Multidisciplinary research into the effects of resistance exercise and whey protein supplementation in healthy older men

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By

Corbin Griffen

PhD

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Certificate of Ethical Approval

Applicant:

Corbin Griffen

Project Title:

Effects of 12 weeks resistance training and whey protein supplementation on multiple indices of sarcopenia in older men

This is to certify that the above named applicant has completed the Coventry University Ethical Approval process and their project has been confirmed and approved as Medium Risk

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PhD Declaration

The work submitted within this thesis has been undertaken during the period of my registration. I declare that this work is my own, conducted by myself with assistance where acknowledged.

Abstract

Introduction: Ageing is associated with declines in skeletal muscle mass, strength and physical function, reductions in components of energy expenditure (EE), and deterioration of metabolic and cognitive function. Two interventions that may mitigate these adverse health outcomes are resistance exercise (RE) and increased dietary protein intake. However, further work is required to determine the synergistic effects compared to each intervention alone. Broadly, the aim of this thesis was to examine the individual and combined effects of RE and whey protein supplementation on components of 24-h energy metabolism, parameters of sarcopenia, and metabolic and cognitive function in healthy older men. Additionally, this thesis aimed to examine potential mechanisms of action.

General methods: Thirty-six healthy older men [(mean ± standard error (SE)) age: 66.9 ± 0.7 y; body mass index (BMI) 25.5 ± 0.4 kg/m^2] participated in a 12-week, 4-arm, double-blind, randomised controlled trial (RCT). Participants were randomised to either control (CON, *n* = 9), whey protein supplementation (PRO, *n* = 9), RE + control (EX+CON, *n* = 9), or RE + whey protein supplementation (EX+PRO, *n* = 9). Resistance exercise was performed twice weekly by participants in the EX+CON and EX+PRO groups. Supplements (PRO, 25 g whey protein isolate; CON, 23.75 g maltodextrin) were consumed twice daily. For the primary analysis, the four intervention groups were compared. Exploratory analyses were also conducted between pooled exercise (EX+CON + EX+PRO, *n* = 18) and non-exercise groups (CON + PRO, *n* = 18), and between pooled whey protein (PRO + EX+PRO, *n* = 18) and control supplement groups (CON + EX+CON, *n* = 18). Three individual study chapters were derived from the 12-week RCT.

Study specific methods and results:

Study 1: Investigated the individual and combined effects of RE and whey protein supplementation on 24-h EE, substrate oxidation, metabolic flexibility, subjective appetite and glucose homeostasis. Participants (*n* = 33) resided in respiration chambers for 24 h pre- and post-intervention. Resistance exercise significantly increased fat-free mass (FFM), resting metabolic rate (RMR), sedentary EE, and 24-h metabolic flexibility compared to non-exercise.

Additionally, RE also elicited within-group increases in insulin sensitivity and subjective hunger, and within-group decreases in the energy cost of step exercise and spontaneous activity. Whey protein supplementation aided maintenance of body mass and reduced FM compared to control supplement groups pooled. Within-group decreases in the energy cost of both spontaneous activity and step exercise in the fasted state were also observed following whey protein supplementation. No negative effects of whey protein supplementation were observed for either total protein or energy intake (EI), or 24-h subjective appetite. However, whey protein supplementation did result in an increase in overnight protein oxidation, resulting in a reduced 24-h protein balance. Consequently, this may be a caveat to longitudinal high protein diets in the elderly. Combined RE and whey protein supplementation did not significantly augment changes in body composition, 24-h EE, substrate oxidation or metabolic flexibility, or glucose homeostasis compared to either RE or whey protein supplementation alone.

Study 2: Examined the individual and combined effects of RE and whey protein supplementation on skeletal muscle/FFM, muscle strength, physical function, and hormonal and inflammatory biomarkers related to sarcopenia. Resistance exercise significantly increased FFM, muscle strength and physical function, and decreased markers of systemic inflammation compared to non-exercise. Whey protein supplementation increased physical function (4 m gait speed) and muscle strength [leg press one repetition maximum (1RM)]. No synergistic effects occurred for any parameter of sarcopenia compared to RE or whey protein supplementation alone. Furthermore, changes in hormonal and inflammatory markers did not correlate with changes in skeletal muscle mass, strength, or physical function. Diurnal salivary cortisol secretion did, however, significantly correlate with multiple parameters of sarcopenia at baseline.

Study 3: Tested the individual and combined effects of RE and whey protein supplementation on cognitive function and explored mechanisms of action. At baseline, parameters of sarcopenia (muscle strength and physical performance) positively correlated with several domains of cognitive function. Following the intervention, RE and whey protein

supplementation alone and combined did not elicit any significant benefits compared to control. However, whey protein supplementation *per se* elicited within-group improvements in global cognitive function, working memory and executive function. When whey protein supplement groups were pooled, there was also a trend towards a greater increase in global cognitive function compared to control supplement groups pooled. Resistance exercise alone elicited within-group improvements in multitasking efficiency but worsened processing speed. No significant additive effects of combined intervention were observed compared to either RE or whey protein supplementation alone. Moreover, changes in cognitive function were not correlated with changes in any neurobiological, inflammatory or insulin sensitivity marker, nor blood pressure or salivary cortisol indices. In contrast, an association was observed between changes in SMI and episodic memory.

Conclusions: Overall, this body of work supports the use of RE as the primary intervention to mitigate age-related declines in EE and parameters of sarcopenia. Increased dietary protein intake via whey protein supplementation may also be recommended as a safe method for older adults to mitigate age-related declines in muscle strength, physical function and sarcopenic obesity, and aid aspects of cognitive functioning. Finally, data presented in this thesis does not support the need for combined RE and whey protein supplementation to curb sarcopenia or age-related declines in metabolic or cognitive function.

Key words: ageing, cognitive function, energy expenditure, metabolic function, resistance exercise, sarcopenia, sarcopenic obesity, whey protein supplementation

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Presentation of results

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Published work whilst conducting this thesis

Dallaway, A., Kite, C., **Griffen, C**., Duncan, M., Tallis, J., Renshaw, D. and Hattersley, J. (2020) 'Age-related degeneration of the lumbar paravertebral muscles: Systematic review and three-level meta-regression'. *Experimental Gerontology* 133, 110856. **Data not presented in this thesis.**

Porumb, M., **Griffen, C**., Hattersley, J. and Pecchia, L. (2020) 'Nocturnal low glucose detection in healthy elderly from one-lead ECG using convolutional denoising autoencoders'. *Biomedical Signal Processing and Control* 62, 102054. **Baseline glucose data from this thesis was used in this paper, but study results are not presented in this thesis.**

Table of Contents

List of Tables

List of Figures

Abbreviations

1RM, one repetition maximum
6MWT, 6-minute walk test
AA, amino acid(s)
ACE, angiotensin converting enzyme
ACSM, American College of Sports Medicine
ADL, activities of daily living
AE, aerobic exercise
AEE, activity energy expenditure
ANCOVA, analysis of covariance
ANOVA, analysis of variance
AS160, Akt substrate of 160 kDa
ASMM, appendicular skeletal muscle mass
ATP, adenosine-5'-triphosphate
AUC, area under the curve
BCAA**,** branched-chain amino acid(s)
BDNF, brain-derived neurotrophic factor
BIA, bioelectrical impedance analysis
BMI, body mass index
BNP, brain natriuretic peptide
BP, blood pressure
CANTAB, Cambridge Neuropsychological Test Automated Battery
CI, confidence interval
CKD, chronic kidney disease
CNS, central nervous system
CON, control group
CRP, C-reactive protein
CT, computed tomography
D3-creatine, deuterated creatine
DIT, diet-induced thermogenesis
DLW, doubly labelled water
DMS, delayed matching to sample
DXA, dual x-ray absorptiometry
EAA, essential amino acid(s)
EDTA, ethylenediaminetetraacetic acid
EE, energy expenditure

eGFR, estimated glomerular filtration rate
EI, energy intake
EIM, electrical impedance myography
ELISA, enzyme-linked immunosorbent assay
ES, energy storage
ESCEO, European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis
EWGSOP, European Working Group of Sarcopenia in Older People
EWGSOP2, European Working Group of Sarcopenia in Older People 2
EX+CON, resistance exercise combined with control group
EX+PRO, resistance exercise combined with whey protein group
FFM, fat-free mass
FFQ, food frequency questionnaire
FM, fat mass
FMI, fat mass index
FSR, fractional synthetic rate
GCP, good clinical practice
GFR, glomerular filtration rate
GH, growth hormone
GLP-1, glucagon-like peptide-1
HbA1c, glycated haemoglobin
HMRU, Human Metabolism Research Unit
HOMA-beta, homeostatic model assessment of beta-cell function
HOMA-IR, homeostatic model assessment of insulin resistance
HPA, hypothalamic pituitary adrenal
HRT, hormone replacement therapy
ICC, intraclass correlation coefficient
IGF-1, insulin-like growth factor 1
IL-6, interleukin-6
IL-10, interleukin-10
MCI, mild cognitive impairment
MET, metabolic equivalent
MMSE, mini-mental state examination
MOT, motor screening task
MPB, muscle protein breakdown
MPS, muscle protein synthesis
MRI, magnetic resonance imaging

mRNA, messenger RNA
MSSE, mini-mental state examination
mTOR, mammalian target of rapamycin
mTORC1, mammalian target of rapamycin complex 1
MTT, multitasking test
MVPA, moderate-vigorous physical activity
n-3 PUFA, omega 3 polyunsaturated fatty acids
NAA, neutron activation analysis
NF-κB, nuclear-factor kappa-light-chain-enhancer of activated B cells
npRQ, non-protein respiratory quotient
NSCA, National Strength and Conditioning Association
OGTT, oral glucose tolerance test
p70S6K, ribosomal protein S6 kinase
PAL, physical activity level
PALT, paired associates learning test
PDH, pyruvate dehydrogenase
PIS, participant information sheet
PRO, whey protein group
PYY, peptide YY
QUCKI, quantitative insulin sensitivity index
QOL, quality of life
RCT, randomised controlled trial
RDA, recommended dietary allowance
RE, resistance exercise
RER, respiratory exchange ratio
RMR, resting metabolic rate
RTI, reaction time
RQ, respiratory quotient
S6K1, S6 kinase 1
SE, standard error
SMD, standardised mean difference
SMI, skeletal muscle index
SMM, skeletal muscle mass
SMR, sleeping metabolic rate
SPPB, short physical performance battery
SST, serum separator tube
SWM, spatial working memory

T2DM, type 2 diabetes mellitus

TEE, total energy expenditure

TGF-β, transforming growth factor β

TNF-α, tumor necrosis factor alpha

UHCW, University Hospitals Coventry and Warwickshire NHS Trust

VAS, visual analogue scale

CHAPTER 1: Introduction

1.1 Rationale

Globally, the population is ageing, with demographic projections indicating individuals aged >60 years will represent 21.3% of the human population by 2050 (Office for National Statistics 2018). Of note, ageing is associated with progressive declines in skeletal muscle mass (SMM), strength and physical function, a phenomenon termed sarcopenia (Cruz-Jentoft et al. 2019). In terms of human health, sarcopenia is associated with an increased risk of falls, hospitalisation, physical disability and poor quality of life (Beaudart et al. 2014). Sarcopenia is also linked with reductions in components of energy expenditure (EE) (Manini 2010, Soysal et al. 2019), which could lead to increased fat mass (FM), sarcopenic obesity and metabolic disease (Wannamethee and Atkins 2015, Westerterp 2018a). Additionally, declines in cognitive function, which may progress to mild cognitive impairment (MCI) and dementia (most commonly Alzheimer's disease) (Alzheimer's Society 2014), has likewise been associated with sarcopenic muscle loss (Chang et al. 2016, Cipolli, Yassuda, and Aprahamian 2019, Peng et al. 2020). As a longer life brings many opportunities at both an individual and societal level, there is a critical need to identify strategies to maintain muscle function and overall health in older adults (Witard et al. 2016).

Resistance exercise (RE) is an effective countermeasure to age-related chronic disease (Mcleod, Stokes, and Phillips 2019). In older adults, meta-analyses have reported increases in fat-free mass (FFM) (Buch et al. 2017, Csapo and Alegre 2016, Peterson, Sen, and Gordon 2011, Yoshimura et al. 2017), muscle strength (Buch et al. 2017, Csapo and Alegre 2016, Martins et al. 2013, Peterson et al. 2010, Yoshimura et al. 2017), and physical function following RE training (Yoshimura et al. 2017). Metabolically, increases in resting metabolic rate (RMR) and 24-h fat oxidation (Hunter, McCarthy, and Bamman 2004, Treuth et al. 1995), reductions in the energetic cost of performing activities of daily living (ADL) (Valenti, Bonomi, and Westerterp 2016), improvements in skeletal muscle glucose metabolism, metabolic flexibility and glucose homeostasis (Bell et al. 2017, Bucci et al. 2016, Consitt, Dudley, and Saxena 2019, Consitt et al. 2016, Flack et al. 2011, Holwerda et al. 2018, Iglay et al. 2007,

Leenders et al. 2013, Miller et al. 1994), and reductions in FM have also been reported (Westcott 2012). On a cognitive level, prior work has demonstrated favourable effects of RE training on executive functioning (Anderson-Hanley, Nimon, and Westen 2010, Best, Nagamatsu, and Liu-Ambrose 2014, Ikudome et al. 2017, Liu-Ambrose et al. 2010, Yoon, Lee, and Song 2018), memory (Best et al. 2015, Cassilhas et al. 2007, Coelho-Júnior et al. 2020, Ikudome et al. 2017, Marston et al. 2019a), and global cognitive function (Coelho-Júnior et al. 2020, Smolarek et al. 2016, Singh et al. 2014).

In addition to RE, increased dietary protein intake may also attenuate sarcopenia and aid body weight management (Drummen et al. 2018, Phillips and Martinson 2019). Meta-analyses have reported reductions in FM (Clifton, Condo, and Keogh 2014, Kim et al. 2016b), and several intervention studies have reported increases in FFM in older adults consuming higher intakes of dietary protein (Bauer et al. 2015, Bell et al. 2017, Bo et al. 2019, Kang et al. 2020, Mitchell et al. 2017, Negro et al. 2019, Norton et al. 2016, ten Haaf et al. 2019)**.** Furthermore, some studies also report improvements in muscle function (Bo et al. 2019, Mitchell et al. 2017, Negro et al. 2019); however, these improvements have not been observed by all (Björkman et al. 2020, Cramer et al. 2016, de Carvalho Bastone et al. 2020, Kim et al. 2012, Kirk et al. 2020, Kukuljan et al. 2009, Verreijen et al. 2017, Zhu et al. 2015). Discrepant findings may be explained by differences in participant health status (i.e., healthy vs. sarcopenic/functionally impaired), habitual protein intake [i.e., inadequate (<1 g/kg/d) vs. adequate (>1 g/kg/d)], and the magnitude of protein deviation from baseline (i.e., ≥0.4 vs. <0.4 g/kg/d).

Regarding the effects on EE, studies in young adults have reported increases in sleeping metabolic rate (SMR) and diet-induced thermogenesis (DIT) (Bray et al. 2015, Martens et al. 2015a, Sutton et al. 2016), RMR (Bray et al. 2012), and total energy expenditure (TEE) (Bray et al. 2012, 2015) following chronic consumption of a high protein diet [25-30% of energy intake (EI)]. However, the effects in older adults is contradictory, with one study reporting increases in RMR and a consequent negative energy balance (Drummen et al. 2018), whilst

others have not reported such effects (Luger et al. 2013, Negro et al. 2019). Disparities might be explained by an insufficient deviation of dietary protein from baseline, as highlighted above. In addition to EE, studies in both young (Bray et al. 2015, Robinson et al. 1990, Pannemans et al. 1995, Martens et al. 2015b), and middle- and older-aged adults (Campbell et al. 1995, Drummen et al. 2020, Hays et al. 2009, Pannemans, Halliday, and Westerterp 1995, Pannemans et al. 1998) have observed increases in 24-h protein oxidation and improved protein balance. Two of these studies also demonstrated decreases in 24-h respiratory quotient (RQ), indicative of increased fat oxidation (Drummen et al. 2020, Martens et al. 2015a).

The effects of dietary protein on glucose homeostasis is controversial with some studies reporting improvements (Acheson et al. 2011, Amirani et al. 2020, Luger et al. 2013, Pal, Ellis, and Dhaliwal 2010, Promintzer and Krebs 2006), whilst others have reported either no changes (Leenders et al. 2011) or an increase in whole-body insulin resistance (Linn et al. 2000, Hattersley et al. 2014, Weickert et al. 2011). Contradictory findings may be explained by differences in the source of dietary protein (Drummen et al. 2018) and protein-induced changes in FM and systemic inflammation (Weickert 2012). Regrettably, data on the effects of dietary protein on metabolic flexibility is limited to one study that reported no effects of increased dairy consumption (Eelderink et al. 2019); however, these findings could have occurred due to an insufficient intervention period.

Alongside increased muscle function and improved body weight management, dietary protein may also assist in curbing age-related declines in cognitive function (van de Rest, van der Zwaluw, and De Groot 2013). Intervention studies of 12-24 weeks in duration have reported protein-induced improvements in reaction time (van der Zwaluw et al. 2014), memory (Charlton et al. 2016) and emotion identification (Charlton et al. 2016, Crichton et al. 2012b, Jakobsen et al. 2011, Lefferts et al. 2020, van der Zwaluw et al. 2014). Conversely, others have not reported improvements, possibly due to an insufficient duration of intervention (Bell

et al. 2019, Zajac et al. 2019) and an inadequate dose of protein supplemented (Moran et al. 2018).

Based on the totality of aforementioned literature, it may be hypothesised that RE combined with dietary protein could synergistically aid attenuation of sarcopenia and sarcopenic obesity, improve metabolic health, and enhance cognitive function in older adults. Indeed, meta-analyses (Cermak et al. 2012, Finger et al. 2015, Liao et al. 2017, Morton et al. 2018a) and randomised controlled trials (RCTs) (Bell et al. 2017, Daly et al. 2014, Junior et al. 2018, Rondanelli et al. 2016, 2020, Tieland et al. 2012b, Verreijen et al. 2015, Yamada et al. 2019, Zdzieblik et al. 2015) have reported augmented effects of dietary protein on the adaptive skeletal muscle response to RE. Additionally, data from a recent meta-analysis suggests RE combined with protein supplementation might also elicit greater decreases in absolute and %FM compared to RE alone (Liao et al. 2017), whilst RCTs have likewise reported synergistic effects on glucose homeostasis (Bell et al. 2017) and cognitive function (Bell et al. 2019, Rondanelli et al. 2020).

However, in contrast to the above, opposing findings have also been reported. For example, several studies have reported no synergistic effects on FFM or muscle function (Arnarson et al. 2013, Candow et al. 2012, Chalé et al. 2013, de Carvalho Bastone et al. 2020, Dulac et al. 2020, Englund et al. 2018, Fielding et al. 2017, Gryson et al. 2014, Hofmann et al. 2016, Holm et al. 2008, Holwerda et al. 2018, Kim et al. 2012, Kirk et al. 2019, 2020, Kukuljan et al. 2009, Leenders et al. 2013, Oesen et al. 2015, Shahar et al. 2013, Thomson et al. 2016, Verreijen et al. 2017), components of EE (Amamou et al. 2017, Campbell et al. 1994, Maltais et al. 2016, Weinheimer et al. 2012), glucose homeostasis (Holwerda et al. 2018, Iglay et al. 2007, Leenders et al. 2013, Maltais et al. 2016, Weinheimer et al. 2012, Verdijk et al. 2009a), or cognitive function (Formica et al. 2020, van de Rest et al. 2014). Similar to that previously mentioned, inconsistent findings may be explained by the population studied, habitual protein intake of participants, and characteristics of the protein intervention.

