revised Black/Carlsen method identified 58% (n=30) (Figure 19). The IUNA NANS method identified fewest with 42% (n=22) participants classed as dietary under-reporters by this method. These findings have clear implications for study design and sample size determinations made through statistical power calculations.

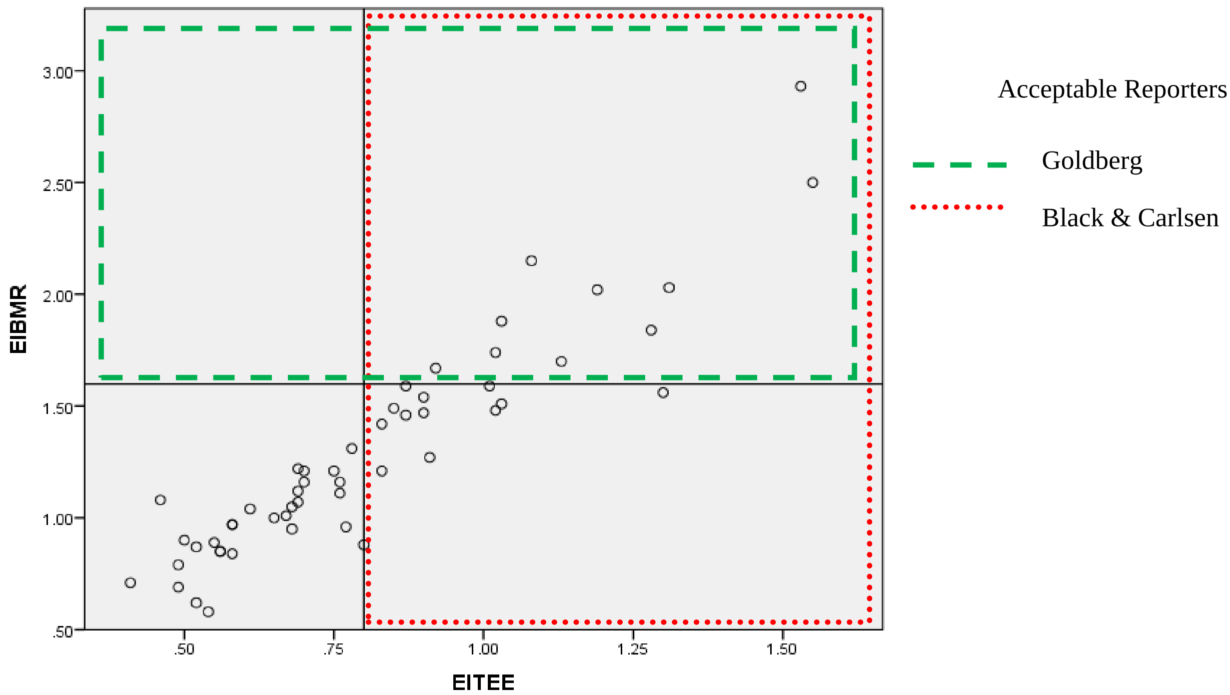


Figure 19 Scatter plot depicting dietary under-reporters by Goldberg method (EI:BMR <1.599) and Black method (EI:TEE <0.8)

Data points above the horizontal line at 1.59 are acceptable reporters using the Goldberg cut-off 2 (n=10)and data points to the right of the vertical line are acceptable reporters using the SWA and Carlsen definition (n=22).

Table 8 Reported mean daily energy and nutrient intakes with different screens applied to identify dietary under-reporting

	Goldberg Method		Black / Carlsen Method		NANS Method		Population w/o exclusions	
	n=10(2♂,8♀)		n=22(4♂,18♀)		n=30(6♂,24♀)		n=52(11♂,41♀)	
	Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
Energy kcal *(kcal)*	2247	600	2098	447	2040	454	1902	572
Protein *g/kgBM*	1.5	0.3	1.4	0.3	1.3	0.3	1.1	0.3
Protein *%TEI*	17%	3%	17%	3%	17%	3%	17%	4%
Carbohydrate *%TEI*	50%	6%	46%	8%	46%	7%	46%	6%
Fat *%TEI*	35%	7%	36%	6%	36%	5%	36%	6%
Calcium *(mg)*	903	219	885	282	829	260	735	337
Vitamin D *(µg)*	3.6	4.0	5.6	5.4	5.2	4.8	4.9	4.7
∑EAAS *(mg/kgBM/d)*	353	121	312	118	281	120	223	94
∑all AAs *(mg/kgBM/d)*	862	294	752	287	680	288	606	128
dietary TAC *(mmol TE)*	13.1	5.3	14.8	7.1	12.5	7.3	9.3	6.4

TEI total energy intake, ∑EAA sum of all essential amino acids, ∑AA sum of all amino acids, TAC total antioxidant capacity

Table 9 Proportion of the included population achieving RDAs for nutrients of interest when screened by different methods

	Goldberg Method	Black / Carlsen Method	NANS Method	Population w/o exclusions
	n=10(2♂,8♀)	n=22(4♂,18♀)	n=30(6♂,24♀)	n=52(11♂,41♀)
	%(n)	***%(n)***	***%(n)***	***%(n)***
Protein *>0.8 g/kgBM*	100% (10)	100% (22)	97% (29)	88% (46)
Protein *>1.2 g/kgBM*	80% (8)	68% (15)	53% (16)	44% (23)
Vitamin *>15µg*	0% (0)	9% (2)	7% (2)	4% (2)
∑EAAS *>214 mg/kgBM*	90% (9)	82% (18)	73% (22)	65% (34)
Leucine *>42 mg/kgBM*	90% (9)	82% (18)	73% (22)	65% (34)
Dietary TAC *>3.2 mmol TE*	100% (10)	95% (21)	90% (27)	88% (46)

∑EAA sum of all essential amino acids, TAC total antioxidant capacity

As is outlined **in Table 8**, the interpretation of population mean daily nutrient intakes is influenced by firstly the application of any screen for dietary misreporting, and secondly the choice of screen. In an elderly population, adequacy of the nutrients listed in **Table 9** is thought to be necessary to prevent age related whole body lean tissue mass loss.. Erroneous conclusions could be drawn from mean daily intakes (MDIs) from the population without any screen for dietary mis-reporters applied. As the sample size decreases with the different UR screens, variable conclusions may be deduced regarding the adequacy at population level.

3.5 Discussion

The present study, conducted in a convenience sample of elderly Irish adults, highlights the influence of choice of method for the screening of dietary under-reporters on both the incidence of under-reporting and the interpretation of self –reported dietary intakes. In the context of this dissertation, the aim of investigating dietary under-reporting was to determine whether choice of screening method for UR would influence the estimation of nutrient intakes in the study population. Those nutrients of particular interest were those discussed in Chapter 2 (protein, essential amino acids (EAA), vitamin D, antioxidant nutrients) and outlined in **Table 8**.

One of the stated aims of this thesis is to ascertain the adequacy of nutrient intakes relative to recommendations. The choice of UR screening method (if any) applied to the study population will clearly influence interpretation of nutrient MDI and may lead to identification of erroneous correlations between nutrient intakes and health.

Dietary under- reporting may have been influenced by a number of sources of error, and these are briefly discussed below.

3.5.1 *Dietary assessment methodology*

An acknowledged likely contributor to dietary UR is the difficulty in the accurate estimation of portion size when recording dietary intakes. Many efforts were made to capture accurate portion estimates; participants were given adapted food atlas photographs and lists of average portion sizes for commonly consumed foods. They were also encouraged to retain and return packaging from goods consumed. However, comparison of reported portion sizes with standard food portions (Ministry of Agriculture Fisheries & Food (MAFF) 2005) often raised questions, where reported intakes differed significantly from standard portions.

Besides errors in the dietary intake estimates, analysis of the nutrient intake of food could also be a source of error. For example the accuracy of the energy content of reported food and drinks are dependent on the nutrient database from which the energy values are derived. The WISP© nutrient databank, based on McCance & Widdosson's Composition of Foods, has been widely used in nutrition research and is a well validated dietary analysis tool, however it has been added to and amended by independent researchers and the accuracy of these amendments is a potential source of error contributing to UR.

In the recording phase of the study participants were asked on each day of recording 'did illness affect your eating today?' Respondents who indicated that illness impacted on dietary intake were not included in analysis. However, this study did not assess or excluded participants who self-reported intentional under-eating or dieting during the dietary recording phase. This may have impacted on incidence of under-reporting.

3.5.2 *Subject characteristics*

Along with the choice of dietary assessment method, the characteristics of the subjects being measured may also affect the validity of the results (Macdiarmid 1998). An increased incidence of under-reporting has been observed among women (de Vries *et al.* 1994; Haraldsdottir and Sandstrom 1994), those with higher BMI (Prentice *et al.* 1986) and smokers (Price *et al.* 1997; Pryer *et al.* 1997). This study population comprised 79% females with only 11 males participating. As previously mentioned, 56% of this total population were overweight and 17% obese. Interestingly a greater incidence of under-reporting was observed in male participants, perhaps due to the small number of men available for inclusion.

3.5.3 *Measurement of TEE*

There is a need to develop accurate devices for measuring daily energy expenditure under free-living conditions. Where high accuracy is required and sufficient resources are available, an open-circuit indirect calorimeter should be used (Johannsen 2010) . Open-circuit indirect calorimeters can employ a mask, hood, canopy or room/chamber for collection of expired air. For short-term measurements, mask, hood or canopy systems suffice. Chamber-based systems are more accurate for the long-term measurement of specified activity patterns but behaviour constraints mean they do not reflect real life (Jakici 2004). Where resources are limited and/or optimum precision can be sacrificed, flexible total collection systems and non-calorimetric methods are potentially useful (Slinde 2003). Portable armband devices, such as the SWA monitors show promise as an acceptable alternative to more invasive techniques. In adult populations the SWA has shown good agreement when validated against the doubly labelled water (DLW) technique (Jakicic 2004, Johannsen 2010). There has been some criticism of using the device to measure activity related energy expenditure (AEE) on an individual level (Slinde 2013). However, acceptable levels of agreement were observed between SWA and criterion measurements of TEE and AEE in older adults (Mackey 2011). A limitation of this study was that TEE was measured for 7 days, whereas the FIR covered only 4 days (2 week days and two weekend days). If the 4 day period when FIR was completed was not representative of food intake over the 7 days of measurement of TEE, they may not be comparable.

3.6 Conclusion

The regrettable fact is that nutritional science has not solved a fundamental problem – measuring accurately how much people truly eat. There is a lack of consensus among the scientific community, with the result that there are no universal guidelines detailing how to screen for, whether to include or how to use and interpret data obtained from under-reporters. As the inclusion of under-reporters can produce erroneously low results for habitual food or nutrient intakes and therefore confound the relationship between dietary intakes and diet-related diseases, it is important to know more about the nature and extent of misreporting. Under-reporting must be taken into account when assessing dietary intake in population based studies as it introduces systematic bias and affects not only the analyses of major components of EI and macronutrients but also micronutrients and the assessment of the contribution of dietary lipids to chronic disease.

Unanimity is required among the scientific and dietetic communities on how best to screen for UR in dietary surveys. The three methods examined here clearly give different results regarding sample size and estimated nutrient intakes. Although the Goldberg cut-offs may lead to bias and reduction in power in predictive models of under-reporting, the method has high predictive value (Black *et al*. 2000). In the absence of objective measures of TEE or physical activity, the Goldberg method is a reasonable approach to characterising under-reporting. However, where feasible a measure of physical activity should be included in study design as highlighted by Black *et al*. (2000). The NANS IUNA method is easily applied but may have poor sensitivity and specificity in identifying under-reporters. It is arguable that the cut-off used may be too low since it has been stated that an EI : BMR of 1.27 is the minimum value for survival and not compatible with long term health (WHO 1985). Goldberg *et al*. (1991) confirmed these results, showing that it is virtually impossible to survive with an EI : BMR $<$ 1.2.

The present study is important as it highlights the necessity to screen FIRs for under-reporting, particularly if accurate conclusions are to be drawn regarding habitual food and nutrient intakes to better inform policy makers of the requirements of an aging population.

Although EI/BMR or EI/EE cut-off points can be used to identify UR, the problem of how to use their data is still unresolved. We cannot yet answer the questions on what to do with databases containing significant numbers of low EI/BMR or EI/EE values. Future work must address the validity of associations made between nutrition and health using databases inclusive of under-reported dietary intakes and also work to achieve unanimity on how best to deal with what has been referred to as “the nutritionists’ guilty secret” (Garrow 1995). However, it should be clear from the present study that simple exclusion of these suspect values would seriously alter the size and nature of the sample, possibly eliminating those who are of most concern in terms of their health risk.

Chapter 4

Development and application of a WISP© - based method to estimate dietary total antioxidant capacity (TAC) from self-reported dietary intakes among an Irish population (aged 50 – 70 years)

A version of this chapter was presented at the Nutrition Society and published as Norton, C., Jakeman, P. (2013) 'A method to estimate dietary total antioxidant capacity among elderly Irish women', Proceedings of the Nutrition Society, 72(OCE4), DOI:10.1017/S0029665113003182

4.1 Introduction

It is widely accepted that a diet rich in fruit, vegetables and other nutrient rich plant-based foods may reduce the risks of oxidative stress-related diseases (Joshipura 1999, Joshipura 2001, Riboli 2003, Stanner 2004, World Cancer Research Fund/American Institute for Cancer Research 2007, Johnson 2004). The health benefits of these foods are attributed, in part, to their antioxidant properties. Where imbalance between concentrations of reactive oxygen species (ROS) and antioxidants (AO) may lead to a physiological condition termed oxidative stress (Figure 20).

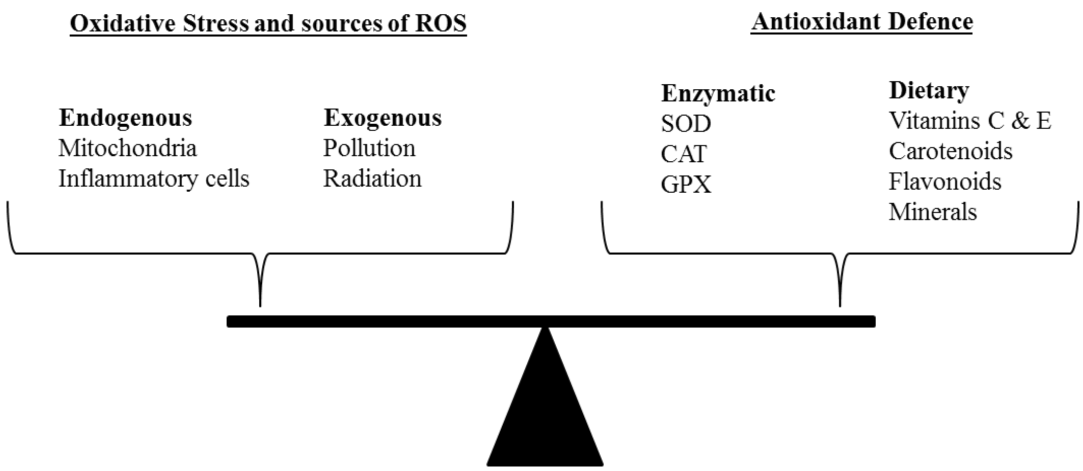


Figure 20 Factors contributing to oxidative stress

ROS are oxygen containing free radicals molecules and highly reactive chemical species with an unpaired electron seeking to pair with another free electron (Halliwell and Gutheridge 1989). AOs are substances able to inhibit the rate of oxidation by free radicals (Clarkson and Thompson 2000) by either donating or accepting the unpaired electron. Biological radicals can be classed as exogenous or endogenous, where exogenous radicals are derived from the external environment (air pollutants, tobacco smoke or radiation). Endogenous radicals are synthesized within the body as by-products of metabolic or inflammatory reactions. Antioxidant enzymes (e.g., catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase) work to maintain a state of balance preventing the transformation of ROS, and to convert them into more stable molecules. Non-enzymatic antioxidants that enhance AO systems include vitamins C and E, glutathione, carotenoids, flavonoids and ubiquinones (Clarkson 2000). Several trace minerals (copper, iron, manganese, selenium and zinc) play important parts by contributing to the AO system as cofactors for antioxidant enzymes (Jensen 2008). Thus the AO defence system can by augmented with appropriate levels of dietary AOs (Berger 2005).

In older age, an accumulation of ROS caused by chronic low-grade inflammatory processes (CLIP) (Beyer 2012), altered dietary habits (Morley 2012) or uncontrolled cellular responses to ROS (Beyer 2012, Chaput et al. 2007) which may lead to oxidative damage and contribute to poor health, including losses of both muscle mass and strength (Kim *et al*. 2010). There is increasing interest in the role of oxidative stress in the aetiology of age-related muscle mass loss; markers of oxidative damage have been shown to predict impairments in physical function in older adults (Semba 2007). Therefore, assessment of the adequacy of antioxidant nutrient intakes in an older population at risk of age related lean tissue mass loss seems prudent.

While assessment of the intake of all nutrient antioxidants is commonly reported (IUNA 2011), it may be equally important to investigate their cooperative / synergistic effects in order to better understand their health benefits. For this reason a *total dietary-based* research approach may elucidate more health effects than those derived from single nutrients. Dietary total antioxidant capacity (TAC), based on the cumulative antioxidant activities of all the antioxidants present in food, has been shown to be inversely associated with risks of chronic diseases (Wang 2012), both in young and older populations (Valtueña 2008, Pellegrini et al. 2006, Del Rio 2011).

Several methods have been developed for measuring the TAC of food and beverages (Pellegrini 2000, Wang 1997, Benzie 1999). These assays differ in their chemistry (generation of different radicals and/or target molecules) and in the way end points are measured. Among the more common are TEAC (trolox equivalent antioxidant capacity), FRAP (ferric reducing/antioxidant power), TRAP (total radical trapping antioxidant power) and ORAC (oxygen radical absorbance capacity). The oxygen radical absorbance capacity (ORAC) assay is the assay of choice in the food and pharmaceutical industries and is often quoted in product literature (Huang *et al*. 2002).

The ORAC assay measures the degree of inhibition of peroxyl-radical-induced oxidation by the compounds of interest in a chemical environment. It measures the value in mmol of Trolox equivalents (TE) and includes both inhibition time and the extent of inhibition of oxidation. The assay is based on pivotal work by Cao (1998a) which was later refined by Prior (1999). This refinement allowed for the differentiation of ORAC from lipophilic components (L-ORAC) (e.g. carotenoids) and hydrophilic components (H-ORAC) (e.g. phenolics). It is reported that the summation of both hydrophilic and lipophilic phases may provide the best indication of total AO capacity of a test compound (Prior *et al*. 2003).

The US Department of Agriculture (USDA 2010) published an ORAC database of selected foods based on the work of Cao and Prior. The authors involved in devising this database expressed a desire to publish data on foods known to contribute significantly to dietary antioxidant capacity (Haytowitz and Bhagwat 2010). This database may provide a means to identify dietary habits which may have potential preventative attributes against oxidative stress. Assessment of dietary TAC has not been reported in an Irish population; it is not currently possible to assess the TAC of a dietary intake using WISP© nutrition analysis software and WISP© has been the preferred nutrition analysis package for national nutrition surveys in Ireland over the last 2 decades (IUNA 2011).

4.2 Objectives

The levels of single antioxidants in food do not necessarily reflect their cumulative dietary TAC. Due to the chemical diversity of antioxidant compounds present in foods, complete databases on food antioxidant content are not yet available. Therefore, the aim of this study was to establish a WISP© (Tinuviel Software, Anglesey, UK) -based method of estimating dietary TAC founded on the USDA ORAC database of Selected Foods (2010) and to apply this assessment method to estimate the dietary total antioxidant capacity in an elderly Irish population.

4.3 Methods

Ethical approval was granted for the study by the Faculty of Education & Health Sciences Research Ethics Committee (EHSREC10/45), University of Limerick. A cohort of elderly people (n=173) recruited from the *Healthy Ageing Study* (Chapter 6) was invited to participate in this study.

4.3.1 *Dietary data collection*

Dietary data collection was as previously described (see Chapter 3). Following collection dietary data were coded and entered to the WISP© nutrition analysis package. It was later transferred to WISP© version 4 (V4) for further analysis and testing of the new functionality described below.

To remove some of the bias introduced by misreporting of food records, under-reporters [defined as having a reported energy intake (EI) to basal metabolic rate (BMR) ratio less < 1.1 (IUNA 2011)] were identified and excluded from analysis. The IUNA cut-off was used to allow comparison between the current work and national datasets. It is acknowledged that this may not be the most appropriate screen to use as it is reported that a EI:BMR cut-off set at 1.1 identifies only 66% of those who under-report. (Medical Research Council (MRC) Dietary Assessment Report, 2014). Following exclusion of invalid eFIRs (30.6%), 120 records (♀ n=94, ♂ n=26) (mean (SD); age 61.0(7.8) y; weight 70.4(13.2) kg; height 164.2 (7.7) cm, BMR 1347(202) kcal/d) were analysed further. Analysis of nutrient intakes was carried out on mean daily nutrient intakes (MDI) and meal level analysis (MLA).

4.3.2 *Development of the WISP© ORAC database*

The analysis phase of this study coincided with a co-operative interaction between researchers at the University of Limerick and Tinuviel Software (manufacturers of WISP© dietary analysis software), whereby Tinuviel required beta-testing of WISP©V4 and the current study necessitated technical assistance in the development of a method of quantification of dietary TAC. In 2007 the USDA released the first database of antioxidant activity for 277 foods using ORAC methodology. It incorporated ORAC values for food samples tested by researchers in the USDA National Food and Nutrient Analysis Program (NFNAP), as well as other analytical data from extant literature. Release 2 of the ORAC database contained additional foods giving a total of 326 food items for which ORAC values were published. The mean ORAC value was determined for each food and weighted by the number of samples reported among the various studies used in collating the database. The database base is sorted by USDA food codes and each food has an associated descriptor identify the characteristics of the food item (Haytowitz 2010) found at http://www.orac-info-portal.de/download/ORAC_R2.pdf. Using the *food database* in WISP©V4 (see Figure 21) it was identified that there was a total of 7374 foods listed. Manual comparisons were made between food item descriptors listed in the USDA database assigned an ORAC score and food descriptors within the UK WISP© nutrient databank. Where foods were similar in description and nutritional composition, it was deemed possible to map ORAC values which were in the USDA database to equivalent foods within the UK databank. The USDA ORAC database contains ORAC information on 326 foods. Sixty four (19.6%) of these foods items were not commonly consumed in the UK or Ireland and did not have a corresponding food descriptor. Food item descriptors had a positive match with only one food code in the WISP©

database in 85 instances (26.0%). There were multiple food descriptor matches made for 177 food items (54.3%). Further details on the matched food codes are presented in **Table 31.** Each food item in either database is assigned a *Long food descriptor* which as the name suggest describes the food item. Commonalities were identified across both datasets to identify foods that had the same / similar long food descriptors (e.g. The USDA ORAC database listed the descriptor *Apples, raw, with skin* along with its associated ORAC scores. This was matched with *Apples, raw, with skin, with and without core* in the WISP database and the associated ORAC scores assigned to the matched food). This manual food mapping process resulted in 6.4% of foods in the WISP© food database having an ORAC score assigned.

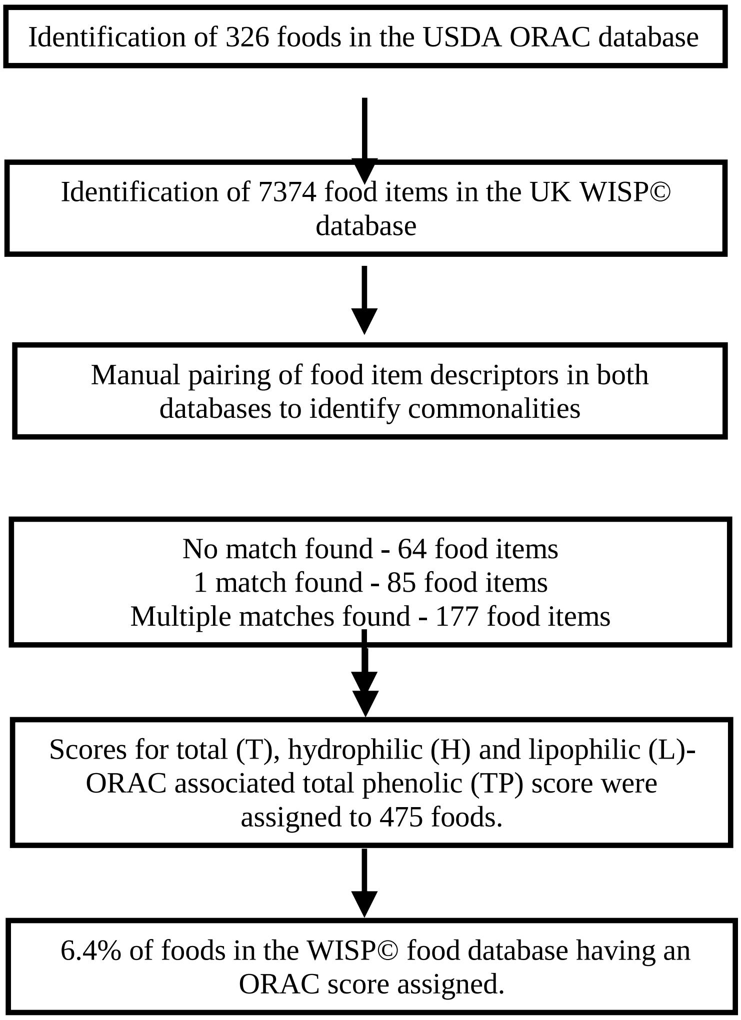


Figure 21 Flow chart outlining methodology for pairing and assigning United States Department of Agriculture Oxygen Radical Absorbance Capacity scores to foods in the UK WISP© database

(More foods were assigned codes than there were codes in the original database, due to multiple matches for some)

Nutrient intake data were subsequently exported from WISP©V4 (as both mean daily intake (MDI) and meal level analysis (MLA)) to determine both individual antioxidant nutrient intakes and the newly appended dietary TAC. This export is a standard function within the analysis package to allow for more extensive investigation in either excel or SPSS packages.

Data manipulation was conducted using SPSS© Version 20.0 for Windows™ (SPSS Inc. Chicago, IL, USA). Variables were described using the mean, median, standard deviation (SD) and inter-quartile range (IQR). Variables were tabulated for total population and split by gender where appropriate.

4.4 Results

4.4.1 *WISP©-based databank of ORAC score*

Of the 7374 foods in the WISP© database 6.4% foods were assigned ORAC scores. The resultant WISP© database contained 475 foods for which a USDA ORAC value was designated with its respective scores for total (T), hydrophilic (H) and lipophilic (L)-ORAC score (mmol Trolox equivalent/100 g; mmol TE), and the associated total phenolic (TP) score (mg Gallic acid equivalents/100 g; mg GAE). **Table 31** (Appendix) provides a list of the 475 assigned WISP© food codes, the original USDA food descriptors, food codes and associated ORAC scores.

The USDA researchers involved in devising the original database expressed a desire to publish data on foods known to contribute significantly to dietary antioxidant capacity (Haytowitz 2010), so while the coverage rate may be low (6.4%), these foods are those considered to contribute greatest to dietary TAC. The latest release of the USDA nutrient database contains 8463 food items, of which 326 have been assigned an ORAC score, giving coverage of 3.6% in the equivalent American database (USDA, 2013).

4.4.2 *Application of the databank in an elderly Irish population*

Following exclusion of invalid eFIRs, 120 records (♀ n=94, ♂ n=26), (mean (SD); age 61.0(7.8) y; weight 70.4(13.2) kg; height 164.2 (7.7) cm, BMR 1347(202) kcal/d) were analysed further. **Table 10** below outlines mean reported daily intake for energy and macronutrients.

Table 10 Reported daily intakes for energy and macronutrients

	Mean	SD
Energy (kcal)	1981	406
Protein (% TEI)	17%	3%
Fat (% TEI)	36%	6%
Carbohydrate (% TEI)	48%	8%

Data are presented as mean ± SD, n=120 (♀ =94, ♂ =26)

As stated previously, mean daily intakes of antioxidant nutrients are commonly reported. Comparison with the relevant RDA indicates the adequacy of individual nutrient intakes (**Table 11**). The Institute of Medicine (IOM) RDA is listed below, along with reported intakes in this elderly population, for each of the nutrients which may act as enhancers or co-factors in enzymatic antioxidant defences.

Table 11 Institute of Medicine Recommended Daily Allowancefor antioxidant nutrients with reported daily intakes for population

Nutrient	IOM RDA 51-70y		Reported Mean Daily Intakes					
	♂	♀	Mean All	SD	Mean ♂	SD	Mean ♀	SD
Vitamin A (µg)º	900	700	na	na	na	na	na	na
Vitamin C (mg)	90	75	101	59	101	77	101	54
Vitamin E (mg)	15	15	7.8	3.5	7.9	3.6	7.8	3.5
Selenium (µg)*	55	55	46.3	22.3	57.4	22.2	43.5	21.5
Manganese (mg)	2.3	1.8	4.1	1.5	4.7	1.7	3.9	1.5
Copper (µg)	900	900	1266	603	1392	452	1235	634
Zinc (mg)	9.4	6.8	9.3	2.5	11.4	2.9	8.8	2.2

*(data are presented as mean ± SD, n= 120 (♀ n=94, ♂ n=26) * not normally distributed,*
º Assessment of vitamin A intakes is not currently possible with WISP©V4

SOURCES: Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); and Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Despite overall energy adequacy of dietary intakes and having macronutrient intakes within recommended ranges (**Table 10**), this sample population failed to meet IOM RDAs for some individual antioxidant nutrients, notably vitamin E and selenium (**Table 11**). The proportion of the population (by gender) who failed to meet IOM RDAs is outlined below (**Table 12**). As stated in the introduction, it may be equally important to investigate the synergistic effects of these nutrients. For this reason a *total dietary-based* research approach was applied using the USDA ORAC database and the new functionality of WISP© V4. The IOM RDA was used to assess adequacy, rather than Irish or European Food Safety Authority (EFSA) recommendations for the purposes of consistency throughout the research. Not all dietary components under investigation currently have EFSA or Irish RDAs (e.g. amino acids or dietary total antioxidant capacity).

Table 12 Percent of population who failed to reach Institute of Medicine Recommended Daily Allowance for antioxidant nutrients

	Male	Female
Vitamin A (μg) º	na	na
Vitamin C (mg)	48%	37%
Vitamin E (mg)	92%	96%
Selenium (μg)	71%	75%
Manganese (mg)	6%	2%
Copper (μg)	23%	25%
Zinc (mg)	53%	16%

(n= 120 (♀ n=94, ♂ n=26)) º Assessment of vitamin A intakes is not currently possible with WISP©V4

Analysis of dietary TAC using the new food scores populating the database resulted in a mean dietary TAC of 9.6(4.9) mmol TE (**Table 13**). TAC was further divided into its constituent hydrophilic (H-ORAC) 97(2)% and lipophilic (L-ORAC) 3(2)% components. Female participants reported a greater intake of dietary TAC across each of the parameters assessed in comparison with male participants. However there was large variability on the intakes reported with minimum reported intake of T-ORAC in the population of 0.20 mmol TE reported and a maximum of 26.35 mmol TE. Similar ranges were observed when the population was considered by gender (Females T-ORAC 0.2 – 26.35 mmol TE and males 1.03 - 21.34 mmol TE).

Table 13 Reported daily intakes of dietary Total Antioxidant Capacity

	Mean			**SD**			**Median**			**IQR**		
	All	♂	♀	**All**	♂	♀	**All**	♂	♀	**All**	♂	♀
T-ORAC mmol TE	9.57	8.38	9.86	4.90	5.38	4.75	9.17	6.91	9.60	6.52	6.70	6.33
H-ORAC mmol TE	7.67	6.71	7.91	4.17	4.25	4.14	6.96	5.99	7.65	5.75	3.69	5.77
L-ORAC mmol TE	0.23	0.19	0.24	0.22	0.24	0.22	0.16	0.11	0.17	0.20	0.15	0.19
Total phenolics mg GAE	2176	1608	2318	2447	2783	2350	1203	556	1398	2810	1112	2891

(data are presented as mean ± SD, n= 120 (♀ n=94, ♂ n=26) total (T-ORAC), hydrophilic (H-ORAC), lipophilic (L-ORAC) ORAC values and total phenolic (TP) scores) ORAC unit (mmol TE), TP unit (mg GAE)

The food groups contributing greatest to dietary TAC (Figure 22) were vegetables (23%), fruit / fruit juices (21%) and other beverages (21%) with a lesser contribution from alcohol (12%) and confectionary (7%).

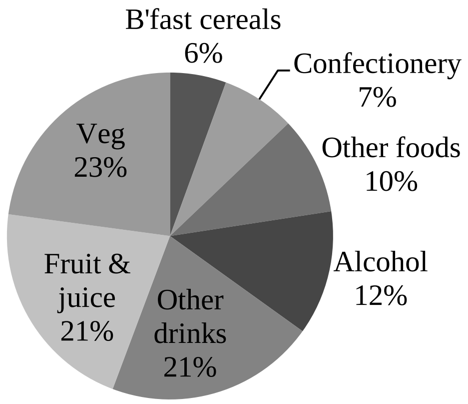


Figure 22 Contribution of food groups to dietary total antioxidant capacity (TAC)

n= 120 (♀ n=94, ♂ n=26)

The relative contributions from fruit and vegetables are to be expected, however, the contribution from 'other drinks' at 21% is notable. This constitutes tea and coffee consumption. An investigation into the distribution of dietary TAC across meal times was carried out (Figure 23). Foods consumed at breakfast and snack occasions contributed greater to dietary TAC than midday and evening meals. Considering the food groups contributing to dietary TAC illustrated in Figure 22, the concurrent consumption of cereals, fruit or juices with tea or coffee at breakfast time may explain the greatest contribution to dietary TAC made by this meal.

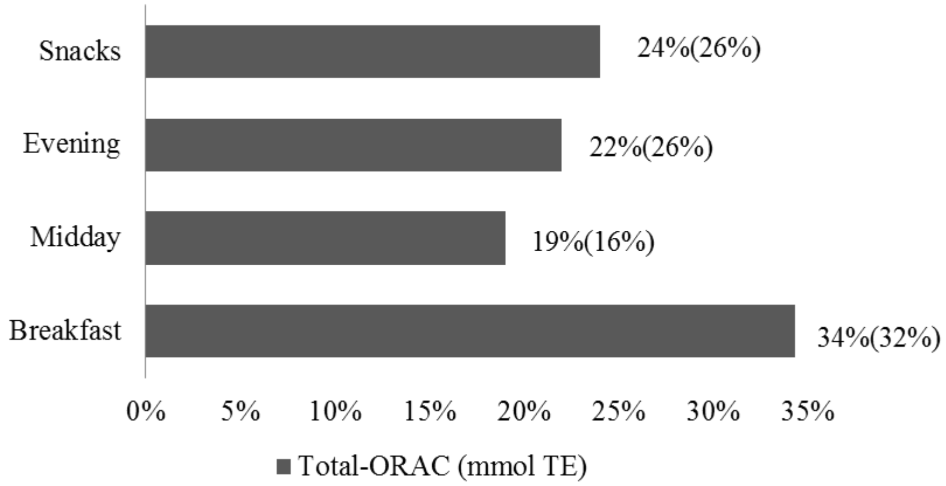


Figure 23 Distribution of dietary TAC across meal times

Percentage of mean daily total ORAC with (SD) (data are presented as mean ± SD, n= 120 (♀ n=94, ♂ n=26)

4.5 Discussion

4.5.1 *WISP©-based databank of ORAC score*

As a result of the present study, there is now a proposed WISP©-based method for assessment of dietary TAC. The methodology in the present study appears to be consistent with the small number of studies that have previously documented methods of adding to or amending nutrition analysis packages (Levis et al. 2011, Neuhouser 2006, Flood 2006), albeit that these studies refer to the addition of glycaemic index / load to databases, similar principles apply.

Certain limitations exist in the current methodology. Several foods in the WISP© data base remain without any ORAC values; therefore, any research involving WISP© analysis of dietary TAC will continue to be limited in its accuracy. By comparison the latest release of the USDA nutrient database contains 8463 food items, of which 326 have been assigned an ORAC score, giving coverage of 3.6% (USDA 2013), where the WISP©-based database developed here has 6.4% coverage. An appreciation of the limitations of both databases is important in the interpretation of results. The statement from the original authors (Hatowitz and Bhagwat 2010) that they wished to provide ORAC scores for those foods that contribute greatest to dietary TAC does provide some security on the accuracy of the proposed toolkit.

Due to a lack of dietary TAC data for foods consumed in an Irish population specifically, it was necessary to use USDA ORAC values. Since it is acknowledged that antioxidant content may vary with geographic location and growing conditions (Rautiainen *et al*. 2012) there may be differences in the AO content of foods evaluated and those. A study by Thomas (2013) investigating variation in the nutrient composition of fruit and vegetable products reports that climatic and soil conditions can alter composition by up to 14% in some species of fruits.

To remove some of the bias introduced by misreporting of food records, under-reporters [defined as having a reported energy intake (EI) to basal metabolic rate (BMR) ratio less < 1.1 (IUNA 2011)] were identified and excluded from analysis. The IUNA cut-off was used to allow comparison between the current work and national datasets. It is acknowledged that this may not be the most appropriate screen to use as it is reported that a EI:BMR cut-off set at 1.1 identifies only 66% of those who under-report. (Medical Research Council (MRC) Dietary Assessment Report, 2014). As addressed in Chapter 3, the method of screening for UR may influence analysis of nutrient intakes. **Table 12** outlines percentages of this study population who failed to meet IOM recommendations for individual micronutrients. Perhaps these figures would be less had a higher EI:BMR cut-off been applied in screening for mis-reporters.

The total antioxidant capacity has not been measured for dietary supplements, thus, these results can only be translated to food items and not to antioxidant supplements. However, to the author's knowledge, this is the first study to describe a method for adding dietary TAC values to the WISP© nutrition analysis software package. Describing such methods will help to increase the utility of dietary TAC in both clinical and research settings, furthering our understanding of its association with health and ageing.

4.5.2 *Application of the dietary TAC tool in an older Irish population (50-70y)*

To date there are no Irish national estimates of dietary TAC, nor are there any guidelines on recommended intakes. Even in the US, there are no well-defined recommendations issued regarding dietary TAC. The US Food & Drug Administration (FDA) is reported to have suggested a daily intake of ~ 7.0 mmol TE (Prior 1999). The USDA recommends an intake of between 1.0 and 5.0 mmol TE/day (Haytowitz 2010). The leading authority and author of pivotal papers on dietary TAC, Prior (1999) suggests an intake of >3.2 mmol TE/day. In this older Irish population 109 participants (91%) achieved dietary TAC intakes in excess of 3.2 mmol TE/day, while sixty five percent of the population (n=78) exceeded 7.0 mmol TE/day.

Prior *et al.* (1999) stated that the USDA laboratory could not be conclusive in defining the optimal dietary antioxidant intake as measured by the ORAC procedure but that work in the area is on-going. Prior estimates that the average T-ORAC from fruits and vegetables may be as low as 1.2 mmol TE/day (Prior 1999). These data were extrapolated from the USDA Continuing Survey on Consumption of Fruits and Vegetables in the United States. In other data from 36 elderly subjects who completed food frequency questionnaires detailing consumption patterns for the previous year, average ORAC was estimated to be 1.64 ± 0.2 mmol TE/day (Cao and Prior 1998a). The number of standard portions of fruit and vegetables consumed by participants in this study was 5 per day, which is higher than the US national average. Extrapolating the data to the national average intake the authors suggest that normal intakes of ORAC are likely in the range 1.2 – 1.6 mmol TE / day. Prior and Cao (1998b) report ORAC intakes as high as 6.0 mmol TE / day in elderly subjects from self-selected diets. The authors describe how increasing ORAC from a reported mean of 1.64 mmol TE / day intake to 3.2 mmol TE /day that serum antioxidant capacity increased by 10 – 15%, an increase of magnitude thought to have physiological relevance (Cao 1998a); hence Prior's recommendation on a daily dietary TAC target. From this, they predict that increasing the ORAC intake from the expected (1.2 – 1.6 mmol TE/day) supplied from normal consumption patterns, by 1.0 -2.5 units per day to reach the intake of >3.2 mmol TE / day, may be needed to improve health outcomes (Prior 1999).

By comparison to the results outlined in the current study, estimates of TAC, based upon USDA's Continuing Survey of Food Intake by Individuals (1994-1996) or more recently intake from food consumption data of NHANES 2001-02, was 5.6 mmol TE (Wu 2004) and 4.7 mmol TE (Prior 2007). The mean dietary TAC (9.6 (4.9) mmol TE/day) in this older Irish population is significantly higher than those values reported in initial pilot studies by Prior and Cao (1998b) and in later studies applied to national nutrient intake databases. One explanation for this is bias introduced by the demographic of the convenience sample recruited for participation in this study. The sample was predominantly female (80%) and comprised largely of retired university staff. The assumption is that participants were educated, affluent and interested in diet as a means of promoting health. This population may not be representative of national dietary intakes.

In an attempt to test the theory that the convenience sample may not represent the national population, comparisons were made on fruit and vegetable consumption. While dietary TAC has not been previously reported in an Irish context, data on fruit and vegetable consumption has (IUNA, 2011). Comparisons on intakes from this food group may allow limited comparisons with IUNA national diet surveys since the dietary TAC in this study was contributed to most by fruit and vegetable.

IUNA have published data from the National Adult Nutrition Survey on the adequacy of fruit and vegetables intakes (IUNA 2011). They report that while fruits and vegetables were consumed by the majority of the study population, the average intake (192 g per day) of fruit and vegetables (excluding juices and composite dishes) falls below the WHO recommendation of 400 g / day (5 x 80 g portions) (Organization 1990). In the present study, the population average intake of fruit and vegetables at 308 g per day (139 g per day from fruit and 169 g per day from vegetables), is above the NANS reported intake but still falls short of WHO recommendations. Based on the 5-a-day WHO recommendation for intake of fruit and vegetables, this population does not meet that requirement with reported intakes of (mean(SD)) 3.9 (1.9) portions of fruit and vegetables. However the difference in habitual intakes from the both the US and Irish national reports outlined above, to the population in this current study regarding fruit and vegetables consumption may explain the difference in reported ORAC units consumed. It also raises the question of whether the health effect of total dietary AO nutrients has the potential to be greater than the sum of their parts. In this population the fruit and vegetable intakes reported are below WHO recommendations, and some of the participants did not achieve the recommendations for single AO nutrients. Regardless of this the calculated mean dietary TAC (T-ORAC 9.6 mmol TE) was three times the level thought to have physiological relevance (T-ORAC 3.2 mmol TE).

Somewhat controversially, in May 2012, the USDA’s Nutrient Data Laboratory (NDL) removed the USDA ORAC Database for Selected Foods from the NDL website. In a statement they expressed concern on the misuse of the database whereby ORAC values were routinely misrepresented by food and dietary supplement manufacturing companies to promote their products, and also by consumers to guide their food and dietary supplement choices. While the statement does not wish to detract in any way from the in vitro assays carried out by the researchers, and confirms that the methods and results for food based ORAC scores is still valid , they continue by stating that there is a lack of unanimity among the scientific community that the values indicating antioxidant capacity have relevance to the effects of specific bioactive compounds, including polyphenols on human health. For these reasons the ORAC Table, previously available on the NDL web site has been withdrawn.

However, Prior, the scientist whose works formed the basis for the original database believes that this argument on a lack of evidence supporting the associations between dietary TAC and health is weak, citing many published articles with evidence of the contrary (Prior 1999). In a study investigating endometrial cancer risk and total phenolic consumption carried out using the ORAC database, it was reported that decreased risk was observed among those with the highest total phenolic intakes compared with the lowest (Gifkins *et al*. 2012). Another recent publication reported that adherence to a Mediterranean-type diet, with emphasis on increasing intakes of foods rich in antioxidants can increase dietary TAC intake and plasma TAC in patients with abdominal obesity (Kolomvotsou *et al.* 2012). Dietary total antioxidant capacity (TAC) has been proposed as a method to assess synergy among antioxidant activities of single antioxidant dietary compounds (Niki 2010). Some authors have debated the applicability of the extrapolation of dietary TAC data to its antioxidant contribution *in vivo* (Ghiselli *et al*. 2007; Sies 2007). Despite this fact the dietary TAC index has been a relevant tool in epidemiological studies (Chrysohoou *et al*. 2007; Yang *et al*. 2011). Increased dietary TAC has been associated with higher diet quality scores (Puchau *et al*. 2009) as well as with improved values concerning glucose metabolism (Psaltopoulou *et al*. 2011) and inflammatory status (Brighent *et al*. 2005; Detopoulou *et al*. 2009) in middle-aged people. In addition, dietary TAC has been recently associated with a lower risk for ischemic stroke in Italian cohort (Del Rio *et al*. 2011). However, the relationship of dietary TAC with biomarkers has been only modestly investigated in young adult people(Hermsdorff et. 2010; Puchau *et al*. 2010). This population requires further investigation as it may provide new information on associations of overall antioxidant intake with metabolic and oxidative biomarkers *in vivo*.

4.6 Conclusion

Further investigation into antioxidant capacity of foods *in vitro* and their *in vivo* effects is necessary to elucidate links that may exist between dietary intakes of AOs (singly or concomitantly) and the prevention of age related morbidity. The development of a WISP©-based method to assess dietary TAC will allow further work in this area, specifically in an

Irish population. The extension of this assessment method to national Irish data are an important progression in estimating dietary TAC, elucidating correlations with health and developing food based dietary guidelines.

Chapter 5

Development and application of a WISP© - based method to estimate essential amino acid (EAA) intakes from self-reported dietary records among an Irish population (aged 50 – 70 years)

A version of this chapter is in preparation for publication as Norton, C., Jakeman, P. (2014) 'A method to estimate dietary amino acid intakes in elderly Irish adults'

5.1 Introduction

There are many issues affecting the conservation of whole body lean tissue mass (LTM) in the elderly. Adequacy of protein intake relative to the recommended daily allowances (RDA) and its constituent amino acid (AA) composition are both factors to consider (Douglas et al. 2004, Paddon-Jones 2013, Volpi 2013, Evans et al. 2013). This chapter proposes a method to describe the adequacy of protein intake by its essential amino acids composition relative to current Institute of Medicine (IOM) recommendations.

Dietary proteins are not created equal. They vary according to their source (plant or animal), their quality (the relative content of essential amino acids) and their digestibility (Boye 2012). Proteins deemed to be of high quality are those that are readily digestible and which contain essential amino acids (EAA) in amounts required to promote or maintain health in humans (Campbell *et al.* 1999). The adequacy of daily amino acid intake is not commonly addressed in research assessing the dietary intakes of Irish populations; rather the adequacy of total daily protein intakes is investigated with no consideration of the AA content of the protein RDA.

In 2005 the IOM (Institute of Medicine 2005) published recommendations on amino acid intakes for American and Canadian populations. In fact, the RDA for EAAs has increased with each iteration of recommendations, reflecting the emerging research on their importance for promotion and maintenance of health. However, to date no such recommendations have yet been developed in Europe.

Quantification of dietary amino acid intake is challenging. McCance and Widdowson's The Composition of Foods contains data on a very limited number of the 7000+ foods contained in the most recent edition. American researchers have published data on habitual AA intakes using the United States Department of Agriculture (USDA) food composition database (United States Department of Agriculture 2013). Similar quantification of AA intakes using the preferred dietary analysis software for national nutrition surveys on the island of Ireland and in the UK (WISP©) is not possible as WISP© does not have the functionality to evaluate amino acid intakes. Clearly there is a requirement to be able to quantify not just total daily protein intake, but also the quality of the daily consumption to inform optimal intake for both general and specific populations.

5.2 Objectives

The EAA content of the RDA for protein ($0.8g.kg^{-1}.d^{-1}$) is one of the dietary factors governing the conservation of lean tissue mass (LTM), yet these data are not widely reported in Irish adults. The aim of this study was to establish a WISP© (Tinuviel Software, Anglesey, UK) - based method of estimating essential amino acid intake and to apply this assessment method to determine whether the EAA intakes satisfied the IOM RDA in an elderly Irish population.

5.3 Methods

Ethical approval was granted for the study by the Faculty of Education & Health Sciences Research Ethics Committee (EHSREC10/45), University of Limerick. A cohort of older people (50-70y) (n=173) recruited from the *Healthy Ageing Study* (Chapter 6) was invited to participate in this study.

5.3.1 *Dietary data collection*

Dietary data collection was as previously described (see Chapter 3). Food intake data were coded and subsequently analysed using WISP© (Tinuviel Software, Anglesey, UK). WISP© uses data from McCance and Widdowson's The Composition of Foods, sixth (Food Standards Agency, 2002) and fifth (Holland *et al.* 1995) editions plus all nine supplemental volumes (Holland *et al*. 1988; Holland *et al*. 1989; Holland *et al*. 1991; Holland *et al*. 1992; Holland *et al*. 1993; Chan *et al*. 1994; Chan *et al*. 1995; Chan *et al*. 1996; Holland *et al*. 1996) to generate nutrient intake data. During this study, modifications were made to the food composition database to include recipes of composite dishes, nutritional supplements, generic Irish foods that were commonly consumed and new foods on the market. All previous modifications to the food composition database were also checked and updated from current manufacturers' information as necessary.

A number of quality procedures were put in place in an attempt to minimise error and ensure consistency throughout the collection and manipulation of food intake data. This was carried out by the same observer on two occasions separated by a minimum of one week and also by another independent researcher following the same data coding and entry procedures. It was stressed to respondents that they should not try to change or 'improve' their diet during the recording period. Random dual entries were performed on multiple food intake records to identify and reduce errors. At the end of the recording period, respondents were asked whether their food intake had been the same as usual, less than usual or more than usual during the recording period and to explain why this might have been. Respondents were also asked if there were any items consumed during the recording period which had not yet been entered in the eFIR. Details on such items were recorded and subsequently entered to food intake records.

Dietary data were coded and entered to the WISP© nutrition analysis package. It was later transferred to WISP© version 4 (V4) for further analysis and testing of the new functionality described below.

To remove some of the bias introduced by misreporting of food records, under-reporters [defined as having a reported energy intake (EI) to basal metabolic rate (BMR) ratio less < 1.1 (IUNA 2011)] were identified and excluded from analysis. The IUNA cut-off was used to allow comparison between the current work and national datasets. It is acknowledged that this may not be the most appropriate screen to use as it is reported that a EI:BMR cut-off set at 1.1 identifies only 66% of those who under-report. (Medical Research Council (MRC) Dietary Assessment Report, 2014). Following exclusion of invalid eFIRs (30.6%), 120 records (♀ n=94, ♂ n=26) (mean (SD); age 61.0(7.8) y; weight 70.4(13.2) kg; height 164.2 (7.7) cm, BMR 1347(202) kcal/d) were analysed further. Analysis of nutrient intakes was carried out on mean daily nutrient intakes (MDI) and meal level analysis (MLA).

5.3.2 *Development of the WISP© essential amino acid database*

Following co-operative interactions with Tinuviel Software during the development of the dietary TAC addition to WISP© (described in Chapter 4), Tinuviel Software decided to consider the possibility of constructing a *mapping database* in WISP©V4 which would link USDA codes to one or more of the UK food codes. Specifically, they looked at each food item in the USDA database and then looked for similar foods within the UK nutrient databank. Where foods were reasonably similar in nutritional makeup, it was deemed possible to map certain nutrients present in the USDA database to equivalent foods within the UK databank. As WISP©V4 has the capacity to store and analyse more than 200 nutrients, slots were populated within the UK product with information taken from the USDA database, including amino acids. An automated system was developed to identify commonalities between both databases to stream line the mapping process, making the time-consuming, manual process described in Chapter 4 obsolete. Where there was a food of similar composition, the mapping database in WISP© V4 was set to link the USDA code to one or more of the UK food codes. The amino acid values from the USDA, factored by the relative amounts of protein in the USDA and UK food (if different) were entered into the UK databank (Figure 24).

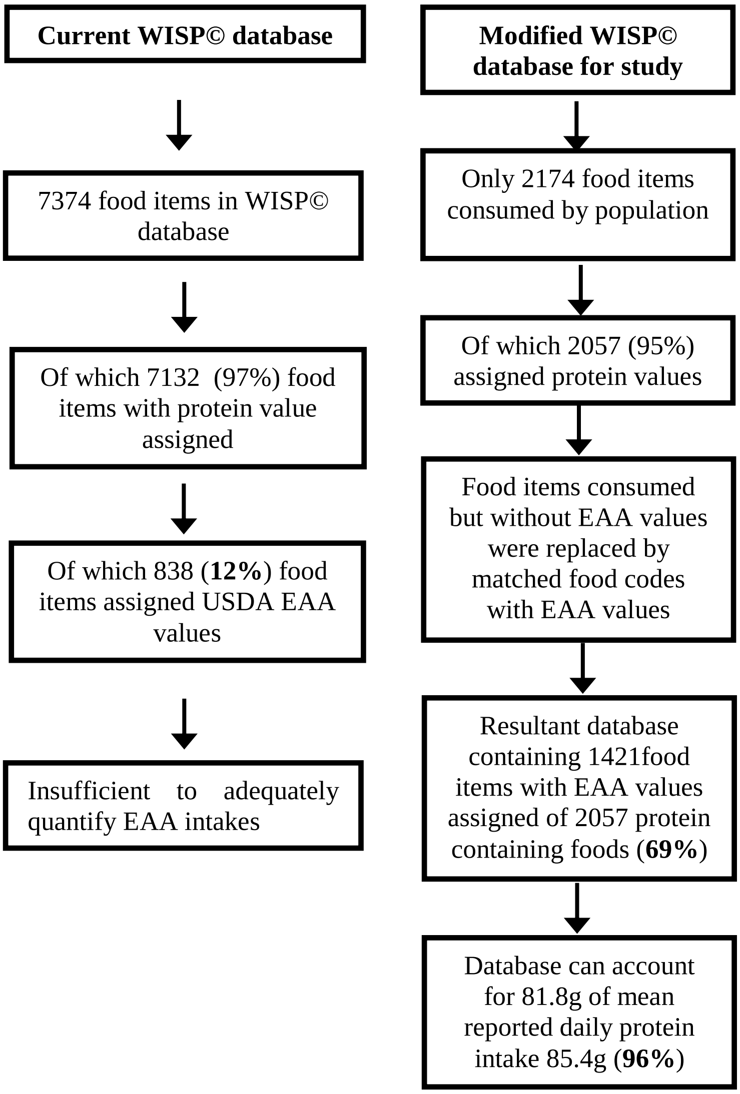


Figure 24 Flowchart outlining the development of the new nutrient database to assess essential amino acid intakes

The limitation of this initially is that there were not very many matches for foods. There are in excess of 7000 USDA foods, many of which are branded or unusual items which are not widely present in Irish diets. The technical support required for this process was carried out by staff at Tinuviel Software (further details in the online manual at http://www.tinuvielsoftware.co.uk/WISP©4.htm).

5.3.2.1 *Refinement of the WISP©V4 essential amino acid database specific to this study population*

Using the *food database* in WISP©V4 it was identified that of the 7374 foods in the database, 7132 foods had a listed known protein content assigned (99% coverage of the total number of foods in the database). However investigation of the *supplementary database* which contains the newly introduced USDA amino acid values revealed an average coverage rate for EAAs of only 12% (n=838 foods). Clearly this was not sufficient to quantify habitual AA intakes.

Using the WISP© V4 *contributions data,* those foods which contributed significantly to group mean total protein intake in this study population were identified, as these were the foods which were deemed most pertinent for quantification of dietary AAs (see Figure 24). Food items were present in the database with varying levels of frequency and priority was given to those food items occurring on greater than ten occasions or those foods with greater than 10 g protein per 100g food. Further interrogations of both the WISP© V4 and USDA databases were carried out to identify potential matches thereby expanding the database.

Using the *code replacer* function, foods without AA values were replaced in the dietary records for foods with matching descriptors but whose AA values were assigned. The resultant database of foods contained 2057 individual food items, of which there were finally 69% (n=1421) foods assigned an AA value.

In order to determine how much of the reported daily protein intake was accounted for with the revised database further analysis of reported intakes was necessary (Figure 25). Any foods in the database for which it was not possible to assign an AA value were deleted from duplicate food intake records to allow comparison with the original reported protein intake. This investigation revealed that the database can account for 96(6) % (mean (SD)) of reported protein intakes (min 70% and max 100%).

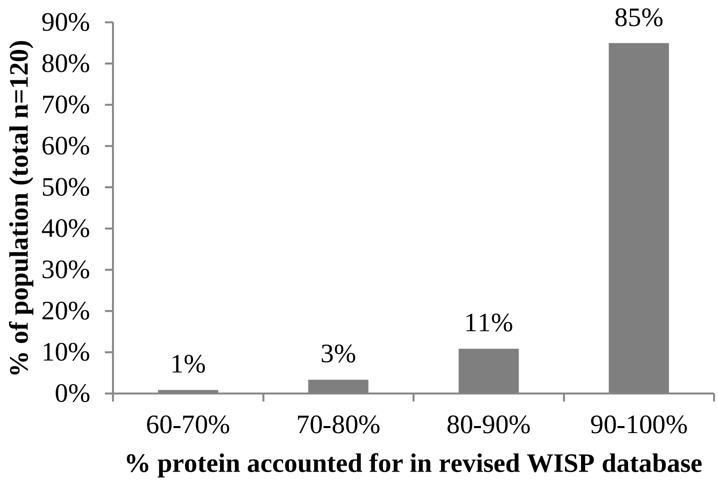


Figure 25 Protein accounted for in revised WISP© database (%)

Nutrient intake data were consequently exported from WISP©V4 (as mean daily intake (MDI)) to determine the adequacy of daily protein intake and quality by comparison of calculated EAA intakes with IOM RDAs.

Data manipulation was conducted using SPSS© Version 20.0 for Windows TM (SPSS Inc. Chicago, IL, USA). Variables were described using the mean, median, standard deviation (SD) and inter-quartile range (IQR). Variables were tabulated for total population and split by gender where appropriate.

5.4 Results

5.4.1 *WISP©-based databank of Essential Amino Acids*

Of the 7374 foods in the WISP© database, 97% contained protein (n=7132). The WISP©V4 data linking system assigned USDA AA values to foods 838 (12%). Further investigation and refinement of the database, by making it specific to the foods (n=2174) consumed by this particular study population, produced a database with 69% cover of AA values by food item. Analysis of the amount of reported daily protein accounted for with the refined database revealed that 96(6) % of protein intake can be accounted for.

Table 14 Number of foods with and without amino acid values assigned in population specific WISP©-database

	n	%
Total number of foods selected by population	2174	
Foods consumed containing protein	2057	95%
AA assigned by Tinuviel mapping system	367	18%
AA assigned manually by researcher	1054	51%
Number of foods with AAs assigned	1421	**69%**
Number of foods without AAs assigned	636	31%

Tables 31 and **32** are presented in the Appendix. **Table 31** contains information on food items assigned EAA values by the Tinuviel mapping system (n=367). **Table 32** displays those food items consumed by this study population which it was possible to manually assign EAA values by matched food descriptors (n=1054).

5.4.2 *Application of the databank an older Irish population (50-70y)*

Following exclusion of invalid eFIRs, 120 records (♀ n=94, ♂ n=26), (mean (SD); age 61.0(7.8) y; weight 70.4(13.2) kg; height 164.2 (7.7) cm, BMR 1347(202) kcal/d) were analysed further. Mean protein intake of 1.2(0.3) $g.kg^{-1}.d^{-1}$ was 17(3) % of mean total energy intake (EI; 1981(406) kcal/d). All bar five subjects (96%) exceeded the RDA for protein intake ($0.8g.kg^{-1}.d^{-1}$). Forty seven percent of the population exceeded the figure many researchers propose as the preferable recommendation for an older population (50-70y); 1.2g/kg BM/d (**Figure** 26).

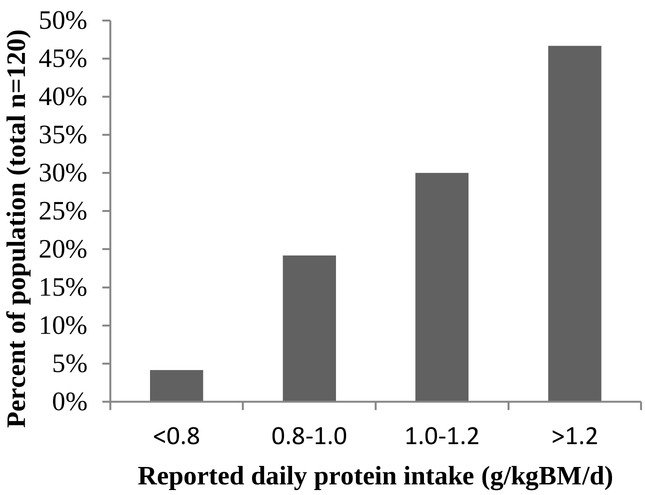


Figure 26 Reported daily protein intakes and percent of the total population achieving these intakes, n= 120 (♀ n=94, ♂ n=26)

Those participants not achieving the protein RDA were female, with no male reporting a daily intake below 0.8 g.kg^{-1}.d^{-1}. Surprisingly, a higher percentage of women than men reported protein intakes of greater than 1.2 g.kg^{-1}.d^{-1} (42% vs 48%, Error! Not a valid bookmark self-reference.).

Table 15 Reported daily protein intakes and numbers percent of population achieving these intakes by gender

Protein intake	ALL		Male (n=26)		Female (n=94)	
	n	% sample	n	% sample	n	% sample
<0.8 g.kg^{-1}.d^{-1}	5	4%	0	0%	5	5%
0.8-1.0 g.kg^{-1}.d^{-1}	23	19%	5	19%	18	19%
1.0-1.2 g.kg^{-1}.d^{-1}	36	30%	10	38%	26	28%
>1.2 g.kg^{-1}.d^{-1}	56	47%	11	42%	45	48%

(n= 120 (♀ n=94, ♂ n=26)

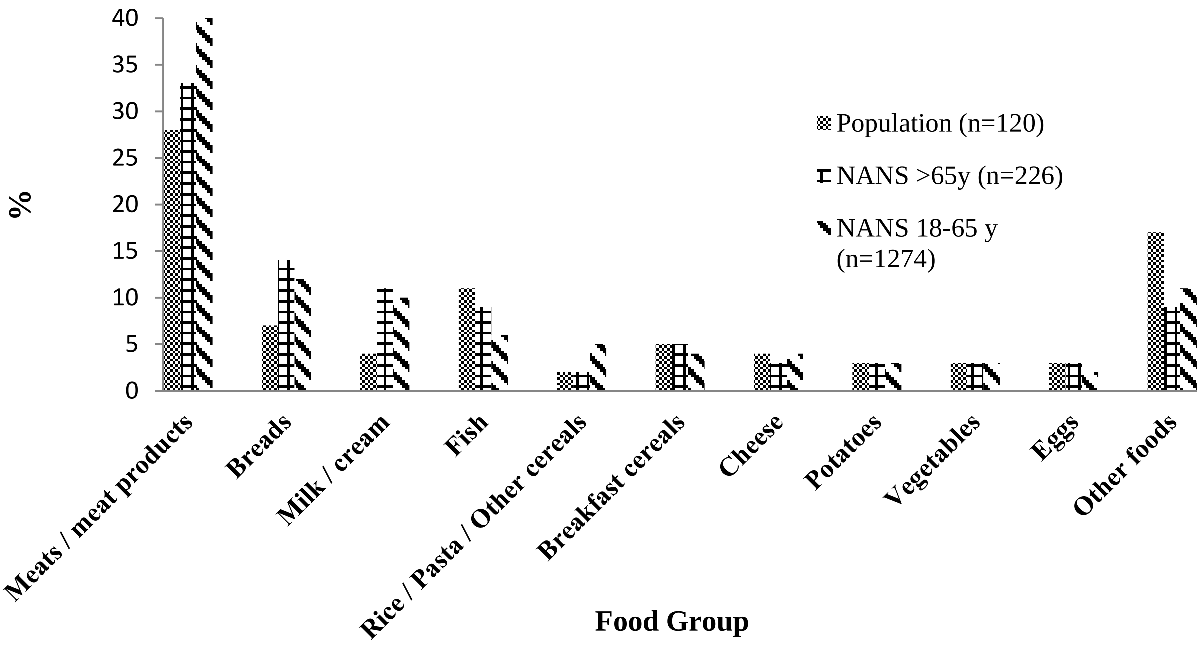


Figure 27 Contribution of food groups to total protein intake (%) for study population, and the National Adult Nutrition Survey (IUNA) populations (18-65y and > 65y)

**Each food consumed by those in the IUNA studies was assigned to one of 77 food groups in the database. For the purposes of reporting, these were reduced to 11 food groups. The same methodology was used in the current* *research. In this instance 'Other foods' contains yoghurt products and this may explain the larger contribution to this food group in the population of the current research.*

The IOM RDA and percentage of the study population achieving or exceeding the RDA for each EAA is provided in **Table 16.** The mean total EAA intake of 276.0(113.5) $mg.kg^{-1}.d^{-1}$ was 128 % of the IOM recommendation. 69% of subjects (n=83) exceeded the sum of total EAA RDA but no subject fulfilled the IOM requirement for all individual EAAs. Of note, is the apparent inadequacy in mean reported daily lysine intake at 8.2(3.7) $mg.kg^{-1}.d^{-1}$ versus the IOM recommendation of 38 $mg.kg^{-1}.d^{-1}$, where no participant in this study achieved this RDA. As with daily protein intakes, a broad range of intakes for EAAs was observed; however, as is highlighted (**Figure** 28), significant numbers of participants in this study exceeded the IOM RDA by greater than 25%

Table 16 Institute of Medicine (IOM) Recommended Daily Allowance (RDA) for amino acids, with reported daily intakes and percent of population achieving the IOM RDA for each EAA and the sum of all EAAs,

EAA	IOM RDA	Mean	SD	%achieving IOM RDA
		($mg.kg^{-1}.d^{-1}$)		
His	14.0	20.4	9.4	77%
Ile	19.0	34.1	14.3	87%
Leu	42.0	56.4	23.1	75%
Lys *	38.0	8.2	3.7	0%
Thr	20.0	27.6	11.6	74%
Trp	5.0	6.4	3.0	66%
Val	24.0	38.7	15.7	86%
Met+Cys	19.0	25.6	11.1	72%
Phe+Tyr	33.0	58.6	22.9	88%
∑EAAS	214.0	276.0	113.5	69%

*Data are presented as mean ± SD, n= 120 (♀ n=94, ♂ n=26) nnd not normally distributed **

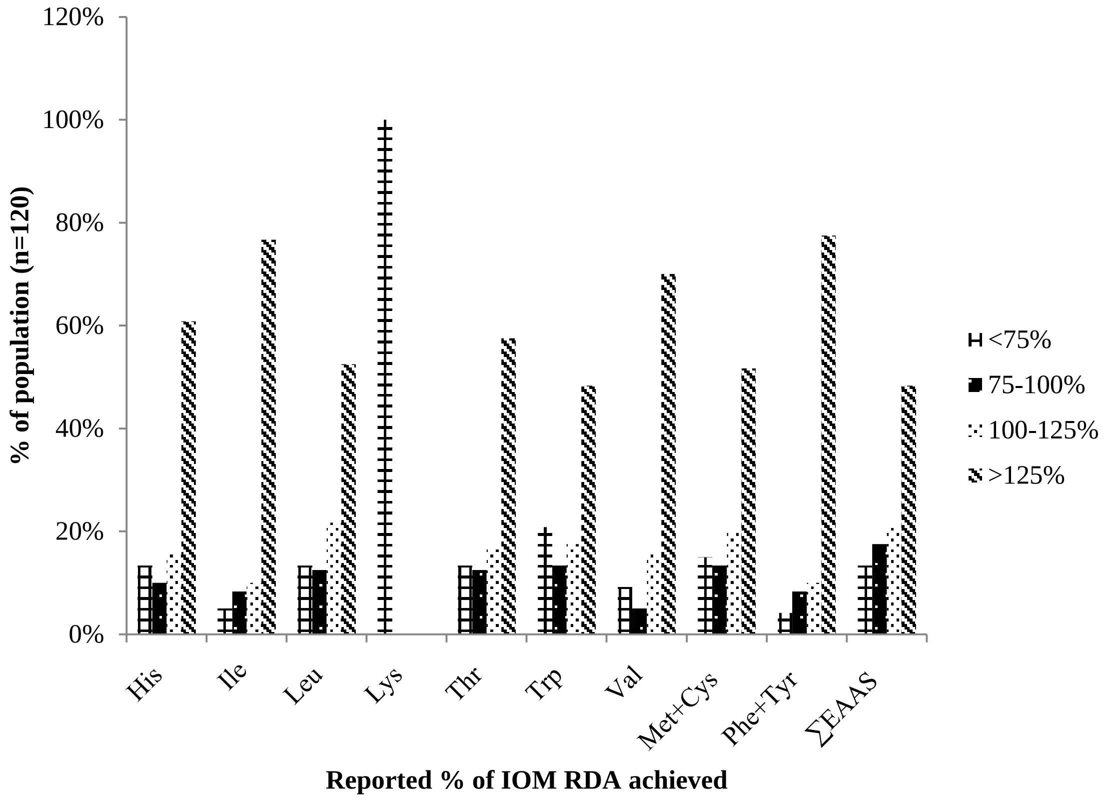


Figure 28 Intakes of essential amino acids compared with Institute of Medicine (IOM) Recommended Daily Allowance (RDA)

5.5 Discussion

5.5.1 *Development of a WISP©-based method to assess EAA intakes*

From the work completed in this study, there is now a proposed WISP©-based method for comprehensive assessment of dietary protein quality by comparison of intakes of EAA with IOM RDAs. The methodology in the present study appears to be consistent with the small number of studies that have previously documented methods of adding to or amending nutrition analysis packages (Levis et al. 2011, Neuhouser 2006, Flood 2006), where the addition of glycaemic index / load to databases was the objective.

While the data reported here represents an advancement of the WISP© database, some limitations exist in this methodology. Several foods in the WISP© data base remain without any EAA values; any research involving WISP© analysis of EAA intake will continue to be limited in its accuracy without further refinement to produce a database specific to a given study population.

Due to a lack of dietary EAA data for foods consumed specifically in an Irish population it was necessary to use USDA values. While the *mapping process* employed by technical staff at Tinuviel Software included a factor for anticipated differences in protein content (if any) between UK and US food items, there may still be some discrepancies. It is widely acknowledged that nutrient content may vary with geographic location and growing conditions (Rautiainen *et al*. 2012) giving rise to variations in the amino acid content of foods. A study by Thomas (2013) investigating variation in the nutrient composition of fruit and vegetable products reports that climatic and soil conditions can alter composition by up to 14% in some species of fruits.

While the limitations of the methodology reported here are acknowledged, this method represents a significant advancement in the efforts to provide detailed analysis of dietary protein and essential amino acid intake in an older Irish population.

To the authors knowledge, this is the first WISP©-based method to assess dietary amino acid intakes. The database available on the most recent release of WISP©V4 contains 12% cover of foods with EAA values assigned when considered by food item containing protein. The development of the refined database described here (specific to this study population) has a cover rate of 69% by food item. By comparison, the USDA database states its coverage by food item as being 58% (United States Department of Agriculture 2013), but they have not published any information on the amount of dietary protein this can account for. The methodology described in refining the database specific to this population is substantially more comprehensive as 96% of reported protein intake can be accounted for. Describing such methods will help to increase the utility of dietary EAA assessment in both clinical and research settings, furthering our understanding of their association with health and ageing.

5.5.2 *Application of the proposed method to assess dietary protein quality (by comparison with Institute of Medicine Recommended Daily Allowances)*

The adequacy of dietary intakes of protein is assessed by comparison with the IOM RDA of 0.8 $g.kg^{-1}.d^{-1}$. Persons with intakes at or above this recommendation are deemed to have an intake that is adequate to "…maintain the body protein mass (assumed to be at a desirable level), in persons at energy balance with modest levels of physical activity and any special needs for growth, reproduction and lactation" (WHO/FAO/UNU 2007). However, this does not consider those sections of the population who may have higher requirements (e.g. athletes (Tipton and Wolfe 2004), those with chronic disease (Bauer *et al.* 2008) or the elderly

(Campbell *et al.* 1994, Wolfe 2013) nor does this definition consider the precise composition of the total dietary protein intake.

Assessment of total protein intake is of paramount importance in certain populations. However, assessment of adequacy is limited by nutrition analysis software. While methodologies reported here are an advancement, they are likely too time consuming for extrapolation in large population based studies without further refinement.

Initial investigation of protein adequacy (comparison with IOM RDA for daily protein $0.8g.kg^{-1}.d^{-1}$) indicated that the majority of participants in this convenience sample meet this requirement (96%). Inspection of food group sources of protein intake suggested that the adequacy of the amino acid composition should be satisfactory given the high relative contribution of food groups deemed to be of high biological value (**Figure** 27). Application of the additional WISP© functionality revealed that the reported intakes of EAA meet or exceed IOM RDAs for a significant percentage of study participants. With the exception of lysine, the reported MDI for EAA exceeded the IOM RDA (by 29 – 79%). While there was a broad range of EAA intakes reported, **Figure** 28 illustrates that for most EAAs, more than 50% of the population achieved intakes well in excess of the IOM RDA.