Whilst numerous studies have investigated the combined effects of RE and dietary protein on multiple aspects of health in older adults, several recurrent methodological limitations warrant further discussion. Firstly, the majority of the aforementioned studies employed a daily protein intake which was less than the required dose (1.6 g/kg/d) reported to maximally accrete skeletal muscle during RE training (Morton et al. 2018a). Secondly, the bulk of studies (31/39 previously cited) failed to include a protein only group. As not older adults are willing or able to perform RE (Dismore et al. 2020), collection of such data is vital to determine the effectiveness of this nutrient compared to both RE alone, and combined with dietary protein. Lastly, only two (Gryson et al. 2014, van de Rest et al. 2014) of the eight studies that investigated the synergistic effects of RE and dietary protein compared to each intervention alone supplemented participants in control groups with either a placebo or energy-matched control supplement. Consequently, the remainder of these studies are at a risk of experimental bias. For this reason, an expert working group has recommended the double-blind RCT as the mainstay of research design for trials investigating the effectiveness of interventions to treat or prevent sarcopenia (Reginster et al. 2016). Based on the abovementioned limitations, a 4-arm (control, protein, RE + control, RE + protein), double-blind RCT is warranted to robustly investigate the effects of RE and dietary protein (at a daily intake of 1.6 g/kg/d) in older adults.

Further to the methodological limitations highlighted above, several unanswered questions also remain regarding the combined effects of RE and dietary protein. For instance, several biochemical pathways are involved in the pathogenesis of sarcopenia, including chronic systemic inflammation [e.g., increases in interleukin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α)] and changes in hormonal function [e.g., reductions in insulin-like growth factor 1 (IGF-1), flattened diurnal cortisol secretion, and increases in myostatin]. Additionally, systemic inflammation, declines in growth and neurotrophic factors [e.g., IGF-1 and brain-derived neurotrophic factor (BDNF)], dysregulation of the hypothalamic pituitary adrenal (HPA) axis, insulin resistance and hypertension are all purported to contribute

to cognitive decline with age (De Felice, Lourenco, and Ferreira 2014, Maass et al. 2016, Macaulay, Fisher, and Schroeder 2020, Walker, Power, and Gottesman 2017). However, the effects of RE and dietary protein on these biomarkers compared to each intervention alone, and the relationship with changes in muscle and cognitive function is relatively unknown. Collection of such data is vital to further understand the mechanisms of how these interventions may synergistically attenuate sarcopenia and cognitive decline.

Moreover, prior studies investigating the combined effects of RE and dietary protein on energy metabolism only captured EE in the resting state (i.e., RMR) (Amamou et al. 2017, Campbell et al. 1994, Maltais et al. 2016, Weinheimer et al. 2012). Twenty-four-hour EE is not constant, and is regulated by numerous factors such as time of day and food intake (Schoffelen and Plasqui 2018); therefore, analysis of solely RMR does not provide a comprehensive analysis of the effects of these interventions on 24-h energy metabolism. Hence, analysis of multiple components of 24-h EE [i.e., RMR, SMR, activity energy expenditure (AEE), DIT and TEE] is warranted. Finally, no study has investigated the synergistic effects on 24-h metabolic flexibility or appetite compared to each intervention alone in older adults. The addition of this data may have important clinical implications for attenuating metabolic dysfunction and aiding energy balance in older adults.

1.2 Aims of the thesis

This thesis aimed to investigate the individual and combined effects of twice weekly RE and twice daily whey protein supplementation (25 g/serving, including 3 g leucine/serving) for 12 weeks in healthy older men. Specifically, this thesis was designed to examine the individual and combined effects on body composition, 24-h energy metabolism, subjective appetite, metabolic flexibility, glucose homeostasis, and muscle and cognitive function. Secondary aims were to explore mechanisms of action relating to sarcopenia and cognitive function. Accordingly, this thesis is comprised of three research studies which contribute novel data

and provide insight into the effects of RE and dietary protein on the abovementioned outcomes.

Study 1 (Chapter 4): Investigated the individual and combined effects of RE and whey protein supplementation on 24-h EE, substrate oxidation and metabolic flexibility, body composition, subjective appetite, and glucose homeostasis.

Study 2 (Chapter 5): Examined the individual and combined effects of RE and whey protein supplementation on skeletal muscle mass, strength, physical function, and hormonal and inflammatory biomarkers related to sarcopenia.

Study 3 (Chapter 6): Tested the individual and combined effects of RE and whey protein supplementation on cognitive function and explored mechanisms of action.

CHAPTER 2: Literature review

2.1 Chapter overview

This literature review is partitioned into three parts. Firstly, age-related declines in SMM, strength and physical function; secondly, changes in body composition, reductions in EE and metabolic dysfunction with age; and finally, age-related declines in cognitive function. Subsequently, the individual and combined effects of RE and dietary protein on these outcomes will be reviewed.

2.1.1 Introduction

The world's population is ageing. By 2050, demographics indicate the number of individuals aged >60 years will increase 2-fold from 900 million reported in 2015, to >2 billion (World Health Organisation 2018). Population ageing – the increasing percentage of elderly individuals within a population, is developing into a global phenomenon and is poised to become one of the most significant social transformations of recent times (World Health Organisation 2018). The considerable demographic shift towards a more elderly population has been driven by an increase in longevity (Office for National Statistics 2018); however, this has not been accompanied by a concomitant reduction in morbidity (Seals, Justice, and LaRocca 2016). Therefore, whilst population ageing is a success of mankind, older adults today are living with greater burden of chronic disease than their ancestors (World Health Organisation 2018). The consequences of population ageing coupled with an increase in morbidity are far-reaching, and incorporate significant societal and healthcare economic burdens (Tolea and Galvin 2015).

2.2 Part 1: Sarcopenia

2.2.1 Skeletal muscle

Skeletal muscle is the largest organ in the human body, accounting for ~45% of FFM (Geisler and Müller 2017). From a mechanical point of view, the main function of skeletal muscle is to exert force, and it does so by attempting to shorten (Winter and Fowler 2009). Metabolically,

skeletal muscle contributes to basal energy metabolism, serves as a reservoir of amino acid (AA) storage, and aids maintenance of glycaemic control (Evans 1995, Wolfe 2006). Furthermore, from a structural perspective, it is important to note that all myofibers are not created equal. Different fibre types are grouped according to the predominant isoform of the myosin heavy chain that they express (Welch, Hayhoe, and Cameron 2020). These include either type I (slow-twitch oxidative), type IIa (fast-twitch oxidative-glycolytic fibres), or type II x (fast-twitch glycolytic) (Welch, Hayhoe, and Cameron 2020).

2.2.2 Origin of sarcopenia

Advancing age is accompanied by a progressive decline in SMM. In 1931, British neurologist Dr MacDonald Critchley wrote that ageing was coupled with a progressive loss of SMM, most noticeably in the hands and feet (Critchley 1931). Several years later in 1989, Dr Irwin Rosenberg stated, “no decline with age is more dramatic, or potentially more functionally significant, than the decline in muscle mass” (Rosenberg 1989). Rosenberg coined the loss of SMM ‘sarcopenia’, derived from the Greek words ‘*sarx*’ and ‘*penia*’, which translates in English to ‘*loss or poverty of flesh*’ (Rosenberg 1989).

2.2.3 Definition and diagnosis

Following its inception by Rosenberg in 1989, defining and classifying a diagnosis of sarcopenia has been at the forefront of research to aid clinical needs. In 2010, The European Working Group of Sarcopenia in Older People (EWGSOP) defined sarcopenia as *a syndrome characterized by progressive and generalised loss of skeletal muscle mass and strength with a risk of adverse outcomes, such as physical disability, poor quality of life, and death* (Cruz-Jentoft et al. 2010). Sarcopenia can be classified as either ‘primary’ (or age-related) or ‘secondary’ when developed depending on other factors such as chronic disease (Cruz-Jentoft et al. 2010). In 2019, based on research demonstrating a stronger relationship between loss of muscle strength and adverse health outcomes as opposed to declines in SMM, the EWGSOP published a revised criteria of sarcopenia (EWGSOP2), highlighting muscle

strength as the primary parameter of sarcopenia (Cruz-Jentoft et al. 2019). For diagnosis of sarcopenia, the EWGOSP2 recommends firstly screening individuals using the SARC-F questionnaire. If sarcopenia is likely, muscle strength, mass and physical function should be measured to confirm diagnosis using the following cut-offs:

- **Low muscle strength**: grip strength <27 kg for men and <16 kg for women, or chair stand >15 s for five raises
- **Low muscle quantity**: appendicular skeletal muscle mass (ASMM) <20 kg for men and <15 kg for women, or ASM/height^2 <7.0 kg/m^2 for men and <5.5 kg/m^2 for women
- **Low physical performance**: either gait speed ≤0.8 m/s, short physical performance battery (SPPB) score ≤8, timed-up and go (TUG) score of ≥20 s, or ≥6 min to complete a 400 m walk.

These cut-offs are also supported by the Australian and New Zealand Society for Sarcopenia and Frailty Research (ANZSSFR) (Zanker et al. 2019). Additionally, alternative definitions of sarcopenia have been proposed by the Asian Working Group for Sarcopenia (Chen et al. 2020) and the International Working Group on Sarcopenia (Fielding et al. 2011). Importantly, although these definitions are different, whereby utilising different criteria and cut-offs, there is agreement in that sarcopenia is defined as a loss of SMM, strength and physical function by all.

2.2.4 Age-related declines in skeletal muscle mass and strength

Cross-sectional studies using bioelectrical impedance analysis (BIA) (Janssen et al. 2000b), dual x-ray absorptiometry (DXA) (Gallagher and Heymsfield 1998, Lynch et al. 1999) and computed tomography (CT) (Borkan et al. 1983, Overend et al. 1992) have demonstrated SMM is relatively well preserved until the fifth decade of life, with notable decreases observed after ~45 years. Longitudinal data has also revealed declines in SMM occur at a rate of ~6% per decade (~0.5-1% per annum) after mid-life (Janssen and Ross 2005), which arises at a

greater rate in men (Delmonico et al. 2009, Goodpaster et al. 2006) and in muscles of the lower extremities (Janssen and Ross 2005). The latter of these findings is of clinical significance, as the lower limbs possess muscles involved in performing ADL (Janssen and Ross 2005).

It is now well-recognised that age-related declines in SMM and strength are not linear, with the decline in muscle strength occurring 2-5 times faster (Manini and Clark 2012). Histologically, these findings may be partly explained by the predominant loss of type II muscle fibres with ageing (Nilwik et al. 2013). Further, age-related declines in muscle strength have been reported to occur earlier than declines in SMM, arising as early as the 4th decade of life (Manini and Clark 2012). However, prominent declines are typically not observed until around ~50 years of age, with more rapid decreases observed after 65 years (Beenakker et al. 2010). Longitudinal studies in older adults, as reviewed by Manini and Clark (2012), have reported strength losses of ~1-3% per annum after mid-life.

2.2.5 Prevalence

The reported prevalence of sarcopenia is varied, ranging from 1-50% (Cruz-Jentoft et al. 2014, Fielding et al. 2011, Patel et al. 2013, Shafiee et al. 2017, Su et al. 2019). Heterogeneity between studies might be explained by differences in the population studied, criteria definition of sarcopenia employed, age of participants, and methods of measuring indices of sarcopenia. In a comprehensive meta-analysis of 35 studies conducted by Shafiee and colleagues (2017) (including 58,404 participants aged ≥60 years), it was estimated that the global prevalence of sarcopenia in both older men and women was 10%. More specifically in the United Kingdom, it has been reported that 4.6% of older men and 7.9% of elderly women met the criteria for sarcopenia (Patel et al. 2013). Taking into account the global prevalence of sarcopenia reported by Shafiee et al. (2017) and a conservative estimate of the current population aged >60 years (~900 million) (World Health Organisation 2018), sarcopenia currently affects ~90

million people worldwide, with the figure likely to rise to ~200 million by 2050 (Cruz-Jentoft et al. 2019).

2.2.6 Clinical implications

Sarcopenia is associated with several adverse health effects, including an increased risk of falls, hospitalisation visits, morbidity and mortality, and poor quality of life (Beaudart et al. 2014). A meta-analysis conducted by Beaudart and colleagues (2017) reported a higher risk of mortality in sarcopenic compared to non-sarcopenic individuals. Beaudart et al. (2017) also reported an association between sarcopenia and incidence of functional disability, and others have observed a higher risk of recurrent falls in sarcopenic compared to healthy older adults (Cawthon et al. 2015). Moreover, sarcopenia has also been associated with cognitive and metabolic dysfunction, leading to increased risk of type 2 diabetes mellitus (T2DM) and Alzheimer's disease (Chang et al. 2016, Scott, de Courten, and Ebeling 2016). These relationships will be reviewed in parts 2 (**section 2.3**) and 3 (**section 2.4.3.2**) of this literature review. Sarcopenia has been recognised as a disease since September 2016, having been awarded an internal classification of disease (ICD-10-CM) code (M62.84) (Cao and Morley 2016).

2.2.7 Clinical cost

The economic burden of sarcopenia to healthcare services globally is highly significant. In the first published economic analysis, it was estimated that the direct cost of sarcopenia to healthcare services in the United States was $18.5 billion in the year 2000 (Janssen et al. 2004). A follow up analysis conducted in 2014 reported an increased expenditure of $40.4 billion (Goates et al. 2019). Likewise, economic analyses conducted in The Netherlands (Mijnarends et al. 2016), Porto (Sousa et al. 2016) and Portugal (Antunes et al. 2017) consistently report ≥50% hospitalisation costs in sarcopenic compared to non-sarcopenic older adults. In the United Kingdom, Pinedo‐Villanueva et al. (2019) reported an annual £2.5

billion estimated healthcare expenditure as a consequence of low muscle strength in older adults.

2.2.8 Mechanisms

The aetiology of sarcopenia is multifactorial, with neurological, immunological, endocrinological, nutritional and physical activity changes all described (Ali and Garcia 2014). Whilst significant research has been undertaken over recent years, a major barrier in the effective management of sarcopenia is the current inadequate understanding of many of these mechanisms (Sakuma, Aoi, and Yamaguchi 2017). The subsequent sections will review the current understanding of the mechanisms of sarcopenia which are directly related to this thesis.

2.2.8.1 Physical inactivity/muscle disuse

Homeostasis of skeletal muscle is maintained through the regulated balance between rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) (Kumar et al. 2009a). For muscle hypertrophy to occur, rates of MPS must exceed MPB, whereas when MPB exceeds MPS, muscle atrophy arises (Kumar et al. 2009a). In healthy young individuals, muscle protein turnover occurs at a rate of 1-2% per day (Wilkinson et al. 2014); however, older adults display disturbances in muscle protein turnover, whereby rates of MPB are chronically increased and rates of MPS are chronically decreased, consequently leading to muscle atrophy (Rennie et al. 2004). Two key factors driving this disturbance are age-related declines in levels of physical activity and an increase in sedentariness (Wall, Dirks, and van Loon 2013). To add, only 10% of older women and 15% of older men have reported complying with current physical activity guidelines (Jefferis et al. 2014), whilst others have shown older adults remain sedentary for ~9.4 hours per day (Harvey, Chastin, and Skelton 2015).

Following muscle disuse, studies have reported up to 40% declines in MPS in older adults (Drummond et al. 2012, Wall et al. 2016). Bed rest studies of 10-42 days have also reported

notable reductions in SMM (~0.5-0.6%) and more variable reductions in muscle strength (0.3-4.2%) (Wall, Dirks, and van Loon 2013). Importantly, substantial muscle atrophy (1.4% and 10% decreases in muscle CSA and myofiber size, respectively) has also been observed following just 4-5 days of disuse (Wall et al. 2014, Suetta et al. 2012). These findings are of clinical significance, as 5-6 days is the typical duration of acute hospitalisation in older adults (Fisher et al. 2010). Further, other circumstances which may lead to muscle disuse of <10 days such as illness that does not require hospitalisation and minor injuries are also likely to occur in the elderly population (Wall, Dirks, and van Loon 2013). Anecdotally, elderly individuals may therefore experience numerous periods of short-term muscle disuse throughout their later years, which could lead to substantial muscle atrophy and sarcopenia if SMM and strength are not preserved.

2.2.8.2 Protein malnutrition

Another factor which contributes to muscle protein imbalance in the elderly is protein malnutrition. Protein ingestion transiently increases MPS and supresses MPB via the effects of insulin, resulting in a positive net protein balance (Rennie et al. 2004). Protein recommendations for adults aged ≥19 years are set according to the recommended dietary allowance (RDA) of 0.8 g/kg body mass/d (Institute of Medicine 2005). However, this *"one-size fits all"* advice does not take into account age-related changes in protein metabolism, hormone concentrations, immunity or progressing frailty (Clegg et al. 2013). More recent data employing the indicator AA oxidation technique has established the current RDA for protein is insufficient for older adults (Rafii et al. 2015, Tang et al. 2014). These findings are supported by a number of consensus groups, who reason that healthy older adults require 1.0-1.2 g protein/kg/d to attenuate sarcopenic muscle loss (Bauer et al. 2013, Deutz et al. 2014). Though, many older adults find consuming dietary protein challenging, with 33% and 67% reported incapable of consuming the RDA and 1.2 g/kg/d, respectively (ten Haaf et al. 2018b, Wolfe and Miller 2008). As a result, a large proportion of older adults do not consume sufficient dietary protein to maintain muscle protein balance.

2.2.8.3 Anabolic resistance

Previous work has demonstrated that older adults display a blunted muscle protein synthetic response to anabolic stimuli (i.e., RE and protein ingestion) compared to their younger counterparts (Breen and Phillips 2011, Burd, Gorissen, and van Loon 2013). For example, Moore et al. (2015) established the dose of dietary protein required to maximally stimulate MPS in younger adults was 0.25 g/kg; however, the same effect was not observed in older adults until 0.4 g/kg was ingested. Likewise, others have shown the MPS response to an acute bout of RE is impaired in the elderly compared to younger adults (Drummond et al. 2008, Kumar et al. 2009b). These blunted responses to anabolic stimuli have been coined anabolic resistance (Burd, Gorissen, and van Loon 2013), which is a multifactorial process incorporating reductions in protein digestion and absorption, postprandial AA delivery and availability, and impairments in the signalling of anabolic proteins (Wilkinson, Piasecki, and Atherton 2018).