One of the few available references on the adequacy of AA intakes was published in an appendix to a WHO publication on dietary reference intakes (DRIs) for energy and macronutrients (WHO 1985). The mean reported intakes of amino acids among 51-70 year olds in NHANES III is outlined in **Table 17** below. Comparison with current IOM RDAs was initially not possible with the NHANES III data as intakes are expressed in g per day rather than $g.kg^{-1}.d^{-1}$, but it does provide context for this current investigation.

Table 17 Dietary intake data on amino acids (mean reported daily intake) from NHANES III (1988-1994) and from current study, by gender

Amino Acid	***NHANES III*** ***51-70 y***		**UL Study** **50-70 y**	
g per day MDI	***Female***	***Male***	**Female**	**Male**
Ala ***g/day***	*2.9*	*4.2*	2.3	3.1
Arg ***g/day***	*3.3*	*4.9*	2.7	3.5
Asp ***g/day***	*5.3*	*7.6*	4.4	6
Cys ***g/day***	*0.8*	*1.2*	0.5	0.7
Glu ***g/day***	*12.1*	*17.1*	8.6	11.7
Gly ***g/day***	*2.6*	*3.8*	2	2.9
His ***g/day***	*1.7*	*2.5*	1.3	1.8
Hydp ***g/day***	*na*	*na*	0.2	0.2
Ile ***g/day***	*2.8*	*4*	2.2	3.1
Leu ***g/day***	*4.8*	*6.9*	3.6	5
Lys ***g/day***	*2.7*	*6*	0.5	0.7
Met ***g/day***	*1.4*	*2*	1.1	1.6
Phe ***g/day***	*2.7*	*3.9*	2.1	2.9
Pro ***g/day***	*4*	*5.7*	2.9	3.9
Ser ***g/day***	*2.8*	*4*	2.2	2.9
Thr ***g/day***	*2.4*	*3.5*	1.8	2.5
Trp ***g/day***	*0.7*	*1*	0.4	0.6
Tyr ***g/day***	*2.2*	*3.1*	1.7	2.3
Val ***g/day***	*3.2*	*4.5*	2.5	3.4

In order to facilitate comparison with IOM RDAs, and to contextualise the current study results, some extrapolations were carried out on the NHANES reported data. Anthropometric data for the same cohort reported mean body mass for these populations at 72.1kg (females) and 85.9kg (males). Using these population mean reported body mass, it was possible to estimate EAA intakes in $mg.kg^{-1}.d^{-1}$ to allow comparison with RDAs (Table 18).

Table 18 Dietary intake data on amino acids extrapolated º from NHANES III (1988-1994) with reported mean daily intakes for EAAs from current study, by gender

Amino Acid MDI (mg.kg^{-1}.d^{-1})	*NHANES III* 51-70 y *º Female*	*NHANES III* 51-70 y *º Male*	UL Study 50-70 y Female	UL Study 50-70 y Male	*IOM RDA (mg.kg^{-1}.d^{-1})*
His	*23.9*	*29.1*	19.8	22.4	*14.0*
Ile	*39.3*	*46.9*	33.3	37.3	*19.0*
Leu	*66.9*	*80.3*	55.3	61.0	*42.0*
Lys	*37.3*	*69.8*	8.0	9.0	*38.0*
Thr	*33.3*	*40.2*	27.0	30.0	*20.0*
Trp	*10.1*	*12.1*	6.2	7.1	*5.0*
Val	*43.8*	*52.5*	37.9	41.7	*24.0*
Met&Cys	*30.7*	*36.9*	25.0	27.9	*19.0*
Phe&Tyr	*67.4*	*81.0*	57.5	63.0	*33.0*
∑ EAAs	*352.6*	*448.9*	270.2	299.3	*214.0*

**∑EAA is the sum of mean group intakes of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. º Extrapolation based on population anthropometric data reporting mean body mass at 72.1 kg and 85.9 kg for females and males respectively to estimate EAA intakes in mg.kg^{-1}.d^{-1}.*

Similarities are observed in intakes across many of the EAAs, with a notable exception for lysine. The anticipated trend of greater intakes among males due to higher total energy intakes is evident in the NHANES data, but not in the data from the present study. This may be due to the low relative contribution males made to the sample population (n=26 (22%)).

It may be warranted to query the application of a dietary reference intake for EAAs derived for an American population to Irish or European communities. Considering the phenotypic differences between these groups, Solomons (2000) states that in a truly just and equitable society, the welfare of all would be fulfilled, but in a "colour blind" society, the health and nutritional needs of few would be satisfied. An interpretation of this could mean that applying nutrient recommendations for an American populous to an Irish sample may not be wise. Perhaps a better approach would be to develop Irish or European DRIs for EAAs.

The anomaly regarding calculated lysine intake in the study population raises questions. No participant achieved the IOM RDA of 38 mg/kg/d. The MDI reported was 8.2 (3.7) mg/kg/d, well below anticipated intakes when compared with the analysis of NHANES nutrient intakes (37.3 – 69.8 mg/kg/d). Interrogation of the methods used to map this nutrient, as well as detailed analysis of the original USDA AA database all suggested that there may have been a technical flaw which caused this surprisingly low level of intake. To clarify this, an individual food intake record was analysed by both the WISP and USDA database. There was good agreement between both nutrient analysis packages for energy, macro nutrients and most amino acids assessed, with the exception of lysine (**Table 19**). This suggests a technical problem with assessment of lysine intakes in the WISP database. This issue has been brought to the attention of the manufacturers, but the resolution of this is beyond the scope of this current research.

Table 19 Comparison of reported MDI for one study participant to assess differences between USDA and WISP software (n=1)

		USDA	**WISP**	**USDA:WISP**
Energy	kcal	2132	2064	0.97
Energy	kJ	8948	8666	0.97
Protein	(g)	103.8	93.6	0.90
CHO	(g)	193.4	222.6	1.15
Fat	(g)	100.6	97	0.96
Calcium	(mg)	839	1013	1.21
Vitamin D	(µg)	2.49	3.29	1.32
Alanine	(mg/kgBM)	84.9	79.0	0.93
Arginine	(mg/kgBM)	98.8	95.8	0.97
Aspartic acid	(mg/kgBM)	156.0	141.9	0.91
Cysteine	(mg/kgBM)	22.5	20.1	0.89
Glutamic acid	(mg/kgBM)	266.9	253.6	0.95
Glycine	(mg/kgBM)	81.5	76.6	0.94
Histidine	(mg/kgBM)	46.8	44.9	0.96
Hydroxyproline	(mg/kgBM)	12.1	11.3	0.93
Isoleucine	(mg/kgBM)	71.1	69.6	0.98
Leucine	(mg/kgBM)	123.1	114.4	0.93
Lysine	(mg/kgBM)	22.5	8.7	**0.38**
Methionine	(mg/kgBM)	38.1	34.3	0.90
Phenylalanine	(mg/kgBM)	65.9	61.9	0.94
Proline	(mg/kgBM)	97.1	95.1	0.98
Serine	(mg/kgBM)	67.6	61.5	0.91
Threonine	(mg/kgBM)	58.9	53.0	0.90
Tryptophan	(mg/kgBM)	12.1	11.6	0.96
Tyrosine	(mg/kgBM)	52.0	46.3	0.89
Valine	(mg/kgBM)	81.5	74.9	0.92

It should be considered that the convenience sample assessed in this study may not have dietary intake patterns representative of the national population. For this reason extension of this study to include the NANS dietary intake data are considered a vital progression in assessing dietary protein quality and elucidating correlations with health, and in developing dietary guidelines specific to an Irish population.

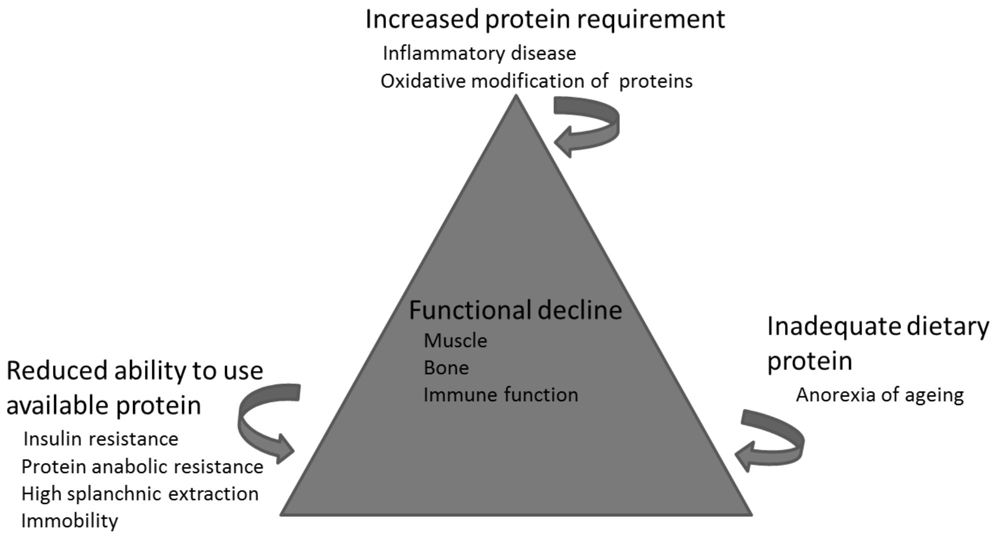


Figure 29 Ageing related causes of protein shortfall

5.6 Conclusion

There are several possible reasons contributing to the failure of 'adequate' total daily protein intake to counteract sarcopaenia, reduced total energy intake or protein combination, composition, distribution or age.

The context of this research is within a programme of work investigating nutrition in age related impairment, specifically the conservation of lean tissue mass in the elderly. Considering the extant literature presented in Chapter 2 describing nutritional solutions to sarcopaenia, further research is of paramount importance in devising both population specific dietary recommendations for EAAs and validated methods for detailed assessment of their daily intake.

This is of particular relevance to an elderly population who may be at increased risk for protein shortfall due to the age related causes illustrated in **Figure** 29. Where it is possible to overcome any of these short comings by simply choosing better sources of protein, surely these strategies must be promoted to avoid functional decline in the elderly.

Chapter 6

A nutrient support to age related lean tissue mass loss: Can supplementing dietary protein intake, with modification to quality and daily distribution, prevent age related whole body lean tissue mass loss in Irish adults (50-70 years)

A version of this chapter is in preparation for publication. Norton, C, McCormack, W, Toomey, C, Francis P, Jakeman, P 2014. A randomised controlled trial investigating the effect of 24 weeks of milk protein supplementation on lean tissue mass in healthy 50-70 year olds.

6.1 Introduction

Age-related lean tissue mass loss or sarcopaenia (a syndrome characterised by progressive and generalised loss of skeletal muscle mass and strength) (Muscaritoli et al. 2010, Rosenberg 1997, Baumgartner et al. 1996, Cruz-Jentoft et al. 2010a, Chumlea et al. 2011) is closely related to poor nutrition, physical inactivity and inflammation. A nutrient solution to the preservation of lean tissue is well founded in the extant literature, but longitudinal studies embracing the specific nutrient deficits and needs of the elderly are few. Due to its safety and ease of application, verification of the efficacy of an innovative nutrient solution taken as a supplement to habitual intakes is necessary and has the potential to impact positively on healthy ageing. This approach is supported by the concluding statement of the report of the European Working Group on Sarcopenia in Older People (EWGSOP) that states that 'based on increased awareness of sarcopaenia in older people and widespread use of tools for screening and assessment, the ultimate goal is to identify dietary strategies, lifestyle changes and treatments that can prevent or delay the onset of sarcopenia', (European Working Group on Sarcopenia in Older People 2010)

Although the mechanisms of sarcopaenia are complex (Figure 30), the roles of both adequate physical activity (Montero-Fernández 2013, Miyachi 2011, Roth 2000) and nutrition (Bauer et al. 2008, Bonnefoy 2008, Campbell 2007, Cruz-Jentoft et al. 2010b, Fujita and Volpi 2004, Kim 2010, Malafarina 2013, Millward 2012, Morley et al. 2010, Volkert 2011) are irrefutable for maintenance of muscle mass and function in older people. It has been proposed that increasing intakes or altering patterns of nutrient consumption may represent effective strategies in preventing age related lean tissue mass loss. To date, there is a lack of agreement among researchers in this field; where some long-term intervention studies show clear benefits of dietary supplementation on skeletal muscle mass and / or function, others have not shown any measurable benefit on lean tissue mass in the elderly (**Table 4**). However, according to a recent systematic review, nutritional supplementation (optimising nutrient type and timing) is effective in improving muscle mass and functionality in old age (Malafarina 2013). To determine the possible benefits of nutritional supplementation on lean tissue mass in an older population, more evidence from well-designed long-term intervention trials is required. Therefore, this study was designed to investigate, in a randomised, single-blind, placebo-controlled manner, the impact of 24 weeks of dietary protein supplementation on whole body lean tissue mass in a group of community-living, older men and women.

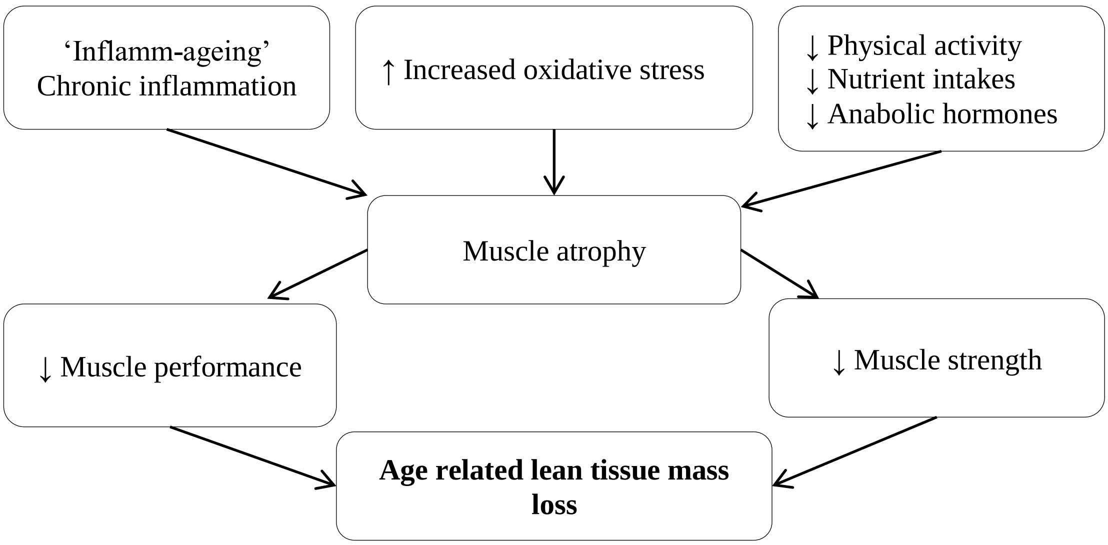


Figure 30 Underlying mechanisms that contribute to sarcopaenia-induced muscle wasting

6.2 Objectives

The aim of this study was to investigate the impact of a 24 week nutrient supplementation (milk protein-based, twice daily with smaller meals) on whole body lean tissue mass in a group of community dwelling Irish adults (50-70 years), through a randomised, single-blind, placebo-controlled trial.

6.3 Methods

Ethical approval was granted for the study by the Faculty of Education & Health Sciences Research Ethics Committee (EHSREC10/45), University of Limerick.

6.3.1 *Subjects*

A convenience sample of 140 healthy adult women and men aged 50-70y were recruited from the University of Limerick’s faculty and surrounding community through campus-wide email invitation, posters and by word-of-mouth. Those who expressed interest in participation were invited to a brief presentation outlining the salient points and participant requirements. Eligible participants provided a full medical history and were screened by a medical doctor (Figure 31). Those defined as healthy, i.e. disease-free based on guidelines detailed by Grieg *et al*. (1994), independent-living, physically mobile and willing to consume a nutrient supplement in addition to their habitual diet, twice per day (b.d.) for 24 weeks were invited to participate and to provide written, informed consent.

6.3.2 *Study Design*

On entry to the study participants were randomly assigned (in a single- blind manner) to receive a food supplement containing either a milk-protein based formulation (FORM) or an iso-caloric non-nitrogenous control (CON). The primary outcome measure was lean tissue mass measured by dual X-ray absorptiometry (DXA).

Completion of a 4 day estimated food intake record (eFIR) (as previousy described – see Chapter 3) and body compositional analysis preceded the 24 week intervention (0 weeks) and was repeated after 12 and 24 weeks of intervention (details of these procedures are provided anon).

Whole body lean tissue mass was the principal outcome variable. Assuming a mean change in total lean tissue mass of ~2% (i.e. ~1 - 1.5kg with the estimated SD of the treatment effect =4.5) over the period of the intervention, statistical power would be >80% at α=0.05. A minimum of 30 participants per treatment group would be required to detect a difference in lean tissue mass. With an anticipated attrition rate of approximately 30% (Mutsaerts 2013), a sample size of 40 participants per group was considered adequate.

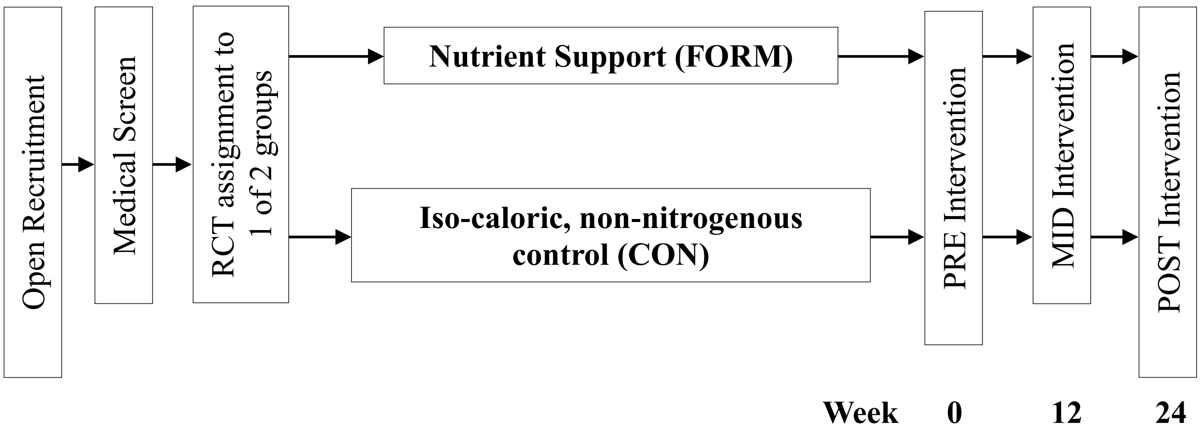


Figure 31 Intervention: study design

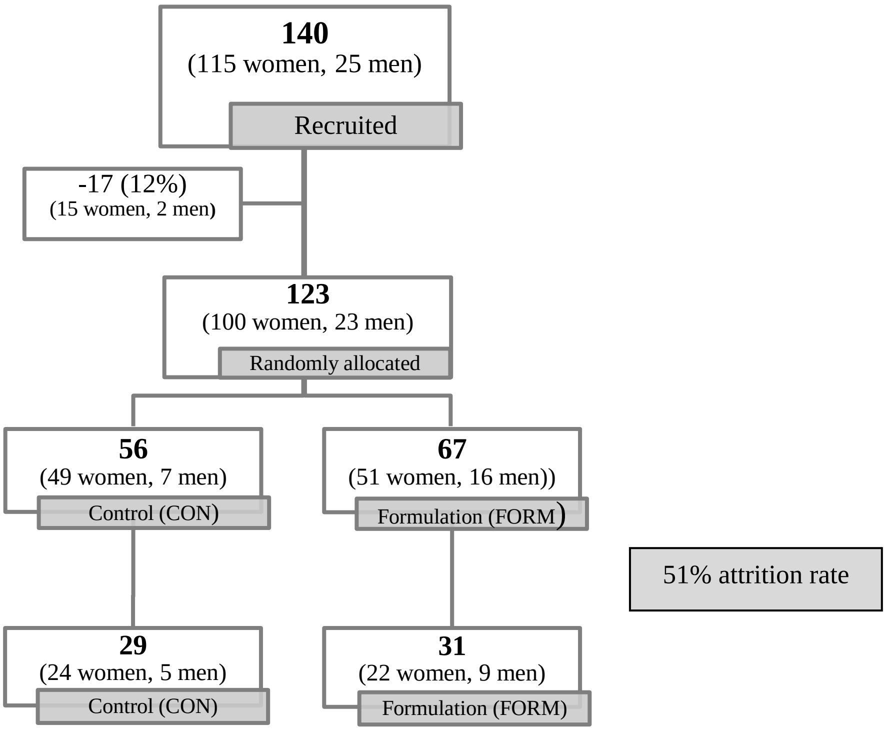


Figure 32 Flowchart of participant numbers from recruitment and design to completion

6.3.3 *Derivation of the nutrient support to age related lean tissue mass loss*

It has been established that dietary protein ingestion stimulates muscle protein synthesis, and inhibits protein degradation resulting in a net lean tissue mass accretion (Malafarina 2013, Evans et al. 2013, Wolfe 2012). The extant literature discussed in Chapter 2 identified the key regulators of muscle protein synthesis to be the essential amino acids, principal of which is leucine (Casperson et al. 2012, Katsanos 2006, Dardevet et al. 2003, Volpi 2013, Bauer et al. 2013). Many researchers have proposed an increase to the RDA for protein in an elderly population to ~1.2 $g.kg^{-1}.d^{-1}$ (Bunker at al. 1987; Campbell *et al*. 2001; Evans *et al*. 2004; Morais *et al*. 2006; Paddon-Jones *et al*. 2008; Breen and Philips 2011; Bauer *et al*. 2013). This is thought necessary to offset the reported phenomenon of anabolic blunting to protein and / or amino acid feeding that has been observed in the elderly (Breen and Phillips 2011; Burd *et al*. 2012; Paddon-Jones *et al*. 2013). Reported observations from an Irish elderly population (IUNA 2011) suggest that the revised recommendation for daily protein intake is not met, particularly in those over 65 years, with SLÁN (2008) reporting mean protein intakes of 93 g/day and 87 g/day respectively for males and females.

A further nutrient positively associated with lean tissue mass and function is vitamin D. Low vitamin D status has been associated with reduced lower-extremity muscle strength, slower walking speed, and impaired balance in the elderly (Bischoff-Ferrari 2004, Visser et al. 2003, Pfeifer 2010, Ceglia and Harris 2013), all of which are reported to improve with vitamin D and/or calcium supplementation (Bischoff-Ferrari 2004). Estimates of the vitamin D intake required to achieve the optimal concentration of 25(OH)D for older adults (50–60 nmol/L) indicate an intake of 15μg or 600 IU/d would suffice (Bischoff-Ferrari et al. 2006). Sato and colleagues (2005) observed increases in the relative number and size of type II muscle fibres and improved muscle strength in elderly women with post-stroke hemiplegia who received 1,000 IU Vitamin D daily. At baseline, serum 25-hydroxyvitamin D levels were in the deficient range in all patients and the researchers conclude that intakes in excess of 1000 IU/d (Sato et al, 2005) are required to have any effect on preventing lean tissue mass loss in healthy populations and those with vitamin D sufficiency.

Studies suggest that a diet supplemented with a combination of antioxidants may possibly increase antioxidant defences, lower muscle oxidative damage, and improve muscle protein balance during senescence (Chaput et al. 2007, Cerullo 2012, Jensen 2008, Jeong-Su Kim et al. 2010). However, the effect of antioxidant supplementation on muscle performance is still largely controversial. Similarly to other nutrient supports proposed in the prevention of muscle mass loss, there are both positive and null results. Consensus in the research community seems to be that caution should be used in recommending high dose antioxidants in the treatment of muscle atrophy and further work is required to substantiate routine use in wasting conditions. However, consideration has been given to whether milk proteins could contribute to dietary antioxidant capacity as several milk derived peptides have been found to possess antioxidant (AO) activity (Pihlanto 2006; Power *et al*. 2013).

Therefore it was hypothesised that an increase in protein intake of $0.33 g.kg^{-1}.d^{-1}$, where 50% of amino acids provided were essential, with a leucine contribution of $0.03 g.kg^{-1}.d^{-1}$, inclusive of antioxidant peptides and fortified with adequate vitamin D to correct deficiency would prevent age related whole body lean tissue mass loss.

Consequently, the protein formulation consisted of a proprietary blend of milk protein concentrates (MPC), two whey protein hydrolysates (WPH) DH32% and DH45% in the ratio 9:2:1 (MPC:DH32:DH45). The relative contribution of the two main milk proteins are 60% intact casein protein, 25% WPH and 15% intact whey protein. MPC was chosen as the ‘core protein’ in the formulation due to its EAA composition, in combination with the fact that the bitter aftertaste associated with protein could be masked more easily than some other proteins.

MPC is also more suitable than whey protein for incorporation into a pH neutral ready to drink beverage, making the formulation more commercially viable, subject to the establishment of its clinical efficacy. The WPHs were included due to their potent antioxidant bioactivity measured *in vitro* (Power *et al.* 2013). The formulation was also fortified with vitamin D (0.25 ug.kg^{-1}.d^{-1}) (**Table 21**). The non-nitrogenous, iso-caloric control substance consisted of maltodextran (**Table 22**). To accommodate phenotypic variation the nutrient support to lean tissue mass loss was prescribed on a per kg body mass (BM) basis **(Table 20**).

Table 20 Daily prescription of nutrient support based on body mass

Body mass range and median (kg) value			45 to 60 (52.5)	60 to75 (67.5)	75 to 90 (82.5)	90 to 105 (97.5)
			n=8	n=29	n=17	n=6
Nutrient	**Unit**	**Unit per kg BM**	**Unit per median kg BM**			
Milk Protein	(g)	0.33	17	22	27	35
Leucine	(g)	0.03	1.6	2.0	2.4	3.0
Vitamin D	(ug)	0.25	13	17	21	24
Milk calcium	(mg)	10	525	675	825	975

Chapter 2 discussed the potential benefits of optimising the distribution of protein intake evenly across eating occasions. In light of the research presented (Paddon-Jones and Rasmussen 2009, Evans et al. 2013, J. S. Kim et al. 2010, Millward 2012, Paddon-Jones et al. 2008) the prescribed dose was divided between the two smaller meals of the day (generally breakfast and lunch) to enhance any effect of the supplement on accrual or preservation of lean tissue mass.

Following discussion with industrial partners (Food for Health Ireland) it was agreed that the greatest utility and ease of application of the nutrient support was as a powder format, presented in single serving sachets to be added to water (consumed as a shake) or sprinkled on food e.g. cereal, soups, yoghurts. The typical nutrient content of an individual sachet for the nutrient support formulation and the iso-caloric, non-nitrogenous control is presented in Tables 21 and 22.

Table 21 Formulation, typical nutritional composition per 100g powder and per individual sachet determined by body mass

FORMULATION					
	100 g powder	12.6 g powder	16.1 g powder	19.7 g powder	23.3 g powder
		A	**B**	**C**	**D**
AA Content *g*					
Alanine	2.4	0.3	0.4	0.5	0.6
Arginine	2.1	0.3	0.3	0.4	0.5
Aspartic acid	5.3	0.7	0.9	1.0	1.2
Cystine	1.0	0.1	0.2	0.2	0.2
Glutamic acid	13.2	1.7	2.1	2.6	3.1
Glycine	1.1	0.1	0.2	0.2	0.3
Histidine	1.9	0.2	0.3	0.4	0.4
Isoleucine	3.4	0.4	0.6	0.7	0.8
Leucine	6.3	0.8	1.0	1.2	1.5
Lysine	5.1	0.6	0.8	1.0	1.2
Methionine	2.0	0.3	0.3	0.4	0.5
Phenylalanine	2.9	0.4	0.5	0.6	0.7
Proline	5.6	0.7	0.9	1.1	1.3
Serine	3.4	0.4	0.5	0.7	0.8
Threonine	3.2	0.4	0.5	0.6	0.8
Tryptophan	0.0	0.0	0.0	0.0	0.0
Tyrosine	2.6	0.3	0.4	0.5	0.6
Valine	4.3	0.5	0.7	0.8	1.0
Sum total AAs	65.9	8.3	10.6	13.0	15.4
Other nutrients					
Protein g	70.3	8.9	11.3	13.8	16.4
Moisture g	5.1	0.6	0.8	1.0	1.2
Fat g	2.3	0.3	0.4	0.4	0.5
Ash g	13.0	1.6	2.1	2.6	3.0
Carbohydrate g	9.4	1.2	1.5	1.9	2.2
Energy kcal	339	43	55	67	79
Calcium mg	3903	492	628	769	909
Sodium mg	427	54	69	84	99
Potassium mg	764	96	123	151	178
Magnesium mg	242	30	39	48	56
Phosphorus mg	2433	307	392	479	567
Vitamin D3 ug	56.3	7.1	9.1	11.1	13.1
Vitamin D3 IU	2250	284	362	443	524
T-ORAC mmol TE	25.2	3.2	4.1	5.0	5.9

Table 22 Control, typical nutritional composition per 100g powder and per individual sachet determined by body mass

	CONTROL				
	100 g powder	11.5g powder L	14.5 g powder M	17.5 g powder P	21.0 g powder H
Carbohydrate g	94.6	10.9	13.7	16.5	19.9
Energy kcal	378	43	55	66	79

6.3.4 *Protein supplementation regime*

Subjects were blinded to treatment allocation, provided with a dietary supplement based on body mass (**Table 20**), in a powdered format and instructed to reconstitute with water, or sprinkle on cooked food and consume twice daily, at breakfast and lunch. Participants were given 60 individual sachets monthly. Each supplement dose was individually packaged in a foil sachet. Six flavours were available (strawberry, apple 'n cinnamon, pineapple, banana, orange 'n mango and cream), to prevent flavour fatigue and improve compliance. Each participant was given a shaker to reconstitute the supplement and a recipe book detailing ideas on how to incorporate the powdered supplement to meals.

6.3.5 *Compliance*

Compliance to the prescribed supplement was assessed by monthly count-back of returned used / unused sachets. Supplements were provided to subjects on a monthly basis with instructions that all used and any remaining unused sachets must be returned to the research centre at the end of each month. This facilitated monitoring of compliance to the supplement programme by count-back.

6.3.6 *Dietary data collection*

A qualified dietitian gave oral and written instructions on recording food types, quantities, cooking methods and meal times. Participants were instructed to record all food and drink consumed for four consecutive days to include two week days and 2 weekend days, detailing information regarding the amount (using household measure and estimates of portion sizes), and types of all foods, beverages and nutritional supplements consumed over the recording period and where applicable, the cooking methods used, brand names of the foods consumed and details of recipes. Data were also collected on the time of each eating or drinking occasion, the respondent's definition of each eating or drinking occasion (e.g. morning snack, lunch) and the location of the preparation of the meal or snack consumed (e.g. home, takeaway). During a subsequent visit, the dietitian checked the diary for completeness and obtained any additional information required to improve the accuracy of the dietary record.

6.3.6.1 Estimation of nutrient intake

Food intake data were coded and subsequently analysed using WISP© (Tinuviel Software, Anglesey, UK). WISP© uses data from McCance and Widdowson's The Composition of Foods, sixth (Food Standards Agency 2002) and fifth (Holland *et al*. 1995) editions plus all nine supplemental volumes (Holland *et al*. 1988; Holland *et al*. 1989; Holland *et al*. 1991; Holland *et al.* 1992; Holland *et al*. 1993; Chan *et al*. 1994; Chan *et al*. 1995; Chan *et al*. 1996; Holland *et al*. 1996) to generate nutrient intake data. During this study, modifications were made to the food composition database to include recipes of composite dishes, nutritional supplements, generic Irish foods that were commonly consumed and new foods on the market. All previous modifications to the food composition database were also checked and updated from current manufacturers' information as necessary.

6.3.6.2 Quality control

A number of quality procedures were put in place in an attempt to minimise error and ensure consistency throughout the collection and manipulation of food intake data. It was stressed to study participants that they should not try to change or 'improve' their diet during the recording period. Random dual entries were performed on multiple food intake records to identify and reduce errors. This was carried out by the same observer on two occasions separated by a minimum of one week and also by another independent researcher following the same data coding and entry procedures. At the end of the recording period, respondents were asked whether their food intake had been the same as usual, less than usual or more than usual during the recording period and to explain why this might have been. Respondents were also asked whether they had been on a weight reducing or weight gain diet whilst taking part in the recording phase, and if there were any items consumed during the recording period which had not yet been written down. Details on such items were recorded and subsequently entered to food intake records. Participants were weighed at the beginning and end of the four day eFIR to assess any change in body mass over the recording phase.

To remove some of the bias introduced by misreporting of food records, under-reporters [defined as having a reported energy intake (EI) to basal metabolic rate (BMR) ratio less < 1.1

(IUNA 2011)] were identified and excluded from analysis. The IUNA cut-off was used to allow comparison between the current work and national datasets. It is acknowledged that this may not be the most appropriate screen to use as it is reported that a EI:BMR cut-off set at 1.1 identifies only 66% of those who under-report. (Medical Research Council (MRC) Dietary Assessment Report, 2014).

6.3.7 *Blood sampling*

A qualified phlebotomist collected blood samples after an overnight fast. Approximately 10 mL of whole blood was collected from an IV Safety-Multifly®-Set (Sarstedt) inserted in the antecubital vein of the dominant arm and divided between serum and plasma vials. Samples were centrifuged (5000 rpm, 4◦C for 10 minutes) and the resultant supernatant aliquot and stored at -80◦C for later analysis.

Analysis of all samples (excluding vitamin D) was carried out in the biochemistry laboratories in University Hospital Limerick. These laboratories are monitored under the Irish External Quality Assessment Scheme (IEQAS), an independent scheme for the objective assessment of analytical performance in laboratory medicine and primary care in Ireland. Plasma insulin was measured by electrochemiluminescence on a Meso Scale Discovery (MSD) QUICKPLEX SQ120 (Meso Scale Discovery, MD, USA). The intra-assay CV was less than 5%. Plasma glucose concentration was measured by glucose oxidase method on a UniCel DxC 800 instrument (Beckman Coulter, Inc. CA, USA). The intra-assay CV was less than 5%. Vitamin D was measured at the University of Limerick using the Elecsys Vitamin D Total (Roche Diagnostics, Mannheim Germany). This assay employs a competitive test principle using recombinant Vitamin D Binding Protein (VDBP) as a capture protein to bind 25OH Vitamin D_3 and 25OH Vitamin D_2, with an intra-assay CV = 4.9%.

6.3.8 *Body composition assessment*

A protocol was established in order to standardise conditions of body composition assessment for all participants. All procedures were in line with the official positions of the International Society for Clinical Densitometry (ISCD) for body composition DXA scanning (Shepherd *et al*. 2013). Each participant was instructed to refrain from strenuous exercise in the 12 hour period before testing, to fast from 22.00 hours the previous night, and to consume 500 mL of water one hour prior to testing. They were also asked to empty their bladder immediately prior to measurement. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Birmingham, UK) with the respondent's head positioned in the Frankfurt Plane. Body mass was measured to the nearest 0.1kg (Tanita MC-180MA Body Composition Analyser, Tanita UK Ltd.) with participants wearing light clothing and without shoes. A Lunar iDXA scanner (GE Healthcare, Chalfont St Giles, Bucks, UK0 with encore ™ 2011v.13.6 software) was used to capture total body composition. Calibration with the use of a phantom spine was carried out daily. Participants were positioned on the scanner bed according to the manufacturer's recommendations. The coefficient of variance (CV) of the iDXA for repeated measures of whole body composition was 0.6%. Participant guidelines were issued regarding the DEXA scan procedures.

6.3.9 *Statistical Analysis*

Statistical analyses were performed using PASW Statistics 20.0 for Windows (SPSS, Inc., Chicago, IL.). Statistical significance (two-tailed) was set at $p<0.05$ for all analyses. All data were tested for normality and homoscedasticity using Shapiro-Wilk and Levene's test respectively. Mean values and standard deviations (SD), median and interquartile range (IQR) are reported, as appropriate, for baseline and repeated descriptive statistics. Differences between groups at baseline were assessed by one-way ANOVA (normally distributed variables) or Kruskal-Wallis test (non-normally distributed variables).The dependent variable (24-week change in lean tissue mass (ΔLTM)) was found to be normally distributed and therefore analysed using a univariate two-way ANOVA with treatment group and gender defined as fixed factors. To account for variance in baseline values of the dependant variables, the relative change from baseline was also analysed. Where significant interaction was evident a pairwise comparison with Bonferroni correction was employed to identify differences between groups. The treatment effect was additionally analysed by two-way ANCOVA with group and gender as fixed factors, with baseline value of the dependent variable as a covariate.