2.2.8.4 Hormone function

Declines in circulating concentrations of hormones of the HPA axis are observed with advancing age (Vitale, Cesari, and Mari 2016). From the fourth decade of life, testosterone concentration in healthy men decline by 1% per annum (Feldman et al. 2002, Morley and Perry 2003), and concentrations of growth hormone (GH) and IGF-1 decline at a rate of ~1.4% per annum (Giustina and Veldhuis 1998, Ryall, Schertzer, and Lynch 2008, Veldhuis et al. 1995). Insulin-like growth factor 1 is a key protein involved in the stimulation of MPS via activation of the mammalian target of rapamycin (mTOR) signalling pathway (Sakuma, Aoi, and Yamaguchi 2017, Sattler 2013). Low concentration has been associated with decreased gait speed, low ASMM and increased sarcopenia risk (Gielen et al. 2015, Volpato et al. 2014). Dysfunction of the HPA axis with age has been shown to increase secretion of glucocorticoids from the adrenal cortex (Vitale, Salvioli, and Franceschi 2013). In skeletal muscle, glucocorticoids inhibit MPS and increase MPB through activation of two target genes, REDD1 and KLF15 (**Figure 2.1**). Glucocorticoid exposure is governed by 11β-hydroxysteroid

dehydrogenase type 1 (11β-HSD1), which converts inactive corticoids into their active form (i.e., cortisone into cortisol) (Vitale, Cesari, and Mari 2016). Generally, the diurnal cycle of cortisol secretion is characterised by a surge of 50-60% 30-40 min upon awakening [known as the cortisol awakening response (CAR)], followed by a decline in the hours subsequent until reaching a nadir in the late evening (Adam et al. 2017). However, in sarcopenic older adults, elevated cortisol production has been observed compared to healthy elderly individuals (Waters et al. 2008). Atypical circadian cortisol secretion (i.e., attenuated morning and elevated pre-sleep concentration) has also been associated with poor physical performance in older adults (Gardner et al. 2011, Sousa et al. 2017).

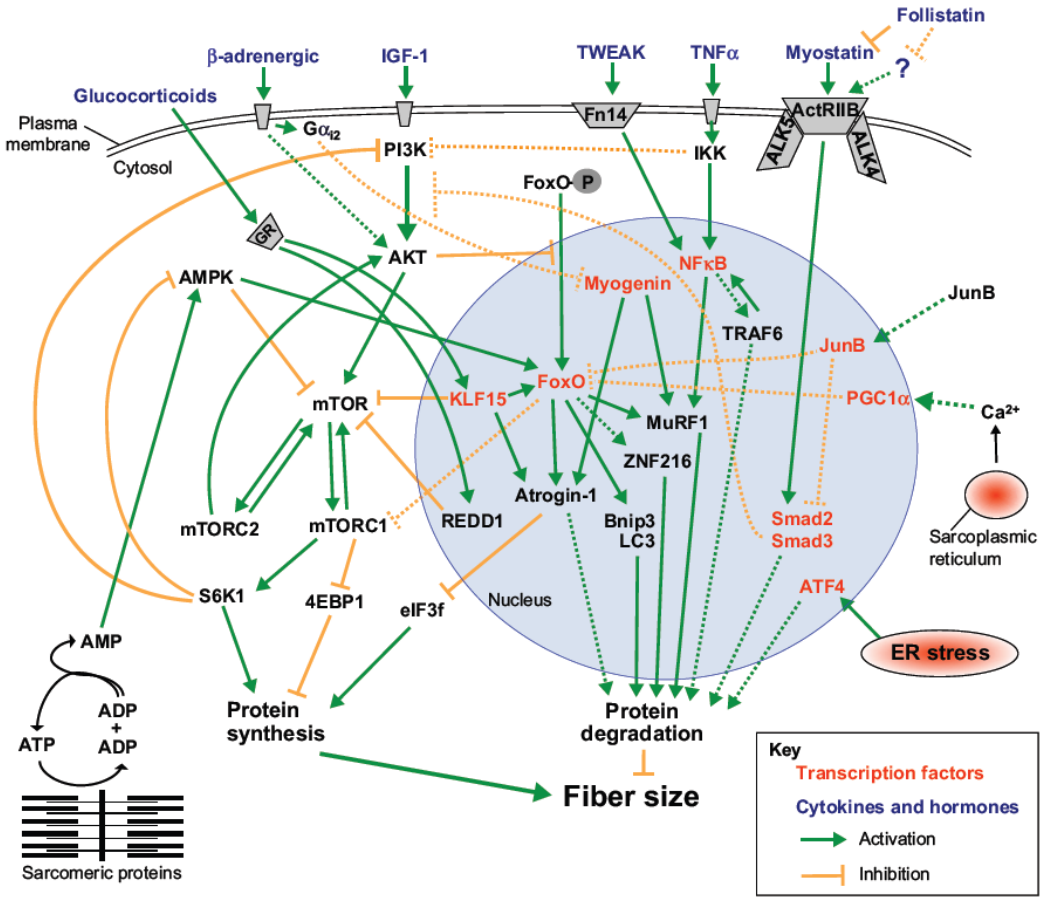

Figure 2.1 Pathways involved in skeletal muscle atrophy. Taken from Bonaldo and Sandri (2013).

Increased circulating myostatin concentration has also been purported to contribute to sarcopenia (Elliott et al. 2012). First characterised by McPherron, Lawler, and Lee (1997), myostatin, a transforming growth factor β (TGF-β) family member, is a potent negative regulator of skeletal muscle. Myostatin promotes protein degradation via phosphorylation of Smad2 and Smad3 by activin-like kinase (ALK) 4 and ALK5 (**Figure 2.1**). Activation of these genes is also thought to blunt MPS via inhibition of the IGF-1/Akt/mTOR pathway (Goodman et al. 2013). Several studies have reported a relationship between increased myostatin concentration and decreased SMM (Léger et al. 2008, Schulte and Yarasheski 2001, Yarasheski et al. 2002) and strength (Han et al. 2011, Patel et al. 2014). In contrast, others have not observed a relationship between myostatin and sarcopenia (Hofmann et al. 2015, Ratkevicius et al. 2011). These discrepancies may be explained in part by measurement difficulties, as myostatin has a close homology with other TGF-β members, making antibody specificity problematic (Consitt and Clark 2018). Nonetheless, as the role of myostatin in the pathogenesis of sarcopenia is still unclear (Consitt and Clark 2018), ongoing research is required to determine methods of disrupting the activity of myostatin and its relation to sarcopenia.

2.2.8.5 Systemic inflammation

Ageing is associated with chronic, low-grade, systemic inflammation, a phenomenon termed inflammaging (Franceschi et al. 2006, Piber et al. 2019). Most notably, circulating concentrations of CRP, IL-6 and TNF-α have been reported to increase with age and have been associated with a greater risk of sarcopenia (Bano et al. 2017, Schaap et al. 2006, 2009, Wang et al. 2017b, Wilson et al. 2017). Previous work has demonstrated chronic systemic inflammation negatively affects skeletal muscle via both direct and indirect pathways (Sakuma, Aoi, and Yamaguchi 2017). Indirectly, TNF-α supresses the expression of GH and IGF-1, inhibiting MPS (Fernández-Celemín et al. 2002). Directly, proinflammatory cytokines potently activate nuclear-factor kappa-light-chain-enhancer of activated B cells (NF-κB)

signalling (Kumar et al. 2004), promoting protein degradation and muscle atrophy (Li, Malhotra, and Kumar 2008).

2.2.9 Measurement of skeletal muscle mass, strength, and physical function

Measurement of SMM, strength and physical function can be performed by a range of methods. These methods have specific advantages and disadvantageous for use in clinical research (**Table 2.1**). For measurement of SMM, methods include body imaging techniques [e.g., magnetic resonance imaging (MRI), CT, DXA, BIA], anthropometry (e.g., skinfold thickness and leg circumference), biochemical markers [e.g., total or partial body potassium, deuterated creatine (D3-creatine) dilution method], and alternative methods such as neutron activation analysis (NAA) and electrical impedance myography (EIM) (Tosato et al. 2017). Both MRI and CT are considered the 'gold standard', predominately due to the ability to measure both SMM and fat infiltration simultaneously (Cesari et al. 2012). Additionally, although not considered gold standard, DXA is also often used as a reference method for identifying low SMM in older adults (Mijnarends et al. 2013). However, whilst CT, MRI and DXA accurately and reliably measure FFM/SMM, they are expensive, not widely available and expose participants to radiation (CT and DXA only) (Heymsfield et al. 2015). As an alternative, BIA is a relatively inexpensive, simple measurement technique, which estimates SMM based on measured impedance (Ω) using the equation of Janssen (2002):

$$\text{Whole body SMM (kg)} = [(\text{height(cm)}^2 / \Omega \times 0.401) + (\text{sex} \times 3.825) + (\text{age} \times -0.071)] + 5.102.$$

Bioelectrical impedance analysis is based on the concept that fluid and electrolyte rich-tissues, particularly skeletal muscle, pose the least impedance to the passage of electrical current compared to those enriched with lipid (e.g., adipose tissue) (Tosato et al. 2017). Prediction equations have been validated for multiple groups including the elderly (Cruz-Jentoft et al. 2010), and measurement of SMM has been cross-validated against MRI in older adults (Janssen et al. 2000a). For these reasons, several expert groups accept measurement of

SMM by BIA in the diagnosis of sarcopenia (Fielding et al. 2011, Chen et al. 2020, Cruz-Jentoft et al. 2019). However, it is important to note that BIA has several limitations, such as nutrition and hydration status besides recent physical activity influencing measurement accuracy (Bauer and Morley 2020). Therefore, control of these factors prior to measurement is essential.

Muscle strength is typically measured by either isometric, isokinetic or isotonic [one repetition maximum (1RM)] testing (Buckner et al. 2017). Isokinetic dynamometry is considered the gold standard; however, its use is limited due to cost and equipment availability (Cooper et al. 2013). Measurement of 1RM offers a reliable alternative, and is strongly correlated with measurement of maximal strength by isokinetic dynamometry (Verdijk et al. 2009b). Furthermore, during 1RM tests, both eccentric and concentric actions are performed, thereby reflecting muscle actions performed during ADL (Grgic et al. 2020). This contrasts with other laboratory tests. Consequently, while testing can be time consuming, many researchers consider 1RM as the gold standard method of determining dynamic strength (Kraemer et al. 2006). Furthermore, handgrip strength, which is an accepted method for diagnosing sarcopenia by numerous working groups including the EWGSOP2 (Cruz-Jentoft et al. 2019), is a simple, inexpensive and non-invasive measure of muscle strength. Low grip strength is also a strong predictor of a number of adverse health outcomes, such as poor quality of life, impaired functional capacity and mortality (Ibrahim et al. 2016, Leong et al. 2015). However, as highlighted by Tieland and colleagues (2015), handgrip strength poorly reflects changes in muscle strength measured by 1RM following RE training. Its use as an outcome measure in RE RCTs is therefore questionable.

Physical performance can be defined as an objectively measured whole-body function related to locomotion (Cruz-Jentoft et al. 2019). This concept is multifaceted, involving muscle and neurological function (Beaudart et al. 2019). Physical function can be assessed by various methods, including measures of balance, gait speed and performance of ADL (Mijnarends et

al. 2013). One of the most widely used tests in the literature is the SPPB, which has been shown to be a reliable and valid method of measuring physical function (Mijnarends et al. 2013), strongly predicts all-cause mortality (Pessini, Barbosa, and Trindade 2016), and is supported for diagnosis of sarcopenia by the EWGSOP2 (Cruz-Jentoft et al. 2019). The SPPB is a composite test including measures of gait speed, standing balance, and standing and sitting chair performance to make up a 12-point scoring system (Guralnik et al. 2000). Of note, a significant limitation of the SPPB is the potential ceiling effect, with 20% of respondents reported achieving the highest or lowest possible score (Bergland and Strand 2019).

In addition to the SPPB, measurement of gait speed, which is also supported by the EWGSOP2, is a quick, safe and reliable test widely used in clinical practice (Bruyère et al. 2016). Gait speed strongly predicts likelihood of numerous adverse outcomes such as falls, mortality and cognitive dysfunction (Cruz-Jentoft et al. 2019). Furthermore, the 6-minute walk test (6MWT) is a timed maximal walking exercise capacity test that requires participants to cover as much distance as possible in six minutes (Crapo et al. 2002). Test-retest reliability has been confirmed in older adults (Rikli and Jones 1998), and poor performance has been associated with an impaired ability to perform ADL (Enright et al. 2003).

Table 2.1 Methods of measuring skeletal muscle mass, strength, and physical function. Adapted from Cruz-Jentoft et al. (2019), Heymsfield et al. (2015), Meijers et al. (2013), Tieland et al. (2015) and Tosato et al. (2017)

Method	Measurement(s)	Advantages	Disadvantages
Skeletal muscle mass			
Anthropometry	Skinfold thickness, circumference (e.g., leg)	Non-invasive, low cost, applicable for large cohort studies	Imprecise, assessor experience and training required, not applicable in the elderly due to increases in intramuscular fat infiltration with age
Bioelectrical impedance analysis (BIA)	Whole-body and segmental impedance, resistance and reactance, phase angle	Relatively inexpensive, portable, and simple to conduct	Measurement specific to participant conditions (e.g., food consumption, hydration status and physical activity). Prediction equations are population specific
Computed tomography (CT)	Cross-sectional area, muscle attenuation	Three-dimensional reconstruction, high resolution, whole-body/regional measurements of lean tissue, fat, and bone mineral content	Participant exposed to radiation, cost, participant size limitations
Deuterated creatine (D3-creatine) dilution method	D3-creatinine quantification from urine	Excellent agreement with MRI for measurement of SMM in humans	Laborious processing (urinary sample processing and D3-creatinine determination), requires sophisticated equipment
Dual X-ray absorptiometry (DXA)	Lean tissue, fat, and bone mineral content	Low radiation, whole-body/segmental measurement	Size restrictions (height and body mass), expensive, cannot specifically determine SMM
Electrical impedance myography (EIM)	Resistance and reactance	Non-invasive, many parameters can be captured (e.g., reductions in myocyte number or size)	Equipment not widely available, requires trained personnel
Magnetic resonance imaging (MRI)	Cross-sectional area, fat infiltration	Three-dimensional reconstruction, high resolution, skeletal muscle quality can be measured using multiple measures	Expensive, not widely available, participant size limitations

Table 2.1 continued

Method	Measurement(s)	Advantages	Disadvantages
Neutron activation analysis (NAA)	Identification and quantification of elements in a given sample. The measurement relies on atom excitation by neutrons, so the treated sample emits gamma rays	Very accurate estimation of overall SMM	High cost, radiation exposure and technical difficulty
Total or partial body potassium	Elemental potassium (K) by either whole- or partial-body scintillation counter, body cell mass can then be derived from total body potassium (TBK) using standard equations	Safe and relatively inexpensive	Equations rely on several assumptions (e.g., nitrogen content, hydration content of FFM)
Ultrasonography	Muscle width and area	Safe, non-invasive, widely available	Technical training and skill required. Measurement of muscle size may be influenced by excess transducer pressure and orientation. Identifying exact location for reproducible measurements critical. Hydration status important
24-h urinary creatinine	24-h urinary creatinine	Safe, non-invasive, reflects muscle cell mass, relatively inexpensive	Requires participant cooperation for accurate results, multiple 24-h samples whilst ingesting a meat-free diet may be required
Muscle strength			
One repetition maximum (1RM)	Maximal weight that can be lifted once whilst maintaining correct form	Strong correlation with isokinetic peak torque in older adults, safe and inexpensive, excellent test-retest reliability. Strongly reflects dynamic muscle actions required for ADL	Potential learning effect if participants are not familiarised with procedures, time consuming
Handgrip dynamometry	Maximum isometric strength of the hand and forearm muscles	Non-invasive, widely used in the literature, strongly associated with adverse health outcomes	Does not strongly reflect changes in strength following RE training

Table 2.1 continued

Method	Measurement(s)	Advantages	Disadvantages
Isokinetic dynamometry	Maximal strength at a constant predetermined velocity throughout a selected joints range of motion	Gold standard for measurement of muscle function, high test-retest reliability	High cost of equipment, generally single joint tests, many familiarisation sessions needed in older adults
Physical function			
3D accelerometery	Walking speed, step and stride time, step-time asymmetry, mediolateral and craniocaudal acceleration	Non-invasive portable method, accurately assesses multiple gait parameters	Regular calibration needed, positioning of the accelerometer introduces bias
400 m walk test	Cover 400 m as quickly as possible with up to two rest stops during the test (endurance and walking capacity)	Can be used to assess sarcopenia according to the EWGSOP2, high reproducibility in older adults	Not applicable for older adults with significant functional limitations, number of stops may influence outcome
6 min walk test (6MWT)	Total distance covered in 6 minutes	High construct and concurrent reliability, simple to administer, well-established method of establishing exercise capacity	Walkway length significantly affects performance due to differences in directional changes
Gait speed	Timed walk at habitual or maximal walking speed over a set distance (typically 4 m)	Quick, safe and highly reliable method to assess sarcopenia, widely used in clinical practice	Differences in test distance influences result due to acceleration. Static or moving start also influences outcome
Physical performance test (4- or 7-item)	Measurement of typical ADL (e.g., lifting a book, simulated dressing, chair raise)	High construct and concurrent validity, scores correlate well with disability and loss of independence	Potential ceiling effect in healthy older adults
Sit-to-stand test	Time to complete either 5 or 10 repetitions, or maximal number of repetitions over 30 seconds	Simple to administer, reliable, high construct validity	Frail/functionally limited older adults may not be able to complete the required repetitions, no consistent chair height used in the literature

Table 2.1 continued

Method	Measurement(s)	Advantages	Disadvantages
Short physical performance battery (SPPB)	Usual 4-m gait speed, balance, and chair stand tests	Widely used test in in the literature, strongly predicts adverse health outcomes, simple to administer, high test-retest reliability	Potential ceiling effect in healthy older adults
Stair climb test	Ascend a flight of stairs as quickly as possible (measures stair climb power)	Clinically feasible and low cost (minimal equipment required), excellent test-retest reliability, high concurrent validity	Stair length may influence what is being measured (e.g., a longer flight of stairs may place greater demand on cardiovascular capacity as opposed to leg power)
Standing balance	Standing balance tests under different conditions (e.g., feet together on firm surface eyes open), balance accelerometery measured during each condition for measurement of postural sway	High test-retest reliability, simple to administer, correlates with adverse physical outcomes	Poor construct validity, does not correlate well with dynamic balance
Timed-up-and-go (TUG) test	Time to stand up from a standard chair, walk to a marker 3 m away, turn around, walk back, and sit down again	Simple to administer, high construct validity for measurement of physical function, extensively used in geriatric medicine	Prediction of future falls in older adults is limited, performance dependent on type of chair used

ADL, activities of daily living; EWGSOP2, European Working Group of Sarcopenia in Older People 2; FFM, fat-free mass; SMM, skeletal muscle mass.

2.2.10 Treatment and prevention

Despite an upsurge of research activity over the last decade, no approved pharmacological interventions are available for treatment or prevention of sarcopenia. Several have been tested, but these have generally been unsuccessful (Borst 2004, Onder, Della Vedova, and Landi 2009). Therefore, effective and safe lifestyle interventions are needed. As briefly highlighted previously, two potent stimulators of MPS are RE and protein ingestion (Wilkinson, Piasecki, and Atherton 2018). The subsequent sections will review the individual and combined effects of RE and dietary protein for the prevention and treatment of sarcopenia and identify areas for further research.

2.2.10.1 Resistance exercise

Although ageing adversely affects skeletal muscle, previous work has demonstrated skeletal muscle can be trained even in older age (Martone et al. 2015). Resistance exercise, which involves the voluntary contraction of targeted skeletal muscle against an external resistance (e.g., body weight, resistance machine, free weights, cables or bands) (Winett and Carpinelli 2001), has been shown to increase SMM, strength and physical function in both old (Hakkinen et al. 1998), and the oldest of old (>90 years) individuals (Fiatarone et al. 1994). However, the effects are attenuated with advancing age (Straight et al. 2020), highlighting the need for earlier prevention to curb sarcopenia. Importantly, RE is well tolerated in both healthy and frail older adults with very few adverse events reported in the literature (Fragala et al. 2019). As a result, RE is considered the most effective preventative treatment of sarcopenia (Phillips and Martinson 2019).

2.2.10.1.1 Effects of resistance exercise on muscle protein turnover

Resistance exercise is a powerful anabolic stimulus for skeletal muscle tissue. A single bout of RE elevates MPS by 40-150%, with rates remaining elevated for up to 48 h post-exercise (Chesley et al. 1992, Phillips et al. 1997). At the molecular level, RE increases rates of MPS

via activation of mTOR complex 1 (mTORC1), phosphorylating several downstream kinases such as ribosomal protein S6 kinase 1 (S6K1), augmenting translational efficiency and capacity (Hodson et al. 2019). Resistance exercise also increases rates of MPB, which remain negative in the absence of food ingestion (Biolo et al. 1995, Phillips et al. 1997). Nevertheless, the concurrent increase in MPS and MPB following RE improves net muscle protein balance (**Figure 2.2**), and when performed chronically, results in accretion of SMM (Joanisse et al. 2020).

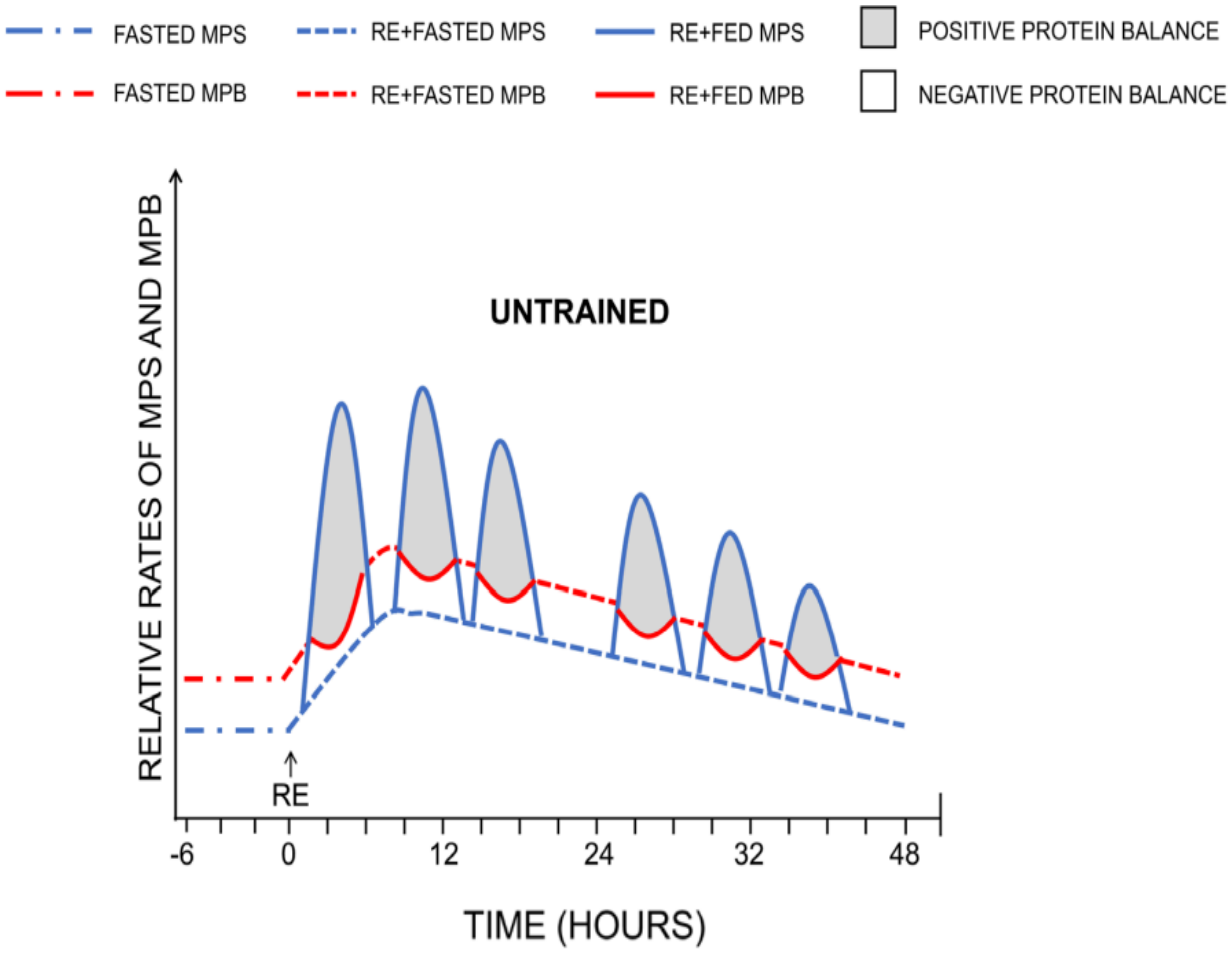

Figure 2.2 Current understanding of changes in muscle protein turnover with resistance exercise training. Taken from Joanisse et al. (2020).

2.2.10.1.2 Effects of resistance exercise on fat-free mass, muscle strength and physical performance

Over time, increases in whole-muscle size (~4.6%), hypertrophy of type I (6.7%), type IIa (12.1%) and type IIx muscle fibres (18.4%), increases in whole-muscle (43.3%) and individual muscle fibre isotonic strength (19.5%), hyperplasia, and an increase in satellite cell content have been reported following RE training (Dankel et al. 2019, Folland and Williams 2007). Increases in muscle strength may be explained, at least in part, by neurological adaptions (Carroll, Riek, and Carson 2001). Such adaptions include an increase in motor unit recruitment and firing frequency, decreased antagonist co-activation, and increased motor unit synchronisation (Folland and Williams 2007).

In older adults, there is a plethora of evidence supporting the positive effects of RE on muscle function (Beckwée et al. 2019). Several meta-analyses, as displayed in **Table 2.2**, have reported increases in FFM (Buch et al. 2017, Csapo and Alegre 2016, Peterson, Sen, and Gordon 2011, Yoshimura et al. 2017), muscle strength (Buch et al. 2017, Csapo and Alegre 2016, Martins et al. 2013, Peterson et al. 2010, Yoshimura et al. 2017) and physical function (Yoshimura et al. 2017). Of note, pooled increases in FFM of 1.1 kg (Peterson, Sen, and Gordon 2011), muscular strength of 25-35% (Peterson et al. 2010), and usual and maximum gait speed of 0.11 m/s and 0.26 m/s, respectively, have been reported following 12-20 weeks of RE (Yoshimura et al. 2017). To add to these findings, a systematic review conducted by Papa, Dong, and Hassan (2017) reported increased TUG and functional reach performance, reporting small to very large effects (Cohen's *d* = 0.49-5.28).

Table 2.2 Summary of meta-analyses investigating the effects of resistance exercise on fat-free mass, muscle strength and physical function in older adults

Study	Measured outcome			Studies (*n*) and participant/RE characteristics	Key findings
	FFM/SMM	Strength	Physical function		
Buch et al. (2017)	x			4 studies (103 participants; age: 64.5 ± 7.4 y); training duration: 39.8 ± 18.1 sessions (~13 weeks); training frequency: 3 ± 1.2 times/week; intensity: 40% 1RM-6RM	↑ upper (1.14 kg overall effect) and lower body strength (11.99 kg overall effect); ↑ FFM (2 kg overall effect)
Csapo and Alegre (2016)	x	x		FFM (7 studies, 213 participants); muscle strength (15 studies, 448 participants; age: 67.8 y); training duration: 154 ± 100 days; training frequency: 3 times/week (all studies); intensity: 40-80% 1RM	↑ muscle size and strength which was greater training at higher (11% and 43%, respectively) than lower intensities (9% and 35%, respectively)
Martens et al. (2013)		x		11 studies (834 participants; age: 60-79 y); training duration: 6-24 weeks (mean: 14.1 ± 7.0 weeks); training frequency: 1-5 times/week (mean: 3.2 ± 0.9); intensity: all resistant band studies, based on target repetitions (6-12)	↑ muscle strength: healthy elderly (SMD: 1.30); older adults with some functional incapacity (SMD: 1.01); older adults with pathology (SMD 0.54)
Peterson et al. (2010)		x		47 studies, 1,079 participants (age: 67.4 ± 6.3 y); training duration: 6-52 weeks (mean: 17.6 ± 8.6 weeks); training frequency: 1-3 times/week (mean: 2.7 ± 0.5 days/week); intensity: 40-50% 1RM (mean: 70 ± 12.7% 1RM)	↑ maximal strength of leg press (29 ± 2%), chest press (24 ± 2%), knee extension (33 ± 3%) and lateral pull (25 ± 2%). ↑ intensity was associated with greater improvements
Peterson, Sen, and Gordon (2011)	x			49 studies, 1,328 participants (age: 65.5 ± 6.5 y); training duration: 10-52 weeks (mean: 20.5 ± 9.1 weeks); training frequency: 2-3 times/week (mean: 2.8 ± 0.4 times/week); intensity: 50-80% 1RM (mean: 74.6 ± 6.9 %1RM)	↑ FFM (1.1 kg pooled estimate). ↑ volume interventions resulted in greater ↑ in FFM
Yoshimura et al. (2017)	x	x	x	3 studies (397 participants with sarcopenia; age: >65 y); training duration: 12 weeks; training frequency: 2 times/week; training intensity: not reported	↑ appendicular SMM (0.38 kg), knee extension strength (0.11 Nm/kg), and usual (0.11 m/s) and maximum walking speed (0.26 m/s)

1RM, one repetition maximum; 6RM, six repetition maximum; FFM, fat-free mass; SMD; standardised mean difference; SMM, skeletal muscle mass.

2.2.10.1.3 Resistance exercise recommendations for older adults

For health-related outcomes, the American College of Sports Medicine (ACSM) recommend older adults should perform RE twice weekly, at a volume of 8-12 repetitions per exercise, and at a moderate-vigorous intensity (60-80% 1RM) (Chodzko-Zajko et al. 2009). However, whilst these guidelines are still applicable, the evidence published by the ACSM in 2009 is somewhat outdated. The following sections will briefly review the current evidence for optimisation of training outcomes in older adults.

2.2.10.1.3.1 Intensity

Resistance exercise intensity is typically prescribed as a percentage relative to maximal strength (Fragala et al. 2019). For optimisation of muscular strength, two meta-analyses have reported RE performed at 70-79% 1RM elicits the largest effects (Borde, Hortobágyi, and Granacher 2015, Steib, Schoene, and Pfeifer 2010). Conversely, for muscle hypotrophy, similar gains have been reported between high (~80% 1RM) and low-moderate intensities (~45% 1RM) when mechanical work (sets x repetitions x intensity) is matched (Borde, Hortobágyi, and Granacher 2015, Csapo and Alegre 2016). More recently, a perhaps surprising finding from a recent meta-analysis by Straight and colleagues (2020) reported low intensity RE resulted in greater increases in type II muscle fibre size. Despite these controversial effects on muscle hypertrophy, as muscle weakness is considered the main determinant of sarcopenia by the EWGSOP2 (Cruz-Jentoft et al. 2019), RE intensities aimed to optimise muscle strength should be utilised. In support, the National Strength and Conditioning Association (NSCA) suggest in healthy adults, RE intensity should achieve 70-85% 1RM to augment strength gains (Fragala et al. 2019).

2.2.10.1.3.2 Volume

Training volume signifies the total amount of weight lifted per session (Fragala et al. 2019). In a meta-analysis by Borde, Hortobàgyi, and Granacher (2015), 2-3 sets per exercise and 7-9 repetitions per set elicited the greatest effects on muscle hypertrophy and strength. The

previously cited meta-analysis by Peterson and colleagues (2010) also reported a greater number of sets per session was associated with superior increases in FFM. Furthermore, training to muscle failure (or very close to) has been suggested to optimise gains in SMM (Schoenfeld and Grgic 2019). However, older adults may experience slower post-exercise recovery compared to that of younger adults, suggesting training to failure for every set of each exercise may be detrimental for this population (Schoenfeld and Grgic 2019). This theory is supported by the NCSA (Fragala et al. 2019).

2.2.10.1.3.3 Frequency

Training frequency represents the total number of sessions per week (Fragala et al. 2019). Results from two meta-analyses suggest 2-3 sessions per week elicits the greatest improvements in muscle strength (Steib, Schoene, and Pfeifer 2010, Borde, Hortobágyi, and Granacher 2015). Additionally, Borde, Hortobàgyi, and Granacher (2015) also reported no additional benefit of training three times per week versus twice-weekly on muscle strength. These findings are supported by more recent studies that reported similar effects on muscle hypertrophy, strength and physical function (Grgic, Schoenfeld, and Latella 2019, da Silva et al. 2017, Kneffel et al. 2020, Stec et al. 2017). Furthermore, out of 94 older adults, a substantial difference has been reported for preference of training frequency, with 26% of older adults preferring to train twice per week, compared to only 1% preferring thrice weekly training (Foley, Hillier, and Barnard 2011). Based on these findings, twice weekly RE may be optimal to aid long-term adherence in this population.

2.2.10.2 Dietary protein

Proteins are essential nutrients for the human body and have an integral role in the growth and repair of all cells and tissues (British Nutrition Foundation 2018). They are natural polymers made up of different monomers called amino acids (AA), linked together by peptide bonds (Litwack 2018). There are 20 AA in total, which can be partitioned into either essential

(EAA, *n* = 9) or non-essential AA (NEAA, *n* = 11). Essential amino acids must be consumed exogenously and are critical to prevent protein malnutrition. Non-essential amino acids are synthesised endogenously predominately from glucose (Litwack 2018).

2.2.10.2.1 Types of dietary protein

Protein occurs in a wide range of foods, including both animal (e.g., meat, fish, dairy products) and plant-based sources (e.g., soy, tofu, lentils) (Devries and Phillips 2014). Of importance, protein derived from dairy products (e.g., milk, yoghurt, cheese) are considered the highest quality of proteins (Devries and Phillips 2015). Whey protein, a by-product of cheese production, contains all nine EAA and is rich in the branched-chain amino acid (BCAA) leucine (~3 g/25 g whey protein), the key AA responsible for stimulation of MPS (Anthony et al. 2000). Following ingestion, whey protein results in a marked increase in blood AA, which are transported across the sarcolemma into the intramyocellular space (West et al. 2011). The increase in intramyocellular EAA concentrations, in particular leucine, subsequently stimulates mTORC1 and ribosomal protein S6 kinase (p70S6K), triggering a cascade of molecular events resulting in elevated rates of MPS (Katsanos et al. 2006). In older adults, acute ingestion of whey protein stimulates MPS [myofibrillar fractional synthetic rate (FSR)] to a greater extent than isonitrogenous amounts (20 g) of casein and soy at both rest (whey: 0.04%/h; casein: 0.02%/h; soy: ~0.03%/h), and following RE (whey: 0.059%/h; casein: 0.035%/h; soy: 0.04%/h) (Burd et al. 2012, Yang et al. 2012b).

2.2.10.2.2 Per meal protein recommendations and protein distribution

As previously mentioned in **section 2.2.8.2**, to attenuate sarcopenia, protein intakes of 1.0-1.2 g/kg/d (Bauer et al. 2013, Deutz et al. 2014), and even up to 1.6 g/kg/d (Phillips, Chevalier, and Leidy 2016) have been recommended. In addition to the total amount of dietary protein, a growing body of evidence also suggests the per meal dose and daily distribution may also be of importance (Murphy, Oikawa, and Phillips 2016). Prior studies have demonstrated a dose-response relationship between dietary protein ingestion and rises in MPS, at least up to

a maximally effective dose (Cuthbertson et al. 2005, Moore et al. 2009a, 2015, Yang et al. 2012a). Beyond this dose, the muscle becomes refractory, and surplus ingestion of AA does not further stimulate MPS (Atherton et al. 2010). In older adults, Moore et al. (2015) established 0.4 g protein/kg (~32 g protein for an 80 kg individual) maximally stimulated myofibrillar FSR (~0.05%/h).

Several studies have examined the pattern in which older adults consume their daily protein intake (Bollwein et al. 2013, Farsijani et al. 2016, 2017, Tieland et al. 2012a, Smeuninx, Greig, and Breen 2020). In agreement, these studies consistently report older adults consume their daily protein intake in a skewed pattern (i.e., high amounts consumed in the evening meal and smaller amounts consumed at breakfast and lunch). For example, Smeuninx, Greig, and Breen (2020) recently published data demonstrating older adults consume ~8, 12 and 75% (or ~0.2, ~0.3 and ~0.5 g/kg) of their daily dietary protein intake at breakfast, lunch and dinner, respectively (**Figure 2.3**). Taking into consideration the previously mentioned findings of Moore and colleagues (2015), these findings suggest the majority of older adults only consume optimal amounts of dietary protein once daily.

Cross-sectional studies have reported an association between evenly distributed daily dietary protein intake and greater retention of leg and appendicular FFM (Jyväkorpi et al. 2020, Loenneke et al. 2016), increases in muscle strength (Loenneke et al. 2016), and improved physical function (ten Haaf et al. 2018a). In contrast, results from RCTs do not support these findings (Arnal et al. 1999, Bouillanne et al. 2013). An inadequate protein intake (0.22-0.33 g/kg) provided at each meal compared to the recommended 0.4 g/kg/meal in older adults may explain these findings. In support, data in young adults providing the recommended dose per meal to stimulate MPS (0.24 g/kg) for 12 weeks resulted in a greater accretion of FFM compared to protein consumed in a skewed fashion (Yasuda et al. 2020). Therefore, although further RCTs are needed to confirm the clinical relevance in older adults, data thus far indicates an evenly distributed daily protein intake may be effective at attenuating sarcopenia.

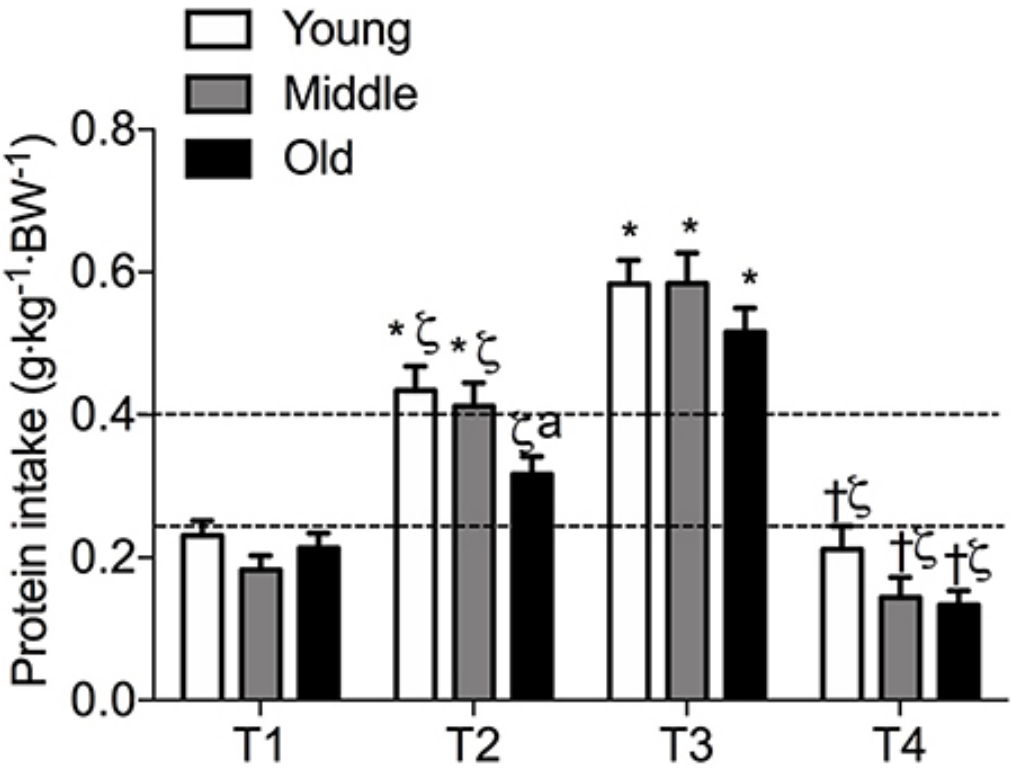

Figure 2.3 Meal-specific relative protein intakes in young, middle and older adults. T1, breakfast; T2, lunch; T3, dinner; T4, snack. Taken from Smeunix, Greig, and Breen (2020).