6.4 Results

6.4.1 *Subjects*

140 volunteers (115 women, 25 men) were recruited and agreed to participate in the study. Seventeen participants (15 women, 2 men; -12%) were excluded following medical screening. Among the reasons for exclusion was renal disease, uncontrolled diabetes, coeliac disease, or being physically unfit to participate in an exercise programme (The data pertaining to the

exercise intervention is not presented in this thesis). The remaining 123 subjects were randomly assigned to either CON (49 women, 7 men) or FORM (51 women, 16 men) (Figure 32**).** From group allocation to completion at week 24, there was a 51% attrition rate. The reasons for discontinuing the intervention are outlined in Figure 33. Thirty seven percent reported intolerance to the supplement as their reason for discontinuing the study. This broad title encompassed dissatisfaction with group allocation and / or dissatisfaction with the palatability of the supplement. At recruitment participants were informed that they could be randomly assigned to a nutrient intervention study (either CON or FORM) or a nutrient with physical activity study arm (FORM+PA). The FORM+PA data are not included in this thesis, however those participants who had hoped to participate in the FORM+PA cited *Tolerance* as their reason for discontinuing.

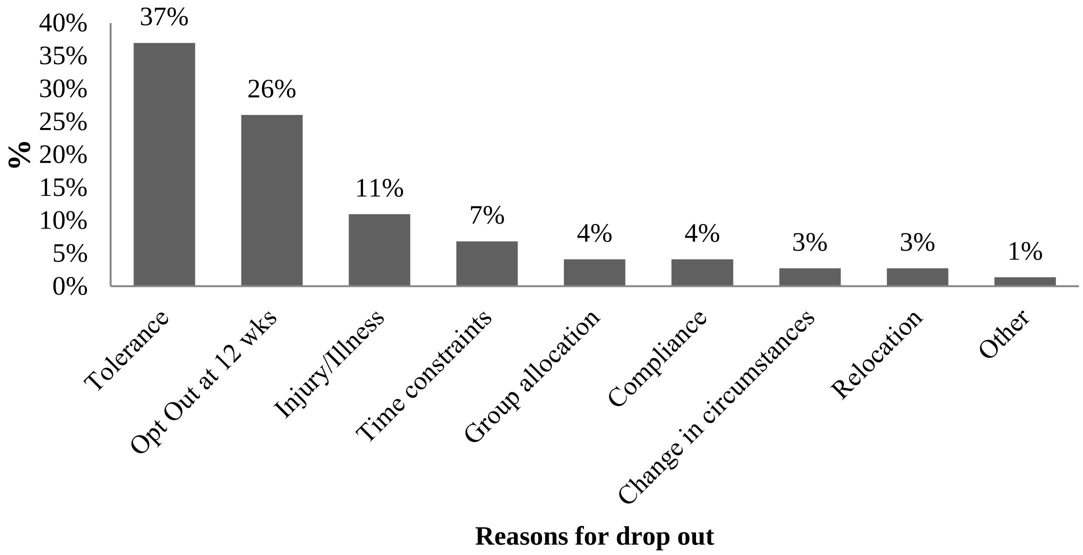


Figure 33 Reasons for discontinuing the 24 week intervention

(n=63, 54 ♀and 9♂) Opt out at 12 weeks – participants chose to cease their involvement at the mid-point asessment (Week 12)

Baseline characteristics for those who completed the 24 week intervention are presented in Table 23 Baseline body composition profile of participants who completed the 24 week intervention.Table 23 and Table 24. . Dietary data are presented in **Table 25, Table 26** and Figure 34, Figure 35, Figure 36 and Figure 37. There were no significant differences between groups for any of the baseline characteristics except for total cholesterol. Between group differences at baseline were examined by One-way ANOVA (normally distributed variables) or Kruskal Wallis test (non-normally distributed variables).

Table 23 Baseline body composition profile of participants who completed the 24 week intervention.

	CON (n = 29)		FORM (n = 31)		
	Median	**IQR**	**Median**	**IQR**	***P value***
Age (y)	60.0	55.1-64.3	63.0	59.0-65.5	0.07
Height (cm)	166.0	162.8-168.3	163.0	160.0-170.2	0.47
Body Mass (kg)	70.0	62.2-79.7	68.4	63.5-82.5	0.91
BMI ($g.cm^{-2}$)	24.9	23.4-27.4	25.6	23.9-28.5	0.91
[1]FTM (kg)	22.8	38.3-45.7	25.0	20.3-30.8	0.99
LTM (kg)	40.2	37 - 66	40.0	36.7-52.8	0.78
[1]Body Fat%	35.0	32.3-42.1	35.1	30.6-40.7	0.91

[1] Normally distributed (Shapiro-Wilk p > 0.05). BMI, Body mass index; FTM, Fat tissue mass; LTM, Lean tissue mass; IQR, interquartile range. Between group differences at baseline examined by One-way ANOVA (normally distributed variables) or Kruskal Wallis test (non-normally distributed variables).

Table 24 Baseline haematological profile of participants who completed the 24 week intervention.

	CON (n = 29)		FORM (n = 31)		
	Median	**IQR**	**Median**	**IQR**	***P value***
Glucose (mM)	4.9	4.7-5.0	4.9	4.6-5.2	0.12
HOMA IR	1.0	0.3-0.7	1.2	0.2-0.6	0.54
[1]Cholesterol (mM)	5.5	5.1-6.2	5.3	4.6-5.9	**0.03**
Albumin (g/L)	42.0	40.3-43.3	41.0	38.7-43.8	0.66
Creatinine (µM)	72.0	44 - 102	65.0	4.6-5.9	0.72
Urea (mM)	4.5	4.1-5.6	4.7	3.9-5.9	0.75
[1]Calcium (mM)	2.3	2.27-2.37	2.3	2.22-2.35	0.05
[1]Vitamin D (nM)	67.0	54.9-80.4	68.0	49.0-79.2	0.85
eGFR	83.0	74.2-96.7	89.0	75.6-98.1	0.67

[1] Normally distributed (Shapiro-Wilk p > 0.05). HOMA IR; Homeostasis model assessment of insulin resistance, eGFR; estimated glomerular filtration rate. Between group differences at baseline examined by One-way ANOVA (normally distributed variables) or Kruskal-Wallis test (non-normally distributed variables).

Table 25 Baseline nutrient intake of participants who completed the 24 week intervention *

	CON (n = 26)		FORM (n = 15)		
	Median	**IQR**	**Median**	**IQR**	***P value***
Energy (kcal)	1777.0	1583.0-2176.0	2149.0	1533.0-2284.5	0.48
[1]Protein ($g.d^{-1}$)	87.0	73.6-99.5	85.0	66.8-96.8	0.84
[1]Fat ($g.d^{-1}$)	72.0	60.1-82.5	73.0	59.4-82.0	0.28
Carbohydrate ($g.d^{-1}$)	199.0	169.8-236.5	227.0	207.1-254.2	0.32
Protein ($g.kg^{-1}$)	1.2	1.0-1.4	1.3	1.1-1.3	0.85
[1]EAA ($g.d^{-1}$)	16.8	9.8-20.1	13.3	8.9-20.0	0.97
Leucine ($g.d^{-1}$)	3.9	2.8-5.1	3.1	2.5-4.8	0.71
Vitamin D ($\mu g.d^{-1}$)	2.6	1.9-3.3	2.8	2.2-4.5	0.81

[1] Normally distributed (Shapiro-Wilk p > 0.05). Between group differences at baseline examined by One-way ANOVA (normally distributed variables) or Kruskal Wallis test (non-normally distributed variables).

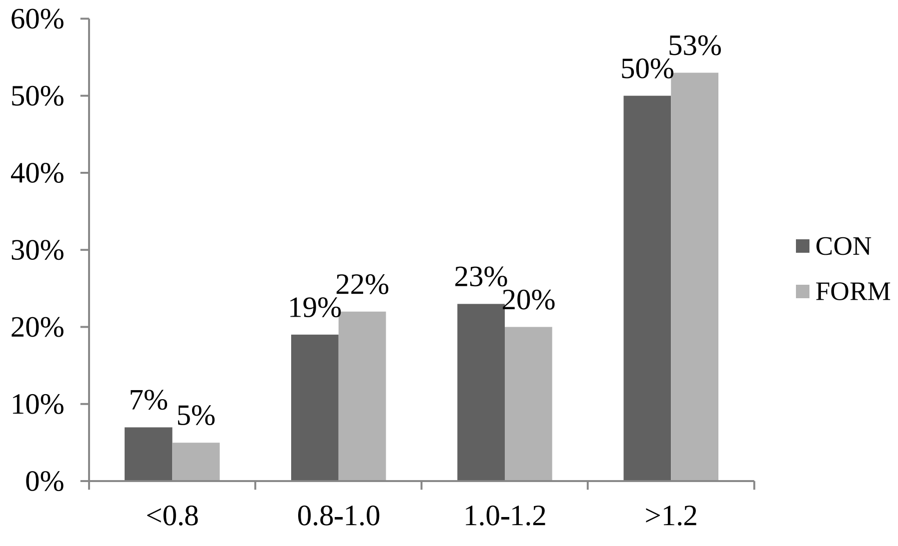


Figure 34 Ranges of protein intakes ($g.kg^{-1}.d^{-1}$) and percent of population (completers to 24 weeks) achieving s (n=41, ♀=32, ♂=9)

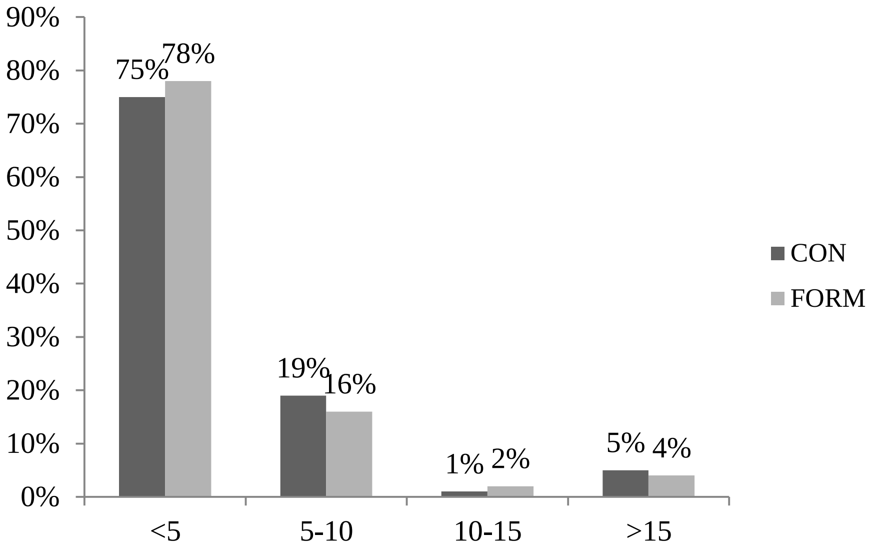


Figure 35 Ranges of vitamin D intakes (µg per day) and percent of population (completers to 24 weeks) achieving same **(n=41, ♀=32, ♂=9)**

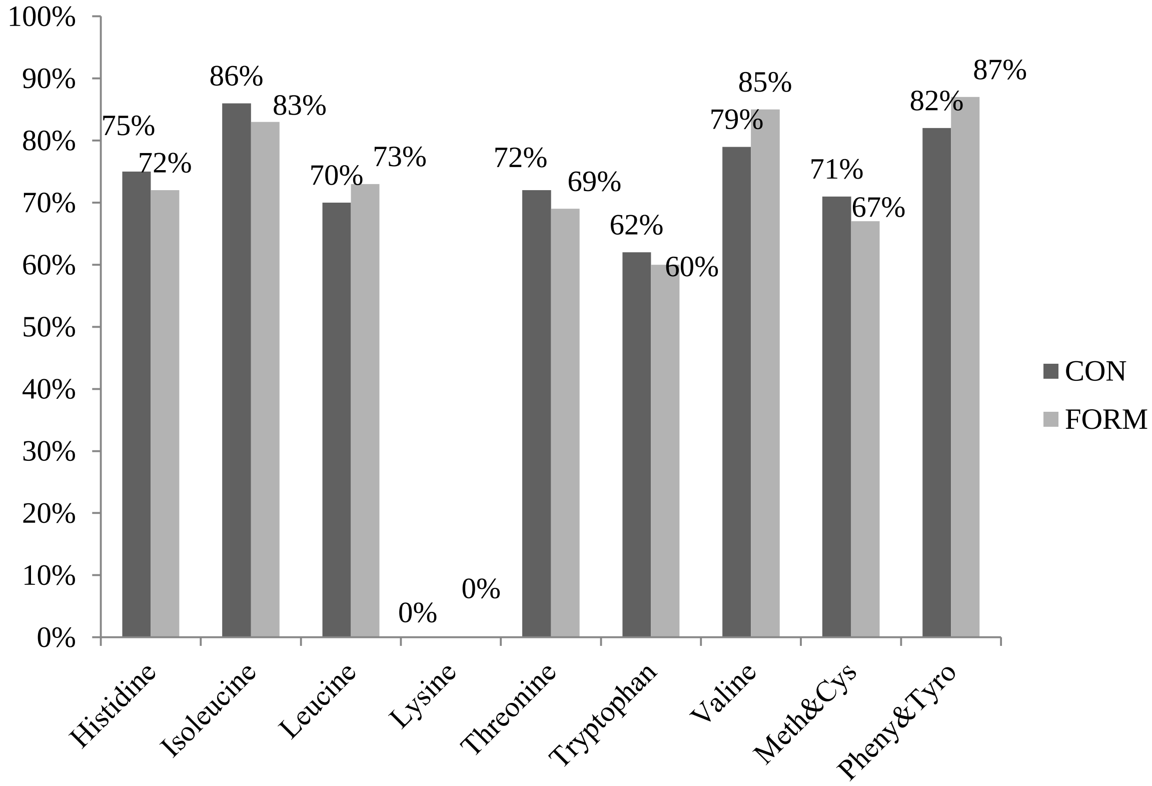


Figure 36 Percent of population (completers to 24 weeks) achieving Institute of Medicine recommended daily allowances for amino acic **(n=41, ♀=32, ♂=9)**

**Numbers within the dietary results section are fewer than other results as these data were screened for under reporting and those misreported intakes were excluded from analysis*

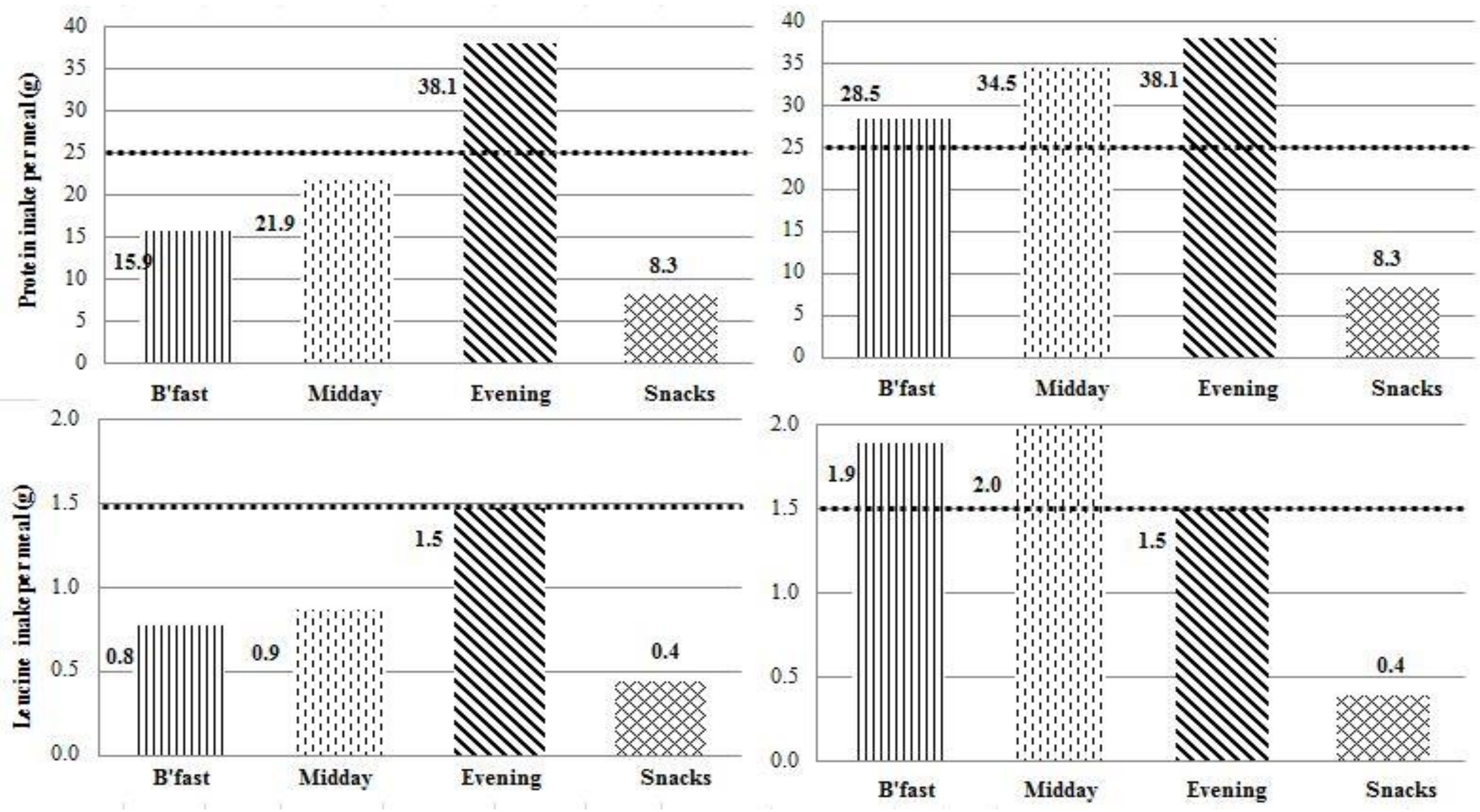


Figure 37 Protein and leucine intake per meal at week 0 (left) and optimised distribution following milk protein supplementation (right).

Dashed line represents the proposed threshold for protein and leucine intake required to overcome "anabolic resistance" in older individuals (Paddon-Jones and Rasmussen 2009). (Completers to 24 weeks, n=41, ♀=32, ♂=9)

Table 26 Baseline meal level analysis of reported protein and amino acid intake

	Protein (g)	[1] Histidine (g)	Isoleucine (g)	Leucine (g)	[1] Lysine (g)	Threonine (g)	Tryptop (g)	Valine (g)	Meth&Cys (g)	Phen&Tyros (g)	∑AAs (g)	∑EAAs (g)
						Breakfast						
Mean	15.9	0.2	0.5	0.8	0.1	0.3	0.1	0.6	4.0	0.9	9.3	3.8
SD	7.5	0.2	0.3	0.6	0.1	0.3	0.1	0.4	2.7	0.6	6.6	2.8
						Midday						
Mean	21.9	0.3	0.5	0.9	0.1	0.4	0.1	0.6	0.4	0.9	10.6	4.3
SD	12.7	0.3	0.5	0.7	0.1	0.4	0.1	0.5	0.3	0.8	9.0	3.8
						Evening						
Mean	38.1	0.6	0.9	1.5	0.2	0.8	0.2	1.0	0.7	1.5	17.4	7.3
SD	15.3	0.4	0.6	0.9	0.1	0.5	0.1	0.6	0.4	0.9	10.5	4.5
						Snacks						
Mean	8.3	0.1	0.3	0.4	0.1	0.2	0.1	0.3	0.2	0.5	5.3	2.1
SD	4.4	0.1	0.2	0.4	0.1	0.2	0.1	0.3	0.2	0.4	4.5	1.9

[1] *Not normally distributed (Shapiro-Wilk $p < 0.05$) (n=41, ♀=32, ♂=9)*

Recommendations for meal level distribution of amino acids are not available. The extant literature suggests a Leucine intake >1.5 g on each eating occasion (Paddon-Jones and Rasmussen 2009)

Optimal meal level apportioning for protein is suggested to be >25 g per meal (Paddon-Jones and Rasmussen 2009)

Table 27 Mean nutrient intake at baseline and Week 24 for CON and FORM groups

	CON (n=26)				
	Baseline		24 week		
	Median	IQR	Median	IQR	*P value*
Energy (kcal)	1777	1583.0-2176.0	1977	1243-2799	0.48
[1]Protein ($g.d^{-1}$)	87.0	73.6-99.5	78.7	69.8-139.9	0.16
[1]Fat ($g.d^{-1}$)	72.0	60.1-82.5	72.9	51.7-94.4	0.98
Carbohydrate ($g.d^{-1}$)	199.0	169.8-236.5	234.0	156.5-326.0	0.09
Protein ($g.kg^{-1}$)	1.2	1.0-1.4	1.1	0.7-3.1	0.28
ORAC (mmolTE)	13.8	8.3-16.6	8.7	6.6-18.8	0.01
Leucine ($g.d^{-1}$)	3.9	2.8-5.1	2.1	2.2-6.9	0.00
Vitamin D ($\mu g.d^{-1}$)	2.6	1.9-3.3	5.0	2.2-19.7	0.25
	FORM (n=15)				
	Baseline		24 week		
	Median	IQR	Median	IQR	*P value*
Energy (kcal)	2149	1533.0-2284.5	1807	1340-2801	0.10
[1]Protein ($g.d^{-1}$)	85.0	66.8-96.8	96.1	66.3-133.2	0.07
[1]Fat ($g.d^{-1}$)	73.0	59.4-82.0	64.7	49.6-93.0	0.39
Carbohydrate ($g.d^{-1}$)	227.0	207.1-254.2	202.9	160.8-292.0	0.07
Protein ($g.kg^{-1}$)	1.3	1.1-1.3	1.4	1.0-1.6	0.19
ORAC (mmolTE)	12.8	9.0-17.6	13.2	8.7-16.5	0.55
Leucine ($g.d^{-1}$)	3.1	2.5-4.8	3.6	2.8-11.0	0.69
Vitamin D ($\mu g.d^{-1}$)	2.8	2.2-4.5	15.5	5.4-22.3	0.00

[1] Normally distributed (Shapiro-Wilk $p > 0.05$). Mean differences at baseline and Week 24 examined by One-way ANOVA (normally distributed variables) or Kruskal-Wallis test (non-normally distributed variables). n=41, CON =26, FORM=15

Dietary results are presented for those participants who completed the nutrient intervention to 24 weeks. Following exclusion of invalid eFIRs (31% based on the NANS IUNA exclusion criteria of EI:BMR <1.1), 82 records (♀=65, ♂=17) remained of which 41 (♀=32, ♂=9) completed the intervention to 24 weeks (mean (SD); age 60.9 (5.4) y; weight 71.2(12.0) kg; height 165.9 (6.9) cm, BMI 25.9(3.6) kg/m^2).

A majority of participants exceeded the IOM RDA for protein ($0.8g.kg^{-1}.d^{-1}$) (94%), with 51% achieving daily intakes of protein above $1.2g.kg^{-1}.d^{-1}$ (Figure 34). The current IOM RDA for vitamin D is 600 IU or 15 µg per day and only 4% of the population exceeded this recommended daily intake. Sixty-two participants (77%) had a daily vitamin D intake below 5 µg per day (Figure 35). There was a large range of intakes for individual EAAs, and there were significant numbers in the population who did not achieve the recommended daily intake for either individual or the sum of all EAAs (∑EAAs) (Figure 36).

Additional assessment was carried out to determine the meal level apportioning of the reported daily nutrient intakes for protein and leucine (Figure 37) in order to determine whether proposed thresholds for protein and leucine intake, required to overcome “anabolic resistance” in older individuals, was achieved. Based on reported compliance figures and median amounts of nutrients delivered by the supplementation programme (FORM) assumed ‘corrected’ meal level apportioning is also presented.

Mean LTM was 39.7 kg for female participants and 57.9 kg for male participants respectively. No participant was classified as sarcopaenic (class II sarcopaenia) based on the European Working Group on Sarcopaenia in Older People (EWGSOP) definition. The change in body composition for participants who completed the 24 week intervention is presented in **Table 29** and individual changes in lean tissue mass are depicted in Figure 38.

Considering the concern that high protein intake may promote renal damage by chronically increasing glomerular pressure and hyperfiltration measurement of estimated glomerular filtration rate (eGFR) and serum creatinine was carried out at 0, 12 and 24 weeks. There was no difference between the groups for either parameter (P=0.72, P=0.67) and no significant change over time (p=0.77, P= 0.19) (– univariate ANOVA (normal distribution) or Kruskal-Wallis test (non-normal distribution) examining changes over time between groups.

6.4.1 *Compliance to supplementation programme*

Compliance to the supplement programme was monitored by monthly count-back of returned empty sachets and any remaining unused supplements. Compliance data pertaining to those subjects who completed 24 weeks of the supplementation programme are outlined in **Table 28**. Male participants had better compliance rates than females in all study arms. Compliance rates were poorest among those randomised to the control arm of the intervention. There was a difference in compliance rates between the control and formulation groups (P=0.150).

Table 28 Compliance to dietary supplementation programme for participants who completed the 24 week intervention

		n	Mean	SD
	All	60	88%	14%
Total	**Females**	46	87%	15%
	Males	14	91%	9%
	All	29	85%	17%
Control	**Females**	24	85%	19%
	Males	5	84%	9%
	All	31	91%	8%
Formulation	**Females**	22	90%	7%
	Males	9	95%	7%

CON (n=29; 24♀and 5♂), FORM (n=31; 22♀and 9♂)

Table 29 Change in body composition from baseline (24 week – 0 week) of participants (n = 41) who completed the 24 week intervention.

	CON		FORM		
	Median	**IQR**	**Median**	**IQR**	***P value***
[1]**Body Mass (kg)**	-0.3	-1.2 - 0.8	0.74	-0.4 - 1.7	0.112
FTM (kg)	-0.03	-0.9 - 1.2	0.41	-0.6 - 0.9	**0.035**
[1]**LTM (kg)**	-0.22	-0.7 - 0.5	0.37	-0.4 - 1.2	**<0.001**
Body Fat %	-0.17	-1.1 - 1.2	0.15	-0.5 - 0.7	**0.003**

[1]Normally distributed (Shapiro-Wilk p > 0.05). FTM, Fat tissue mass; LTM, Lean tissue mass; SD, standard deviation; IQR, interquartile range. Between group differences at baseline examined by One-way ANOVA (normally distributed variables) or Kruskal Wallis test (non-normally distributed variables).

At 24 weeks the mean LTM increased by 0.45 (1.06) kg in the FORM group compared to a mean decrease of 0.16 (0.88) kg in the CON group **(Table 29** and **Table 30**) (ANOVA; F3,59= 4.619; P = 0.006, 1-ß=0.87, $ŋp^2$=0.2). Treatment effect was confirmed using a two-way ANCOVA to investigate the influence of baseline LTM on results between groups (ANCOVA; F4,59=1241.343, P<0.000, 1-ß=0.989, $ŋp^2$=1.0). A linear regression model was run, and no other variable apart from gender was found to influence delta LTM and therefore were not included in the ANCOVA model. These variables include: age, baseline protein intake, compliance to supplement and body mass index. At 24 weeks the mean change in LTM resulted in a net difference of 0.6 kg between CON and FORM. [ANOVA; $F_{(5,84)}$ = 5.08, P < 0.001, 1-ß = 0.98, η_p^2 = 0.23]. Post-hoc analysis revealed a statistically significant difference between the CON and FORM (P = 0.004). Expressed as a percentage, the change in LTM at 24 weeks resulted in a significant group interaction [ANOVA; $F_{(5,84)}$ = 3.23, P =

0.01, 1-ß = 0.87, η_p^2 = 0.16]. Post-hoc analysis revealed a statistically significant difference between the CON and FORM (*P* = 0.037). Expressed as a percentage, the increase in LTM at 24 weeks was +0.91 (2.4) % in the FORM group compared to a decrease of -0.3 (2.1) % in the CON group (ANOVA; $F_{3,59}$= 2.616, P=0.06, 1-ß=0.861, ŋp2=0.12). The 1.2% mean difference in response between the treatment and control group was statistically significant (P=0.011).

Table 30 Two-way ANOVA describing the change (∆) in LTM after 24 weeks of formulation (FORM) or control (CON) supplementation in 50-70 year olds

	Mean (SD) kg ∆	Range (kg)	P value	Mean (SD) % ∆	Range %	P value
FORM	+0.45 (1.06)	-3.75		+0.91 (2.4)	-9.19	
			0.006			0.06
CON	-0.16 (0.88)	-3.38		-0.3 (2.1)	-8.06	

CON, Control group; FORM, Formulation group; LTM, Lean tissue mass; SD, standard deviation.

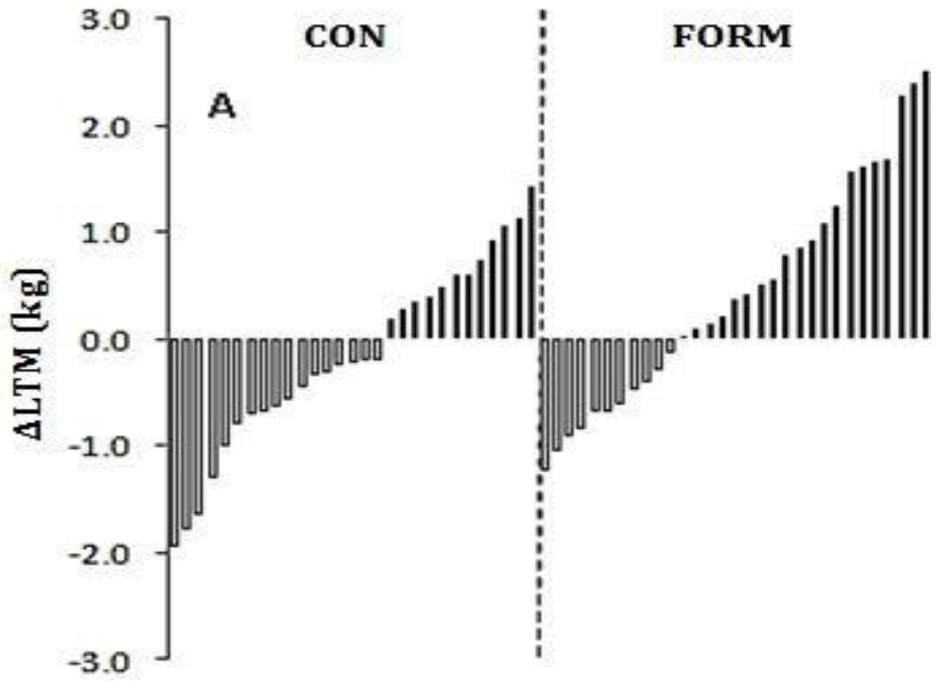


Figure 38 Individual change in lean tissue mass sorted from low to high response for the control (CON) and formulation supplemented (FORM)

6.5 Discussion

The present study indicates that supplementation of dietary protein, rich in essential amino acids, with optimised apportioning of the daily intake, increases whole body lean tissue mass in healthy, community dwelling adults (50-70y). Considerable inter-individual variability was evident in the LTM response to supplementation (Figure 38) (individual losses and gains of greater than ± 1 kg were evident in each of the experimental groups). The mean change in lean tissue mass in the FORM group was +0.45 (1.06) kg over the 24 week intervention. An accrual of lean tissue mass of this magnitude would be considered physiologically significant and of clinical benefit over the course of a number of years (Cruz-Jentoft et al. 2010a).

An accurate measure of nutrient intake was required to determine the effect of the supplement on basal nutrient intakes. Dietary intake was recorded by estimated food intake record (eFIR) at week 0. The repeated measure of dietary intakes at 24 weeks (**Table 27**) was intended to assess any changes to habitual consumption resulting from the supplementation programme and to confirm that a true supplementation (and not a substitution) had been achieved. Analysis of the repeated eFIRS concluded that assessment of changes of the magnitude which we were trying to detect was not possible with the instruments used. The day to day variation in intakes, validity of the record and poor sensitivity of the instrument used to assess dietary intakes meant that detecting changes of $0.33 g.kg^{-1}.d^{-1}$ protein, $0.03 g.kg^{-1}.d^{-1}$ leucine or $0.25\ ug.kg^{-1}.d^{-1}$ vitamin D (FORM group), on either a meal level or mean daily analysis, was not possible. Repeatability of a dietary method is important in determining the quality of nutritional data. It is a limitation of this study that nutrient intake at week 24 did not reflect the known supplemented nutrient intake due to the poor repeatability of the four day estimated dietary records in this population. Reported compliance to supplement was >88% and a known quantity of each nutrient was consumed daily on this basis. This anticipated increase in nutrients (e.g. $0.33 g.kg^{-1}.d^{-1}$ protein, $0.03 g.kg^{-1}.d^{-1}$ leucine or $0.25\ ug.kg^{-1}.d^{-1}$ vitamin D) in the FORM group was not evident in the week 24 dietary records (**Table 27**). Either this means that consumption of the supplement (both the CON and FORM) resulted in changes to reported habitual intakes at baseline (i.e. the intervention was a substitution rather than a supplement), or that the assessment tool was not sensitive enough to assess changes of this magnitude. The four day FIR was chosen as a means of collecting dietary data to allow comparison with national studies conducted in similar populations (NANS). It has previously been shown to have good repeatability (Toeller et al. 1997), however the authors suggest that repeatability test should be carried out in the population under investigation. There is a belief among some researchers that the degree of dietary misreporting might increase with repeated dietary assessment in the same subjects (Westerterp 2009). A study conducted by Francis and Stevenson (2013) investigated the test-retest reliability of a dietary questionnaire and concluded that it may be a useful screen in epidemiological studies to assess macronutrient intakes due to its reported validity and reliability. However they did not feel it was sensitive enough to assess micronutrient intakes. Wong et al (2013) reported similar results; using a food frequency questionnaire (FFQ) they concluded that it exhibited good test-retest reliability and reasonable validity in ranking intakes of the food groups, but not micronutrient intakes. Specific to this study population (free-living older people, aged 50-70y) the FFQ has been reported to have reasonable repeatability and validity in ranking nutrient intakes (Jia et al. 2008). The use of web-based FFQs has been reported to be more accurate in the assessment of all nutrient intakes due to the complex skip patterns, portion size estimation based on food pictures, and real-time error checking that web based systems allow (Kristal et al. 2014). Perhaps this method may make the assessment of all nutrient intakes and dietary patterns more accurate as the 4 day eFIR was not adequate for this study.

There was nothing remarkable in the reported dietary intake of the population at week 0; these intakes are in-line with current IOM recommendations (Institute of Medicine 2005) and comparable with mean daily reported intakes from the NANS (IUNA, 2010) (n=226, >65 y men and women). The convenience sample used in the present study had a similar contribution of energy from different macronutrients (protein -18.5%TEI, carbohydrate - 45.6%TEI and fat - 35.9%TEI) to a national sample. However, mean daily energy intake of 1768 kcal in the NANS population was lower than that reported in this sample by ~200 kcal. Mean daily reported protein intake was 1.2 (0.3) g with 94% of those who completed the 24 week intervention reporting intake of ≥ 0.8 mg.kg-^{1}.d^{-1} at week 0 and 51% achieving intakes ≥ 1.2 mg.kg^{-1}.d^{-1}.

The current recommendation for protein intake are on a per kg body mass basis. There was an increase over the duration of the intervention in total mass in the FORM group of 0.8(1.9) kg. Lean tissue mass constituted 0.4(1.1) kg of this increase but 0.2(1.4) kg was fat tissue mass (FTM). Feeding additional protein on a per kg actual body weight (ABW) may have led to this increase. It may be warranted to investigate prescribing the nutrient formulation based on ideal body weight (IBW) or on a per unit lean tissue mass basis but there is no precedence for this in the extant literature.

To the author's knowledge, assessment of amino acid intakes has not been previously reported in an Irish population. Using the expansions to the WISP© database outlined in Chapter 5 it was possible to estimate these intakes and compare them to the IOM RDAs. There was no significant difference between the two groups (CON n=26, FORM n=15) in the intervention, or between either study group and the larger Healthy Ageing Population (n=120). Comparison of the mean daily intake (MDI) of the groups suggest adequacy of AA intake for all except lysine. Reported mean daily intakes of leucine (56.4 (23.1) $mg.kg^{-1}.d^{-1}$) , acknowledged as the most potent of all amino acids as a regulator of muscle protein synthesis (MPS), was above the IOM RDA (42 $mg.kg^{-1}.d^{-1}$) with 72% of those who completed the intervention to 24 weeks meeting or exceeding the recommendation. However there was great variation in individual intakes as is evident in Figure 36. Comparisons with one of the few available reports on EAA intakes reported on a US population (NHANES III) displayed in **Table 18** (Chapter 5) show similarities across many of the EAA intakes with the exception of lysine. There is no obvious explanation as to why estimated intakes of lysine are inadequate in this study population. Dietary sources include eggs, meat (specifically red meat, lamb, pork, and poultry), soy, beans and peas, cheese (particularly Parmesan), and certain fish (such as cod and sardines). Of note is the fact that the RDA for lysine was increased in 2007 from 12 $mg.kg^{-1}.d^{-1}$ to 38 $mg.kg^{-1}.d^{-1}$ (WHO/FAO/UNU 2007); however, comparison with either RDA indicates inadequacy in mean daily reported intakes (8.2(3.7) $mg.kg^{-1}.d^{-1}$) for the population.