2.2.10.2.3 Adverse effects of high protein diets

Although high protein diets (>1.0-1.2 g/kg/d) have been recommended to attenuate sarcopenia, they are often discouraged due to purported harmful effects on renal function (Devries et al. 2018). The basis of this hypothesis stems from data demonstrating lower protein diets (0.6-0.8 g/kg/d) improved outcomes and delayed mortality in patients with chronic kidney disease (CKD) (Fouque et al. 2000, Levey et al. 1999). However, these findings do not provide direct evidence that high protein diets affect renal function in healthy adults. Therefore, Devries and colleagues (2018) conducted a comprehensive meta-analysis and meta-regression (28 studies; 1358 participants) to answer this research question. The findings reported no effect on change in glomerular filtration rate (GFR), suggesting high protein diets do not adversely affect renal function in healthy adults.

Opponents of high protein diets are also apprehensive about the effects on calcium homeostasis and bone health (Phillips, Chevalier, and Leidy 2016). This theory originated from the acid-ash hypothesis, coined from findings demonstrating that diets high in protein and grains yield a greater dietary acid load (Eisenstein et al. 2002, Fenton et al. 2009). According to the hypothesis, large protein-induced acid loads increase urinary calcium excretion, resulting in the release of skeletal calcium (Fenton et al. 2009). However, no casual association between acid load and osteoporotic bone disease has been reported (Fenton et al. 2009, 2011). In fact, recent research suggests dietary protein may aid bone health when sufficient calcium is consumed (Mangano, Sahni, and Kerstetter 2014). Hence, based on literature cited in this section, evidence supports the safe consumption of high protein diets in healthy older adults.

2.2.10.2.4 Effects of dietary protein on fat-free mass, muscle strength and physical function

Observational data over a 3-year period has reported an association between increased dietary protein intake and a greater retention of FFM (Houston et al. 2008). Others have shown consumption of >1 g protein/kg/d can preserve grip strength (McLean et al. 2016, Mishra et al. 2018) and physical function (Mustafa et al. 2018). In support of epidemiological studies, several RCTs ranging from 6-24 weeks in duration, as shown in **Table 2.3**, have established beneficial effects of dietary protein on FFM, muscle strength and physical function (Bauer et al. 2015, Bell et al. 2017, Bo et al. 2019, Sardeli et al. 2018, Kang et al. 2020, Mitchell et al. 2017, Negro et al. 2019, Norton et al. 2016, Park, Choi, and Hwang 2018, Shahar et al. 2013, ten Haaf et al. 2019, Tieland et al. 2012c). However, not all studies have observed such effects (Björkman et al. 2020, Cramer et al. 2016, de Carvalho Bastone et al. 2020, Kim et al. 2012, Kirk et al. 2020, Kukuljan et al. 2009, Verreijen et al. 2017, Zhu et al. 2015). The lack of protein-induced effects in these studies, with the exception of de Carvalho Bastone et al. (2020), may be partially explained by participants habitually consuming ample amounts (~1.0-1.2 g/kg/d) of dietary protein at baseline (Bauer et al. 2013, Deutz et al. 2014). Consequently, this may

have masked any additional effects of supplementation. Nonetheless, it must be noted that declines in FFM have been reported in older adults consuming 1.2 g/kg/d of dietary protein (Norton et al. 2016). Of note, Norton et al. (2016) also demonstrated a significant increase in FFM following protein supplementation in older adults habitually consuming this level of dietary protein. These findings suggest that some healthy older adults may still be sensitive to the anabolic properties of protein supplementation whilst already consuming adequate dietary protein. Consequently, increased dietary protein may still be a promising strategy to attenuate sarcopenia in these individuals.

As a result of the hypothesis proposed above, the lack of protein-induced effects reported in several studies may be explained by an insufficient increase in dietary protein from baseline. Reported deviations in these studies ranged from 0-0.35 g/kg/d (Björkman et al. 2020, Cramer et al. 2016). In contrast, other studies that reported beneficial effects deviated dietary protein by ≥0.4 g/kg/d (Bauer et al. 2015, Bell et al. 2017, Mitchell et al. 2017, Norton et al. 2016, Park, Choi, and Hwang 2018, ten Haaf et al. 2019). As previously mentioned, Moore et al. (2015) demonstrated a dose of 0.4 g protein/kg might be required to maximally stimulate MPS in older adults. Hypothetically, a deviation of ≥0.4 g protein/kg/d may therefore be required to stimulate muscle hypertrophy in older adults. This theory is supported by others (Park, Choi, and Hwang 2018).

Whilst numerous studies have reported advantageous effects of dietary protein in older adults, the majority report effects on only FFM (Bauer et al. 2015, Bell et al. 2017, Sardeli et al. 2018, Kang et al. 2020, Mitchell et al. 2017, Negro et al. 2019, Norton et al. 2016, Park, Choi, and Hwang 2018, Shahar et al. 2013, ten Haaf et al. 2019, Tieland et al. 2012c). Several of these studies that also examined muscle strength and physical function observed no effect on either one or both of these outcomes (Bauer et al. 2015, Bell et al. 2017, Kang et al. 2020, ten Haaf et al. 2019). These findings are supported by a recent meta-analysis that reported a positive effect of increased dietary protein on FFM, but not muscle strength (Wirth, Hillesheim, and

Brennan 2020). As the EWGSOP2 highlights muscle strength as the primary index of sarcopenia (Cruz-Jentoft et al. 2019), further work is required to establish a mechanism of how dietary protein may increase muscle strength and physical function. Although, it may be that to obtain clinically significant improvements in these parameters in older adults, dietary protein may need to be combined with RE.

Table 2.3 Summary of intervention studies investigating the effects of dietary protein on fat-free mass, muscle strength and physical function in older adults

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Bauer et al. (2015)	380 sarcopenic older adults (age: 77.7 y; 65% women) 13-week multicentre double-blind RCT	**Active:** 20 g whey protein-based supplement (twice daily) **Control:** isocaloric carbohydrate supplement (twice daily)	**Active:** 1.0 (0-9-1.2) **Control:** 1.0 (0.8-1.2)	Active group ↑ appendicular FFM and chair stand test performance greater than control group. No differences observed between groups for handgrip strength or SPPB. Protein intake ↑ to 1.5 g/kg/d (0.5 g/kg/d deviation from baseline) in the active group
Bell et al. (2017)	49 healthy older men (age: 73 ± 1 y) 20-week double-blind RCT split into 2 phases: (6-weeks nutrition and 12-weeks exercise + nutrition)	**SUPP:** 30 g whey protein, 1500 mg n−3 PUFA, 2.5 g creatine, 500 IU vitamin D and 400 mg calcium (twice daily) **CON:** 22 g maltodextrin (twice daily)	**SUPP:** 1.1 ± 0.3 **Control:** 1.2 ± 0.3	SUPP group ↑ FFM and strength greater than the control group. No differences in physical function observed between groups. **Key findings from the exercise phase are presented in Table 2.4**
Björkman et al. (2020)	218 older community-dwelling adults with sarcopenia (age: >74 y) 12-month RCT	**Whey protein:** 20 g whey protein (twice daily) **Placebo:** isocaloric placebo (twice daily) **Control:** no supplement	**Whey protein:** 1.1 ± 0.5 **Placebo:** 1.0 ± 0.4 **Control:** 1.0 ± 0.3	No differences between groups for SMM or physical performance (SPPB). No changes in protein intake occurred during the intervention in the whey protein group
Bo et al. (2019)	60 sarcopenic older adults (age: 60-85 y) 24-week double-blind RCT	**Protein:** 22 g protein supplement (twice daily) **Placebo:** isocaloric carbohydrate supplement (twice daily)	**Protein:** 0.76 ± 0.32 **Placebo:** 0.71 ± 0.36	Skeletal muscle index and handgrip strength ↑ greater in the protein than the placebo group. Protein intake ↑ by 0.25 g/kg/d in the protein group

Table 2.3 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Cramer et al. (2016)	330 sarcopenic malnourished older adults (mean age: 77 y) 24-week multicentre double-blind RCT	**Experimental:** 330 kcal supplement (20 g protein; 499 IU vitamin D3; 1.5 g CaHMB) **Control:** 330 kcal supplement (14 g protein; 147 IU vitamin D3)	**Experimental**: 0.94 (95% CI 0.70, 1.20) **Control:** 0.97 (95% CI 0.73, 1.30)	No differences in isokinetic peak torque, muscle quality, grip strength or gait speed occurred between groups. In participants with mild-moderate sarcopenia, strength ↑ greater in experimental compared to control group. Protein intake ↑ to ~1.3 g/kg/d in the experimental group
de Carvalho Bastone et al. (2020)	69 older adults with dynapenia (age: 75.9 ± 6.7 y) 12-week RCT	**RE + supplement:** home-based RE 3 times/week (80% 1RM, 3 sets of 8 repetitions) + 40 g protein supplementation once daily **RE:** home-based RE 3 times/week (80% 1RM, 3 sets of 8 repetitions) **Supplement:** 40 g protein supplementation once daily **Control:** maintained habitual diet and physical activity	<1.0 for all participants	No significant differences between supplement and control groups occurred for any outcome. **Results of RE vs. RE + supplement presented in Table 2.4**
Kang et al. (2020)	120 community-dwelling older adults (age: >65 y) 12-week double-blind RCT	**Intervention:** 20 g leucine-enriched protein supplement **Control:** isocaloric carbohydrate supplement	~0.93 for both groups	FFM ↑ greater in the intervention compared to control group. No differences occurred between groups for grip strength or physical function. Protein intake did not change during the intervention

Table 2.3 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Kim et al. (2012)	151 sarcopenic older women (age: >75 y) 12-week RCT	**Exercise + AA:** strength training twice weekly (8 repetitions; intensity: 12-14 on Borg scale) + 3 g EAA twice daily **Exercise:** strength training twice weekly (8 repetitions; intensity: 12-14 on Borg scale) **AA:** 3 g EAA twice daily **Control (health education):** health education once a month	Not reported	No effect of EAA on SMM, strength or physical function. **Results of exercise + EAA are reported in Table 2.4**
Kirk et al. (2020)	100 community-dwelling healthy older adults (52% women; age: 69 ± 6 y) 16-week RCT	**Exercise + protein:** twice weekly RE, once weekly functional performance + individualised whey protein supplementation to achieve 0.5 g/kg/meal **Exercise:** twice weekly RE, once weekly functional performance **Protein:** individualised whey protein supplementation to achieve 0.5 g/kg/meal **Control:** maintained habitual dietary and physical activity levels	**Exercise + protein:** 1.16 ± 0.4 **Exercise:** 1.10 ± 0.4 **Protein:** 0.99 ± 0.2 **Control:** 0.98 ± 0.3	No effects of protein supplementation without exercise on SMM, strength or physical function. **Results of exercise + protein are reported in Table 2.4**
Kukuljan et al. (2009)	180 community-dwelling older men (50-79 y) 18-month RCT	**Exercise + milk**: RE 3 days/week (2 sets x 8-12 repetitions at 80-85% 1RM) + 2 x 200 mL/d fortified milk **Exercise:** 3 days/week (2 sets x 8-12 repetitions at 80-85% 1RM) **Milk:** 2 x 200 mL/d fortified milk **Control:** Maintain habitual physical activity and diet	**Exercise + milk:** 1.26 ± 0.32 **Exercise:** 1.32 ± 0.32 **Milk:** 1.23 ± 0.28 **Control:** 1.33 ± 0.31	Milk had no effect on SMM, strength or physical function. Protein intake ↑ by 0.16 g/kg/d (to 1.39 g/kg/d) at 12 months but did not change at 18 months in the milk group. **Results of exercise + milk reported in Table 2.4**

Table 2.3 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Mitchell et al. (2017)	29 men (age: >70 y) 10-week RCT	**2 x RDA protein:** diet containing 1.6 g/kg/d protein **RDA protein:** diet containing 0.8 g/kg/d protein	**2 x RDA:** 1.1 ± 0.3 **RDA:** 1.2 ± 0.4	2 x RDA group ↑ whole-body and appendicular FFM and knee extension power greater than RDA group
Negro et al. (2019)	38 healthy elderly subjects (age: 68.9 ± 4.6 y) 12-week double-blind RCT	**SUPP:** EAA-based multi-ingredient supplement containing ~10 g protein (5 g EAA, 1.4 g leucine, 1500 mg creatine, 1000 IU vitamin D) twice daily **Placebo:** maltodextrin (twice daily)	Not reported	SUPP group ↑ appendicular FFM, maximal voluntary contraction and peak power greater than placebo
Norton et al. (2016)	60 healthy older men and women (age: 61 ± 5 y) 24-week double-blind RCT	**Protein:** 0.165 g/kg of median body mass protein supplement (twice daily) **Con:** isoenergetic maltodextrin supplement (twice daily)	**Protein:** 1.2 ± 0.3 **Con:** 1.2 ± 0.3	Total and appendicular FFM ↑ greater in the protein compared to control group. Protein intake ↑ to 1.6 ± 0.3 g/kg/d in the protein group (deviation of 0.4 g/kg/d from baseline)
Park, Choi, and Hwang (2018)	120 prefrail and frail elderly individuals (age: 70-85 y) 12-week double-blind RCT	**Diet containing 1.5 g/kg/d protein** **Diet containing 1.2 g/kg/d protein** **Diet containing 0.8 g/kg/d protein**	**1.5 g/kg/d:** 0.80 ± 0.21 **1.2 g/kg/d:** 0.77 ± 0.24 **0.8 g/kg/d:** 0.84 ± 0.28	Appendicular FFM, SMI and gait speed ↑ in the 1.5 g/kg/d group greater than the 0.8 g/kg/d group

Table 2.3 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Shahar et al. (2013)	65 sarcopenic older adults (age: 60-74 y) 12-week RCT	**Exercise + protein:** resistance band exercise twice weekly at moderate intensity + soy protein drink (20 g/d for men and 40 g/d for women) to achieve 1.5 g/kg/d protein **Exercise:** resistance band exercise twice weekly at moderate intensity **Protein:** soy protein drink (20 g/d for men and 40 g/d for women) to achieve 1.5 g/kg/d protein **Control:** relaxation programme twice weekly	**Exercise + protein:** 0.85 **Exercise:** 0.82 **Protein:** 0.92 **Control:** 0.91	Protein supplementation alone ↑ upper body strength greater than control. **Results of exercise + protein is detailed in Table 2.4**
ten Haaf et al. (2019)	116 physically active older adults (age: 69 y) 12-week double-blind RCT	**Protein:** 31 g milk protein supplement (twice daily) **Placebo:** isocaloric carbohydrate placebo (twice daily)	**Protein:** 0.86 ± 0.23 **Placebo:** 0.92 ± 0.24	FFM ↑ in the protein group greater than placebo group. No differences between groups for strength or physical function
Tieland et al. (2012c)	65 frail elderly participants (age > 65 y) 24-week double-blind RCT	**Protein:** 15 g milk protein supplement (twice daily) **Placebo:** energy-matched placebo (no protein; twice daily)	**Protein:** 1.0 ± 0.0 **Placebo:** 1.0 ± 0.0	No differences occurred between groups for SMM but physical performance ↑ in the protein group greater than the placebo group. Muscle strength also tended to ↑ greater in the protein group compared to placebo

Table 2.3 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Verreijen et al. (2017)	100 older adults who were overweight or with obesity (55–80 year) 10-week RCT	**Exercise + protein:** RE 3 days/week (2-3 sets of 50 s per exercise) + dietary advice to achieve 1.3 g protein/kg/d **Exercise:** RE 3 days/week (2-3 sets of 50 s per exercise) + dietary advice to achieve 0.8 g protein/kg/d **Protein:** dietary advice to achieve 1.3 g protein/kg/d **Control:** dietary advice to achieve 0.8 g protein/kg/d **All groups:** followed a hypocaloric diet (-600 kcal/d)	**Exercise + protein:** 1.00 ± 0.31 **Exercise:** 0.93 ± 0.30 **Protein:** 0.92 ± 0.34 **Control:** 0.95 ± 0.36	No effect of dietary protein on FFM, handgrip strength or gait speed. Protein intake during the intervention was lower than targeted (1.02 ± 0.36 and 1.02 ± 0.35 g/kg/d in the protein and exercise plus protein groups respectively). **Results of exercise + protein are shown in Table 2.4**
Zhu et al. (2015)	181 postmenopausal older women (age: 74.3 ± 2.7 y) 2-year double-blind RCT	**Protein:** 30 g/d milk-based protein supplement **Placebo:** skimmed milk-based supplement (2.1 g protein)	**Protein:** 1.2 ± 0.3 **Placebo:** 1.1 ± 0.3	No differences between groups for SMM, strength or physical function. Protein intake ↑ by 20 g/d to ~1.47 g/kg/d (0.27 g/kg/d deviation from baseline) in the protein group

↑, increased; CaHMB, calcium β-hydroxy-β-methylbutyrate; EAA, essential amino acids; FFM, fat-free mass; n-3 PUFA, omega 3 polyunsaturated fatty acids; RE, resistance exercise; RCT, randomised controlled trial; SMM, skeletal muscle mass; SPPB, short physical performance battery.

2.2.10.3 Resistance exercise combined with dietary protein

Previous work has shown acute ingestion of dietary protein during recovery of RE stimulates a greater and more sustained increase in rates of MPS (Moore et al. 2009b, Pennings et al. 2011), and mitigates post-exercise MPB to a greater extent than RE without protein ingestion (Biolo et al. 1995, 1997). Resistance exercise also sensitises skeletal muscle to the anabolic properties of protein ingestion for up to 24 h post exercise (Burd et al. 2012). Accordingly, older adults chronically participating in RE training and consuming higher amounts of dietary protein may habitually increase their net muscle protein balance, resulting in a greater attenuation of sarcopenia. The synergistic effects of RE and dietary protein will be subsequently reviewed.

2.2.10.3.1 Effects of resistance exercise combined with dietary protein on fat-free mass, muscle strength and physical function

The findings of the majority (Cermak et al. 2012, Finger et al. 2015, Liao et al. 2017, Morton et al. 2018a), but not all meta-analyses (Thomas et al. 2016) including both younger and older adults suggest dietary protein may augment the adaptive response of skeletal muscle to RE. In contrast, whilst several individual RCTs in older adults have reported significantly greater increases in FFM, muscle strength and physical function following RE combined with dietary protein compared to RE alone (Bell et al. 2017, Daly et al. 2014, Kang et al. 2019, Junior et al. 2018, Rondanelli et al. 2016, 2020, Tieland et al. 2012b, Verreijen et al. 2015, Yamada et al. 2019, Zdzieblik et al. 2015), the majority of studies, as displayed in **Table 2.4**, have not observed such effects (Arnarson et al. 2013, Candow et al. 2006, Chalé et al. 2013, de Carvalho Bastone et al. 2020, Dulac et al. 2020, Englund et al. 2018, Fielding et al. 2017, Gryson et al. 2014, Hofmann et al. 2016, Holm et al. 2008, Holwerda et al. 2018, Kim et al. 2012, Kirk et al. 2019, 2020, Kukuljan et al. 2009, Leenders et al. 2013, Maltais, Ladouceur, and Dionne 2016, Oesen et al. 2015, Ottestad et al. 2017, Shahar et al. 2013, Thomson et al. 2016, Verdijk et al. 2009a, Verreijen et al. 2017).

Disparities between the aforementioned studies might be explained in part by differences in the population studied and habitual protein intake of participants. To elucidate, several studies that observed synergistic effects included sarcopenic or frail older adults with habitual protein intakes ≤1 g/kg/d (Kang et al. 2019, Rondanelli et al. 2016, 2020, Tieland et al. 2012b, Yamada et al. 2019, Zdzieblik et al. 2015). Contrary to these studies, others that failed to observe augmented effects included healthy older adults whom habitually consumed ≥1 g protein/kg/d (Arnarson et al. 2013, Candow et al. 2006, Dulac et al. 2020, Holm et al. 2008, Holwerda et al. 2018, Hofmann et al. 2016, Kirk et al. 2019, 2020, Kukuljan et al. 2009, Leenders et al. 2013, Oesen et al. 2015, Thomson et al. 2016, Verdijk et al. 2009a). These findings imply a relatively good health status and an adequate habitual dietary protein intake may mask the supplemental effects in older adults performing RE. Differential effects between sarcopenic/frail and healthy older adults may be related to the higher chronic systemic inflammatory activity observed in frail older adults, resulting in dietary protein augmenting a greater anti-inflammatory effect in these individuals (Breen and Phillips 2011, Degens 2010, Toth et al. 2005).

In opposition of the health status and habitual protein intake hypotheses above, augmented effects have been observed in healthy older adults habitually consuming both adequate (1.0-1.1 g/kg/d) (Bell et al. 2017, Daly et al. 2014) and inadequate (0.85 ± 0.10 g/kg/d) amounts of dietary protein (Junior et al. 2018). Furthermore, no effects have also been reported in sarcopenic/functionally impaired older adults (Chalé et al. 2013, de Carvalho Bastone et al. 2020, Englund et al. 2018, Fielding et al. 2017, Kim et al. 2012, Maltais, Ladouceur, and Dionne 2016, Ottestad et al. 2017, Shahar et al. 2013). Similar to that of the effects of dietary protein alone, these findings indicate participant health status and/or habitual protein intake do not fully explain the discrepancies between studies.

Accordingly, inconsistent findings within the literature may also be explained by the characteristics of the protein intervention. Specifically, the magnitude of a) protein deviation

from baseline, and b) daily protein intake. In support, as frequently reported in this thesis, the breakpoint for maximal protein-induced stimulation of MPS in older adults has been established at 0.4 g/kg (Moore et al. 2015), suggesting this dietary modification may be required to augment the adaptive response to RE in older adults (Park, Choi, and Hwang 2018). Indeed, two studies that demonstrated synergistic effects in healthy older adults deviated dietary protein by 0.5-0.55 g/kg/d (Bell et al. 2017, Junior et al. 2018). This is in contrast to those that failed to observe additive effects, of whom deviated dietary protein by ≤0.3 g/kg/d (Arnarson et al. 2013, Dulac et al. 2020, Gryson et al. 2014, Holwerda et al. 2018, Kirk et al. 2019, 2020, Kukuljan et al. 2009, Leenders et al. 2013, Maltais, Ladouceur, and Dionne 2016, Verdijk et al. 2009a).

Furthermore, studies in sarcopenic/functionally impaired older adults that reported null findings also deviated dietary protein intake by ≤0.4 g/kg/d (Chalé et al. 2013, Maltais, Ladouceur, and Dionne 2016, Ottestad et al. 2017), or those studies where dietary intake data was not reported, supplemented dietary protein either only post-exercise on training days (Englund et al. 2018, Fielding et al. 2017), or daily at a dose of ≤20 g/d [Kim et al. 2012, Shahar et al. 2013 (for men only)]. These dosing regimens were likely insufficient to elicit synergistic effects. A meta-regression conducted by Morton and colleagues (2016) also reported the dose of dietary protein required for maximal accretion of SMM during RE training in healthy adults to be 1.6 g/kg/d. This level of dietary protein was only achieved by healthy participants in the study by Bell et al. (2017). Taking together the totality of evidence, a dietary modification of ≥0.4 g protein/kg/d and a protein intake of 1.6 g/kg/d may be required to elicit synergistic effects in healthy older adults. Further research is required to confirm this theory.

Limitations of many of the aforementioned studies was the failure to include a protein only group. In fact, only 7/33 studies cited investigated the synergistic effects compared to both RE and dietary protein alone over a ≥10-week period (de Carvalho Bastone et al. 2020, Gryson et al. 2014, Kim et al. 2012, Kukuljan et al. 2009, Shahar et al. 2013, Kirk et al. 2020, Verreijen

et al. 2017). Since dietary protein was increased by ≤0.3 g/kg/d in these studies, the synergistic effects compared to each intervention alone utilising the optimal protein dose is currently unknown. Recently, it has been reported not all older adults are willing or able to perform RE (Dismore et al. 2020); thus, collection of such data is vital to determine the effectiveness of this nutrient in these groups. Furthermore, only one of the abovementioned 4-arm RCTs included a placebo or energy-matched control product (Gryson et al. 2014), which has been recommended by an expert working group for trials investigating the effectiveness of interventions to treat or prevent sarcopenia (Reginster et al. 2016). Lack of such experimental control significantly increased the risk of bias in these studies (i.e., participants in the non-protein groups potentially increasing their dietary protein intake). A 4-arm, double-blind, RCT is therefore warranted to robustly investigate the synergistic effects of RE and dietary protein (at a daily intake of 1.6 g/kg/d) compared to RE alone, dietary protein alone, and control in healthy older adults.

Table 2.4 Summary of intervention studies investigating the effects of resistance exercise combined with dietary protein on skeletal muscle/fat-free mass, muscle strength and physical function in older adults

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Arnarson et al. (2013)	161 older adults (age: 65–91 y)	**Whey protein:** 20 g protein, 20 g carbohydrate, 1 g fat (after each RE session only) **Carbohydrate:** 40 g carbohydrate, 1 g fat (after each RE session only) **Both groups performed:** RE 3 times/week (75-80% 1RM, 6-8 repetitions per set)	**Whey protein:** 1.0 ± 0.3 **Carbohydrate**: 0.9 ± 0.3	FFM, maximal strength and physical function ↑ after RE but whey protein did not provide an additive effect. Protein deviation from baseline was 0.06 g/kg/d in the whey protein group
Bell et al. (2017)	49 healthy older men (age: 73 ± 1 y) 20-week, double-blind RCT split into 2 phases: 6-weeks nutrition only + 12-weeks exercise + nutrition	**SUPP:** 30 g whey protein, 1500 mg n−3 PUFA, 2.5 g creatine, 500 IU vitamin D and 400 mg calcium (twice daily) **CON:** 22 g maltodextrin (twice daily) **Both groups performed:** twice weekly RE at 80% 1RM (8 repetitions per set) + HIIT training once weekly (10 x 60 s intervals at 90% max HR)	**SUPP:** 1.1 ± 0.3 **Control:** 1.2 ± 0.3	No augmented effect of whey protein supplementation on FFM in phase 2 (exercise). Upper-body strength ↑ following 12-weeks exercise greater in the SUPP group compared to the CON group. Protein intake during the intervention ↑ to 1.6 ± 0.4 g/kg/d in the SUPP group (0.5 g/kg/d deviation from baseline)
Candow et al. (2006)	38 older men (age: 59-76 y) 12-week double-blind RCT	**Protein before training:** 0.3 g/kg protein before training + 0.63 g/kg maltodextrin after training **Protein after training:** 0.63 g/kg maltodextrin before training + 0.3 g/kg protein after training **Placebo before and after training:** 0.63 g/kg maltodextrin before and after training **All groups performed:** 3 days/week RE (3 x 10 repetitions at 70% 1RM)	Not reported	Protein supplementation before or after RE had no effect on SMM or strength

Table 2.4 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Chalé et al. (2013)	80 mobility-limited adults (age: 70–85 y) 24-week RCT	**Whey protein:** 20 g whey protein, 25 g maltodextrin, 1 g fat (twice daily) **Control:** isocaloric supplement containing 45 g maltodextrin and 1 g fat (twice daily) **Both groups performed:** RE (3 sets of 12 repetitions at 80%1RM) 3 times/week	**Whey protein:** ~0.97 **Control:** ~1.0	FFM, muscle CSA and stair climbing performance ↑ in both groups. Whey protein did not elicit additional benefits. Protein intake ↑ to 1.17 g/kg/d (0.2 g/kg/d spread from baseline) in the whey protein group.
Daly et al. (2014)	100 women living in retirement villages (age: 60–90 y) 4-month cluster randomized controlled trial	**Meat:** 160 g (cooked) lean red meat 6 days/week **Control:** 1 serving of pasta or rice per day **Both groups performed:** RE twice weekly (14-16 'somewhat hard' on Borg scale, 3 sets of 8-12 repetitions)	**Meat:** ~1.04 **Control:** ~1.11	Greater ↑ in FFM and muscle strength were observed in the meat compared to the control group. Protein intake during the intervention was 1.29 ± 0.30 g/kg/d (deviation from baseline of ~0.25 g/kg/d) in the meat group
de Carvalho Bastone et al. (2020)	69 older adults with dynapenia (age: 75.9 ± 6.7 y) 12-week RCT	**RE + supplement:** home-based RE 3 times/week (80% 1RM, 3 sets of 8 repetitions) + 40 g protein supplementation once daily **RE:** home-based RE 3 times/week (80% 1RM, 3 sets of 8 repetitions **Supplement:** 40 g protein supplementation once daily **Control:** maintained habitual diet and physical activity	<1.0 for all participants	RE ↑ handgrip strength, gait speed and sit-to-stand test performance, but no differences occurred between RE groups
Dulac et al. (2020)	60 elderly men (age: 69 ± 7 y) 12-week double-blind RCT	**Fast protein supplementation:** 10 g whey 3 times/day **Slow protein supplementation:** 10 g casein 3 times/day **Placebo:** isocaloric amount of maltodextrin **All groups performed:** mixed power training: RE: 3 times/week (80% 1RM, 3 sets of 10-12 repetitions) and functional exercises (3 sets of 10 repetitions, 8 intensity on Borg scale)	**Fast protein:** 1.40 ± 0.31 **Slow protein:** 1.34 ± 0.45 **Placebo:** 1.48 ± 0.36	All groups ↑ FFM, MQ and functional capacity but no differences were observed between groups. Protein intake ↓ in both the fast and slow protein groups during the intervention (1.24 ± 0.40 and 1.22 ± 0.40 g/kg/d at 12 weeks, respectively)

Table 2.4 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Englund et al. (2018)	149 mobility limited (SPPB ≤9) older adults (age: 78.5 ± 5.4 y) 24-week double-blind RCT	**Supplement:** 20 g whey protein, 800 IU vitamin D and 350 mg of calcium post-exercise on training days **Placebo:** non-nutritive sweetened drink providing 30 kcal per serving **Both groups performed:** strength exercises 3 times/week (intensity: 15–17 on Borg Scale)	Not reported	Both groups ↑ muscle strength, FFM and thigh composition. No differences occurred between groups
Fielding et al. (2017)	*Same as above	*Same as above	Not reported	No differences in physical function (SPPB or gait speed) occurred between groups
Gryson et al. (2014)	48 healthy sedentary men (age: 60.8 ± 0.4 y) 16-week double-blind RCT	**Exercise + leucine enriched fortified milk:** RE + AE 3 times/week (RE: 50-80% 1RM, 3 sets of 8-12 repetitions; AE: 80% max HR) + fortified drink containing 10 g soluble milk proteins rich in leucine **Exercise + fortified milk:** RE + AE 3 times/week (RE: 50-80% 1RM, 3 sets of 8-12 repetitions; AE 80% max HR) + fortified drink containing 10 g milk protein **Exercise + placebo:** RE + AE 3 times/week (RE: 50-80% 1RM, 3 sets of 8-12 repetitions; AE 80% max HR) + placebo drinking containing 4 g milk protein **Fortified milk:** Fortified milk containing 10 g soluble milk proteins rich in leucine **Placebo:** placebo drink containing 4 g milk protein	Not reported	↑ in appendicular and dominant leg FFM, and quadricep strength occurred in both the RE + fortified milk and RE + placebo groups. No differences were observed between groups
Holm et al. (2008)	29 older women (55 ± 1 y) 24-week double-blind RCT	**Nutrient:** supplement containing 10 g whey protein, 31 g carbohydrate, 1 g fat, 200 IU vitamin D and 250 mg calcium after RE **Placebo:** 6 g carbohydrate and 12 mg calcium after RE **Both groups performed:** RE 3 times/week (3-5 sets x 8-15 reps at 8-10RM)	**Nutrient:** ~1.04 **Placebo:** ~0.89	FFM, muscle fibre CSA and strength ↑ in both groups but no significant differences occurred between groups. The nutrient supplement was ineffective at increasing dietary protein intake

Table 2.4 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Holwerda et al. (2018)	41 healthy older men (age: 70 ± 1 y) 12-week double-blind RCT	**Protein:** 21 g whey protein (twice daily) **Placebo:** Energy matched placebo (24.5 g carbohydrate, 5.8 g fat) (twice daily) **Both groups performed:** RE 3 times/week at 80% 1RM (10 repetitions per set)	**Protein:** 1.14 ± 0.05 **Placebo:** 1.19 ± 0.06	Maximal strength and FFM ↑ after RE but protein supplementation did not augment the effects. Protein intake during the intervention was 1.43 ± 0.04 g/kg/d (0.29 g/kg/d deviation from baseline) in the protein group
Hofmann et al. (2016)	91 older women (age: 65.0–92.2 y) 6-month RCT	**RE + nutritional supplementation:** RE twice/week at moderate-high intensity using TheraBand®, 2 x 15 repetitions per exercise) + 20.7 g protein every morning and after each session **RE:** RE twice/week at moderate-high intensity using TheraBand®, 2 x 15 repetitions per exercise) **Control:** cognitive training twice weekly	Not reported	MQ and physical performance ↑ following RE but no differences occurred between groups
Junior et al. (2018)	31 resistance-trained older women (age: ≥60 y) 12-week RCT	**Protein:** 35 g whey protein after each RE session **Placebo:** 35 g maltodextrin after each RE session **Both groups performed:** RE 3 times/week (3 sets x 8-12 RM)	**Protein:** 0.85 ± 0.1 **Placebo:** 0.81 ± 0.1	Greater ↑ in SMM and total strength were observed in the protein group. Protein intake ↑ to 1.4 ± 0.1 g/kg/d at 12 weeks in the protein group (0.55 g/kg/d deviation from baseline)
Kang et al. (2019)	115 pre-frail and frail older adults mean age: 77.3 y) 12-week multicentre case-control parallel group study	**Active:** home-based RE (no intensity or frequency reported) + 32.4 g whey protein daily **Control:** home-based RE (no intensity or frequency reported)	Not reported	Active group ↑ handgrip strength, gait speed and chair raise performance greater than the control group.

Table 2.4 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Kim et al. (2012)	151 sarcopenic older women (age: >75 y) 12-week RCT	**Exercise + AA:** exercise including strength training twice weekly (8 repetitions, 12-14 Borg scale intensity) + 3 g EAA twice daily **Exercise:** exercise including strength training twice weekly (8 repetitions, 12-14 Borg scale intensity) **AA:** 3 g EAA twice daily **Control (health education):** health education once a month	Not reported	Exercise ↑ leg muscle mass, usual and maximum gait speed, and knee extension strength but no additive effects were observed following EAA
Kirk et al. (2019)	46 community-dwelling healthy older adults (age: 68 ± 5 y) 16-week RCT	**Exercise + protein:** twice weekly RE, once weekly functional exercise + individualised whey protein supplementation to achieve 0.5 g/kg/meal protein (supplement 3 times/d) **Exercise:** twice weekly RE, once weekly functional exercise	**Exercise + protein:** 1.2 ± 0.4 **Exercise:** 1.10 ± 0.4	Maximal strength and physical function ↑ following RE but no additive effects were observed following protein supplementation. Protein intake during the intervention ↑ to 1.5 ± 0.7 g/kg/d (0.3 g/kg/d deviation from baseline) in the protein group
Kirk et al. (2020)	100 community-dwelling healthy older adults (age: 69 ± 6 y) 16-week RCT	**Exercise + protein:** twice weekly RE, once weekly functional exercise + individualised whey protein supplementation to achieve 0.5 g protein/kg/meal **Exercise:** twice weekly RE, once weekly functional exercise **Protein:** individualised whey protein supplementation to achieve 0.5 g protein/kg/meal **Control:** maintained habitual dietary and physical activity levels	**Exercise + protein:** 1.16 ± 0.4 **Exercise:** 1.10 ± 0.4 **Protein:** 0.99 ± 0.2 **Control:** 0.98 ± 0.3	Exercise ↑ myoelectrical muscle fatigue but not SMM. Protein supplementation had no additive effects. Protein intake during the intervention ↑ to 1.5 ± 0.7 g/kg/d (0.3 g/kg/d deviation from baseline) in the exercise + protein group
Kukuljan et al. (2009)	180 community-dwelling older men (age: 50-79 y) 18-month RCT	**Exercise + milk:** RE 3 days/week (2 sets x 8-12 repetitions at 80-85% 1RM) + 2 x 200 mL/d fortified milk **Exercise:** RE 3 days/week (2 sets x 8-12 repetitions at 80-85% 1RM) **Milk:** 2 x 200 mL/d fortified milk **Control:** maintained habitual physical activity and diet	**Exercise + milk:** 1.26 ± 0.32 **Exercise:** 1.32 ± 0.32 **Milk:** 1.23 ± 0.28 **Control:** 1.33 ± 0.31	RE ↑ muscle strength, gait speed and muscle CSA. Milk did not elicit supplemental effects. Protein intake ↑ by 0.26 g/kg/d (to 1.52 g/kg/d) at 12 months but did not change at 18 months in the exercise + milk group