The anomaly regarding calculated lysine intake in the study population raises questions. Interrogation of the methods used to map this nutrient, as well as detailed analysis of the original USDA AA database all suggested that there may have been a technical flaw which caused this surprisingly low level of intake. To clarify this, an individual food intake record was analysed by both the WISP and USDA database. There was good agreement between both nutrient analysis packages for energy, macro nutrients and most amino acids assessed, with the exception of lysine (**Table 19**). This suggests a technical problem with assessment of lysine intakes in the WISP database. This issue has been brought to the attention of the manufacturers, but the resolution of this is beyond the scope of this current research.

Further assessment of the meal level distribution of the reported intakes of protein and leucine reveal that where the recommendation for mean daily intake may be satisfied by a majority of participants, recommended intakes at an individual meal level are not (Figure 37 and **Table 26)**. Intakes of protein and leucine at both smaller meals in the day are reported as 15.9 (7.5) g and 0.8 (0.6) g at breakfast and 21.9 (12.7) g and 0.9 (0.7) g at the midday meal. It has been suggested that 25 – 30g of protein per meal and in excess of 1.5g of leucine is required to facilitate stimulation of muscle protein synthesis (Symons 2009, Pennings 2011, Breen and Phillips 2011, Paddon-Jones and Rasmussen 2009). Ingestion of smaller portion sizes has been reported to attenuate the MPS response in an elderly population (Symons 2009). One aim of this study was to increase dietary protein at both breakfast and lunch with the intention of maximising opportunities for increasing muscle protein synthesis (MPS), decreasing muscle protein breakdown (MPB) and resulting in an accrual of lean tissue mass after 24 weeks of intervention. With compliance to the supplementation programme in excess of 88%, it was expected that intakes of protein and leucine at breakfast and the midday meal would increase from the portions reported at baseline to mean intakes of 28.5g protein and 1.9 g leucine (breakfast) and 34.5 g protein and 2.0 g leucine (midday meal) (Figure 37). These intakes surpass the thresholds estimated for both nutrients to stimulate MPS (Breen and Phillips 2011). Where these intakes depict anticipated changes in MPS in just one day, the extrapolation of this theory to habitual patterns of intake could have very significant benefits for the preservation of lean tissue mass in the elderly. Over a six-month period with optimal protein apportioning there would be 546 (3 meals daily x 7 days weekly x 26 weeks) opportunities to maximally stimulate post-prandial MPS. If the recommendation for protein dose at meal level is not achieved at one eating occasion daily this decreases the number of opportunities to 364, with a further decrease to 182 occasions on which MPS is optimally stimulated if the recommendations are met at only one meal daily. Redistributing protein foods throughout the day to optimise protein apportioning in the context of MPS seems a logical approach to preventing age related lean tissue mass loss. This strategy could increase the opportunities to stimulate post-prandial MPS from the meal level analysis reported at baseline, by 33-66%.

Hypovitaminosis D, encompassing both vitamin D insufficiency and deficiency is highly prevalent in the general population and has potentially deleterious musculoskeletal effects (Bischoff-Ferrari *et al*. 2006). The current IOM RDA for vitamin D is 600 IU or 15 µg per day (The Irish Dietary Reference Value (DRV) is 7.5 µg per day for adults). Only 4% of the population exceeded the IOM recommended daily intake. Sixty-two participants (77%) had a daily vitamin D intake below 5 µg per day. The dose of vitamin D contained in the supplement (0.25 $\mu g.kg^{-1}.d^{-1}$) was intended to correct for suspected inadequacies in dietary intake (Bischoff-Ferrari *et al*. 2004). The intention was to supplement inadequate dietary intakes to the IOM RDA rather than increase vitamin D intakes to those levels reported as necessary to have a therapeutic effect on sarcopaenia (25 µg or >1000 IU per day) (Candow *et al*. 2012). 25(OH) Vitamin D concentration was considerably higher than previously reported for a group of older community dwelling women in Ireland. Anderson *et al.* (2005) reported that the average 25(OH) vitamin D concentration for a sample of forty-three women with an average age of 72 years was 44 nmol/L (95% C.I. 17, 89 nmol/L). This value is 33% lower than the average baseline concentration measured in the present study. However, the analytical platform used to measure vitamin D concentration was different between the two investigations. Another possible explanation for the discrepancy is the time of year that vitamin D was measured (during the winter months in the Anderson *et al.* (2005) report while baseline vitamin D concentration was measured during the summer months during this trial). Substantial inter-individual variability was observed in the serum vitamin D concentration and a limitation of the study was that exposure to sunlight (measurement via electronic dosimeter badges or polysulfone film) was not undertaken in this instance.

Compliance of the participants to the intervention, providing either formulation or control twice daily, was acceptable. After 24 weeks of supplementation 88(14) % of the supplements provided were consumed (CON 85(17) % and FORM 91(8) %). This indicates that the prescribed supplementation programme was well tolerated among those who completed the programme. Since the ultimate aim of any prescribed therapy is to achieve certain desired outcomes, compliance or more importantly non-compliance can have very serious implications for the interpretation of the results of an intervention. Therapeutic compliance not only includes patient compliance with medication but also with diet, exercise, or life style changes. Jing *et al*. (2008) carried out an extensive meta-analysis on compliance in order to evaluate the possible impact of therapeutic non-compliance on clinical outcomes. It was estimated that the compliance rate of long-term medication therapies was between 40% and 50%. The rate of compliance for short-term therapy was much higher at between 70% and 80%, while the compliance with lifestyle changes was the lowest at 20%–30%. Although it must be acknowledged that this is still controversial, in relation to good medication compliance, it has commonly been defined as taking 70 to 120% of the medication prescribed (Jing J 2008). The mean compliance to the dietary supplementation programme, of those who completed to 24 weeks, was 88 (14)%. In an intervention study of similar design to the present one, Tieland and colleagues (2012), reported the average adherence to the treatment at ≥92%. This was based on ticked calendars and non-consumed returned product. They reported no difference between the formulation and control groups.

While this nutritional intervention was not intended as protein / energy supplementation for the treatment of malnutrition, the intervention necessitated consumption of an oral nutritional product (approximately 200 mL) twice daily. To discuss attrition and compliance rates, comparison with oral nutrition supplementation programmes in a more clinical context seems warranted. The issue of compliance with oral nutritional supplements (ONS) has been extensively discussed in the literature. A number of studies in the community have reported similar rates of compliance during studies ranging from three to six months duration. Volkert and colleagues (1996) studied compliance with ONS in a group of older females (> 75 years of age) living in their own homes, recently discharged from hospital. Patients in the study were asked to consume one 200 mL container (providing 250 kcal) of a milk based supplement per day and compliance was reported as 55% after 6 months. In another study where patients were asked to consume two 235 mL containers of ONS per day for three months, a 68% compliance rate was reported (Gray-Donald 1995). The subjects included in the study by Gray-Donald (1995) and colleagues were older people (> 60 years) living in their own homes who were receiving home help services. In a later study involving a mixed gender group of older persons (> 65 years) with a range of diagnoses living in their own homes, compliance was reported as 55%. During the study older persons were asked to take two 235 ml cans of a milk based ONS per day (Payette *et al*. 2002). There is some evidence in more recent studies to suggest that studies where patients have been asked to consume smaller container sizes of ONS (i.e. less than 200 mL) may have higher compliance rates. A study by Wouters-Wessling and colleagues (2003) reported a rate of 85% compliance at six months in a group of male and female older persons (> 60 years) living in sheltered accommodation who were asked to take two 125 mL containers of a nutritional supplement daily. Similarly, in a study by Steiner and colleagues (2003), there were high rates of compliance (97.5%) with ONS seen in a group of patients with chronic obstructive airways disease (mean age of 66 years). Patients were asked to take three 125 mL containers of a nutritional supplement providing 570 kcal daily for seven weeks (Steiner 2003). Hubbard and colleagues (2010) presented preliminary findings of a systematic review of compliance with ONS. The mean compliance rate reported was 78% over an approximate follow-up period of 6 weeks. The authors reported that there was better compliance with ONS by patients in community based studies than in hospital based studies.

Reasons for non-compliance which have been suggested in the literature include patients' taste perceptions, loss of appetite, taste fatigue, effects of ageing and lack of recognition of

ONS as a food product (Dunstan 2005). Personal preferences of patients and lifestyle factors are also thought to have an important role (Lad 2005). Problems with adverse events such as nausea and GI discomfort were reported in 18/24 (75%) of the studies reviewed by Milne and colleagues (2009).

In the present study the attrition rate from week 0 (n=123) to week 24 (n=60) was 51%. The most frequently cited reason for drop-out was the time commitment required to complete the intervention study, among the other reasons outlined in the Methods section. The anticipated attrition rate was 30% (Mutsaerts 2013), and although this was taken into consideration in the study design, there was a higher drop-out rate than was expected for a lifestyle intervention. Many participants reported intolerance to the supplement and it is pertinent to note that a group of 50-70 year olds are not habituated to the consumption of powdered formulae. The participants were also blinded as to the contests of the sachets. This may have impacted on their willingness to consume the supplement over the duration of the study. Packaging the formulation into a ready to drink (RTD) format is one option to improve tolerance but the expense of this operation was prohibitive in this instance. The success of an RTD format is evident from the Kukuljan study (2001) as the attrition rate owing to the supplement was much lower with a total number of drop-outs of just 8 of 180 (4.4%).

A standardised approach exists for DXA scanning to ensure the random (non-biological) error that is inherent in all quantitative medical testing is kept to a minimum. Calculation of this precision error is critical in serial measurement of body composition or bone mineral density to monitor the change in body composition through longitudinal or intervention studies. Common sources of variation between scans that affect measurement precision include poor or inconsistent positioning and incomplete data acquisition. A second source of potential variation is inconsistent placement during scanning. Measurement precision has important implications on study design, such as sample size determination and optimisation of monitoring intervals between measurements. To accurately interpret serial measurements, knowledge of the least significant change (LSC) is necessary to govern confidence that true loss or gain in body composition parameters has occurred. Precision error for whole body fat tissue mass, lean tissue mass and bone tissue mass has been estimated as <1% (Toombs *et al* 2012).

While the average LTM response to nutrient supplementation was positive relative to the isocaloric maltodextrin control, considerable inter-individual variability was evident. This variability is consistent with previous intervention studies examining the change in LTM in response to protein and/or AA supplementation (Kukuljan et al. 2009, Verdijk et al. 2009, Verhoeven et al. 2009, Dillon et al. 2009). There are several reasons why the individual response varies to such a degree, including but not limited to, training status, habitual exercise training and physical activity levels, dietary macro/micro nutrient intake, general health and wellbeing, age and gender. It is a limitation of this study that no measure of habitual physical activity or exercise training was captured. Consideration was given to this issue in study design, but both the resources (physical and time) required to measure physical activity over 24 weeks was beyond the scope of this research. Considering that habitual activity levels will influence response to the treatment, it is regrettable not to have any information on this parameter. Notwithstanding the variability in response the overall outcome was the offsetting of the age-related decline in LTM resulting from milk protein supplementation.

Analysis was carried out on those who completed the intervention to 24 weeks. Those who did not complete the intervention are not included in the results, based on a non-intention to treat analysis (NITT). The intention-to-treat principle (ITT) implies that the primary analysis should include all randomized subjects, regardless of whether or not they completed the intervention. It is important to recognize that the ITT and NITT strategies differ not only in terms of the estimation procedure, but also in terms of the underlying research goal. Given the distinction between the effect of treatment "*as assigned*" corresponding to the ITT approach and the effect of treatment "*as received*" addressed by the NITT strategies, careful consideration was given to which treatment effect was the primary research goal for this specific study. The as-received treatment effect of the NITT approaches attempts to measure the effect of the experimental treatment relative to the control condition when all participants adhere to the assigned treatment condition. Such an effect is usually the primary research goal for the development of new treatments. In contrast, the as-assigned treatment effect of the ITT analysis is usually more pertinent for the evaluation of the effectiveness of the treatment in terms of the public health benefits of administering the treatment in the community in light of inevitable treatment non-adherence (Gupta 2011). For proof of concepts studies such as this, the NITT analysis was deemed more appropriate.

The appropriateness of the population recruited for investigation is contentious. The open recruitment employed in this study resulted in a predominantly female cohort (76%). This gender bias influenced the final outcome and was accounted for through inclusion of gender as a fixed factor in the statistical analysis. In essence the male participants responded to a greater degree in both experimental groups (on average males lost more LTM than females in the CON group and gained more LTM in the FORM group). It may have been advisable to recruit an exclusively female or male cohort to potentially reduce the variability of the response as per other research groups (Koopman et al. 2008, Koopman et al. 2009, Verdijk et al. 2009, Verhoeven et al. 2009, Pennings et al. 2012, M. Tieland et al. 2012). However, this study was designed to be applicable to as wide a target population as possible. It could not be foreseen at the outset that the open recruitment would be so biased towards females. The decision was taken not to redress the gender balance during recruitment and target males exclusively as to do so would have violated the open recruitment principle of the trial. Additionally, the convenience sample recruited to participate in this study comprised largely of well educated, affluent, recently retired university staff. The characteristics and habitual dietary and physical activity patterns may not well represent the national behaviours. For this reason, expansion of this study (certainly the cross sectional analysis) must be applied to a more representative national database such as the IUNA NANS or the TILDA data.

6.6 Conclusion

The nutrient support proposed in this study as a solution to age related lean tissue mass loss is a dairy derived ingredient containing whey protein, whey protein hydrolysate with bioactive anti-oxidant properties, and milk minerals fortified with vitamin D. When consumed as part of a regular diet, taken daily at breakfast and lunch over 24 weeks, the formulation leads to an increase in whole body lean tissue mass. There is great appeal in the use of a food grade supplement as a solution to age related lean tissue mass loss due to its ease of application and safety profile.

Chapter 7

Thesis Summary, Future Work, Clinical Applications and Public Health Implications

7.1 Thesis Summary

Age-related loss of lean tissue mass is recognised as one of the major determinants affecting physical impairment and disability (Cruz-Jentoft 2013). Lifestyle interventions are attractive potential strategies to offset the expected trajectory of loss of muscle mass and function with age (Scott *et al*. 2011) due to their safety profiles and ease of application.

This thesis examined the relationship between dietary practices, nutrient intakes and age related whole body lean tissue mass loss. In order to do this with confidence, modifications and expansions to existing dietary assessment methods and tools were necessary.

An exploration of traditional (Goldberg *et al*. 1991) and more recently recommended methods (Black 2000) of screening for dietary under-reporters was outlined in chapter 3. This was in an effort to increase the validity of estimated nutrient intakes and highlighted the importance of unanimity among the scientific and dietetic communities on how best to screen and use under-reported dietary records.

The innovative additions to WISP©-based dietary assessment methods (chapters 4 & 5) facilitated more accurate descriptions of nutrient intakes pertinent to age related whole body lean tissue mass loss (dietary TAC and EAAs). To the author's knowledge, these have not been previously reported in an Irish or UK population and bring to the fore valuable knowledge pertaining to nutrient intakes implicated in the aetiology of sarcopaenia, and other diseases.

Based on the extant literature discussed in chapter 2, a novel nutrient support to age related lean tissue mass loss was developed (chapter 6). This formulation was then used in a 24 week nutrient RCT investigating the effect of the supplement programme on whole body lean tissue mass loss in a convenience sample of 'healthy' Irish adults (50 – 70 y). The intervention study has shown that this nutrient formulation and the prescribed pattern of intake can attenuate age related muscle mass loss in this population.

7.1.1 *Dietary intake and age related lean tissue mass loss*

In all circumstances the prerequisite for muscle protein synthesis is dietary protein (Wolfe 2012). Dietary intake of protein is vital to support muscle protein synthesis, as proteins act not only as a substrate for synthesis, but also as a regulator. The quantities, quality and apportioning of daily protein intake are all important considerations in this regard (Paddon-Jones *et al*. 2008; Paddon-Jones and Rasmussen 2009).

It has been proven that adequate dietary protein intake is necessary to attenuate and treat age related lean tissue mass loss in the elderly. However, in developed countries of community dwelling populations the adequacy of protein intakes relative to the IOM RDA (0.8 $g.kg^{-1}.d^{-1}$) is undeniable, with many researchers reporting intakes at or above the RDA (Stookey *et al*. 1999; Fulgoni 2008; IUNA 2011; Tieland 2012; Wolfe 2013). This trend held true in the convenience population of this current study also where reported mean daily protein intake was 1.2 (0.3) $g.kg^{-1}.d^{-1}$. Since the phenomenon of sarcopaenia persists despite the apparent adequacy of protein intakes, various dietary protein intake strategies have been investigated, including the timing and quality of dietary protein.

7.1.2 *How much protein do we need?*

The RDA for protein for adults older than 18 y is 0.8$g.kg^{-1}.d^{-1}$, which is an estimate of the amount of protein necessary to maintain protein balance. Several experimental studies, however, suggest that the RDA for protein may not be adequate for older people to maintain skeletal muscle. Accordingly, a daily protein intake between 1.2 and 1.5 $g.kg^{-1}.d^{-1}$ has been recommended by many researchers to reduce the risk for adverse health outcomes, such as sarcopaenia and frailty (Cesari and Pahor 2008; Paddon-Jones and Rasmussen 2009; Morley *et al*. 2010; Rolland *et al*. 2011; Evans *et al*. 2013; Wolfe 2013). Based on the emerging evidence, it is suggested that the RDA for protein for the elderly should be reconsidered. With a modification of the RDA for protein to 1.2–1.5 g/kgBM/d, the protein intake of an average weighted individual (75 kg) would be 90–110 g per d. This amount of protein intake would facilitate the further recommendation that the protein intake of all main meals should increase to 25–30 g, if optimally distributed.

7.1.3 *Timing of protein intake*

Observations in this thesis and supported by other research (Breen and Phillips 2011; Lanham-New 2011; Tieland 2012; Bollwein 2013; Bouillanne 2013; Mamerow 2014) show that daily protein intake is not equally distributed over the various main meals consumed throughout the day. Breakfast and lunch are particularly low in protein (chapter 6). Interestingly, evidence suggests that the post-prandial muscle protein synthetic response to smaller amounts of amino acids is attenuated in older subjects when compared with young controls (Cuthbertson 2005; Katsanos 2005), resulting in a negative net muscle protein balance and degradation of muscle tissue. The attenuated muscle protein synthetic response to lesser protein intakes becomes of greater clinical relevance over the course of many years. Consequently, it has been suggested that 25–30 g of dietary protein per main meal is required to allow an appropriate stimulation of post-prandial muscle protein synthesis and to overcome the attenuated protein synthetic response (Koopman 2009; Paddon-Jones and Rasmussen 2009; Pennings 2011; Volpi 2013). Correcting for this skewed apportioning of protein intakes in the intervention study (chapter 6) may have had a significant role in arresting whole body lean tissue mass loss in this population.

7.1.4 *Dietary sources of protein*

An increase in essential amino acid (EAA) availability represents the main anabolic signal responsible for stimulating postprandial muscle protein synthetic rates (Volpi 2003). Therefore, protein sources rich in EAA, i.e. milk, whey, casein, meat, egg, fish, as well as some vegetable protein sources, are most potent in stimulating muscle protein synthesis. However, the effect on postprandial muscle protein synthesis rates differs among these food sources, despite a relatively high proportion of EAA. The extant literature suggests that ingestion of whey protein results in greater postprandial protein retention when compared with ingestion of casein (Boirie 1997; Dangin 2001; Pennings 2011). The greater anabolic properties of whey versus casein protein have been attributed to the faster digestion and absorption kinetics of whey, resulting in a greater increase in postprandial plasma amino acid availability, thereby further stimulating muscle protein synthesis in elderly people (Boirie 1997; Dangin 2001; Pennings 2011). Furthermore, whey protein has a high content of leucine, an essential amino acid. Earlier studies showed that a leucine-enriched mix of EAA increase muscle protein synthesis to a greater extent than other forms of protein (Rieu 2003; Katsanos 2006; Wall 2013). Consequently, it is suggested that increasing the leucine content of a meal represents an effective strategy to attenuate lean tissue mass loss in the elderly. In long-term intervention studies, however, there are mixed results for leucine supplementation with some researchers reporting no effect of increasing leucine intake in an elderly population (Verhoeven 2009; Leenders *et al*. 2012). These trials suggest that a complete mix of EAA, i.e. protein fractions or food products, is more likely to be beneficial than one single amino acid. Milk protein, i.e. 80% casein and 20% whey protein, is suggested to be a very potent protein source to stimulate muscle mass gain (Phillips 2005). Previous studies have shown that milk stimulated protein accretion to a greater extent than an iso-nitrogenous quantity of soy proteins (Bos 2003; Morens 2003). The intervention study detailed in chapter 6 showed a significant increase in lean body mass after 24 weeks of milk protein supplementation. This corroborates other research concluding that dairy based proteins are very potent dietary protein sources to stimulate muscle synthesis and elicit improvements in muscle hypertrophy in the elderly.

7.1.5 *Dietary total antioxidant capacity*

Pihlanto (2006) and Power *et al*. (2013) both reported the discovery of milk derived peptides with antioxidant activity. Inclusion of these bioactive peptides in the nutrient formulation seemed worthwhile in light of the likely improvements to health associated with increasing antioxidant intake, as measured by dietary TAC. In the intervention study, the total ORAC score per 100 g of formulation prescribed was 25.2 mmol TE, with the median portion providing 4.1 mmol TE twice daily. Prior (1999) estimates from extensive research in the area of dietary TAC and ORAC that a daily intake of >3.2 mmol TE is required to affect health outcomes. The convenience sample observed in chapter 4 reported a mean daily intake of 9.6 mmol/ TE T-ORAC, well in excess of the assumed levels required to support health, and significantly higher than those values reported in initial pilot studies by Prior (1999) and Cao (1998a), and in later studies applied to national nutrient intake databases (Wu 2004; Cao 1998a; Prior 1999).

7.1.6 *Vitamin D*

Low vitamin D status has been associated with reduced lower-extremity muscle strength, slower walking speed, and impaired balance in the elderly (Visser *et al*. 2003; Bischoff-Ferrari 2004; Pfeifer 2010; Ceglia and Harris 2013). While the extant literature has shown improvements in muscle function from vitamin D supplementation, to date there has not been an intervention presenting increases in lean tissue mass. The purpose of including vitamin D to IOM recommendations in the nutrient formulation in the intervention study presented here was to simply correct for likely deficiencies in the elderly population recruited. The intention was not to test the hypothesis that increasing beyond the IOM RDA would attenuate lean tissue mass loss. Estimates of the vitamin D intake required to achieve the optimal concentration of 25(OH)D for older adults (50–60 nmol/L) indicate an intake of 15µg or 600 IU/d would suffice. Raising the intake to in excess of 1000 IU (25.0 µg) vitamin D/day would be necessary to observe changes in muscle mass or function (Bischoff-Ferrari *et al*. 2006).

In the intervention study, the vitamin D content per 100 g of formulation prescribed was 56.3 μg, with the median portion providing 9.1 μg twice daily. At the end of the 24 week intervention, no participant of those who completed the formulation arm had a serum vitamin D indicative of deficiency and only 6% had levels suggesting insufficiency. However, this study did not account for subcutaneous vitamin D production from UV exposure. Many studies describe the use of the plastic film, polysulphone, as a dosimeter for monitoring personal exposure to both natural and artificial sources of ultraviolet radiation (Hill *et al*. 2004; McCarthy 2006), but this study did not account for UV exposure. Further research in this area is warranted.

7.1.7 *Population*

Age related whole body lean tissue mass loss is associated with a sedentary lifestyle and suboptimal habitual dietary intakes. The work presented in this thesis suggests a benefit in altering the dietary habits of 'healthy', 'young' elderly people, of 50 – 70 years. It could be speculated that the benefits of nutrient supplementation may be even more evident in a compromised, frail, elderly population where lifestyle factors may have an even greater role to play. No participant in the study cohort was suffering from age-related sarcopaenia based on the EWGSOP definition. The open recruitment employed in this study resulted in a predominantly female cohort (78% in cross sectional analysis and 76% in the intervention study). This gender bias influenced the final outcome and was accounted for through inclusion of gender as a fixed factor in the statistical analysis of the 24 week intervention. The decision was taken not to redress the gender balance during recruitment and target males exclusively as to do so would have violated the open recruitment principle of the trial. Additionally , the convenience sample recruited to participate in this study comprised largely of well educated, affluent, recently retired university staff. The characteristics and habitual dietary and physical activity patterns may not well represent the national behaviours. For this reason, expansion of this study (certainly the cross sectional analysis) must be applied to a more representative national database such as the IUNA NANS or the TILDA data.

7.2 Future research

As extensively discussed, protein intake has been identified as the primary dietary factor influencing muscle protein synthesis, with amino acids, particularly the essential amino acids (chief among these is leucine) the most potent stimulators. Since this study was a nutrient formulation to age related lean tissue mass loss, with an emphasis placed on correcting protein dose, source and apportioning, but also containing vitamin D and bioactive peptides with antioxidant functions, we can surmise that the effect was due to the dominant fraction of the formulation. However, further single shot studies would be recommended to further elucidate the use, singly and concomitantly, of nutrient supports to sarcopaenia.

The investigation of amino acid intakes and dietary total anti-oxidant capacity, using the novel additions to the WISP© database, provided new information on habitual consumption of these nutrients. Considering the limitations of the sample previously discussed, the expansion of these investigations to national datasets is an important development in the validation of these proposed assessment methods.

Serum samples have been retained and are in storage at -80ºC for potential further investigation of plasma anti-oxidant status. This was beyond the scope of the current research. In light of the emerging interest in dietary TAC and the requirement to validate the use of ORAC in terms of physiological value it is hoped to conduct this work in the future. No single component of serum antioxidant complex could fully reflect the protective efficiency of human blood, probably because of interactions that occur in vivo among different antioxidant compounds (Suresh *et al.* 2009). As further studies using total antioxidant capacity assays are reported, the usefulness and predictive value of these tests should become more apparent. However, at present it appears that as a global indicator of oxidative stress in biological fluids TAC assays by themselves are inadequate (Young 2001). A better approach may be to use a range of measurements of individual antioxidants and markers of oxidative damage, with TAC perhaps used as one of these tests. Serum malondialdehyde (MDA), vitamin E, vitamin C and superoxide dismutase (SOD) enzyme activity are all established proposed methods of assessing antioxidant capacity (Young 2001). Correlations between TAC results (dietary or plasma) and clinical outcomes are lacking and further work in this area is required.

Assessment of dietary intake is fraught with uncertainties. The aim of the study on dietary under-reporting was to compare the incidence of dietary under-reporting (UR) from self-reported estimated food intake records using three different methods. An additional aim was to determine the influence on the estimated nutrient intakes of using different screening methods from self-reported food intake records in the study population. Future research must work to achieve unanimity on how best to deal with “the nutritionists’ guilty secret” first described by Garrow in 1995. The long-standing problem of how to accurately capture dietary intake information and best manage data pertaining to under-reported records as yet remains unsolved.

Tieland (2012) concludes from his research on protein and physical activity in preventing sarcopaenia that “a multi-nutrient supplement with at least 15 g of protein provided at breakfast and lunch during resistance-type exercise training in a compromised elderly population might be a very promising strategy to augment muscle mass and improve physical performance”. Having completed the nutritional aspect of this recommendation and found positive results, investigating which of the multi-nutrient components has the most potent effect is now necessary. Investigating whether the addition of a resistance training programme to the nutrient support may augment the increases in MPS observed would also be of great interest.

7.3 Public health implications

In an increasing elderly population, the prevention of age related lean tissue mass loss may reduce the risk for disability, dependence, institutionalisation, morbidity and mortality (Rexach 2006). Nutrition has an important role to play in the prevention or treatment of sarcopaenia (Paddon-Jones and Rasmussen 2009), and the work presented in this thesis suggests that age related lean tissue mass loss may be reversible or preventable with adequate lifestyle intervention. The data presented demonstrate that prescribing a milk protein based nutrient formulation, rich in AAs, to supplement both smaller meals in the day can augment lean tissue mass. This not only reduces the cost to the individual (impairment, disablement, disability and mortality) but also societal costs which are significant in monetary terms (Cruz-Jentoft *et al*. 2010a). The cost to society of not intervening is unfathomable, where the measure of a civilisation is how it treats its weakest members (Mahatma Ghandi).

7.4 Clinical applications

The research in this thesis and future research directions are not only important in the context of the preservation of whole body lean tissue mass loss in the elderly, but could also have more far reaching health impacts. Increasing muscle mass may well reduce risks for various other disease states including diabetes (Morley 2008, Waters and Baumgartner 2011) and obesity (Waters and Baumgartner 2011), may increase survival rates during oncology treatments (Boddaert 2006) and accelerate post-operative recovery and subsequent hospital discharge (Evans 2010). The food for special medical purposes (FSMP) market-leaders, Nutricia and Abbott, have already launched products in this treatment area, but have targeted those in secondary care institutions. The intervention study presented here suggests that the preservation of muscle mass can be achieved with a food grade supplement in a 'healthy' elderly population. Surely this would be a preferable product to a medically prescribed FSMP and in the context of 'keeping healthy people healthy' it seems prudent to intervene at an earlier phase than to wait until institutionalised elderly have succumbed to frailty and disability.

7.5 Translation of research to practice

It may take as long as one or two decades for original research to be put into routine clinical practice (AHRQ 2001). Unfortunately, there are many barriers to the translation. Despite its increasing prevalence and the high costs to both the individuals and societies, screening for age related muscle mass loss is rare. **Figure** 41 illustrates the expanding interest in sarcopaenia and age related muscle wasting in the scientific community; there has been consistent annual increase in the number of publications in this area. Unfortunately, this interest is not matched by clinicians. There is little evidence in the literature or in practice of either screening or clinical interventions aiming to manage age related lean tissue mass loss. On the other hand, there is a wealth of knowledge on treating frailty and disablement, particularly in a frail institutionalised population. The premise of this Healthy Ageing Project is "keeping healthy people healthy", and assessment or intervention at long-term care institutions seems too little too late.

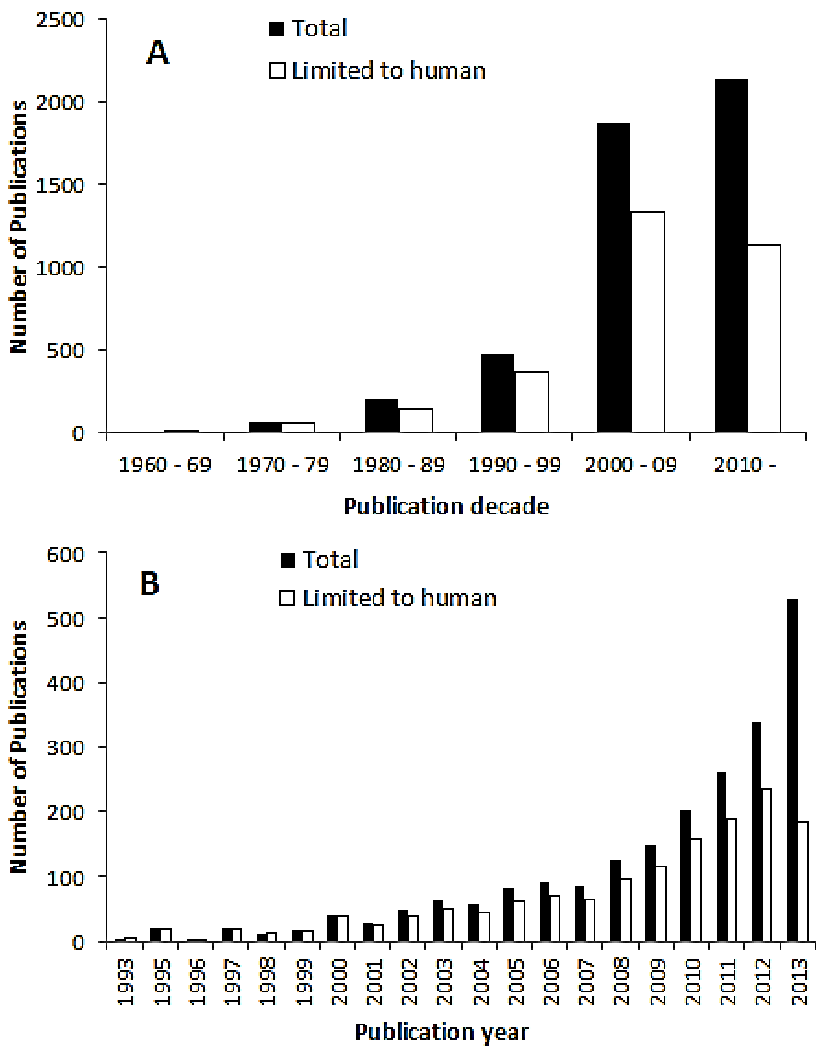


Figure 39 Publications per decade (A) and per year (B) based on a bibliographical search on 7 February 2014 of the online database National Library of Medicines PubMed Central Medline (1950 to present) using the Medical Library Subject Heading (MeSH) terms "Muscle Wasting" AND sarcopenia (A) (1960 – 2013) and Sarcopenia (B) (1993 – 2013). Closed bar – total number of publications, open bar – limited to humans.

While food has traditionally been viewed as a root cause, it is emerging as a potential solution to many ills. Registered dietitians are recognised as food and nutrition experts and are well positioned to communicate the benefits of foods or dietary patterns. The dietary advice itself is quite straightforward. Unnecessary weight loss should be avoided; adequate energy must be consumed to avoid weight loss. Where weight loss is clinically recommended, guidance on adequate protein intake and appropriate resistance exercise to maintain lean tissue mass is required. In light of the lack of consensus on optimal protein intakes for the elderly, it seems reasonable to ensure an intake of at least 0.8 – 1.2 $g.kg^{-1}.d^{-1}$, particularly for those at risk of sarcopaenia. Daily protein intake should be optimally apportioned and of adequate quality to meet recommendations for amino acids. An adequate supply of vitamin D to prevent deficiency seems prudent in light of the research supporting optimal physical performance and fall prevention. Appropriate dietary intake of anti-oxidant nutrients may contribute to antioxidant defence, reduce oxidative damage and reduce functional decline. Since loss of peak muscle mass occurs from the age of 40 years, strategies to preserve mass should occur throughout life.

The difficulty in translating these research findings to practice arises when we consider the unprecedented pressures on healthcare spending, and dietetic services at both community and hospital settings. Dietetic services are under resourced resulting in a shortage of staff and limited access to technologies required to identify those at risk and intervene appropriately. Access to DXA to diagnose muscle mass loss is inadequate. Co-operative work with exercise scientists or physiotherapists to assess loss of muscle function is uncommon. Due to the inadequacy in the number of dietetic posts, priority will be given to the treatment rather than the prevention of illness. Aspects of this translational hurdle could be overcome by using a wide range of strategies for implementing research in practice: provider reminder systems, local opinion leaders, computer decision support systems, collaborative research projects or even financial incentives. Dietitians of Canada launched an innovative approach to overcome the difficulty of translating the science of functional foods to their members by producing a toolkit and information booklet entitled 'Functional Foods for Healthy Aging: A Toolkit for Registered Dietitians (Duncan 2012). Perhaps this is something health care professionals should implement as routine in the quest for continuing professional development.

The benefits of early and appropriate nutrition intervention and support in the management of chronic disease are clearly established; dietitians must champion this message consistently in community and acute settings and become drivers of change to bridge the gap between research and clinical practice.