Table 2.4 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Leenders et al. (2013)	53 healthy older adults (age: 70 ± 1 y) 24-week double-blind RCT	**Protein:** 15 g milk protein supplement daily after breakfast **Placebo:** 7.13 g lactose (not isocaloric) daily after breakfast **Both groups performed:** RE 3 times/week at 75-80% 1RM (8-10 repetitions)	1.2 ± 0.1 in women and 1.1 ± 0.0 in men	Maximal strength, FFM and physical performance ↑ in both groups with no differences between groups. Protein intake did not change during the intervention
Maltais, Ladouceur, and Dionne (2016)	26 sarcopenic elderly men (age: 60-75 y) 12-week double-blind RCT	**Milk protein:** 13.53 g protein, 7 g EAA, 37.5 g carbohydrate, 3.8 g fat (270 kcal) after each exercise session **EAA:** soy beverage enriched with EAA powder (12 g protein, 7 g EAA – 3.5 g from leucine, 39 g carbohydrate, 5.3 g fat - 252 kcal) after each exercise session **Control:** rice milk (59.5 g carbohydrate, 3.75 g fat – 290 kcal) after each exercise session **All groups performed:** RE 3 times/week (80% 1RM, 8 repetitions per set, 3 sets per exercise)	**Milk:** 1.04 ± 0.20 **EAA:** 1.26 ± 0.20 **Control:** 1.32 ± 0.45	FFM and maximal strength ↑ in all groups with no differences between groups. Protein intake did not change over the duration of the study in the EAA group (week 12:1.21 ± 0.3 g/kg/d – deviation from baseline, -0.05 g/kg/d) or the milk group (week 12: 0.95 ± 0.3 g/kg/d – deviation from baseline, -0.09 g/kg/d)
Oesen et al. (2015)	171 older adults (age: 82.8 ± 6.0 y) 6-month RCT	**RE + nutrient supplementation:** elastic band RE 2 times/week (1 set x 15 repetitions) + 20.7 g protein supplement every morning and after each training session **RE only:** elastic band RE 2 times/week (1 set x 15 repetitions) **Control**: cognitive training twice weekly	Not reported	RE ↑ physical function compared to control. No differences were observed between groups

Table 2.4 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Ottestad et al. (2017)	36 healthy, community-dwelling older adults with reduced strength and gait speed (age: ≥70 y) 12-week double-blind RCT	**Protein-enriched milk:** 2 x 0.4 L/d milk (2 x 20 g/d protein) **Control:** isocaloric carbohydrate drink (2 x 0.4 L/d) **Both groups:** performed 30 min/d physical activity including strength exercises	**Protein-enriched milk:** 1.0 ± 0.3 **Control:** 1.0 ± 0.3	No additive effect of milk supplementation on SMM, strength or physical function occurred. Protein intake ↑ to 1.4 ± 0.5 g/kg/d at 12 weeks (0.4 g/kg/d deviation from baseline) in the milk group
Rondanelli et al. (2016)	130 sarcopenic older adults (age: 80.3 y) 12-week double-blind RCT	**Intervention:** 22 g whey protein, 10.9 g EAA and 100 IU vitamin D (once daily) **Placebo:** isocaloric amount of maltodextrin (once daily) **Both groups performed:** 20 min moderate intensity (12-14 on Borg scale) RE programme (using bands) 5 times/week	**Intervention:** ~0.93 **Placebo:** ~0.97	Whole-body FFM, relative SMM and handgrip strength ↑ greater in the intervention compared to placebo group
Rondanelli et al. (2020)	140 sarcopenic older adults (81 ± 6 y) 4-8-week RCT (minimum 4 weeks, maximum 8 weeks)	**Experimental:** 20 g whey protein, 2.8 g of leucine, 9 g of carbohydrate, 3 g of fat, 800 IU of vitamin D **Control:** isocaloric amount of maltodextrin **Both groups:** completed a physical rehabilitation programme including strength exercises	**Experimental:** 0.79 **Control:** 0.75	Whey protein resulted in greater ↑ in gait speed, SMM, SPPB, chair stand test performance and handgrip strength compared to control
Shahar et al. (2013)	65 sarcopenic older adults (age: 60-74 y) 12-week RCT	**Exercise + protein:** resistance band exercise twice weekly at moderate intensity + soy protein drink (20 g/d for men and 40 g/d for women) to achieve 1.5 g protein/kg/d **Exercise:** resistance band exercise twice weekly at moderate intensity **Protein:** soy protein drink (20 g/d for men and 40 g/d for women) to achieve 1.5 g protein/kg/d **Control:** relaxation programme twice weekly	**Exercise + protein:** 0.85 **Exercise:** 0.82 **Protein:** 0.92 **Control:** 0.91	No additive effect of protein supplementation was observed for FFM or muscle strength

Table 2.4 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Tieland et al. (2012b)	62 frail older adults (age: 78 ± 1 y) 24-week double-blind RCT	**Protein:** 15 g milk protein supplement (twice daily) **Placebo:** energy matched placebo (no protein – twice daily) **Both groups performed:** twice weekly RE at 75% 1RM (8-10 repetitions, 3-4 sets per exercise)	**Protein:** 1.0 (0.9-1.1) **Placebo:** 1.0 (0.9-1.1)	FFM ↑ greater in the protein compared to placebo group. Maximal strength ↑ in both groups but no additive effect was observed with protein supplementation
Thomson et al. (2016)	179 healthy older adults (age: 61.5 ± 7.4 y) 12-week RCT	**High dairy protein diet:** 1.0 g/kg/d protein diet + ~27 g/d dairy based protein shake **High non-dairy protein diet:** 1.0 g/kg/d protein diet + ~27 g/d soy protein shake **Usual protein diet:** 1.0 g/kg/d protein diet + carbohydrate energy matched foods **All groups performed:** RE 3 days/week (3 sets of 8-12 repetitions at 8-12RM)	~1.0 maintenance in all diets	Additional protein (from dairy or soy) did not provide additive benefits on strength, FFM or physical function. Protein intake ↑ to 1.42 ± 0.14 and 1.45 ± 0.14 g/kg/d in the high and non-dairy groups, respectively
Verdijk et al. (2009a)	26 healthy older men (age: 72 ± 2 y) 12-week double-blind RCT	**Protein:** 10 g protein pre- and post-session) **Placebo:** water only (pre- and post-session)	**Protein:** 1.1 ± 0.1 **Placebo:** 1.1 ± 0.1	Maximal strength and muscle mass ↑ in both groups but protein supplementation provided no augmented effects. Protein intake did not change over the intervention period (1.1 ± 0.1 g/kg/d at 12 weeks in the protein group)
Verreijen et al. (2015)	80 older adults with obesity (age: ≥55 y) 13-week double-blind RCT	**Intervention:** high whey-protein- (20.7g), leucine- (2.8g) and vitamin D-enriched supplement once daily on non-exercise days + 3 times/day on exercise days **Control:** isocaloric carbohydrate supplement **Both groups:** received a hypocaloric diet (-600 kcal/d) and performed RE 3 times/week (3 sets x 20 repetitions – intensity not reported)	N/a	Appendicular FFM ↑ greater in the intervention compared to the control group. Muscle strength and physical performance ↑ in both groups without differences between groups. Protein intake during the intervention was 1.11 ± 0.28 g/kg/d in the intervention group and 0.85 ± 0.24 g/kg/d in the control group

Table 2.4 continued

Study	Participants, duration, and design	Groups	Habitual protein intake (g/kg/d)	Key findings
Verreijen et al. (2017)	100 older adults who were overweight or with obesity (age: 55–80 year) 10-week RCT	**Exercise + Protein:** RE 3 days/week (2-3 sets of 50 s per exercise) + dietary advice to achieve 1.3 g protein/kg/d **Exercise:** RE 3 days/week (2-3 sets of 50 s per exercise) + dietary advice to achieve 0.8 g protein/kg/d **Protein:** dietary advice to achieve 1.3 g protein/kg/d **Control:** dietary advice to achieve 0.8 g protein/kg/d **All groups:** followed a hypocaloric diet (-600 kcal/d)	**Exercise + protein:** 1.00 ± 0.31 **Exercise:** 0.93 ± 0.30 **Protein:** 0.92 ± 0.34 **Control:** 0.95 ± 0.36	No differences in FFM, handgrip strength or gait speed occurred between groups. Protein intake during the intervention was lower than targeted (1.02 ± 0.36 and 1.02 ± 0.35 g/kg/d in the protein and exercise + protein groups, respectively)
Yamada et al. (2019)	112 older adults with sarcopenia or dynapenia (age: 84.2 ± 5.5 y) 12-week RCT	**Exercise + Nutrition supplementation:** supervised body weight RE twice weekly + instructed to perform daily exercise at home (3 sets of 30 repetitions) + 10 g whey protein and 800 IU vitamin D once daily **Exercise:** supervised body weight resistance exercise twice weekly + instructed to perform daily exercise at home (3 sets of 30 repetitions) **Nutrition supplementation:** 10 g whey protein and 800 IU vitamin D once daily **Control:** maintained habitual diet and physical activity	Not reported	Exercise + nutrition supplementation ↑ muscle quality greater than exercise and nutrition supplementation alone
Zdzieblik et al. (2015)	53 older men with class I or class II sarcopenia (age: 72.2 ± 4.7 y) 12-week double-blind RCT	**Treatment:** 15 g/d collagen peptides **Placebo:** silicon dioxide **Both groups performed:** RE 3 times/week, 8-10 RM	**Mean sample:** 0.91	Treatment group ↑ FFM, isokinetic quadriceps strength and ↓ FM greater than the placebo group

↑, increased; ↓, decreased; AE, aerobic exercise; FFM, fat-free mass; HIIT, high intensity interval training; HR, heart rate; MQ, muscle quality; n-3 PUFA, omega 3 polyunsaturated fatty acids; RCT, randomised controlled trial; RE, resistance exercise; SMM, skeletal muscle mass; SPPB, short physical performance battery.

2.2.10.4 Effects of resistance exercise and dietary protein on hormone function and systemic inflammation

It has been proposed that the sarcopenic attenuating effects of RE and dietary protein may be partially explained by changes in circulating hormones and reductions in systemic inflammation (Xia et al. 2017, Ziaaldini et al. 2017). The following sections will review the effects of these interventions on endocrine and inflammatory markers related to this thesis.

2.2.10.4.1 Insulin-like growth factor 1

Studies conducted on both young (Borst et al. 2001, Gregory et al. 2013, Kraemer et al. 1990) and older adults (Cassilhas et al. 2010, de Souza Vale et al. 2009, Singh et al. 1999, Formica et al. 2020) have observed increases in circulating IGF-1 concentration following RE. Acutely, RE has been shown to increase serum IGF-1 concentration by ~15% (Gregory et al. 2013, Tsai et al. 2018). These findings are supported by a recent meta-analysis of longitudinal studies in older adults (Jiang et al. 2020). However, there is also evidence demonstrating either no change (Walsh et al. 2015) or a decrease in IGF-1 following RE in older adults (Arnarson et al. 2015, Ogawa et al. 2010). The lack of increase in two of these studies might be explained by an insufficient RE intensity (<70% 1RM) (Ogawa et al. 2010, Walsh et al. 2015), as studies that have reported increases in IGF-1 have generally employed a high intensity RE intervention (75-85% 1RM) (Stein et al. 2018). In the other study, the authors hypothesised that the decrease in circulating IGF-1 was due to a redistribution of IGF-1 into skeletal muscle (Arnarson et al. 2015).

In addition to RE, several RCTs in older adults have observed increases of 7-12% in circulating IGF-1 concentration following increased dietary protein intake (Bauer et al. 2015, Bo et al. 2019, Mehlsen et al. 2017, Zhu et al. 2011). Furthermore, when combined with RE, some (Daly et al. 2014, Rondanelli et al. 2016), but not all studies (Formica et al. 2020, Holwerda et al. 2018, Shahar et al. 2013), have reported supplemental increases compared to RE alone. Disparities in these studies may be explained by synergistic (or lack thereof) effects on SMM,

as studies that reported greater increases in IGF-1 also reported additive effects on SMM (Daly et al. 2014, Rondanelli et al. 2016). This is in contrast to studies that did not observe additive increases in IGF-1 (Formica et al. 2020, Holwerda et al. 2018, Shahar et al. 2013).

Whilst evidence demonstrates an increase in IGF-1 following RE alone and combined with dietary protein, recent studies suggest these increases may not be required nor reflect the incremental activation of intracellular muscle signalling and the subsequent adaptive response of skeletal muscle (Morton et al. 2016, West et al. 2012). For example, in healthy young adults, changes in IGF-1 were not related to changes in muscle hypertrophy or strength following RE training, but muscle androgen receptor content was (Morton et al. 2018b). Data from this study suggests the relative increase in SMM following RE training may be underpinned by local intramuscular factors. Nevertheless, as the above study was conducted in young adults, further work in older adults is required to determine whether muscle hypertrophy following RE is related to changes in circulating IGF-1 concentration.

2.2.10.4.2 Cortisol

The effects of RE on the adaptative cortisol response in older adults is ambiguous. Kraemer et al. (1999) reported declines in fasting cortisol concentration after 3 and 10 weeks of RE. In agreement, two studies reported decreases in fasting cortisol during RE training in older adults (Häkkinen et al. 2002, Izquierdo et al. 2003). However, it is important to note these decreases were observed only during the intervention [i.e., after 16 but not 24 weeks post-intervention (Häkkinen et al. 2002), and only in the final 8 weeks of RE (Izquierdo et al. 2003)], but no significant differences were observed between pre- and post-intervention concetrations. The lack of pre-post intervention change is consistent with other studies in older adults (Ahn and Kim 2018, Häkkinen et al. 2000, 2001). Equivocal findings may be explained by methological issues, particularly the timing of cortisol measurement (Hayes et al. 2016). As preivously discussed in **section 2.2.8.4**, cortisol secretion rises 50-60% 30-40 min after awakening, known as the CAR (Anderson and Wideman 2017). Thus, minor differences in the timing of

cortisol measurement between studies might have resulted in a different component of the CAR being analysed.

In addition to RE, the macronutrient composition of the diet may also influence cortisol secretion. Data from two acute studies suggests dietary protein may increase cortisol concentration (Slag et al. 1981, Gibson et al. 1999). In contrast, others have demonstrated either no differences (Lemmens et al. 2011) or a lower cortisol concentration following a high protein compared to a high carbohydrate meal/diet (Vicennati et al. 2002, Martens et al. 2010). Additionally, longitudinal data has reported no effects of oral AA supplementation, or RE plus protein supplementation, on fasting cortisol concentration (Chaborski et al. 2015, Park et al. 2019). Although further longitudinal research is needed, particularly in older adults, available data thus far suggests dietary protein does not negatively affect the HPA axis.

A significant limitation of studies investigating the effects of RE and dietary protein on the adaptive cortisol response is the sole measurement of fasting cortisol concentration. It is well-known cortisol secretion follows a diurnal rhythm, and recent studies have shown elevated evening (2000 h) cortisol concentration in particular is associated with sarcopenia prevalence (Gonzalez et al. 2018). Further, increases in the magnitude of the fasting CAR may actually be beneficial for health-related outcomes in older adults (Anderson and Wideman 2017). Therefore, further research examining the diurnal cortisol response following RE and dietary protein is warranted to fully understand the physiological response to these interventions.

2.2.10.4.3 Myostatin

Several studies have reported decreases in fasting concentrations of both plasma and serum myostatin following RE training in middle- and older-aged adults (Bagheri et al. 2019, 2020, Binns et al. 2017, Negaresh et al. 2019, Shabani and Izaddoust 2018). For example, serum myostatin has been shown to decrease by 0.36 ng/mL in middle-aged adults following 8 weeks of RE (Bagheri et al. 2019). The same research group also reported decreases in sarcopenic

older men following RE over the same timeframe (Bagheri et al. 2020). However, not all studies have observed reductions in myostatin (Hofmann et al. 2016, Kim et al. 2007, Planella-Farrugia et al. 2019), which may be explained by an insufficient RE intensity (Hofmann et al. 2016, Planella-Farrugia et al. 2019), and due to extreme pre-post test myostatin variability amongst participants (Kim et al. 2007).

Following an acute bout of RE, protein supplementation has been shown to hinder the decrease in myostatin messenger RNA (mRNA) in middle and older-aged men (Hulmi et al. 2008). Another study by the same research group confirmed these findings in young adults (Hulmi et al. 2009). More recently, Sniijders et al. (2014) demonstrated acute dietary protein restriction decreased myostatin mRNA expression for a longer duration following a single bout of RE compared to when ample protein was consumed. The authors speculated these findings may signify a compensatory mechanism to allow sufficient muscle reconditioning during times of protein restriction. In addition to acute studies, longitudinal RCTs in both young (Paoli et al. 2015) and older adults (Planella-Farrugia et al. 2019) have reported either increases or no change in plasma and serum concentrations following increased dietary protein intake during RE training, respectively. Taken together, when dietary protein is sufficient, current evidence suggests that the mechanistic reduction in myostatin concentration may not be required because whey protein aids net protein balance following RE (Tipton et al. 2007). Further longitudinal studies in older adults are required to confirm this hypothesis.

2.2.10.4.5 Systemic inflammation

The effects of RE on markers of systemic inflammation, particularly in the elderly, is controversial. Intervention studies (Nahas, Maestá, and Burini 2014, Nunes et al. 2016, Ogawa et al. 2010, Tomeleri et al. 2016) and a recent meta-analysis (Sardeli et al. 2018) have reported significant decreases; however, others have not observed these effects (Azizbeigi et al. 2015, Bell et al. 2018, Grosicki et al. 2020, Hangelbroek et al. 2018). Inconsistent findings might be explained by differences in participant characteristics (i.e., age, sex, health status

and baseline inflammatory profile), the RE training protocol (i.e., training intensity, volume and duration) and changes in body composition components (i.e., increases in SMM and reductions in FM). In support of the latter, a sub-analysis in the meta-analysis by Sardeli et al. (2018) reported a larger effect size (-1.26 vs. -0.32) for decreased CRP when skeletal muscle hypertrophy occurred compared to when it was maintained. Several studies have also reported associations between RE-induced changes in SMM and markers of systemic inflammation (Mavros et al. 2014, Ogawa et al. 2010, Tomeleri et al. 2018). Furthermore, others have reported a relationship between reductions in FM and markers of systemic inflammation following RE (Mavros et al. 2014, Tomeleri et al. 2016, Wanderley et al. 2013), and only studies that observed a reduction in FM in the meta-analysis by Sardeli et al. (2018) also observed reductions in TNF-α. Based on these findings, RE may be a potent stimulus to reduce systemic inflammation, but the effects may be mediated by changes in body composition.

Several studies have investigated the independent effects of dietary protein on markers of systemic inflammation in older adults (Bell et al. 2018, de Aguilar-Nascimento, Silveira, and Dock-Nascimento 2011, Liberman et al. 2019, Sugawara et al. 2012, Solerte et al. 2008, Wright et al. 2018). All but one of these studies reported a reduction in inflammatory markers (Wright et al. 2018). For example, twice daily consumption of a vitamin D- and leucine-enriched whey protein supplement for 13 weeks attenuated age-related increases in inflammation in sarcopenic older adults (Liberman et al. 2019). Furthermore, Bell and colleagues (2018) reported modest (~1-3%), but significant reductions in IL-6 and TNF-α following 6 weeks consumption of a multi-ingredient whey protein-based supplement. Mechanistically, protein-induced reductions in MPB and the subsequent anti-inflammatory effect may decipher these beneficial effects (Bordoni et al. 2017).