Appendices

Table 31 USDA food codes, descriptors and ORAC scores with matched WISP© food codes

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
01103	Milk, chocolate, fluid, commercial, reduced fat	1263	1263		58	56600, 56680
02044	Basil, fresh	4805	4805		264	13804
02003	Spices, basil, dried	61063	56685	4378	4489	13805
02006	Spices, cardamom	2764	2764		167	13809
99591	Spices, szechuan pepper, dried	118400	86100		118400	13813
02011	Spices, cloves, ground	290283	111490	178793	16550	13816
02014	Spices, cumin seed	50372	47600	3933	849	13820
02045	Dill weed, fresh	4392	4392		243	13824
02020	Spices, garlic powder	6665	6523	143	42	13830
02021	Spices, ginger, ground	39041	9154	29887	669	13832
99438	Marjoram, fresh	27297	27297		964	13835
02024	Spices, mustard seed, yellow	29257	28759	498	1844	13839
99115	Oregano, fresh	13970	13970		491	13841
02027	Spices, oregano, dried	175295	165712	22582	3789	13842
02029	Spices, parsley, dried	73670	86417	5253	2244	13845
02031	Spices, pepper, red or cayenne	19671	8400	11271	1130	13847
02033	Spices, poppy seed	481	406	75	20	13849
99116	Sage, fresh	32004	32004		901	13853
99117	Tarragon, fresh	15542	15542		643	13857
02049	Thyme, fresh	27426	27426		1734	13859
02048	Vinegar, Apple	564	564		23	1185, 17339
02068	Vinegar, Red wine	410	410		26	1185, 17339
99442	Vinegar, Apple and Honey	270	270		27	1185, 17339
99443	Vinegar, Honey	225	225		21	1185, 17339
02009	Spices, chili powder	23636	21827	1808	1713	838, 13812
02010	Spices, cinnamon, ground	131420	143264	3326	4533	839, 13815
02015	Spices, curry powder	48504	24981	23523	1075	840, 13822-13823
02025	Spices, nutmeg, ground	69640	12600	42625	567	844, 13840
02028	Spices, paprika	21932	13750	8182	1643	845, 13843
02030	Spices, pepper, black	34053	10205	23323	287	847, 13846
02032	Spices, pepper, white	40700	3200	37500		848, 13848
02036	Spices, rosemary, dried	165280	112200	53080	4980	849, 13851
02038	Spices, sage, ground	119929	98714	21214	4520	850, 13854
02042	Spices, thyme, dried	157380	137720	19660	4470	851, 13860
03116	Babyfood, fruit, applesauce, strained	4123	4123		612	17391
03131	Babyfood, fruit, peaches, junior	2551	2551		673	17391

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
03165	Babyfood, fruit, apple and blueberry, junior	4822	4822		563	17391
43408	Babyfood, juice, pear	414	414			17391
97017	Babyfood, fruit, bananas	2658	2658		590	17391
97019	Babyfood, fruit, peaches	6257	6257		916	17391
04042	Oil, peanut, salad or cooking	106	106		0	17040, 326
99423	Olive oil, extra-virgin	372	372		113	324, 17038
99424	Olive oil, extra-virgin, w/basil, home prepared	684	684		8	324, 17038
99425	Olive oil, extra-virgin, w/garlic, home prepared	557	557		10	324, 17038
99426	Olive oil, extra-virgin, w/garlic and red hot peppers, home prepared	219	219		9	324, 17038
99427	Olive oil, extra-virgin, w/parsley, home prepared	766	766		9	324, 17038
06164	Sauce, salsa, ready-to-serve	1001	966	35	245	30790
99450	Cereals ready-to-eat, granola, low-fat, with raisins	2294	2168	126	367	2049
99452	Cereals ready-to-eat, oat bran	2183	1971	212	167	11489
97097	Cereals, ready-to-eat, corn flakes	2359	2302	57	842	11130, 11490, 69
08147	Cereals ready-to-eat, wheat, shredded, plain, sugar and salt free	1303	1222	81	94	11148, 20068, 83, 11499
08049	Cereals ready-to-eat, QUAKER, QUAKER OAT LIFE, plain	1517	1422	95	117	20079, 20078, 11017, 11018, 6
97103	Cereals, oats, old fashioned, uncooked	1708	306		163	20079, 20078, 11017, 11018, 6
99455	Cereals, oats, quick, uncooked	2169	1763	406	183	20079, 20078, 11017, 11018, 6
08122	Cereals, oats, instant, fortified, plain, dry	2308	2026	282	183	20079, 20078, 11017, 11018,6
09226	Papayas, raw	300	300		54	13311
09003	Apples, raw, with skin	3049	3016	32	250	14012
99416	Apples, dried to 40% moisture (purchased in Italy)	6681	6681		324	14016
97070	Apples, Granny Smith, raw, with skin	3898	3859	39	341	14021
99417	Apricots, dried to 40% moisture (purchased in Italy)	3234	3234		248	14031
09083	Currants, European black, raw	7957	7880	81	1202	14053

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
09078	Cranberries, raw	9090	8888	202	503	14073
09088	Elderberries, raw	14697	14500	197	1950	14089
99586	Mangosteen, raw	2510	2510		85	14151
99418	Peaches, dried to 40% moisture (purchased in Italy)	4222	4222		283	14185
99421	Pears, dried to 40% moisture (purchased in Italy)	9496	9496		679	14196
99044	Currants, red, raw	3387	3260	127	540	14249
09160	Lime juice, raw	823	823		117	14279
09442	Pomegranate juice, bottled	2681	2681		356	14288
09294	Prune juice, canned	2036	2036			14289
09019	Applesauce, canned, unsweetened, without added ascorbic acid (includes USDA commodity)	1965	1965		217	17288
43382	Cranberry juice, unsweetened	1452	1452		159	17537
99432	Juice, cranberrry, 100% - cranberry blend, red	865	865			17537
99436	Juice, red grape	1788	1788			17537
09370	Peaches, canned, heavy syrup, drained	436	436		73	941, 14189
09016	Apple juice, canned or bottled, unsweetened, without added ascorbic acid	414	414		38	1087, 14272, 14271
09152	Lemon juice, raw	1225	1225		175	1090, 14277, 14278,
09207	Orange juice, canned, unsweetened	703	703		67	1091, 14301, 14823,
09273	Pineapple juice, canned, unsweetened, without added ascorbic acid	568	568			1092, 14286
09176	Mangos, raw	1300	1300		101	13273, 14148, 916, 917, 14149,14294
97068	Apples, Golden Delicious, raw, without skin	2210	2204	5	217	14014, 858, 14015, 859
97071	Apples, Red Delicious, raw, without skin	2936	2929	7	232	14014, 858, 14015, 859
09004	Apples, raw, without skin	2573	2567	6	225	14015, 858, 859,14014
97066	Apples, Fuji, raw, with skin	2589	2568	21	210	14017, 14018, 856, 14012

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
97067	Apples, Gala, raw, with skin	2828	2793	35	262	14017, 14018, 856, 14012
97072	Apples, Red Delicious, raw. with skin	4275	4234	41	347	14017, 14018, 856, 14012
97069	Apples, Golden Delicious, raw, with skin	2670	2644	26	248	14019, 14020, 14021, 14022, 856, 14012
09021	Apricots, raw	1110	1078	32	79	14025, 14026, 860, 862, 14036, 861
97080	Avocados, Hass, raw	1922	1371	552	142	14042, 14041
09040	Bananas, raw	795	730	66	155	14045, 14046, 14045, 867
09087	Dates, deglet noor	3895	3863	32	661	14084, 886, 14083
09089	Figs, raw	3383	3200	183	960	14095, 890, 14091
09111	Grapefruit, raw, pink and red and white, all areas	1640	1640		71	14105, 899, 900, 14106,14292
09112	Grapefruit, raw, pink and red, all areas	1548	1512	35	214	14105, 899, 900, 14106,14292
09139	Guavas, common, raw	1422	1422		136	14119, 905, 906, 14118
09148	Kiwi fruit, (chinese gooseberries), fresh, raw	862	838	24	211	14123, 908, 14124, 909, 14293
97079	Kiwi, gold, raw	1210	1159	51	366	14123, 908, 14124, 909, 14293
09159	Limes, raw	82	82		12	14131, 14132
09326	Watermelon, raw	142	123	19	59	14166, 14296, 14165, 925
09200	Oranges, raw, all commercial varieties	2103	2103		57	14176, 932, 931, 14175, 14298, 16199
09202	Oranges, raw, navels	1819	1785	34	1819	14176, 932, 931, 14175, 14298, 16199
09206	Orange juice, raw	726	726			14182, 14281
97075	Pears, green cultivars, with peel, raw	2201	2145	56	178	14190, 942, 14191, 1419, 943
97076	Pears, red anjou, raw	1746	1711	35	214	14190, 942, 14191, 1419, 943

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
97077	Plums, black diamond, with peel, raw	7581	7546	35	478	14213, 950, 14300, 14221, 14220
09279	Plums, raw	6100	6083	17	332	14220, 14221, 14300, 950, 14213, 14214, 951, 14300
99050	Juice, white grape	793	793			14237, 1088
99419	Raisins, white, dried to 40% moisture (purchased in Italy)	4188	4188		372	14242, 958
09218	Tangerines, (mandarin oranges), raw	1627	1620	7	192	14267, 971, 970, 14266
09286	Pomegranates, raw	4479	4479		338	14277, 14226
09421	Dates, medjool	2387	2360	27	572	14806, 14085
99583	Goji berry (wolfberry), raw	3290	3173	117		2006, 2108, 3208
09050	Blueberries, raw	4669	4633	36	311	2111, 2009, 3203
97085	Blueberries, wild, raw	9621	9621		429	2111, 2009, 3203
09150	Lemons, raw, without peel	1346	1346		51	31073, 910, 14129, 14130
09128	Grapefruit juice, white, raw	1238	1238		336	55951, 14275, 1089, 30023, 14276
09042	Blackberries, raw	5905	5802	103	477	869, 14048
09070	Cherries, sweet, raw	3747	3730	17	259	876, 877, 14062, 14061
09107	Gooseberries, raw	3332	3302	30	517	895, 14103, 14100
97074	Grapes, red, raw	1837	1837		170	903, 14110, 904, 14109
99047	Grapes, white or green, raw	1018	1018		145	903, 14110, 904, 14109
99048	Grapes, black	1746	1746			903, 14110, 904, 14109
99428	Guava, red-fleshed	1990	1990			905, 14118
99429	Guava, white-fleshed	2550	2550			905, 14118
09181	Melons, cantaloupe, raw	319	306	13	92	919, 14156, 14157, 920, 14158,14295
09184	Melons, honeydew, raw	253	242	11	56	923, 14162, 14163, 924, 14164

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
09191	Nectarines, raw	919	889	29	96	927, 14297, 14172, 14171, 928
09236	Peaches, raw	1922	1872	50	133	938, 14183, 939, 14184, 14299
09266	Pineapple, raw, all varieties	385	373	12	81	947, 14209, 14208
09429	Pineapple, raw, traditional varieties	562	520	42	122	947, 14209, 14208
09430	Pineapple, raw, extra sweet variety	943	916	27	165	947, 14209, 14208
09291	Plums, dried (prunes), uncooked	8059	7880	179	938	957, 14239, 14240
09297	Raisins, golden seedless	10450	10450			958, 14242
09298	Raisins, seedless	3406	3371	35	1065	958, 14242
09302	Raspberries, raw	5065	4927	138	5065	959, 14244
99411	Raspberries, black	19220	19220			959, 14244
09316	Strawberries, raw	4302	4266	36	332	967, 14260,
11312	Peas, green, frozen, unprepared	600	505	95	187	13132
11052	Beans, snap, green, raw	799	745	55	92	13143
11054	Beans, snap, green variety, canned, regular pack, solids and liquids	290	206	84	61	13144
11001	Alfalfa seeds, sprouted, raw	1510	1510		53	13148
11007	Artichokes, (globe or french), raw	6552	6552		373	13153
11094	Broccoli, frozen, spears, unprepared	496	496		68	13173
11113	Cabbage, red, boiled	3145	3145		321	13191
11115	Cabbage, savoy, boiled	2050	2050		119	13193
11137	Cauliflower, frozen, unprepared	620			55	13218
11216	Ginger root, raw	14840	14840		227	13247
11250	Lettuce, butterhead (includes boston and bibb types), raw	1423	1321	103	100	13267
11251	Lettuce, cos or romaine, raw	1017	855	162	73	13268
11993	Mushrooms, maitake, raw	669	572	96	71	13293
11268	Mushrooms, shiitake, dried	752	668	84	52	13294
11987	Mushroom, oyster, raw	664	596	68	51	13295
11921	Peppers, sweet, red, sauteed	847	847		564	13321
11951	Peppers, sweet, yellow, raw	1043	974	69	216	13322
99440	Peppers, sweet, yellow, grilled	694	974	69	216	13322

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
99451	Peppers, sweet, orange, raw	984	908	76	543	13322
11178	Corn, sweet, yellow, frozen, kernels cut off cob, unprepared	522	447	75	174	13370
99051	Tomatoes, plum, raw	546	546		36	13388
11110	Cabbage, boiled	856	856		59	13444
11109	Cabbage, raw	529	508	21	202	13468
11156	Chives, raw	2094	2094		85	13814
11165	Coriander (cilantro) leaves, raw	5141	5141		151	13817
11578	Vegetable juice cocktail, canned	548	548			17376
11463	Spinach, frozen, chopped or leaf, unprepared	1687	1687		91	17417
11821	Peppers, sweet, red, raw	821	797	24	255	13321, 805, 804, 13320
11540	Tomato juice, canned	486	486		323	1093, 13382
11353	Potatoes, russet, flesh and skin, raw	1322	1272	51	122	13009, 664, 13020, 13019, 13018, 13017,
11354	Potatoes, white, flesh and skin, raw	1058	1010	49	163	13009, 664, 13020, 13019, 13018, 13017,
11355	Potatoes, red, flesh and skin, raw	1098	1060	38	138	13009, 664, 13020, 13019, 13018, 13017,
11452	Soybeans, mature seeds, sprouted, raw	962	962		47	13116, 722, 13115, 721
11333	Peppers, sweet, green, raw	935	921	14	160	13119, 803, 802, 13318
11339	Peppers, sweet, green, sauteed	615	615		437	13119, 803, 802, 13318
99362	Artichokes, Ocean Mist, boiled	9416	9221	195	703	13153, 13154^{-} ^{1}3155^{-1}3156
99363	Artichokes, Ocean Mist, Microwaved	9402	9332	70	881	13153, 13154^{-} ^{1}3155^{-1}3156
99587	Asparagus, white, raw	296	296			13157, 737
11091	Broccoli, boiled	2160	2128	32	213	13171, 13172
11112	Cabbage, red, raw	2496	2476	20	231	13190, 13468
11124	Carrots, raw	697	649	47	35	13200, 754, 13446
11125	Carrots, boiled	326	310	18	88	13201, 755, 13202, 13447

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
11960	Carrots, baby, raw	436	355	81	45	13203, 756, 13448
11967	Cauliflower, green, cooked	1387			65	13217, 13216, 760
11136	Cauliflower, boiled	739			57	13218, 13217
11957	Fennel, bulb, raw	307	307		26	13242, 770, 771, 13241
11246	Leeks, (bulb and lower leaf-portion), raw	569	569		47	13263, 775, 13466
11253	Lettuce, green leaf, raw	1532	1391	141	90	13266, 13267, 778, 13453
11282	Onions, raw	913	900	12	23	13304, 792
99055	Onions, red, raw	1521	1521		48	13304, 792
99056	Onions, white, raw	863	863		27	13304, 792
11422	Pumpkin, raw	483	414	69	157	13327, 810, 809, 13326
11429	Radishes, raw	1750	1724	26	79	13330, 13331, 13332, 812
11477	Squash, summer, zucchini, includes skin, raw	180	180		26	13356, 13355
11485	Squash, winter, butternut, raw	396	396		26	13356, 13355
11507	Sweet potato, raw	902	858	44	74	13363, 13364, 822, 13464,13362, 13463, 821, 133665
11508	Sweet potato, baked in skin	2115	2085	30	233	13363, 13364, 822, 13464,13362, 13463, 821, 133666
11510	Sweet potato, cooked, boiled, without skin	766	729	37	120	13363, 13364, 822, 13464,13362, 13463, 821, 133667
11167	Corn, sweet, yellow, raw	728	593	135	211	13372, 13368
11549	Tomato products, canned, sauce	694	652	42	177	13387, 832, 13461
11530	Tomatoes, red, ripe, cooked	423	389	34	94	13467, 13386, 831, 827, 13467, 13386, 836, 830, 13419, 829, 828, 13385, 13419

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
11935	Catsup	578	535	43	249	31145, 30036, 1172, 17338, 17513
11959	Arugula (rocket), raw	1904	1904		125	3260, 3267, 3236
11356	Potatoes, Russet, flesh and skin, baked	1680	1652	28	176	665, 13010
11357	Potatoes, white, flesh and skin, baked	1138	1098	40	136	665, 13010
11358	Potatoes, red, flesh and skin, baked	1326	1304	22	179	665, 13010
11011	Asparagus, raw	2252	2150	102	106	737, 13157
11012	Asparagus, cooked, boiled, drained	1644	1644		159	738, 13442, 13159, 13158
11209	Eggplant, raw	932	932		63	739, 13161
11080	Beets, raw	1776	1767	9	188	741, 13164
11090	Broccoli, raw	1510	1351	159	316	744, 13170
11135	Cauliflower, raw	870	833	37	93	759, 13215
11965	Cauliflower, green, raw	136			70	759, 13215, 13218
11143	Celery, raw	552	512	40	42	761, 13221, 13451
11205	Cucumber, with peel, raw	232	203	28	29	767, 13233
11206	Cucumber, peeled, raw	140	126	14	22	767, 13233
11215	Garlic, raw	5708	5541	400	92	772, 13244
11252	Lettuce, iceberg (includes crisphead types), raw	438	406	33	50	779, 13269
11260	Mushrooms, white, raw	691	640	51	64	783, 13284, 13289, 13288
11265	Mushrooms, portabella, raw	968	919	49	75	783, 13284, 13289, 13288
11266	Mushrooms, brown, Italian, or Crimini, raw	951	900	51	89	783, 13284, 13289, 13288
11457	Spinach, raw	1513	1513		205	813, 13456, 13343, 814, 815, 13344, 13457, 13345, 13346
11170	Corn, sweet, yellow, canned, brine pack, regular pack, solids and liquids	413	361	52	169	824, 13371, 13459
11529	Tomatoes, red, ripe, raw, year round average	387	364	23	80	827, 13384, 13460
11297	Parsley, raw	1301	1301		77	846, 13844

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
12155	Nuts, walnuts, english	13541	13057	484	1556	14850, 14851, 998, 997, 14879
12087	Nuts, cashew nuts, raw	1948	1505	443	269	14811, 14812, 976
12142	Nuts, pecans	17940	17524	416	2016	14837, 14838, 991
12147	Nuts, pine nuts, dried	720	443	277	68	14839, 992
12151	Nuts, pistachio nuts, raw	7675	7295	379	1657	14840, 14841, 993
12061	Nuts, almonds	4454	4282	172	418	972, 973, 14801, 14802, 14803, 14870
12078	Nuts, brazilnuts, dried, unblanched	1419	862	557	310	975, 974, 14808, 14809, 14871
12120	Nuts, hazelnuts or filberts	9645	9275	370	835	980, 981, 14821, 14822, 14874
12132	Nuts, macadamia nuts, dry roasted	1695	1443	252	156	982, 14823
14192	Cocoa mix, powder	485			486	12082
14355	Tea, brewed, prepared with tap water	1128			1128	17165
99070	Tea, green, brewed	1253			1253	17171
99343	Tea, green, ready-to-drink	520	520			17171
99439	Alcoholic beverage, wine, table, rose	1005	1005		42	1108, 17229
99341	Tea, black, ready-to-drink, plain and flavored	313	313			17165^{-1}7166^{-}^{1}7167
14096	Alcoholic beverage, wine, table, red	3607	3607		228	17228, 1107
14097	Alcoholic Beverage, wine, table, red, Cabernet Sauvignon	4523	4523		203	17228, 1107
14102	Alcoholic Beverage, wine, table, red, Zinfandel	2400	2400			17228, 1107
14602	Alcoholic Beverage, wine, table, red, Merlot	2670			2670	17228, 1107
14106	Alcoholic beverage, wine, table, white	392	392		20	17230, 17231, 1110, 1111
16042	Beans, pinto, mature seeds, raw	8033	7610	423	618	13106
16043	Beans, pinto, mature seeds, boiled	904	846	57	128	13107

USDA Nutrient Database Number	Long Description	T-ORAC umol TE/100 g	H-ORAC umol TE/100 g	L-ORAC umol TE/100 g	TP mmol GE/100g	Corresponding WISP© Food Code
16062	Cowpeas, common (blackeyes, crowder, southern), mature seeds, raw	4343	3707	636	647	13062, 700
16069	Lentils, raw	7282	7282		628	13089, 13091, 712, 710
16056	Chickpeas (garbanzo beans, bengal gram), mature seeds, raw	847	847		90	13107, 30539, 13076, 13074, 704
16108	Soybeans, mature seeds, raw	5409	5409		266	13116, 13115, 721, 722
16085	Peas, split, mature seeds, raw	524	524		74	13142, 13141
16098	Peanut butter, smooth style	3432	3127	305	536	14829, 14876, 721
16087	Peanuts, all types, raw	3166	2893	273	396	14877, 14878, 989, 14833, 14831, 14877, 987, 988, 14832
16032	Beans, kidney, red, mature seeds, raw	8606	8410	196	637	716, 13109
18035	Bread, Multi-Grain (includes whole-grain)	1421	1298	123	171	11113, 11114, 11115 ,11116, 11117, 11611, 57
19353	Syrups, maple	590			590	17067
19078	Baking chocolate, unsweetened, squares	49944	49093	850	3508	17086
19080	Candies, semisweet chocolate	18053			1238	17087
19120	Candies, milk chocolate	7519	6612	907	477	1015 , 17089
19165	Cocoa, dry powder, unsweetened	55653			3663	1050 , 12082
99412	Candies, chocolate, dark	20816			1297	17090, 1016, 17491
19034	Snacks, popcorn, air-popped	1743	1535	208	117	17131
19444	Snacks, tortilla chips, low fat, made with olestra, nacho cheese	1858	1733	125	329	17497
97089	Snacks, tortilla chips, low fat, made with olestra	1549	1486	64	278	17497

Table 32 WISP© nutrient database food codes, food descriptors, protein content, frequency of food selection among study population, number of amino acid values assigned by Tinuviel mapping system (n=367)

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
11456	Brown bread average	7.9	656	11
11099	White bread average	8.4	299	11
11073	Brown bread toasted	10.4	287	11
11475	White bread toasted	9.7	287	11
12138	Cheese Cheddar Irish	25.1	226	11
11071	Brown bread large sliced	8.1	198	11
11611	Wholemeal bread toasted	11.2	192	11
11476	Wholemeal bread average	9.4	159	11
19003	Bacon rashers back grilled	23.2	150	11
19080	Pork sausages chilled grilled	14.5	113	11
11510	Cream crackers	9.5	101	11
17495	Potato crisps	5.7	86	11
11468	White bread sliced	7.9	80	11
19079	Pork sausages chilled fried	13.9	75	11
14842	Pumpkin seeds	24.4	66	11
12810	Eggs chicken poached	12.5	61	11
14827	Mixed nuts	22.9	60	11
14879	Walnuts	14.7	60	11
12808	Eggs chicken fried with fat	13.6	51	11
11465	Pitta bread white	9.1	50	11
14870	Almonds	21.1	49	11
16339	Tuna canned in brine drained	23.5	48	11
19002	Bacon rashers back dry-fried	24.2	43	11
18331	Chicken meat average roasted	27.3	39	11
12812	Eggs chicken scrambled without milk	13.8	37	11
17493	Kit Kat	7.5	35	11
16239	Prawns boiled	22.6	33	11
11114	Wholemeal bread large	9	32	11
11577	Fruit cake rich	3.9	32	11
14871	Brazil nuts	14.1	31	11
529	Sausages pork grilled	13.3	31	11
11482	White rolls crusty	9.2	31	11
438	Chicken roast meat only	24.8	30	11
12356	Cheese Feta	15.6	28	11
12351	Cottage cheese plain	12.6	28	11
18066	Beef sirloin steak fried lean	28.8	27	11
14834	Peanuts roasted and salted	24.5	27	11

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
12809	Eggs chicken fried without fat	15	23	11
11483	White rolls soft	9.3	23	11
11481	Hamburger buns	9.1	22	11
18330	Chicken light meat roasted	30.2	21	11
14877	Peanuts plain	25.6	21	11
11213	Sponge cake jam filled	4.2	20	11
14811	Cashew nuts plain	17.7	19	11
12355	Edam	26.7	18	11
14874	Hazelnuts	14.1	17	11
19010	Bacon rashers back smoked grilled	23.4	16	11
14876	Peanut butter smooth	22.6	16	11
11521	Semi-sweet biscuits	6.7	16	11
12344	Cheese Brie	20.3	15	11
11110	White bread Vienna	9.3	14	11
19012	Bacon rashers back 'tendersweet' grilled	26.4	13	11
19015	Bacon rashers middle grilled	24.8	13	11
11116	Wholemeal bread small unsliced	9.3	13	11
11349	Pizza	9	13	11
11091	Rye bread	8.3	13	11
11480	Croissants	8.3	13	11
19008	Bacon rashers back fat trimmed grilled	25.7	12	11
19017	Bacon rashers streaky fried	23.8	12	11
16002	Bass Sea raw	19.3	12	11
14843	Quinoa	13.8	12	11
11202	Fruit cake rich retail	4.9	12	11
11471	White bread French stick	9	11	11
11072	Brown bread large unsliced	8.8	11	11
11339	Couscous	5.7	11	11
11211	Sponge cake	6.4	10	11
11606	Stuffing sage and onion	6.1	10	11
12171	Cheese Parmesan	39.4	9	11
18072	Beef sirloin steak grilled well-done lean	33.9	9	11
16230	Tuna canned in oil drained	27.1	9	11
237	Edam	26	9	11
16210	Salmon red canned in brine flesh only drained	21.6	9	11

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
12345	Cheese Camembert	21.5	9	11
11484	Wholemeal rolls	10.4	9	11
11507	Chocolate biscuits cream filled full coated	6.4	9	11
11246	Eclairs fresh	4.1	9	11
354	Bacon rasher lean and fat fried middle	24.1	8	11
625	Salmon canned	20.3	8	11
16331	Crab boiled	19.5	8	11
238	Feta	15.6	8	11
19082	Pork sausages frozen fried	13.8	8	11
13090	Lentils green and brown whole dried boiled in salted water	8.8	8	11
19006	Bacon rashers back dry-cured grilled	28.4	7	11
18070	Beef sirloin steak grilled medium-rare lean	26.6	7	11
14878	Peanuts dry roasted	25.5	7	11
11068	Breadcrumbs homemade	11.6	7	11
12921	Omelette plain	10.9	7	11
346	Bacon gammon joint lean only boiled	29.6	6	11
18470	Beef mince stewed	21.8	6	11
16217	Sardines canned in tomato sauce	17	6	11
526	Sausages beef grilled	13	6	11
11538	Danish pastries	5.8	6	11
11532	Sponge cake with dairy cream and jam	4.3	6	11
16338	Salmon pink canned in brine flesh only drained	23.5	5	11
19001	Bacon rashers back raw	16.5	5	11
16291	Fish fingers cod fried in sunflower oil	13.2	5	11
16273	Crabsticks	10	5	11
650	Fish cakes fried	9.1	5	11
11470	White bread farmhouse or split tin freshly baked	9	5	11
11242	Doughnuts jam	5.7	5	11
11214	Sponge cake with butter icing	4.5	5	11
18255	Pork loin chops roasted lean	37.5	4	11

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
18033	Beef fore-rib/rib-roast roasted lean	33.3	4	11
19018	Bacon rashers streaky grilled	23.8	4	11
19311	Hamburger takeaway	13.9	4	11
11452	Spaghetti white raw	12	4	11
13308	Onions dried raw	10.2	4	11
17131	Popcorn plain	6.2	4	11
11519	Sandwich biscuits cream filled	5.9	4	11
247	Parmesan	39.4	3	11
352	Bacon rasher lean only fried average	32.8	3	11
18334	Chicken drumsticks roasted meat only	26.6	3	11
353	Bacon rasher lean and fat fried back	24.9	3	11
14830	Peanut butter wholegrain	24.9	3	11
16160	Whiting steamed	20.9	3	11
14812	Cashew nuts roasted and salted	20.5	3	11
16326	Salmon raw	20.2	3	11
443	Chicken leg quarter roast meat only weighed with bone	15.4	3	11
19083	Pork sausages frozen grilled	14.8	3	11
232	Cottage cheese plain	13.8	3	11
10214	American muffins chocolate FSA Sept 2004	5.5	3	11
11520	Crunch biscuits cream filled	5.2	3	11
18341	Whole chicken roasted meat and skin	26.3	2	11
18344	Chicken corn-fed roasted light meat only	25.8	2	11
14833	Peanuts dry roasted	25.5	2	11
18345	Chicken corn-fed roasted meat only	24.9	2	11
18329	Chicken dark meat roasted	24.4	2	11
16262	Scallops steamed	23.2	2	11
227	Camembert	20.9	2	11
226	Brie	19.3	2	11
16324	Mackerel raw	18.7	2	11
12805	Eggs chicken yolk raw	16.1	2	11

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
20047	Bran wheat	14.8	2	11
16288	Fish fingers cod grilled	14.3	2	11
19091	Pork and beef economy sausages chilled fried	12.3	2	11
13842	Oregano dried ground	11	2	11
11489	Oat Bran Flakes with raisins	10	2	11
16281	Fish cakes grilled	9.9	2	11
11098	Wheatgerm bread average	9.2	2	11
11113	Wholemeal bread average	9.2	2	11
14837	Pecan nuts	9.2	2	11
15252	Pizza cheese and tomato	9.1	2	11
12161	Full fat soft cheese	8.6	2	11
13110	Red kidney beans dried boiled in unsalted water	8.4	2	11
11474	White bread premium	8.3	2	11
17497	Tortilla chips	7.6	2	11
17135	Potato crisps jacket	6.4	2	11
17139	Potato crisps thick crinkle-cut	6.1	2	11
17134	Potato crisps crinkle cut	5.6	2	11
20014	Wild rice cooked	5.3	2	11
19004	Bacon rashers back grilled crispy	36	1	11
18009	Beef braising steak braised lean and fat	32.9	1	11
18251	Pork loin chops grilled lean	31.6	1	11
387	Beef topside lean only roast	29.2	1	11
18011	Beef braising steak slow cooked lean and fat	29	1	11
368	Beef forerib lean only roast	27.9	1	11
12137	Cheese Cheddar English	25.5	1	11
357	Bacon rasher lean and fat grilled back	25.3	1	11
19005	Bacon rashers back microwaved	24.2	1	11
243	Gouda	24	1	11
16211	Salmon red canned in brine flesh and bones drained	23.5	1	11
985	Mixed nuts	22.9	1	11
18291	Chicken meat average raw	22.3	1	11
18488	Chicken meat only average raw	22.3	1	11

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
569	Cod frozen grilled steaks	20.8	1	11
16251	Clams canned in brine drained	16	1	11
19013	Bacon rashers middle raw	15.2	1	11
16235	Crayfish raw	14.9	1	11
11614	Pizza cheese and tomato thin base	14.4	1	11
13805	Basil dried ground	14.4	1	11
13116	Soya beans dried boiled in unsalted water	14	1	11
14839	Pine nuts	14	1	11
19077	Beef sausages chilled grilled	13.3	1	11
19088	Pork and beef sausages chilled grilled	13.3	1	11
16289	Fish fingers cod fried in blended oil	13.2	1	11
525	Sausages beef fried	12.9	1	11
13812	Chilli powder	12.3	1	11
20030	Wheat flour wholemeal	11.6	1	11
12176	Cheese Ricotta	9.4	1	11
63	White rolls soft	9.2	1	11
11473	White bread 'with added fibre' toasted	9	1	11
12804	Eggs chicken white raw	9	1	11
20009	Brown rice basmati raw	8.9	1	11
11442	Brown rice raw	6.7	1	11
10246	Egg noodles fine cooked FSA Sept 2004	5.8	1	11
11247	Eclairs frozen	5.6	1	11
10215	American muffins not chocolate FSA Sept 2004	5	1	11
11173	Gingernut biscuits homemade	4.4	1	11
15187	Lasagne vegetable	4.1	1	11
12184	Whole milk yogurt plain	5.7	164	10
13244	Garlic raw	7.9	65	10
11572	Wholemeal crackers	10.1	58	10
11529	Fruit cake plain retail	5.1	48	10
13815	Cinnamon ground	3.9	47	10
13029	Oven chips frozen baked	3.2	31	10
11514	Gingernut biscuits	5.6	30	10
13433	Hummus	7.6	20	10
11591	Mince pies individual	4.2	20	10

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
11580	Sponge cake	6.3	19	10
11508	Chocolate chip cookies	5.8	18	10
12360	Cheese Mozzarella	18.6	16	10
11597	Fruit pie pastry top and bottom	3.1	16	10
11531	Madeira cake	5.4	12	10
12396	Cheesecake fruit individual	6.1	6	10
16329	Sardines canned in oil drained	23.3	5	10
17371	Stuffing mix	9.9	5	10
13843	Paprika	14.8	4	10
11455	Spaghetti wholemeal boiled	4.7	4	10
13030	Oven chips thick cut frozen baked	3.2	4	10
16209	Salmon pink canned in brine flesh and bones drained	23.5	3	10
20049	White rice long grain cooked	2.8	3	10
11003	Barley pearl boiled	2.7	3	10
11553	Pizza cheese and tomato frozen	11.5	2	10
11258	Mince pies individual	4.3	2	10
12132	Cheese Caerphilly	23.2	1	10
144	Mince pies individual	4.3	1	10
13846	Pepper black	10.9	156	9
11279	Apple pie pastry top and bottom	2.9	100	9
13044	Baked beans canned in tomato sauce re-heated	5.2	14	9
11453	Spaghetti white boiled	3.6	12	9
17373	Stuffing sage and onion homemade	5.2	8	9
13170	Broccoli green raw	4.4	8	9
17516	Tomato puree	5	6	9
11608	Muffins American style chocolate chip	6.3	5	9
13066	Broad beans boiled in unsalted water	5.1	4	9
11548	Lemon meringue pie	2.9	4	9
13845	Parsley dried	15.8	1	9
10236	Egg noodles dried FSA Sept 2004	12	1	9

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
13429	Butter beans canned re-heated drained	5.9	1	9
12327	Milkshake thick takeaway	3.7	1	9
11598	Fruit pie pastry top and bottom blackcurrant	3.1	1	9
12313	Semi-skimmed milk pasteurised	3.5	2051	8
12316	Whole milk pasteurised	3.3	1166	8
12009	Semi-skimmed milk pasteurised average	3.3	397	8
12312	Semi-skimmed milk average	3.4	148	8
13172	Broccoli green boiled in unsalted water	3.1	148	8
12387	Ice cream dairy vanilla	3.6	82	8
13043	Baked beans canned in tomato sauce	4.8	75	8
13439	Peas boiled in unsalted water	6.7	63	8
13171	Broccoli green boiled in salted water	3.1	62	8
12375	Whole milk yogurt fruit	4	61	8
13010	Old potatoes baked flesh and skin	3.9	50	8
13440	Peas frozen boiled in unsalted water	6	43	8
12380	Yogurt low fat fruit	4.2	35	8
12383	Yogurt virtually fat free/diet plain	5.4	34	8
12010	Semi-skimmed milk pasteurised fortified plus SMP	3.7	32	8
11443	Brown rice boiled	2.6	31	8
17364	Mustard smooth	7.1	29	8
12218	Cheesecake	3.7	29	8
13128	Peas boiled in salted water	6.7	27	8
12392	Ice cream dairy premium	3.9	26	8
12205	Ice cream dairy flavoured	3.5	20	8
12382	Yogurt virtually fat free/diet fruit	4.8	18	8
13370	Sweetcorn kernels boiled in unsalted water	4.2	17	8
12310	Skimmed milk UHT	2.8	16	8
12112	Cream fresh half	3	13	8

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
13456	Spinach raw	2.8	13	8
14803	Almonds toasted	21.2	10	8
12353	Cream cheese	3.1	9	8
684	Chips fine cut frozen fried in blended oil	4.5	7	8
13441	Peas canned re-heated drained	5.3	5	8
13407	Chips homemade fried in blended oil	3.9	5	8
17365	Mustard wholegrain	8.2	4	8
13374	Sweetcorn on-the-cob whole boiled in unsalted water	2.5	4	8
20011	Brown wholegrain rice cooked	3.6	3	8
13368	Sweetcorn kernels raw	3.4	3	8
17532	Drinking chocolate powder made up with semi-skimmed milk	3.6	2	8
12185	Whole milk yogurt fruit	5.1	1	8
17529	White sauce sweet made with whole milk	3.9	1	8
12319	Whole milk sterilised	3.5	1	8
12317	Whole milk pasteurised summer	3.4	1	8
12328	Goats milk pasteurised	3.1	1	8
12388	Ice cream non-dairy vanilla	3	1	8
12121	Cream UHT half	2.8	1	8
19	Brown rice boiled	2.6	1	8
13373	Sweetcorn on-the-cob whole boiled in salted water	2.5	1	8
12307	Skimmed milk pasteurised	3.5	863	7
11569	Porridge made with water	1.4	183	7
12306	Skimmed milk average	3.4	148	7
12379	Yogurt low fat plain	4.8	102	7
13217	Cauliflower boiled in unsalted water	2.9	63	7
14031	Apricots dried	4.8	43	7
13173	Broccoli green frozen boiled in unsalted water	3.3	35	7
12333	Cream fresh whipping	2	30	7
13422	Chips retail fried in vegetable oil	3.2	27	7
677	Chips retail fried in blended oil	3.2	26	7

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
13216	Cauliflower boiled in salted water	2.9	16	7
13844	Parsley fresh	3	12	7
13442	Asparagus boiled in salted water	3.4	11	7
12123	Cream UHT whipping	2	9	7
13465	Peas frozen boiled in salted water	6	7	7
13144	Sugar-snap peas boiled in salted water	3.1	5	7
13804	Basil fresh	3.1	4	7
13316	Peppers capsicum chilli green raw	2.9	4	7
13215	Cauliflower raw	3.6	1	7
13462	Watercress raw	3	1	7
13235	Curly kale boiled in salted water	2.4	1	7
12119	Cream frozen whipping	1.9	1	7
12042	Soya milk plain	2.9	78	6
13281	Mixed vegetables frozen boiled in salted water	3.3	57	6
13179	Brussels sprouts boiled in unsalted water	2.9	13	6
12303	White sauce packet mix made up with whole milk	3.9	12	6
13458	Spinach frozen boiled in unsalted water	3.1	6	6
681	Chips straight cut frozen fried in blended oil	4.1	4	6
13178	Brussels sprouts boiled in salted water	2.9	4	6
13443	Brussels sprouts frozen boiled in unsalted water	3.5	3	6
13180	Brussels sprouts frozen boiled in salted water	3.5	2	6
13421	Old potatoes boiled in unsalted water	1.8	298	5
13447	Carrots old boiled in unsalted water	0.6	259	5
13003	New potatoes boiled in unsalted water	1.5	134	5

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
13013	Old potatoes boiled in salted water	1.8	106	5
13402	Old potatoes mashed with butter	1.8	87	5
13002	New potatoes boiled in salted water	1.5	86	5
12331	Soya non-dairy alternative to milk unsweetened	2.4	57	5
13352	Spring onions bulbs and tops raw	2	37	5
13204	Carrots young boiled in salted water	0.6	30	5
13403	Old potatoes mashed with hard margarine	1.8	19	5
13446	Carrots old raw	0.6	19	5
17510	Mayonnaise retail	1.1	12	5
13814	Chives fresh	2.8	7	5
13344	Spinach boiled in salted water	2.2	7	5
13122	Mange-tout peas raw	3.6	3	5
13004	New potatoes in skins boiled in salted water	1.4	2	5
13205	Carrots young boiled in unsalted water	0.6	1	5
14045	Bananas	1.2	618	4
17316	Mayonnaise	1.1	168	4
13201	Carrots old boiled in salted water	0.6	103	4
17513	Tomato ketchup	1.6	25	4
14239	Prunes ready-to-eat	2.5	23	4
13317	Peppers capsicum chilli red raw	1.8	9	4
13466	Leeks raw	1.6	5	4
14092	Figs dried	3.6	4	4
13365	Sweet potato steamed	1.1	3	4
13202	Carrots old boiled in unsalted water	0.6	3	4
13218	Cauliflower frozen boiled in unsalted water	2	2	4
865	Avocado average	1.9	2	4
13224	Chard Swiss boiled in unsalted water	1.9	1	4

WISP© Nutrient Database Code	Long Description	Protein (g/100g)	Occurrences within the sample population	Number of AA values assigned
14242	Raisins	2.1	68	3
14231	Prunes	2.8	66	3
14037	Avocado average	1.9	44	3
13457	Spinach boiled in unsalted water	2.2	19	3
13452	Leeks boiled in unsalted water	1.2	14	3
13158	Asparagus boiled weighed as served	1.6	6	3
13264	Leeks boiled in salted water	1.2	6	3
13282	Mixed vegetables canned re-heated drained	1.9	1	3
13284	Mushrooms common raw	1.8	26	2
13230	Courgette raw	1.8	3	2
14091	Figs raw	1.3	2	2
13206	Carrots frozen boiled in unsalted water	0.4	2	2
13012	Old potatoes baked flesh only weighed with skin	1.5	1	2
17274	Oxtail soup dried as served	1.4	1	2
17485	Butter	0.6	1464	1
17073	Jam fruit with edible seeds	0.6	331	1
14293	Kiwi fruit	1.1	143	1
17283	Vegetable soup	0.9	68	1
13164	Beetroot raw	1.7	34	1
13321	Peppers capsicum red boiled in salted water	1.1	25	1
14073	Cranberries	0.4	15	1
13231	Courgette boiled in unsalted water	2	14	1
14284	Orange juice concentrate unsweetened	2.9	11	1
13319	Peppers capsicum green boiled in salted water	1	9	1
13356	Squash butternut baked	0.9	6	1
13468	Cabbage raw average	1.7	4	1
14124	Kiwi fruit weighed with skin	1	2	1
14123	Kiwi fruit	1.1	1	1
13268	Lettuce Cos raw	1	1	1

Table 33 WISP© nutrient database food codes, food descriptors, protein content, frequency of food selection among study population, and new WISP© codes assigned manually by researcher (n=1054)

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
11348	Pilau rice	20049	2.7	4
11047	White rice glutinous boiled	20049	1.7	1
11038	Red rice boiled	20014	1.8	2
20016	Thai fragrant rice cooked	20014	2.3	1
20002	White rice basmati cooked	20007	2.8	49
11446	White rice easy cook boiled	20007	2.6	41
11050	White rice polished boiled	20007	2.2	14
20004	White rice basmati easy cook cooked	20007	3.5	10
11610	White rice fried	20007	2.5	2
20023	Savoury rice ready cooked eg. Tilda/Rizazz/Uncle Ben's Express	20007	3.5	1
11620	Savoury rice cooked	20007	2.9	1
20001	White rice basmati raw	20005	8.1	1
19087	Pork and beef sausages chilled raw	19093	10.2	1
19094	Premium sausages chilled fried	19091	15.8	1
19086	Pork sausages reduced fat chilled/frozen grilled	19083	16.2	4
19103	Garlic sausage	19083	15.9	1
528	Sausages pork fried	19079	13.8	23
57203	Pork Sausage Fried In Olive Oil (UCC)	19079	14.1	8
57204	Pork Sausage Fried In Sunflower Oil (UCC)	19079	14.1	1
19031	Beefburgers homemade fried	19037	23.8	27
19033	Beefburgers homemade grilled	19037	24.9	7
19029	Beefburgers chilled/frozen fried	19037	28.5	6
19030	Beefburgers chilled/frozen grilled	19037	26.5	6
19034	Beefburgers homemade grilled with bun	19037	21.9	5
30874	80% Pure Beef Burger (BB-003536)	19037	15.1	1
439	Chicken roast meat and skin	18341	22.6	13

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
18325	Chicken breast grilled meat and skin	18341	28.9	4
18367	Whole turkey roasted	18341	30.9	1
18327	Chicken portions deep-fried meat and skin	18334	26.9	1
30625	Chicken Breast Fillet Cooked Skinless (BB-032396)	18331	21.8	51
19126	Chicken slices	18331	23.2	29
18324	Chicken breast grilled with skin meat only	18331	29.8	13
30418	Chicken Meat - Cooked (BB-001095)	18331	25.3	2
30581	Chicken Breast Fillet (BB-030672)	18330	24	37
18323	Chicken breast grilled without skin meat only	18330	32	26
30428	Chicken Breast Fillets (BB-003025)	18330	24	8
30549	Turkey Breast Sliced (BB-029175)	18330	19.2	8
56852	Chicken Breast Cooked In Sunflower Oil Homemade (UCC)	18330	31.2	6
30862	Cooked Diced Chicken Breast Meat (BB-030722)	18330	24.1	6
19120	Chicken breast marinated w/garlic and herbs chilled/frozen baked	18330	24	6
30416	Chicken Breast Meat (BB-001069)	18330	23.7	5
464	Turkey roast light meat	18330	29.8	4
30614	Chicken Fillet Roast Skinless (BB-032279)	18330	26	4
56855	Chicken Breast Cooked In Blended Oil Homemade (UCC)	18330	31.2	3
18357	Turkey strips stir-fried	18330	31	3
30562	Chicken Breast Strips Cooked (BB-030137)	18330	25.1	3
30556	Sliced Cooked Chicken Breast (BB-029189)	18330	17.7	2
31007	Cooked Turkey Breast Saddle (BB-030311)	18330	26.3	1

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
31105	Thick Cut Roast Turkey Breast (BB-070013)	18330	23.2	1
30927	Frozen Diced Chicken (BB-003116)	18330	20.6	1
30629	Chicken Burger (BB-032491)	18330	19.5	1
30936	Cooked Skewered Chicken Yakitori (BB-003371)	18330	19.4	1
19158	Turkey steaks in crumbs frozen grilled	18330	17.6	1
19239	Lemon chicken	18330	16.4	1
30657	Breaded Chicken Chunk (BB-032785)	18330	16.2	1
57799	Chicken Chasseur Homemade (UCC)	18330	16	1
57305	Chicken In Batter (birds Eye Crispy) (UCC)	18330	15	1
30816	Chicken Strips (BB-032783)	18330	14.3	1
19121	Chicken fingers baked	18330	12.5	1
19118	Chicken breast in crumbs chilled fried	18329	18	2
18326	Chicken breast strips stir-fried	18300	29.7	26
19148	Pork slices	18255	23.3	9
18208	Pork belly joint roasted lean and fat	18255	25.1	7
18262	Pork loin joint roasted lean	18255	30.1	6
18485	Pork leg joint lean and fat roast	18255	30.9	4
18266	Pork loin steaks fried lean and fat	18255	27.5	4
19147	Pork roast frozen cooked	18255	26.1	4
30634	Pork Roasting Joint Boneless (BB-032524)	18255	18.9	4
18288	Pork steaks stewed lean and fat	18255	31	3
18233	Pork hand shoulder joint roasted lean	18255	30.8	3
18484	Pork leg joint lean only roast	18255	33	2
18242	Pork leg joint roasted well done lean	18255	34.7	1
18260	Pork loin joint pot-roasted lean	18255	31	1

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
18234	Pork hand shoulder joint roasted lean and fat	18255	27.5	1
425	Pork leg lean and fat roast	18255	26.9	1
18273	Pork spare rib chops braised lean and fat weighed w/bone	18255	21.6	1
30815	Roast Pork (BB-071269)	18255	21.5	1
19260	Pork spare ribs 'barbecue style'	18255	20.6	1
18278	Pork spare ribs raw lean and fat	18255	18.7	1
19264	Pork spare ribs in black bean sauce	18255	15	1
18268	Pork mince stewed	18251	24.4	2
18219	Pork diced casseroled lean	18251	31.7	1
18215	Pork chump steaks fried lean and fat	18251	27.9	1
19266	Pork stir-fried with vegetables	18251	12.1	1
18285	Pork steaks grilled lean	18250	33.9	11
18271	Pork spare rib chops braised lean	18248	30.5	3
19262	Pork spare ribs 'barbecue style' chilled/frozen reheated	18248	26.3	1
339	Bacon fat only cooked average	18205	9.3	1
338	Bacon fat only raw average	18205	4.8	1
400	Lamb cutlets lean and fat grilled	18196	23	10
18119	Lamb chump chops fried lean and fat	18196	24.7	5
18117	Lamb chump chops fried lean	18196	28.1	3
18477	Lamb loin chops lean and fat grilled	18196	26.5	2
18124	Lamb leg chops grilled lean and fat	18196	28.3	1
18109	Lamb best end neck cutlets grilled lean and fat	18196	24.5	1
18105	Lamb best end neck cutlets barbecued lean and fat	18196	24.3	1
395	Lamb chops loin lean and fat grilled	18195	23.5	18
18149	Lamb loin chops roasted lean	18195	34.4	3

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
18107	Lamb best end neck cutlets grilled lean	18195	28.5	1
397	Lamb chops loin lean only grilled	18194	27.8	12
402	Lamb cutlets lean only grilled	18194	27.8	7
18147	Lamb loin chops microwaved lean and fat	18194	27.5	1
398	Lamb chops loin lean only grilled weighed with fat and bone	18194	15.3	1
18480	Lamb leg lean and fat roast	18192	28.1	6
18122	Lamb chump steaks fried lean and fat	18192	24.8	3
18114	Lamb breast roasted lean and fat	18192	22.4	2
18168	Rack of lamb roasted lean	18192	27.1	1
19129	Doner kebabs meat only	18192	23.5	1
18113	Lamb breast roasted lean	18191	26.7	13
18156	Lamb loin joint roasted lean and fat	18191	25.3	10
18100	Lamb average trimmed fat cooked	18191	15	7
18132	Lamb leg joint roasted lean and fat	18191	29.7	6
18126	Lamb leg half fillet braised lean	18191	27.3	6
18479	Lamb leg lean only roast	18191	29.7	5
393	Lamb breast lean only roast	18191	25.6	5
19134	Lamb roast frozen cooked	18191	24.3	4
30578	Sliced Roast Lamb In Gravy (BB-030610)	18191	11.9	1
392	Lamb breast lean and fat roast	18189	19.1	4
18169	Rack of lamb roasted lean and fat	18189	23	3
18136	Lamb leg whole roasted medium lean and fat	18189	28.1	2
30436	Lamb Burger (BB-003409)	18159	13.7	2
409	Lamb scrag and neck lean only stewed	18159	27.8	1
18185	Stewing lamb pressure cooked lean and fat	18159	25.7	1

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
18098	Lamb average trimmed fat raw	18158	13.3	3
18153	Lamb loin joint raw lean and fat	18158	16.9	1
18067	Beef sirloin steak fried lean and fat	18072	26.8	11
18091	Beef topside roasted well-done lean and fat	18072	32.8	3
18073	Beef sirloin steak grilled well-done lean and fat	18072	31.8	3
18062	Beef sirloin joint roasted lean	18072	32.4	1
18020	Beef fillet steak grilled lean	18070	29.1	16
18071	Beef sirloin steak grilled medium-rare lean and fat	18070	24.8	4
18068	Beef sirloin steak grilled rare lean	18070	26.4	3
18046	Beef rump steak barbecued lean and fat	18070	29.5	1
18019	Beef fillet steak fried lean and fat	18066	28	9
18075	Beef sirloin steak from steakhouse lean and fat	18066	29.6	1
56856	Steak Lean Fried In Sunflower Oil (UCC)	18066	29.4	1
57321	Steak Lean Fried In Olive Oil (UCC)	18066	29.4	1
18023	Beef fillet steak from steakhouse lean and fat	18066	28.5	1
18048	Beef rump steak fried lean and fat	18066	28.4	1
18080	Beef stewing steak stewed lean	18038	32	2
30548	Roast Beef Sliced (BB-029174)	18033	18.9	16
18034	Beef fore-rib/rib-roast roasted lean and fat	18033	29.1	3
18056	Beef silverside pot-roasted lean	18033	34	1
18029	Beef fore-rib/rib-roast raw lean and fat	18028	18.8	2
18018	Beef fillet steak fried lean	18016	28.2	21
18021	Beef fillet steak grilled lean and fat	18011	28.7	6

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
19112	Beef slices	18010	25.3	16
18027	Beef flank pot-roasted lean and fat	18009	27.1	1
56800	White Sauce Dry Mix Made Up With Milk Added Mushroom And Leek (UCC)	17529	3.4	1
17333	Sauce tomato base homemade	17516	1.4	4
17486	Butter spreadable	17485	0.5	38
1173	Tomato sauce	17338	2.2	9
17317	Mayonnaise homemade	17316	2.1	3
56738	Soup vegetable Homemade (UCC)	17283	0.9	48
17276	Potato and leek soup	17283	1.5	11
17249	Carrot and orange soup	17283	0.4	6
56792	Soup vegetable (carrot Onion Celery Parsnip Turnip) Homemade (UCC)	17283	0.6	4
56736	Soup Mushroom Homemade (UCC)	17283	1.3	3
56798	Soup Cream Of vegetable Homemade (UCC)	17283	1.2	3
2027	Cully and Sully vegetable Soup	17283	0.6	3
56795	Soup Pumpkin Homemade (UCC)	17283	1.1	2
10223	Premium chilled carton soup carrot and coriander FSA Sept 2004	17283	0.9	2
56753	Soup vegetable (carrot Celery Tomato Onion) Homemade (UCC)	17283	0.7	2
56817	Soup Lentil Onion And Carrot Homemade (UCC)	17283	5.3	1
56810	Soup Potato And Sweetcorn Homemade (UCC)	17283	2	1
44081	TOMATO SOUP (AP-324656)	17283	0.9	1
56794	Soup Tomato And Red Pepper Homemade (UCC)	17283	0.7	1
56809	Soup Tomato And Lentil Homemade (UCC)	17264	11.3	1
17074	Jam stone fruit	17073	0.4	16

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
56405	Jam (fruitfield Extra Fruit Red Sugar Jam/danish Orchard Red Sugar Jam) (UCC)	17073	0.3	5
30032	Mixed Fruit Jam (BB-033739)	17073	0.2	3
31092	Jam Portions (BB-034022)	17073	0.3	2
17071	Fruit spread	17073	0.7	1
16197	Mackerel canned in brine drained	16324	19	10
16297	Mackerel pate smoked	16324	13.4	3
16021	Cod in batter fried in blended oil	16291	16.1	8
30170	Cod Fish Finger Battered (BB-030231)	16290	10	2
652	Fish fingers fried in lard	16290	13.5	1
16166	Whiting in crumbs fried in sunflower oil	16289	18.1	3
651	Fish fingers fried in blended oil	16289	13.5	2
653	Fish fingers grilled	16288	15.1	4
31045	Cod Fillet Fish Fingers (BB-032554)	16288	12.4	1
30433	Cod Fish Cakes (BB-003280)	16285	7.9	1
56284	King Prawns In Batter Homemade (UCC)	16239	14.1	3
16222	Swordfish grilled	16221	22.9	2
16327	Salmon grilled	16203	34.2	73
10203	Salmon baked farmed FSA Sept 2004	16203	25.2	48
16205	Salmon steamed	16203	21.8	21
56227	Salmon Baked In Butter (UCC)	16203	21.6	18
56228	Salmon Fried In Olive Oil (UCC)	16203	25.6	5
56275	Salmon Fried In Blended Oil (UCC)	16203	20.6	1
16140	Rock Salmon/Dogfish in batter fried in sunflower oil	16203	14.7	1
16304	Salmon en croute retail	16203	11.8	1
16207	Salmon smoked	16202	25.4	58
30875	Smoked Salmon Slices (BB-071082)	16202	18.6	31

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
14298	Oranges	16199	1.1	238
14291	Clementines	16199	0.9	55
30544	Mandarins Whole Segments (BB-021141)	16199	0.5	20
14297	Nectarines	16199	1.4	11
14175	Oranges	16199	1.1	1
16196	Mackerel smoked	16191	18.9	22
16188	Kipper grilled	16187	20.1	2
16182	Herring canned in tomato sauce	16177	12.8	1
16006	Bream Sea raw	16002	17.5	3
15331	Vegeburger retail grilled	15333	16.6	1
56155	Lasagne Vegetarian (linda Mccartney) (UCC)	15188	5.1	2
30566	Lasagne vegetable Verdi (BB-030279)	15187	4.6	4
14845	Sunflower seeds	14842	19.8	49
14301	Orange juice unsweetened	14284	0.5	136
14281	Orange juice freshly squeezed	14284	0.6	108
14257	Satsumas	14284	0.9	24
30746	Orange Juice From Concentrate (BB^{-1}00243)	14284	0.5	22
14282	Orange juice freshly squeezed weighed as whole fruit	14284	0.3	5
14263	Sultanas	14242	2.7	22
17296	Cranberry sauce	14073	0.2	6
14025	Apricots raw	14031	0.9	13
13444	Cabbage boiled in unsalted water average	13468	1	84
13184	Cabbage boiled in salted water average	13468	1	45
56143	Potatoes Mashed With Low-fat Milk (UCC)	13403	2.1	9
56059	Potatoes Mashed With Added Fat (UCC)	13403	1.7	9
30474	Potato - Mashed (BB-003945)	13403	1	3
13391	Turnip boiled in unsalted water	13392	0.6	60
13390	Turnip boiled in salted water	13392	0.6	12
13360	Swede boiled in salted water	13392	0.3	2

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
13367	Sweetcorn baby canned drained	13370	2.9	11
13363	Sweet potato baked	13365	1.6	9
13464	Sweet potato boiled in salted water	13365	1.1	8
13453	Lettuce average raw	13268	0.8	381
15380	Salad green	13268	0.7	110
13269	Lettuce Iceberg raw	13268	0.7	41
777	Lettuce average raw	13268	0.8	5
3363	Rocket (UL)	13268	2.6	2
13267	Lettuce butterhead raw	13268	0.9	2
3260	Rocket (UL)	13268	2.6	1
13266	Lettuce average raw	13268	0.8	1
13270	Lettuce Webbs raw	13268	0.8	1
13143	Sugar-snap peas raw	13144	3.4	3
15320	Tofu spread	13119	4.6	5
13071	Butter beans dried boiled in unsalted water	13072	7.1	2
30009	Baked Beans (BB-000186)	13043	4.3	3
30538	Baked Beans (BB-011758)	13043	5.1	1
56176	Potato Chips Heinz Weight Watchers Oven Chips (UCC)	13036	2.8	2
3258	Walkers Baked Cheese and Onion Crisps	13036	6.4	1
10228	Potato wedges oven baked FSA Sept 2004	13010	2.8	18
12814	Eggs duck boiled and salted	12920	14.6	1
12926	Eggs chicken scrambled with milk	12812	10.9	44
55722	Eggs Scrambled With Added Fat And Semi-skimmed Milk (UCC)	12812	12.1	12
17414	Egg white dried	12804	73.8	1
12192	Low calorie yogurt	12383	4.3	13
55534	Yogurt Drinking 0% Fat (danone) (UCC)	12383	2.8	6
12371	Fromage frais virtually fat free natural	12382	7.7	11
255	Low fat yogurt plain	12379	5.1	120
12315	Whole milk average	12316	3.3	808
12304	White sauce packet mix made up with semi-skimmed milk	12303	4	3

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
17322	Pasta sauce white	12299	7.8	2
55632	Yoghurt Drinking (yoplait Essence Low Blood Pressure & Cholesterol) (UCC)	12188	2.7	9
2063	Danone Danacol	12188	3.3	5
12376	Yogurt greek style plain	12184	5.7	22
55503	Yogurt Hazelnut (average Of Sno & Yoplait) (UCC)	12184	4.8	16
12177	Cheese Roquefort	12164	19.7	3
12367	Cheese Stilton blue	12161	23.7	8
12156	Cheese Emmental	12154	28.7	6
12181	Cheese Stilton white	12154	19.9	4
3381	Cream Cheese low fat (UL)	12150	8.2	3
12346	Cheese Cheddar average	12138	25.4	284
12368	White cheese average	12138	23.7	6
12347	Cheese Cheddar vegetarian	12138	25.5	5
30765	Mature White Cheddar (BB-071144)	12138	25.4	3
55506	Cheese Processed Avonmore Light (UCC)	12138	31	1
55524	Cheese Strings (golden Vale) (UCC)	12138	28	1
55525	Cheese Light (babybel) (UCC)	12138	26	1
230	Cheese Cheddar-type reduced fat	12134	31.5	19
31123	Healthier Choices Grated Cheese (BB-071951)	12134	30.8	1
12338	Dairy cream UHT canned spray	12123	1.9	1
12122	Cream UHT single	12121	2.6	1
12332	Cream fresh single	12115	3.3	50
17089	Chocolate milk	12094	7.7	139
2002	CHOCOLATE CHIP BREAD & BUTTER PUDDING	11608	4.9	15
11546	Crumble fruit	11597	2.4	9
11195	Chocolate cake	11580	7.4	42
11260	Muffins	11541	10.1	8
11261	Muffins bran	11541	7.8	6
30426	English Muffin (BB-002815)	11541	10.6	2
30455	Dairy Cream Sponge (BB-003721)	11532	5.2	3

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
11176	Homemade biscuits wholemeal	11521	13.4	9
30669	Biscuits Digestive Round (BB-033977)	11521	6.4	2
30668	Biscuits Custard Creams (BB-033976)	11519	5.4	32
11524	Wafer biscuits filled	11519	4.7	7
11516	Sandwich biscuits jam filled	11519	5.6	4
11182	Sandwich biscuits	11519	5	2
11512	Digestive biscuits chocolate	11507	6.8	174
17491	Chocolate plain	11507	5	83
17088	Chocolate fancy and filled	11507	4.9	50
1018	Chocolates fancy and filled	11507	4.1	30
11506	Chocolate biscuits full coated	11507	7.3	27
17084	Chocolate covered bar with fruit/nut wafer/biscuit	11507	8.7	25
55306	Biscuit Tesco Rich Tea Milk Chocolate (UCC)	11507	6.3	23
55193	Chocolate Biscuit Cake Homemade (UCC)	11507	5	15
55345	Biscuit Chocolate Chip Cookies (weight Watchers) (UCC)	11507	4.7	12
55190	Brownies Homemade (UCC)	11507	4.9	10
11509	Wafers filled chocolate full coated	11507	6.9	9
55323	Biscuit Bar Mcvities Riva Chocolate (UCC)	11507	6	9
30667	Biscuits Bourbon Cream (BB-033975)	11507	5.4	9
55311	Biscuit Jacob's Chocolate Kimberly (UCC)	11507	4.6	9
55340	Biscuit Chocolate Chip / Chocolate Wafer (shs Loprofin) (UCC)	11507	0.3	7
10230	Cake bars chocolate FSA Sept 2004	11507	5	3
17492	Chocolate covered caramels	11507	5	3
56433	Cadbury's Caramello / Caramel Bar (UCC)	11507	6.2	2
55348	Cadbury’s Magi Milk Bar (fortified) (UCC)	11507	5.2	2
17086	Chocolate cooking	11507	4.8	2

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
56400	Chocolate Bar Low Fat (e.g. Flyte) (UCC)	11507	3.5	2
56415	Cadbury Byte (UCC)	11507	4.4	1
20058	Rice Krispies	11497	6	8
20050	Corn Flakes	11490	7.1	76
20071	Fruit and Fibre	11489	8	8
55156	Breakfast Cereal Bran Flakes (dunnes Stores) (UCC)	11489	10.2	3
11461	Granary bread	11476	9.6	66
55181	Bread White Soda Homemade (UCC)	11470	6.9	17
55197	Bread Brown Soda Homemade (UCC)	11456	8.5	503
11092	Soda bread	11456	7.7	172
55185	Bread Brown Soda Made With Egg Homemade (UCC)	11456	10.2	1
3237	Spelt Bread (UL)	11456	10	1
55203	Bread Brown Soda With Sunflower Seeds Homemade (UCC)	11456	7.9	1
11357	Spaghetti canned in tomato sauce	11356	1.9	1
55411	Pizza Pepperoni Frozen (thin N Crispy) (UCC)	11349	12.5	3
20043	Cous Cous cooked	11339	7.2	13
58514	Salad Couscous (UCC)	11339	2.9	2
30480	Strawberry Cheesecake (BB-003992)	11288	3.8	1
30786	Apple Pie (BB-004070)	11279	9.9	17
11280	Apple pie wholemeal one crust	11279	2.4	5
11539	Doughnuts ring	11244	6.1	2
30198	Jam Doughnuts (BB-004557)	11242	5.4	2
55038	Doughnut Jam With Added Cream (UCC)	11242	4.7	1
30497	Puff Pastry (BB-004308)	11224	5.6	1
55188	Cake Coffee Sponge With Cream Homemade (UCC)	11214	6.6	21
55196	Cake Fairy/queen With Added Chocolate (UCC)	11214	5.3	8
44166	APPLE CAKE (AP-324815)	11211	3	1
11513	Digestive biscuits plain	11183	6.3	145
11511	Crispbread rye	11168	9.4	91

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
55324	Crackerbread Original/cracotte Original (UCC)	11168	10.3	46
55321	Carrs Table Water Crackers (UCC)	11167	10.4	20
30422	Cream Crackers (BB-001474)	11167	9.3	5
20079	Porridge Oats cooked	11143	5.4	18
55144	Breakfast Cereal Corn Flakes (super Valu/dunnes Stores) (UCC)	11130	7.3	4
2062	Kelkin Gluten Free Corn Flakes	11130	8.2	1
20059	Coco Pops	11129	4.6	2
20065	All-Bran	11126	12.4	36
20064	Bran Flakes	11126	9.6	8
55093	Breakfast Cereal Weetabix Alpen Crunchy Bran (UCC)	11126	11.5	1
55146	Breakfast Cereal Bran Flakes (aldi Harvest Morn /lidl Little Man) (UCC)	11126	11	1
11477	Brown rolls crusty	11125	10.3	23
55195	Bread Brown Yeast Homemade (UCC)	11125	9.2	14
11478	Brown rolls soft	11125	9.9	4
11115	Wholemeal bread small sliced	11113	9.3	45
55189	Bread Wholemeal Homemade (UCC)	11113	8	28
55152	Breakfast Cereal Corn Flakes (lidl Little Man) (UCC)	11113	7.5	12
30674	Bread Sliced Wholemeal Loaf (Thick) (BB-050149)	11113	10.3	4
31059	Panini Baguette (BB-033097)	11107	8.9	6
30574	7.5" Baguette (BB-030515)	11107	8.7	6
30679	Bread White Thick Sliced Bloomer (BB-052160)	11100	10	2
30856	White Sandwich Baguette (BB-054626)	11099	9.3	6
55157	Bread White Sliced Pan (johnston Mooney & O'brien Butterkrust) (UCC)	11099	8.4	3
3295	Spelt Bread (UL)	11070	10	17

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20048	Wheatgerm	11034	27.3	4
20045	Bulgar Wheat	11007	10.6	3
11132	Farmhouse Bran	11005	12.9	4
75	Oat and Wheat Bran	11005	10.6	3
11059	Noodles plain boiled	10246	2.4	9
11056	Noodles egg boiled	10246	2.2	2
30876	Medium Egg Noodles (BB-071064)	10246	7.7	1
10248	Rice noodles fine cooked FSA Sept 2004	10246	1.9	1
30719	Blueberry Muffin (BB-087556)	10215	4.8	4
30971	Giant Blueberry Muffin (BB-004659)	10215	4.2	2
30825	Blueberry Muffins (BB-004655)	10215	3.8	1
30465	English Mustard (BB-003849)	1180	6.4	3
17334	Soy sauce	1171	3	17
17527	White sauce savoury made with whole milk	1154	4.2	11
17521	Cheese sauce made with whole milk	1154	8.1	1
10225	Premium chilled carton soup tomato FSA Sept 2004	1129	0.9	5
17278	Tomato soup cream of canned	1129	0.8	5
56779	Soup Fresh Tomato And Basil Fortified (knorr) (UCC)	1129	0.8	3
56796	Soup Chicken And vegetable Homemade (UCC)	1125	6.3	3
57772	Chicken In Canned Soup With vegetable s (UCC)	1125	10.9	1
12034	Flavoured milk	1065	3.6	1
30723	Flavoured Milk Drink Banana (BB-088516)	1065	3.1	1
3249	Kelkin Multigrain Corn Cakes	1035	12	17
3421	Kelkin Multigrain Corn Cakes	1035	12	15
3223	Kelkin Multigrain Corn Cakes	1035	12	1
14844	Sesame seeds	994	18.2	37

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13366	Sweetcorn baby fresh and frozen boiled in salted water	825	2.5	3
13459	Sweetcorn kernels canned re-heated drained	824	2.9	3
13369	Sweetcorn kernels boiled in salted water	824	4.2	2
13351	Spring onions bulbs only raw	818	0.9	18
13342	Shallots raw	818	1.5	1
13320	Peppers capsicum red raw	805	1	83
30868	Sliced Mixed Peppers (BB-004793)	803	0.9	5
13322	Peppers capsicum yellow raw	801	1.2	16
13318	Peppers capsicum green raw	801	0.8	16
30515	Mixed vegetable (BB-004789)	782	2.3	21
30432	vegetable Mixed (BB-003235)	782	2.3	7
30520	Mixed vegetable (BB-004855)	782	2.6	2
17359	Garlic puree	772	3.5	11
56199	Colcannon (curly Kale And Onions) (UCC)	769	1.9	2
13415	Courgette fried in blended oil	765	2.6	10
13232	Courgette fried in corn oil	765	2.6	3
13414	Courgette fried in butter	765	2.6	2
13449	Carrots young boiled in unsalted water	757	0.6	59
13448	Carrots young raw	754	0.7	19
13190	Cabbage red raw	749	1.1	7
13193	Cabbage Savoy boiled in salted water	749	1.1	5
13445	Cabbage white raw	749	1.4	1
13185	Cabbage boiled in unsalted water average	749	1	1
13220	Celeriac boiled in salted water	749	0.9	1
13166	Beetroot pickled drained	741	1.2	29
13165	Beetroot boiled in salted water	741	2.3	21
13437	Mushy peas canned re-heated	733	5.8	6
13140	Processed peas canned re-heated drained	733	6.9	4

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13138	Petit pois frozen boiled in unsalted water	731	5	2
13142	Split peas dried boiled in unsalted water	731	8.3	1
13131	Peas dried boiled in unsalted water	731	6.9	1
13436	Mange-tout peas boiled in salted water	725	3.2	16
13065	Broad beans boiled in salted water	702	5.1	4
30672	Oven Chips (BB-036141)	687	2.1	1
13025	Chips straight cut frozen fried in corn oil	681	4.1	1
13423	Chips French fries retail	680	3.3	29
13411	Chips retail fried in sunflower oil	680	3.2	15
13024	Chips crinkle cut frozen fried in corn oil	677	3.6	2
13027	Chips thick cut frozen fried in corn oil	677	3.6	2
13410	Chips retail fried in corn oil	677	3.2	2
679	Chips retail fried in vegetable oil	677	3.2	1
13408	Chips homemade fried in sunflower oil	674	3.9	28
13021	Chips homemade fried in corn oil	674	3.9	4
13406	Chips homemade fried in dripping	674	3.9	1
56194	Potatoes Mashed With Flora Light (UCC)	670	1.7	15
56189	Potatoes Mashed With Dairygold Light (UCC)	670	1.8	14
13015	Old potatoes mashed with margarine	670	1.8	7
13405	Old potatoes mashed with polyunsatd. margarine	670	1.8	7
56179	Potatoes Mashed With Milk And Low Low Spread (UCC)	670	2	1
56190	Potatoes Mashed With Golden Olive (UCC)	670	1.7	1
56186	Potatoes Mashed With Milk And Fat (UCC)	669	2.1	8

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30429	Potato Mashed (BB-003034)	669	1.6	4
44149	MASHED POTATO (AP-324787)	669	1.7	2
44123	BOILED POTATOES (AP-324752)	668	1.8	32
13011	Old potatoes baked flesh only	665	2.2	13
30770	Potatoes Baked British Baby (BB-032651)	665	1.7	3
44145	JACKET POTATOES (AP-324778)	665	2.1	1
13009	Old potatoes average raw	664	2.1	10
13001	New potatoes average raw	664	1.7	3
13420	New potatoes in skins boiled in unsalted water	661	1.4	4
16245	Shrimps boiled	643	23.8	4
16241	Prawns frozen raw	642	18.3	1
16238	Prawns raw	642	17.6	1
56278	Prawns With Garlic And Butter (UCC)	639	16.5	1
30879	Norwegian Prawns (BB-031571)	639	15	1
16093	Monkfish grilled	637	22.7	4
30524	Tuna Chucks In Brine (BB-005365)	632	23.5	10
30547	Tuna Chunks In Brine (BB-028283)	632	27.1	3
16228	Tuna raw	632	23.7	3
30532	Tuna Chunks In Brine (BB-006731)	632	23	1
2034	John West Tuna with a twist french dressing	631	15.2	3
16328	Sardines canned in brine drained	627	21.5	7
16212	Sardines raw	627	20.6	4
16213	Sardines grilled	627	25.3	2
16325	Mackerel grilled	617	20.8	7
618	Mackerel fried	617	21.5	6
16192	Mackerel fried in blended oil	617	24	2
607	Whiting in crumbs fried	605	18.1	2
16164	Whiting in crumbs fried in dripping	605	18.1	2

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16189	Kippers grilled weighed with bones	605	12.7	1
16330	Trout rainbow grilled	587	21.5	6
16103	Plaice grilled	587	20.1	6
629	Trout brown steamed	587	23.5	4
16085	Lemon sole steamed	587	20.6	2
595	Plaice steamed	587	18.9	2
16113	Plaice in batter fried in sunflower oil	587	15.2	2
16106	Plaice frozen grilled	587	25.7	1
599	Plaice in crumbs fried fillets	587	18	1
16114	Plaice in crumbs fried in blended oil	587	18	1
16115	Plaice in crumbs fried in lard	587	18	1
30527	Skinless Breaded Whiting (BB-006298)	587	18	1
30849	Plaice Fillets (BB-002644)	587	16.7	1
592	Lemon sole in crumbs fried	587	16.1	1
16118	Plaice in crumbs froz fried sunflower oil w/bones and skin	587	10.5	1
16120	Plaice goujons fried in blended oil	587	8.5	1
16080	John Dory raw	586	19	3
16013	Cod baked	569	21.4	32
16071	Hake grilled	569	22.2	28
564	Cod baked fillets	569	21.4	12
56207	Cod Fillets Breaded Baked (donegal Catch) (UCC)	569	13.8	10
16068	Haddock smoked steamed	569	23.3	6
16017	Cod steamed	569	18.6	6
16020	Cod frozen grilled	569	20.8	4
16055	Haddock in batter fried in sunflower oil	569	17.1	3
16340	Coley steamed	569	23.3	2
16066	Haddock smoked poached	569	18.7	2
16070	Hake raw	569	18	2
16023	Cod in batter fried in retail blend oil	569	16.1	2
16060	Haddock in flour fried in sunflower oil	569	21.1	1
16108	Plaice frozen steamed	569	19.6	1
16065	Haddock smoked raw	569	19	1

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30935	Smoked Haddock Fillets (BB-003365)	569	19	1
16024	Cod in batter fried in sunflower oil	569	16.1	1
16030	Cod in parsley sauce frozen boiled	569	12	1
16064	Haddock in crumbs frozfried blended oilw/bones and skin	569	11.8	1
30444	Pork Cocktail Sausages (BB-003578)	529	14.1	7
19095	Premium sausages chilled grilled	526	16.8	2
18337	Chicken leg quarter roasted meat and skin	443	20.9	12
30417	Chicken Drumsticks - Cooked (BB-001087)	443	26.1	7
18335	Chicken drumsticks roasted meat and skin	443	25.8	4
19204	Chicken wings marinated chilled/frozen barbecued	443	27.4	3
18319	Chicken thighs diced casseroled meat only	443	25.6	2
18339	Chicken wing quarter roasted meat and skin	443	24.8	2
18313	Chicken leg quarter casseroled meat only	443	25	1
30631	Chicken Roasting Joint Boneless (BB-032520)	443	21.6	1
18317	Chicken thighs casseroled meat and skin	443	21.5	1
18294	Chicken leg quarter raw meat and skin weighed with bone	443	12.6	1
444	Chicken breaded fried in vegetable oil	441	18	6
19123	Chicken kiev frozen baked	441	18.6	4
18358	Turkey dark meat roasted	441	39.4	1
18359	Turkey light meat roasted	440	33.7	8
56851	Chicken Breast Cooked In Olive Oil Homemade (UCC)	440	31.2	7
436	Chicken boiled light meat	440	29.7	7
30530	Chicken Breast Strips (BB-006615)	440	25	5

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19119	Chicken breast in crumbs chilled grilled	440	17.5	4
31016	Breaded Chicken Goujons (BB-030827)	440	18.2	3
19122	Chicken goujons chilled/frozen baked	438	19.4	20
18361	Turkey meat average roasted	438	31.2	8
435	Chicken boiled meat only	438	29.2	8
18307	Chicken breast casseroled meat only	438	28.4	8
463	Turkey roast meat and skin	438	28	3
18356	Turkey breast fillet grilled meat only	438	35	1
420	Pork chops loin lean and fat grilled	423	28.5	12
18286	Pork steaks grilled lean and fat	423	32.4	11
422	Pork chops loin lean only grilled	423	32.3	7
18212	Pork chump chops fried lean and fat	423	24.6	6
18265	Pork loin steaks fried lean	423	31.5	3
31085	Healthier Choices Breaded Pork Loin Steaks (Smartcrumb) (BB-033935)	423	20.8	3
18226	Pork fillet slices grilled lean	423	33.6	2
18256	Pork loin chops roasted lean and fat	423	31.9	1
18263	Pork loin joint roasted lean and fat	423	26.3	1
30632	Beef Roasting Joint Boneless (BB-032522)	387	16.5	3
386	Beef topside lean and fat roast	387	26.6	2
381	Beef sirloin lean and fat roast	382	23.6	1
373	Beef rump steak lean and fat grilled	375	27.3	2
18052	Beef rump steak strips stir-fried lean	374	32.3	6
19045	Grillsteaks beef chilled/frozen fried	374	25.1	2
18047	Beef rump steak fried lean	374	30.9	1

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56854	Beef Mince Fried In Olive Oil (UCC)	370	26.3	4
19128	Corned beef canned	370	25.9	4
57600	Beef Meatballs (UCC)	370	14.5	4
19352	Bolognese sauce with meat	370	11.8	4
30942	99% Beefburgers (BB-003535)	370	15.1	2
384	Beef stewing steak lean and fat stewed	370	30.9	1
18041	Beef mince extra lean stewed	370	24.7	1
57546	Bolognese Sauce (beef Mince Onions) Homemade (UCC)	370	16.8	1
57725	Bolognese Sauce (beef Mince And Jarred Sauce Only) Homemade (UCC)	370	15.3	1
19242	Minced beef stewed	370	11.1	1
18090	Beef topside roasted well-done lean	368	36.2	6
56853	Beef Roasted In Sunflower Oil (UCC)	368	27.8	2
358	Bacon rasher lean and fat grilled middle	357	24.9	2
19014	Bacon rashers middle fried	353	23.4	42
56903	Bacon Rasher Fried In Olive Oil (UCC)	353	23.2	8
56902	Bacon Rasher Fried In Sunflower Oil (UCC)	353	23.2	3
30941	Cooked Smoked Streaky Bacon (BB-003527)	353	20.2	1
356	Bacon rasher lean only grilled average	348	30.5	2
19019	Bacon loin steaks grilled	348	25.9	1
19308	Ham	346	18.4	482
342	Bacon collar joint lean and fat boiled	346	20.4	40
343	Bacon collar joint lean only boiled	346	26	37
19021	Ham gammon joint boiled	346	23.3	24
31104	Sliced Gammon Ham (BB-070008)	346	20.5	11
19026	Ham premium	346	21.2	10
19025	Ham Parma	346	27.2	9

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345	Bacon gammon joint lean and fat boiled	346	24.7	8
30658	Gammon Steak (BB-032786)	346	19.2	2
31113	Honey Roast Ham (BB-071050)	346	22.7	1
30551	Honey Roast Ham (BB-029178)	346	20.3	1
55723	Omelette With 5 vegetable (UCC)	301	6.5	1
12806	Eggs chicken boiled	295	12.5	227
55721	Egg Fried In Olive Oil (UCC)	294	12.5	33
12919	Eggs chicken fried in vegetable oil	294	13.6	30
55720	Egg Fried In Sunflower Oil (UCC)	294	12.5	12
55606	Ice Cream Romantica/viennetta Biscuit (hb) (UCC)	268	3.3	4
12391	Ice cream bar chocolate coated	268	5	3
30509	Soft Scoop Chocolate Flavour Ice Cream (BB-004523)	268	3.2	3
30785	Ice Cream Chocolate Tub (BB-051010)	268	3	1
12210	Ice cream with cone	267	3.5	16
30420	Vanilla Ice Cream (BB-001234)	267	2.8	9
12211	Ice cream with wafers	267	3.5	3
30675	Ice Cream Vanilla Tub (BB-050985)	267	3.2	3
2031	HB Magnum Classic	267	3.5	2
12385	Frozen ice cream desserts	267	3.5	2
30662	Ice Cream Vanilla Flavour Brickettes (BB-032836)	267	2.9	2
31031	Vanilla Flavour Ice Cream Cups (BB-031947)	267	3.4	1
17408	Yogurt fruit baby	261	13.1	2
2016	Muller Corner Yogurt with Strawberry	261	3.8	2
12377	Yogurt greek style fruit	261	4.8	1
12186	Whole milk yogurt 'organic'	260	4.3	25

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3204	Activia Vanilla (S)	260	5	18
55537	Yoghurt Danone Activia Cereal (UCC)	260	3.7	18
12193	Drinking yogurt	260	3.1	15
12194	Greek yogurt cows	260	6.4	12
2074	Activia pouring yogurt	260	3.9	4
12189	Low fat yogurt flavoured	257	3.8	63
2113	Actimel (Strawberry)	257	2.7	10
55544	Yoghurt Low Fat (danone Vitalinea/shape) (UCC)	257	4.3	8
55530	Yoghurt Drinking Muller Vitality (UCC)	257	2.6	3
3225	Muller light strawberry	257	4.2	2
12349	Cheese spread plain	242	11.3	18
12350	Cheese spread reduced fat	242	15	10
12354	Cheese Danish blue	242	20.5	5
12364	Spreadable cheese soft white full fat	242	7.5	3
12365	Spreadable cheese soft white medium fat	242	9.8	2
12334	Cream fresh double	214	1.6	13
12113	Cream fresh single	211	2.6	3
12114	Cream fresh soured	211	2.9	2
12336	Creme fraiche half fat	211	2.7	2
12321	Breakfast milk pasteurised average	190	3.5	1
55522	Milk Avonmore Super (fortified) (UCC)	187	3.5	49
3331	Supermilk Avonmore Low fat	187	3.4	9
12025	Calcium-fortified milk Vital	187	4	2
3419	Supermilk Avonmore Low fat	187	3.4	2
2045	Avonmore Lactose Free Milk	187	3	1
17372	Stuffing mix made up with water	178	2.8	3
55460	Stuffing Bread Homemade (UCC)	177	5.3	15
56180	Potato Stuffing With Onion Homemade (UCC)	177	1.7	9
30050	Sage & Onion Stuffing (BB-028340)	177	9.4	1
11556	Pizza meat topped	170	13.2	2

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
55461	Pizza Cheese And Tomato (thin N Crispy) With Added Ham And Mushroom (UCC)	170	9.4	2
31079	12" Deep Pan Pizza Base (BB-033839)	170	9	2
11350	Pizza frozen	170	7.5	2
55408	Pizza Cheese And Tomato Frozen (thin N Crispy) (UCC)	170	13.8	1
11557	Pizza vegetarian	170	10.8	1
55401	Pizza Ham And Pineapple Deep Pan Frozen (UCC)	170	10.2	1
30461	Tomato & Cheese Pizza (BB-003789)	170	7	1
55482	Pizza Mushroom Homemade (UCC)	170	7	1
11281	Apple pie wholemeal pastry top and bottom	157	3.8	6
11293	Crumble apple	157	1.8	5
44188	APPLE PIE (AP-324859)	157	2.6	3
11284	Blackcurrant pie pastry top and bottom	157	3.1	1
30500	Tangy Lemon Cake (BB-004340)	122	4.8	18
55000	Chocolate Gateaux Cake Butter Icing (cadbury’s Swiss Gateaux) (UCC)	122	5	13
11528	Fancy iced cakes individual	122	3.8	10
11574	Battenburg cake	122	5.6	9
11196	Chocolate cake with butter icing	122	5.7	6
55116	Cake Madeira With Added Icing (UCC)	122	4.9	2
11305	Flan sponge with fruit	121	2.8	4
30771	Fruit Sponge (BB-033007)	121	7.2	2
30379	Sponge Strawberry (BB-030249)	121	3	1
55072	Cake Fairy/queen With Added Icing (UCC)	119	4.7	26
30609	Apple Sponge Cake (BB-032004)	119	4.7	6
30961	Lemon Sponge (BB-004200)	119	5.2	5
11307	Flan case sponge	119	9.8	2

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
55047	Cake Almond Slice (mr Kipling And Other Brands) (UCC)	119	6.3	1
11329	Spotted dick	114	4.2	2
55007	Barm Brack (rankins) (UCC)	113	4.5	28
55191	Cake Fairy/queen With Apple Homemade (UCC)	113	4.9	6
11579	Fruit cake wholemeal	113	6	5
55204	Cake Apple Homemade (UCC)	113	3.6	3
30721	Rich Tea Biscuits (BB-088476)	104	7.3	29
11517	Oat based biscuits	104	7.6	23
11522	Short-sweet biscuits	104	6.2	11
55316	Biscuit Mcvities Café Noir (UCC)	104	4.5	9
11523	Shortbread	104	6	7
31094	Shortcake (BB-034067)	104	6.2	5
55307	Biscuit Mcvities Boasters (UCC)	104	7.1	3
55313	Biscuit Jacob's Shortbread Creams (UCC)	104	4.7	3
11187	Water biscuits	104	10.8	1
55326	Biscuit Mcvities Go Ahead Golden Crunch (UCC)	99	7.7	8
2017	Cracottes Crisp bread	95	8.5	13
55328	Crackerbread High Fibre/cracotte Wholemeal (UCC)	95	11.8	8
55164	Breakfast Cereal Aldi Frosted Flakes (UCC)	71	5.9	1
30188	4" Sesame Seed Bun (BB-003608)	64	10.5	1
11479	Granary rolls	64	10	1
55177	Bread Rolls White Soft Fortified (irwins) (UCC)	63	10.8	14
30590	Hot Dog Rolls (BB-031298)	63	10.1	1
30190	5" Whole Flour Bap (BB-004581)	63	9.7	1
30191	10" White Sandwich Baguette (BB-003490)	62	9.1	4
30450	Croissant Butter Fully Baked (BB-003647)	60	9.3	1

WISP© Nutrient Database Code	Long Description	New WISP© Code Assigned	Protein (g/100g)	Occurrences within the sample population
31032	Fully Baked Croissant (BB-031995)	60	9.3	1
11083	Hovis toasted	57	12.1	2
2020	Juvela Gluten free Fibre Bread	55	3.4	2
31025	Fully Baked French Baguette (BB-031809)	53	8.9	2
30445	Pitta Bread (BB-003600)	45	9.1	5
20027	Wheat flour soft white plain	16	9.1	4
20028	Wheat flour strong white	16	11.3	1
11439	Wheat flour white plain	16	9.4	1

Poster presentations and publications

Centre for Interventions in Infection Inflammation & Immunity

Leahy S, Toomey C, Francis P, McCormack W, Norton C, Jakeman P
Human Body Composition Research Group,
University of Limerick, Limerick, Ireland.

University of Limerick

A reference base for age-related decrease in lean tissue mass: whole body and segmental lean tissue mass of 1616 Irish men and women 18 to 76y

Ref No: H&WB-A.120:110511

INTRODUCTION

Ageing is accompanied by a progressive loss of lean tissue mass. Sarcopenia defines the age-related loss in skeletal muscle mass estimated to affect ~30% of people older than 60y and >50% of those older than 80y. The magnitude of change in lean tissue mass (LTM) with ageing is referenced to race and gender specific healthy young adults[1]. The UL Body Composition Study[2] is a prospective study that seeks to establish age- and gender-specific reference ranges for whole-body and segmental body composition based on a convenience sample of the Irish population residing within the UL community. The present report provides a reference base for whole body and segmental lean tissue mass established from an analysis of 889 men and women aged 18 to 29y.

AIMS

•To describe whole body and segmental lean tissue mass in healthy young Irish adults aged 18-29 years

• Using criteria derived from the young adult population, to investigate the prevalence of sarcopenia in a convenience sample of older adults, aged 50-76 years.

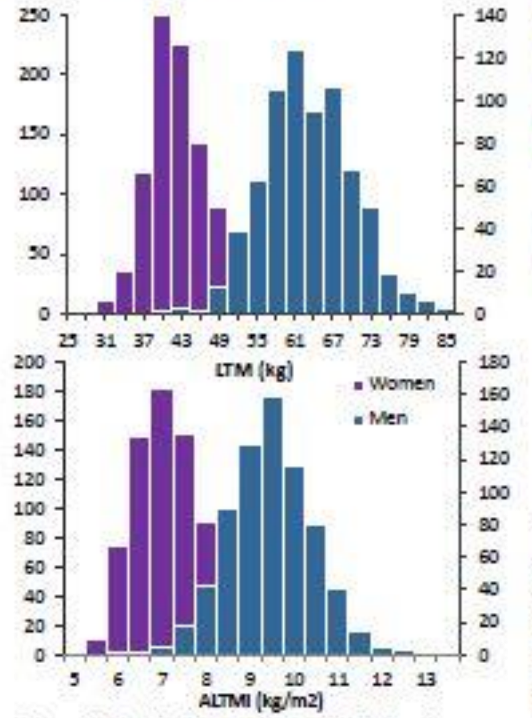

Figure 1. Distribution of LTM and ALTMI and prevalence of sarcopenia in men and women.

METHODS

With ethical approval (ULREC 08/07) and informed, written consent height, weight and body composition was measured by dual energy X-ray absorptiometry (DXA; IDXA™; GE Healthcare, Chalfont St Giles, Bucks., UK) in 497 men and 392 women aged 18-30y and an additional 194 men and 512 women aged 30-76y. Total body lean tissue mass (LTM) was segmented into appendicular (legs + arms) lean tissue mass and expressed relative to stature squared generating an appendicular lean tissue mass index (ALTMI; Table 1). Two criteria for defining sarcopenia were investigated; total body LTM and ALTMI.

Table 1. Descriptive Statistics of young adults [Mean (SD)]

Subjects	Age (y)	Height (m)	Mass (kg)	BMI (kg/m²)	LTM (kg)	ALTM (kg)	ALTMI (kg/m²)
Men (n=497)	22.3 (2.8)	1.80 (0.07)	80.6 (11.7)	24.8 (3.2)	62.4 (6.9)	30.3 (4.0)	9.3 (1.0)
Women (n=392)	22.4 (3.1)	1.67 (0.06)	64.6 (9.5)	23.2 (3.3)	41.6 (4.5)	19.0 (2.4)	6.8 (0.74)

RESULTS

The data revealed predicted sex specific differences in the distribution of total body (LTM) and appendicular lean tissue mass index (ALTMI; Figure 1).

Class I sarcopenia, defined as LTM or ALTMI < 1SD below the young adult mean, corresponded to an **LTM < 56.6kg or ALTMI < 8.3 kg/m² in men;**

an LTM < 37.1kg or ALTMI < 6.1 kg/m² in women.

Class II sarcopenia, defined as LTM or ALTMI > 2SD below the young adult mean, corresponded to an **LTM < 48.8kg or ALTMI < 7.3 kg/m² in men;**

an LTM < 32.6kg or ALTMI < 5.4 kg/m² in women.

Figure 1 and Table 2 demonstrate the prevalence of sarcopenia in older adults according to these criteria.

Table 2. Prevalence of sarcopenia in older adults aged 50-76 years

		Men (n=106)		Women (n=359)	
Criteria		50-60y	60-76y	50-60y	60-76y
LTM	Class I	29%	51%	23%	3%
	Class II	5%	6%	3%	4%
ALTMI	Class I	27%	32%	18%	18%
	Class II	2%	9%	1%	2%

CONCLUSION

The data from the young adult population establish reference criteria for sarcopenia in Irish men and women. These criteria can be applied to the general population to estimate the prevalence of age-related sarcopenia and to monitor the efficacy of strategies for prevention and/or treatment.

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CARDI Conference, Dublin, 3-5th November 2011.

WP8: Muscle Metabolism for the Healthy Ageing

Establishing the Prevalence of Sarcopenia in the Study of Healthy Ageing

Peter Francis[1], Clodagh Toomey[1], Will McCormack[1], Catherine Norton[1], Giuseppe De Vito[2], Phil Jakeman[1].

Food for Health Ireland: [1]University of Limerick, and [2]University College Dublin.

Work programme description

To promote a nutritional solution supported by a physical activity program to preserve lean tissue mass and improve muscle metabolism in the healthy ageing

***Keeping the healthy ageing healthy:* delaying age-related loss of muscle mass and function through diet & exercise**

Introduction

Sarcopenia is used to define the age-related loss in skeletal muscle mass estimated to affect 30% of people older than 60y and >50% of those older than 80y. With a prevalence in the 7th decade of life (60–70y) of 5–13% sarcopenia currently affects >50 million people and will affect >200 million by 2050[1]. Accurate reference data is therefore required to diagnose and investigate the prevalence of sarcopenia and its impact on health and function. In this respect, sarcopenia Class I is defined as loss in muscle mass between –1 *SD* and –2 *SD* and Class II being greater than -2 *SD* below the sex-specific mean of the reference young adult population[2]. Reference criteria from which to determine the prevalence of sarcopenia in the Irish population have yet to be established.

Objectives:

- To analyse data from the UL Body Composition Study[2] to establish reference criteria for total body (LTM) and appendicular lean tissue mass (ALTM) for Irish adult men and women aged 18-29y.
- To evaluate the prevalence of Class I and Class II sarcopenia in the cohort of subjects aged 50+y recruited to the FHI Healthy Ageing project to June 2011.

Expected outcome:

To establish the prevalence of Class I and Class II sarcopenia in the first cohort of subjects recruited to engage in the FHI study of a nutrient solution for healthy ageing.

Methodology

With ethical approval (ULREC 08/07) and informed, written consent height, weight and body composition was measured by dual energy X-ray absorptiometry (DXA; IDXA™; GE Healthcare, Chalfont St Giles, Bucks., UK) on 497 men and 392 women aged 18-30y. Total body lean tissue mass (LTM) was segmented into appendicular (legs + arms) lean tissue mass and expressed relative to stature squared generating an appendicular lean tissue mass index (ALTMI; Table 1). Two criteria for defining sarcopenia were then investigated, total body LTM and ALTMI, in 58 men and 171 women aged 50-70y screened for the FHI programme in healthy ageing.

Table 1. Descriptive Statistics of young adults [Mean (SD)]

Subjects	Age (y)	Height (m)	Mass (kg)	BMI (kg/m^2)	LTM (kg)	ALTM (kg)	ALTMI (kg/m^2)
Men (n=497)	22.3 (2.8)	1.80 (0.07)	80.6 (11.7)	24.8 (3.2)	62.4 (6.9)	30.3 (4.0)	9.3 (1.0)
Women (n=392)	22.4 (3.1)	1.67 (0.06)	64.6 (9.5)	23.2 (3.3)	41.6 (4.5)	19.0 (2.4)	6.8 (0.74)

Results

The data revealed predicted sex specific differences in total body (LTM) and appendicular lean tissue mass index (ALTMI; Figure 1).

Class I sarcopenia, corresponded to:

an LTM < 55.5kg or ALTMI < 8.3 kg/m^2 in men;

an LTM < 37.1kg or ALTMI < 6.1 kg/m^2 in women.

Class II sarcopenia, corresponded to:

an LTM < 48.6kg or ALTMI < 7.3 kg/m^2 in men;

an LTM < 32.6kg or ALTMI < 5.4 kg/m^2 in women.

Figure 1 and Table 2 illustrate the prevalence of sarcopenia in older adults screened under these criteria in the FHI Healthy Ageing Study.

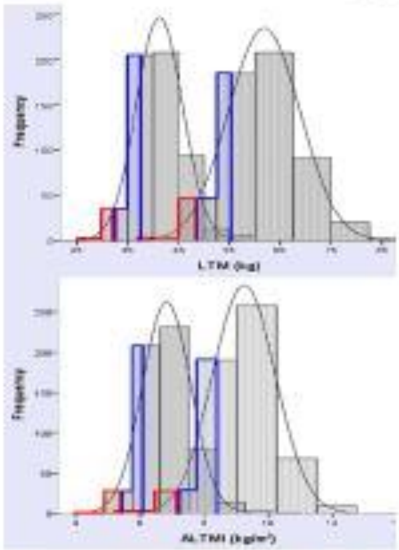

Figure 1. Distribution of LTM and ALTMI and prevalence of sarcopenia in men and women

Table 2. Prevalence of sarcopenia in older adults aged 50-70 years

Criteria		Men 50-60y	Men 60-70y	Women 50-60y	Women 60-70y
LTM	Class I	35%	27%	15%	19%
	Class II	3%	18%	2%	3%
ALTMI	Class I	32%	50%	29%	37%
	Class II	5%	9%	5%	7%

References:

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM *et al.* (2010) *Age and Ageing* 39(4), 412-23. Baumgartner RN, Koehler KM, Gallagher D *et al.* (1998) *Am J Epidemiol* 147(8), 755-763.
2. Faculty of Education and Health Sciences (2009) University of Limerick – Body Composition Study. www.ul.ie/bodycompositionstudy

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REVIEW

Towards a Sustainable Dairy Sector: The underappreciated role of dairy protein in the preservation of lean tissue mass in the elderly

CATHERINE NORTON* and PHILIP JAKEMAN

Faculty of Education & Health Sciences, University of Limerick, Limerick, Ireland

Sarcopenia has been attributed to a diminished postprandial muscle protein synthetic response. Differences in digestion and absorption kinetics of dietary protein and/or its amino acid composition have been suggested to modulate postprandial muscle protein accretion. The amino acid composition of dairy protein, especially whey, has the potential to confer advantages above other nondairy protein sources in prevention of lean tissue mass loss. This may be attributable to its relative high concentration of the essential amino acid leucine.

Keywords Protein, Dairy proteins, Body composition, Muscle mass, Sarcopenia, Elderly.

INTRODUCTION

Ageing is accompanied by a progressive decline in lean tissue mass, termed sarcopenia (Sehl and Yates 2001). With an estimated increase in the older population (>60 years) to exceed two billion by 2050 and a prevalence of sarcopenia of 5–13% within this age group, there will be >200 million sarcopenic adults by the year 2050 (Cruz-Jentoft *et al.* 2010). The causes of sarcopenia are multifactorial (Figure 1) and include compromised nutrition, inactivity, anabolic resistance and inflammation (Millward 2012). This short review focuses on the role of dietary protein intake, specifically dairy protein, in the prevention of age-related sarcopenia.

DIETARY PROTEIN INTAKE AND AGE-RELATED SARCOPENIA

Dietary protein provides amino acids that are necessary for the synthesis of lean tissue mass (LTM). The absolute amount of protein consumed (Volpi *et al.* 2001), the essential amino acid composition of the protein intake (Volpi *et al.* 2003) and the distribution of protein intake in the daily diet (Paddon-Jones and Rasmussen 2009; Symons *et al.* 2009) are implicated in age-related decline in lean tissue mass. Recent data indicate postprandial skeletal muscle protein synthetic response is impaired leading to the term anabolic resistance in the elderly. Dietary proteins from different sources differ in their capacity to stimulate muscle protein synthesis (MPS). This postprandial muscle protein synthetic response depends on the quantity and type of protein that is ingested. For example, whey protein and bovine milk (Wilkinson *et al.* 2007) promote greater increases in MPS than does consumption of an equivalent amount of plant-based soy protein despite the fact that these protein sources have protein digestibility-corrected amino acid scores (PDCAAS) above 1.0. The mechanism responsible for these differences is not entirely clear but may relate to the bioavailability in relation to the kinetics of digestion/absorption and/or amino acid profile. Whey protein is acid soluble and is associated with a rapid, transient increase in postprandial amino acid availability (Power *et al.* 2009) and postprandial muscle protein accretion while casein coagulates and precipitates when exposed to stomach acid resulting in a moderate, but sustained rise in circulating amino acids (Pennings *et al.* 2011). The faster digestion and absorption kinetics of whey are deemed, in part, to account for a greater increase in postprandial plasma amino acid availability and muscle protein synthesis in the elderly (Dangin *et al.* 2002).

The current recommended daily allowance (RDA) for adults of 0.8 g/kg body mass/day was established by the Institute of Medicine

*Author for correspondence. E-mail: catherine.norton@ul.ie

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Figure 1 Causes of sarcopaenia.

(IOM) from short-term nitrogen balance studies in young adults (Trumbo *et al.* 2002; Rand *et al.* 2003). It is, as yet, unclear whether the lower total energy intake and postprandial protein anabolic resistance in the ageing adult require an increase in the daily dietary protein requirement. Several recent studies argue for a moderate increase in daily protein intake of 1.0–1.3 g kg body mass (Campbell *et al.* 2001; Evans 2004; Morais *et al.* 2006; Wolfe *et al.* 2008; Paddon-Jones and Rasmussen 2009), but this has not received universal support. An alternative approach has been to pursue a process of discovery as to the efficacy of 'meal-like' quantities of dietary proteins to stimulate postprandial MPS and effect a change in lean tissue mass in the elderly (Millward 2012; Phillips *et al.* 2005).

THE CASE FOR DAIRY PROTEIN

The greater anabolic effect of milk protein is probably related to the amino acid composition, specifically the high relative composition of essential amino acids (EAAs) compared to other common dietary protein sources (Table 1). Fifteen gram of EAAs would have to be provided from dietary protein for an average of 70 g adult to comply with the IOM recommendation. Because of the high relative composition of EAAs, the IOM RDA for EAA is satisfied by the ingestion of 30 g of milk protein. Other protein sources within the diet require a higher total protein intake (Table 1).

Among the EAAs, branched chain amino acids, and especially leucine, are potent stimulants of muscle protein synthesis. The ability of leucine to influence MPS can be part explained by the fact that leucine acts as a substrate for and regulator of MPS. Leucine's independent role as a regulator of MPS is via the activation of mTOR (mammalian target of rapamycin), an intracellular protein kinase and transcriptional regulator of protein synthesis (Anthony *et al.* 2000; Crozier *et al.* 2005; Atherton *et al.* 2010). It has recently been reported that MPS is directly related to the extracellular concentration of leucine and the suggestion that a postprandial 'leucine threshold' must be surpassed to optimally stimulate MPS (Breen and Phillips 2011). In an elderly population, a higher leucine threshold is required to elicit the same anabolic response (Katsanos *et al.* 2005; and Rieu *et al.* 2006). With increasing age, muscle may become resistant to the stimulatory effects of normal postprandial concentrations of leucine. Because of the high relative leucine composition in dairy protein, greater emphasis on dairy protein in the elderly may partly offset this effect. In older humans, the addition of leucine to a mixed nutrient meal results in a significant increase in muscle protein synthesis (Rieu *et al.* 2006).

HOW HAS THE DAIRY INDUSTRY RESPONDED TO THE CHALLENGE?

The dairy industry in Ireland work collaboratively with University and state funded research centres under the aegis of the Food for Health Ireland (FHI) innovative research

Table 1 Institute of Medicine (IOM) guideline recommended daily intake of essential amino acids (EAA) and the relative EAA composition of selected dietary proteins. Data for average 70 kg body mass.

	IOM		*Milk Whey*			*Milk Casein*			*Wheat Grain*			*SOY Protein*		
EAA	*mg/kg/d*	*g/d*	*g/100*	*g*	*IOM%*	*g/100*	*g*	*IOM%*	*g/100*	*g*	*IOM%*	*g/100*	*g*	*IOM%*
His	14	0.98	2.1	0.62	63	3.1	0.99	101	2.3	1.04	107	2.8	1.05	107
ISO	19	1.33	7.2	2.12	160	4.9	1.57	118	3.7	1.68	126	4.4	1.65	124
Leu	42	2.94	11.2	3.30	112	9.9	3.16	108	6.8	3.09	105	6.2	2.32	79
Lys	38	2.66	8.8	2.59	98	7.9	2.52	95	2.8	1.27	48	6.2	2.32	87
Meth+Cyst	19	1.33	2.2	0.65	49	3.1	0.99	74	3.5	1.59	119	2	0.75	56
Phe+Tyr	33	2.31	3.1	0.91	40	5.9	1.88	82	6.4	2.90	126	8.8	3.30	143
Thre	20	1.4	8	2.36	169	3.8	1.21	87	2.9	1.32	94	3.2	1.20	86
Try	5	0.35	1.9	0.56	160	1.5	0.48	137	0.21	0.10	27	1	0.37	107
Val	24	1.68	6.3	1.86	111	6.8	2.17	129	4.4	2.00	119	5.4	2.02	120
Total	214	14.98	50.8	14.98	107	46.9	14.98	105	33.01	14.98	97	40	14.98	101
Protein (g)				29.49			31.94			45.38			37.45	

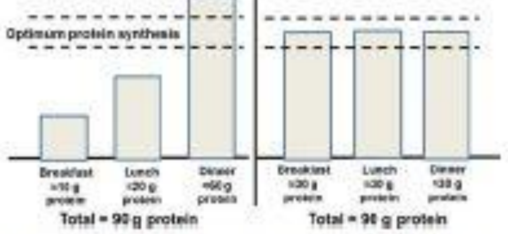

Figure 2 Re-distribution of dietary protein intake throughout the day may improve MPS independent of an increase in absolute protein intake.

programme to 'mine' for bioactive properties within milk-related proteins, peptides and derivatives (further information at www.fhi.ie). An FHI work-package in healthy ageing is currently investigating the role of protein intake and protein quality as a nutritional strategy to offset the age-related decline in lean tissue mass. A supplement containing selected milk-based proteins, peptides and their derivatives with enhanced bioactive properties to stimulate postprandial muscle protein synthesis, assessed *in vitro* and *in vivo*, is currently under evaluation in healthy men and women aged 50–75 years. The nutrient support supplies milk protein amounting to 0.33/kg BM and is supplemented with vitamin D and dairy calcium. Participants take the supplement in two doses with their two smaller meals in the day to optimise the protein synthetic effect in Figure 2 (Paddon-Jones and Rasmussen 2009). Outcome measures include change in lean tissue mass, muscle strength (isokinetic dynamometry) and function (activities of daily living).

CONCLUSIONS

The absolute amount of protein required by the elderly to offset age-related lean tissue mass loss may not need to change. However, current patterns of consumption should be addressed to optimise muscle protein synthesis. The emerging research on the quality of the protein consumed, in terms of EAA composition, places emphasis on the role of dairy protein in the prevention of sarcopaenia.

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A method to estimate dietary total antioxidant capacity among elderly Irish women

C. Norton and P. Jakeman

Human Science Research Unit, Faculty of Education & Health Sciences, University of Limerick, Limerick, Republic of Ireland

Antioxidant intake is potentially protective against cell oxidative damage and related metabolic complications yet little is known of the dietary total antioxidant capacity (TAC) that might inform an RDA.

The aim of this study was to establish a WISP©(Tinuviel Software, Anglesey, UK) -based method of estimating dietary TAC founded on the USDA Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods[1] and to use this method to estimate the total dietary antioxidant capacity of a sample of elderly Irish women.

The descriptors of 326 foods listed in the USDA database were matched with foods in the WISP© database and their respective scores for total (T), hydrophilic (H) and lipophilic (L)-ORAC score (mmol Trolox equivalent/100 g; mmolTE), and the associated total phenolics score (mg Gallic acid equivalents/100 g; mgGAE) were assigned to 475 foods.

Using an estimated food intake record (eFIR) 75 healthy, elderly women provided 4-day food and drink consumption data that were analysed by WISP© (Tinuviel Software, Anglesey, UK). Following exclusion of invalid eFIRs, 53 records (mean (SD); age 61.1(5.3) y; BMI 24.9(4.6) kg/m^2; BMR 1287(132) kcal/d) were analysed further.

Analysis of the nutrient intake showed mean TAC of 9.3(5.1) mmol TE (Table 1). TAC was further divided into its constituent hydrophilic 97(2)% and lipophilic 3(2)% components. The food groups contributing most to TAC were vegetables (23%), fruit / fruit juices (21%) and other beverages (21 %) with a lesser contribution from alcohol (12%) and confectionary (7 %). By comparison, estimates of TAC, based upon USDA's Continuing Survey of Food Intake by Individuals (1994–1996) or more recently intake from food consumption data of NHANES 2001–02, was 5.6 mmol TE and 4.7 mmol TE[2].

Table 1.

		Mean	Median	SD	5th percentile	95th percentile
T-ORAC	(mmol TE)	9.3	9.2	5.1	2.3	15.8
H-ORAC	(mmol TE)	7.2	6.9	4.1	2.3	13.2
L-ORAC	(mmol TE)	0.2	0.1	0.2	0	0.7
Total Phenolics	(mg GAE)	2310	1331	2414	99	7067
% H of T-ORAC	%	97 %	98 %	2%	93%	99%
% L of T-ORAC	%	3%	2 %	2%	1%	7%

Oxidative stress is a key issue in many of the diseases of aging e.g. sarcopaenia, even if it is not a causative factor. There is continued growing interest in dietary TAC and *in vivo* antioxidant status and effects on health outcomes. Dietary TAC may be a potential marker of diet intake quality, providing a novel approach to assess the role of antioxidant intake on health, particularly among the elderly.

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