Regrettably, the combined effects of RE and dietary protein on markers of systemic inflammation has not been well studied in older adults. Nevertheless, studies that have been

conducted have reported synergistic reductions in both healthy (Bell et al. 2017, Daly et al. 2014) and sarcopenic older adults (Rondanelli et al. 2016). Although, one recent study did not report augmented effects (Formica et al. 2020). The authors hypothesised this null finding may have been due to a high habitual protein intake (1.24 g/kg/d) of participants. Therefore, based on the abovementioned evidence, RE and dietary protein may synergistically attenuate age-related systemic inflammation. However, whilst synergistic effects have been observed compared to RE alone, these studies did not include a protein only group, highlighting an area for further research.

2.3 Part 2: Body composition, energy metabolism and metabolic health

2.3.1 Introduction

As reported in part 1 of this literature review (**section 2.2.4**), ageing is associated with sarcopenic muscle loss, which occurs at a rate of ~0.5-1% per annum after ~45 years of age (Janssen 2010). In addition to declines in SMM, ageing is accompanied by an increase in FM (St-Onge 2005), particularly visceral and intramuscular adiposity (Demerath et al. 2007, 2011). From around 20 years of age, %FM increases at a rate of ~0.2% per annum until the seventh decade of life and decreases thereafter (Imboden et al. 2017, Westerterp 2018a). Since 1980, obesity prevalence has increased by ~35-60% in middle- and older-aged adults (Flegal et al. 2012, Gutiérrez-Fisac et al. 2012, Howel 2012, Oreopoulos et al. 2009). Subsequently, the prevalence of metabolic disease has also risen (Wannamethee and Atkins 2015), which has resulted in a 30% increase in healthcare expenditure (König et al. 2015, Withrow and Alter 2011). The coexistence of sarcopenia and obesity, termed sarcopenic obesity (Baumgartner 2000), is of great concern for the health of the elderly population as they act synergistically, exacerbating the negative effects of one another (Batsis et al. 2015, Lee et al. 2016, Scott et al. 2018). Hence, preventative interventions that preserve SMM and attenuate adiposity are of critical importance for public health.

2.3.2 Energy balance

Body composition changes with age are ascribed to alterations in energy balance (St-Onge and Gallagher 2010). Obeying the first law of thermodynamics, energy balance [or rate of energy storage (ES)] is equal to the difference between rates of EI and EE (Shook et al. 2018). Energy intake comprises the chemical energy from food and drink consumption, which incorporates the three major macronutrients with the following energy densities: carbohydrate (~4 kcal/g), protein (~4 kcal/g) and fat (~9 kcal/g), and alcohol, which has an energy density of ~7 kcal/g (Hall et al. 2012). Energy expenditure refers to energy used for any work performed and heat lost via conductive, radiant, convective and evaporation processes (Hall et al. 2012). Maintenance of energy balance and a healthy body mass throughout the ageing process is key, as a major drawback of significant weight loss is a concomitant loss of SMM, subsequently accelerating the development of sarcopenia (Houston, Nicklas, and Zizza 2009, Waters, Ward, and Villareal 2013). Additionally, weight loss leads to adaptive thermogenesis, that is, a reduction in EE greater than predicted based on reductions in body composition, thereby making long-term weight maintenance difficult (Müller, Enderle, and Bosy-Westphal 2016, Camps, Verhoef, and Westerterp 2013). Age-related declines in EE has been implicated as a significant risk factor for energy imbalance and increased FM with age (Trouwborst et al. 2018, Westerterp 2018a). The subsequent sections will review the effects of age on components of EE.

2.3.3 Total Energy Expenditure

Total energy expenditure is comprised of three major constituents: RMR, AEE and DIT (**Figure 2.4**). Resting metabolic rate consists of both the energy expended during sleep and through arousal (Manini 2010, Schoffelen and Westerterp 2008). Activity EE is subdivided into the energy expended through both volitional exercise (e.g., walking or jogging) and spontaneous physical activity (SPA) (e.g., fidgeting) (Manini 2010). Diet-induced thermogenesis is defined as the metabolic cost of processing food (Westerterp, Wilson, and Rolland 1999). Over the human lifespan, TEE increases 2-fold in the first two decades of life,

plateaus between 17-40 years of age, and dramatically decreases by ~160 kcal/decade in men and by ~100 kcal/decade in women following ~40 years of age. Age-related declines in TEE is a consequence of a decline in all components of TEE (Roberts and Rosenberg 2006); however, age-related declines in AEE explains the vast majority of the decrease (Roberts et al. 1995, Westerterp and Meijer 2001).

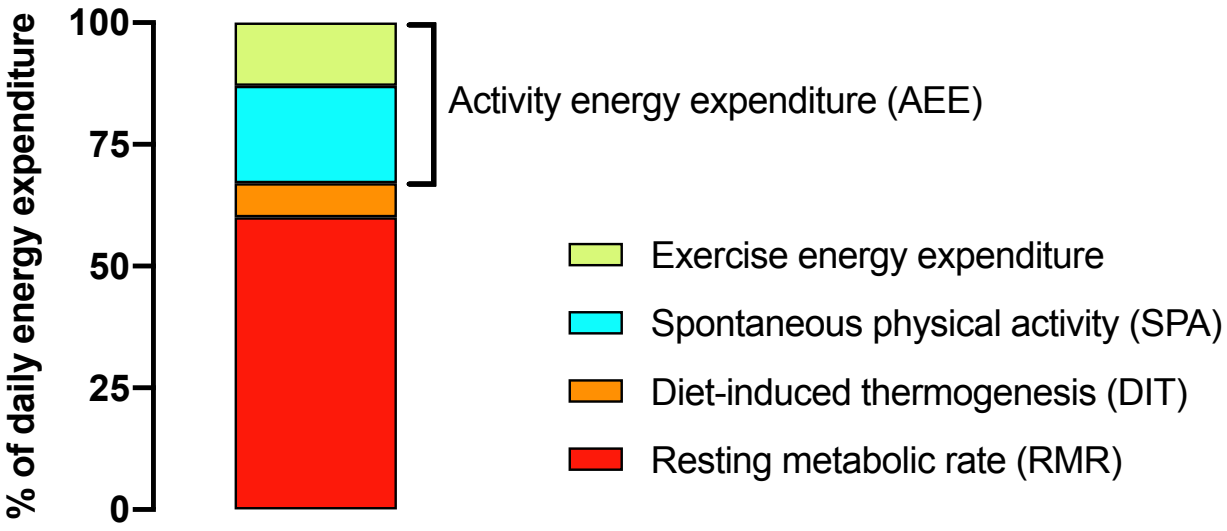

Figure 2.4 Constituents of TEE. Resting metabolic rate accounts for 60-80%, DIT uses ~10%, and AEE, which is divided into SPA and volitional exercise, is the most variable component, comprising 20-50%. Adapted from Manini (2010).

2.3.4 Resting metabolic rate

Resting metabolic rate is defined as the energy required for maintenance of organismal homeostasis and is the largest component of TEE, accounting for 60-80% (Manini 2010). Declines in RMR begin around the third decade of life and occur at a rate of 1-2% per decade (Geisler et al. 2016a, Geisler and Müller 2017). Fat-free mass accounts for 50-70% of the variance in RMR (Bosy-Westphal et al. 2003, Gallagher et al. 2000). Metabolically, FFM is heterogeneous, comprised of both chemical (mineral content, glycogen, protein and water) and anatomical (organ/tissue level) aspects (Geisler and Müller 2017). Historically, it was assumed that the mass and metabolic rates of specific organs remained constant throughout

adulthood (Elia 1992). This assumption has more recently been shown to be incorrect, as both the chemical and anatomical components of FFM decline with age (He et al. 2009, Müller et al. 2013). This led to age-predicted, organ-specific equations to be developed (Wang et al. 2010). Recently, Geisler and Müller (2017) published data demonstrating SMM accounts for 46.5% and 43.3% of FFM in young and older adults, respectively, accounting for ~20% of RMR. Interestingly, RMR normalised to FFM was lower in older compared to younger adults (Geisler and Müller 2017). Age-related changes in the chemical as opposed to anatomical components of FFM explained this difference. When anatomical and chemical components of FFM were combined, the variance increased to 82.7%; however, 17.3% was still unexplained (Geisler and Müller 2017). Differences in rates of MPS, thyroid hormones, body temperature, heart rate, sympathetic nervous system activity and sleep quality may explain the remaining variance (Geisler and Müller 2017). Thus, current evidence suggests age-related declines in RMR can mostly be explained by FFM quality, which includes both anatomical and chemical aspects.

2.3.5 Activity energy expenditure

Activity-induced EE is the most variable component of TEE, comprising 20-50% (Manini 2010). Spontaneous physical activity contributes the majority of AEE (Westerterp 2013). Typically, physical activity EE is measured as a physical activity level (PAL), calculated by dividing TEE by RMR (Westerterp 2018a). As previously stated in part 1 of this literature review (**section 2.2.8.1**), physical activity levels decline with age, whereas sedentary behaviour increases (Westerterp 2000, Meijer et al. 2001). Between the ages of 15-50, PAL has been reported to be between ~1.7-1.8, with gradual decreases observed from 50-80 years to ~1.4 (Westerterp 2018a). Of concern, a lower PAL has been associated with reduced mobility and a higher mortality risk in elderly individuals (Xue et al. 2012). Furthermore, whilst PAL decreases with age, the energetic cost of ADL increases (Schrack, Simonsick, and Ferrucci 2010, Schrack et al. 2012, 2016a). For example, Schrack et al. (2016b) established advancing age was associated with an increased metabolic cost of walking, which contributed

to declines in gait speed. Problematically, an increased metabolic cost of ADL contributes to less body movement, resulting in less AEE (Westerterp 2013), and weight gain (Schoeller, Shay, and Kushner 1997, Weinsier et al. 2002).

2.3.6 Diet-induced thermogenesis

Diet-induced thermogenesis represents the metabolic cost of digestion, absorption, transport, metabolism and storage of food (Westerterp, Wilson, and Rolland 1999). Food ingestion therefore increases EE above basal metabolism, with DIT representing ~10-15% of total daily EI in healthy young individuals consuming a typical Western diet in energy balance (Westerterp 2004). In elderly individuals, several (Du et al. 2014, Hawley et al. 2020, Jones et al. 2004, Morgan and York 1983, Schwartz, Jaeger, and Veith 1990), but not all studies (Bloesch et al. 1988, Vaughan, Zurlo, and Ravussin 1991, Visser et al. 1995) have reported decreases in DIT. Discrepancies might be explained by difficulties in measuring DIT and lack of statistical power. It is well-known that inter-individual variation in measurement of DIT is high [coefficient of variation (CV) 30-48%] (Tataranni et al. 1995, Weststrate 1993); thus, studies with small sample sizes were likely underpowered to detect changes between young and older adults. Additionally, differences in the duration of DIT measurement and macronutrient composition of the meal may also explain inconsistencies between studies (Du et al. 2014). Whilst age-related declines in DIT may be small (Du et al. 2014), the thermogenic implications may significantly contribute to increased FM. To explain, based on the findings of Du and colleagues (2014), consumption of 2,500 kcal/d would yield a DIT deficit of 22.5 kcal/d in older compared to younger adults. Extrapolated over a year, EE would be ~8,000 kcal less from purely DIT if activity and FFM were matched.

2.3.7 Substrate oxidation

In comparison to EE, age-related changes in substrate oxidation has received little research attention (Welch, Hayhoe, and Cameron 2020). This is perhaps surprising considering skeletal muscle is essential to the metabolism and oxidation of carbohydrate, fat and protein (Welch,

Hayhoe, and Cameron 2020). Nevertheless, research groups that have examined the effects of age on substrate oxidation have reported age-related reductions in basal and 24-h fat oxidation (Calles-Escandon et al. 1995, Levadoux et al. 2001, Solomon et al. 2008), decreases in exercise-induced carbohydrate oxidation and glucose utilisation (Dubé et al. 2016, Malone et al. 2019, Sial et al. 1996), and increases in 24-h protein oxidation and declines in protein balance (Davy et al. 2001). In contrast, others have reported either higher (Melanson et al. 2007) or no age-related declines in basal or 24-h fat oxidation (Davy et al. 2001, Siervo et al. 2016).

Inconsistencies may be explained by differences in participant body composition characteristics. In particular, FM, which is associated with reductions in 24-h fat oxidation (Siervo et al. 2016, Zurlo et al. 1990), was notably higher in elderly compared to younger participants in two studies that observed age-related declines (Levadoux et al. 2001, Solomon et al. 2008). Contrary to these studies, others that did not observe differences matched body composition variables between groups (Davy et al. 2001, Melanson et al. 2007). Further, although Calles-Escandon et al. (1995) reported declines in basal fat oxidation with age, this study also reported an association between fat oxidation and FFM. These findings suggest ageing *per se* does not cause declines in fat oxidation, but age-related changes in body composition, especially increases in intramuscular fat infiltration which has been shown to increase with age (Delmonico et al. 2009) and impair mitochondrial fatty acid oxidation (Batsis and Villareal 2018), may.

Age-related declines in exercise-induced carbohydrate oxidation and glucose utilisation might be explained declines in glucose transporter type 4 (GLUT-4) protein and adenosine 5'-monophosphate-activated protein kinase (AMPK) activity, which have been reported in older adults with reduced insulin sensitivity (Dela et al. 1994, Morris, Rivas, and Fielding 2010, Qiang et al. 2007). The increased protein oxidation reported by Davy and colleagues (2001) could be due to diminished AA uptake with age as a consequence of declines in SMM,

resulting in greater amounts of exogenous dietary protein being oxidised (Welch, Hayhoe, and Cameron 2020).

2.3.8 Metabolic flexibility

Metabolic flexibility is defined as the ability to adjust rates of substrate oxidation to changes in fuel availability (Kelley and Mandarino 2000). It represents the ability to switch between high rates of fat oxidation (or low RQ) under fasted resting conditions, to supressing fat oxidation and increasing carbohydrate oxidation (higher RQ) under insulin- and exercise-stimulated conditions (Goodpaster and Sparks 2017). Metabolic inflexibility has been linked with a number of health risks and pathologies such as obesity, insulin resistance and T2DM, cardiovascular disease (CVD) and cancer (Goodpaster and Sparks 2017, Rynders et al. 2018, Smith et al. 2018). Although data is limited, recent work suggests metabolic flexibility is impaired in the elderly (Smith et al. 2018). At present, the mechanisms are not fully understood; however, it is thought a more sedentary lifestyle, deleterious changes in body and muscle fibre composition, mitochondrial dysfunction, systemic inflammation and endocrinological changes with age all contribute towards metabolic inflexibility (Calçada et al. 2014, Smith et al. 2018).

2.3.9 Glucose homeostasis

Impairments in glucose homeostasis have been frequently observed with age (DeFronzo 1981, Ferrannini et al. 1996, Shimokata et al. 1991). Elevations in 2-h post oral glucose tolerance test (OGTT) glucose concentrations (Dubowitz et al. 2014), increased glycated haemoglobin (HbA1c) (Davidson and Schriger 2010, Dubowitz et al. 2014), reduced insulin sensitivity following a mixed meal (Basu et al. 2003) and during a hyperinsulinemic-euglycemic clamp (DeFronzo 1979), and increased 24-h glucose variability (Wijsman et al. 2013) have all been associated with age. However, these impairments cannot be explained by ageing *per se* (Chee et al. 2016). Similar to that of metabolic flexibility, unfavourable changes in body composition, including decreases in SMM, increases in visceral adiposity which produces

proinflammatory cytokines such as IL-6 and TNF-a, and adipokines such as leptin, all of which are associated with insulin resistance and sarcopenic muscle loss (Batsis and Buscemi 2011, Coon et al. 1992, Karakelides et al. 2010, Manoy et al. 2017), and physical inactivity (Flack et al. 2011), collectively contribute towards age-related impairments in glucose homeostasis.

2.3.10 Measurement of energy metabolism

Energy expenditure can be measured by either *direct calorimetry* (rate of heat loss from the body); *indirect calorimetry* [measuring the rate of oxygen consumption ($\dot{V}O_2$) and/or carbon dioxide production ($\dot{V}CO_2$) and converting to EE using formulae, e.g., Brouwer (1957), with or without measurement of urinary nitrogen excretion]; or *non-calometric* techniques (predicting EE from extrapolation of physiological measurements and observations) (Levine 2005). The variability of such methods with regards to the accuracy, reproducibility and reliability of measurement is extensive (Westerterp 2019). Likewise, cost and complexity of methods differ substantially. A summary of methods used to measure EE within the literature is provided in **Table 2.5**.

Table 2.5 Summary of methods used to measure energy expenditure. Adapted from Lam and Ravussin (2016) and Westerterp (2019)

Method	Measurement	Duration of use	Advantages	Disadvantages
Direct calorimetry	Direct measurement of heat production	Hours to several days	Researcher able to completely control environmental factors, high accuracy, and precision	High cost, technically demanding, participant refined to a small area, requires substantial technical support
Metabolic cart (using facemask, mouthpiece, or ventilated hood)	Indirectly measures EE via breath-by-breath collection of O_2 consumption and CO_2 production	Hours	Fast response time, easy to operate in clinical settings, high precision for RMR	Facemask and mouthpiece can only be tolerated for a limited amount of time. Ventilated hood only applicable whilst supine, restricts participant mobility
Douglas bag	Indirectly measures EE by collection of expired O_2 consumption and CO_2 production	Hours	High accuracy and precision, low cost	Laborious method, each bag can only collect a certain volume, rapid changes in ventilation cannot be measured
Respiration chamber	Indirectly measures EE by collection of expired O_2 consumption and CO_2 production	Hours to several days	Minute-by-minute measurement in real time, allows measurement of multiple components of 24-h EE and substrate oxidation, high accuracy, and precision	Participant restricted to a confined environment, high cost, requires technical support
Doubly labelled water	Indirectly measures EE by CO_2 production	4-21 days	Able to assess habitual free-living TEE, high accuracy, and precision	High cost due to isotope expense, no time-course of data, different components of EE unable to be differentiated
Physical activity logs	EE calculated by multiplying energy equivalent values for activities undertaken (estimated from prediction equations)	3-7 days	Simple administration, low cost	Underreporting likely, data quality poor due to participant burden, low accuracy, and precision

Table 2.5 continued

Method	Measurement	Duration of use	Advantages	Disadvantages
Kinematic measures	Activity counts translated into EE using specific equations	Flexible	Able to assess habitual free-living TEE, easy to administer	Requires participant compliance to monitor wear and non-wear times, prediction equations age specific, low accuracy and precision
Heart rate monitoring	Predicts EE based on the relationship between heart rate and $\dot{V}O_2$	Flexible	Administration simple, unbiased	Loss of data likely, individualised calibration required, low to moderate accuracy and precision
Ventilation monitoring	Predicts EE based on the relationship between $\dot{V}O_2$ and EE	Hours	Low-to-moderate cost, less sensitive to physical cofounders	Not applicable in free-living, low to moderate accuracy and prevision

EE, energy expenditure; TEE, total energy expenditure.

Indirect calorimetry calculates EE by measurement of the energy released by oxidisation of carbohydrate, fat and protein (Lam and Ravussin 2016). For accurate measurement, measurement of $\dot{V}O_2$, $\dot{V}CO_2$ and urinary nitrogen is required (Westerterp 2019). Examples of equations used to calculate EE and substrate oxidation include Astrup (1990), Brouwer (1957), Frayn (1983), and Weir (1949). Differences relate to the chemical values used for assumptions on gaseous exchange and energy released from carbohydrate, fat and protein (Westerterp 2019). For this thesis (**Chapter 4**), the Brouwer equation was used due to its established significant agreement with direct calorimetry (Webb et al. 1988).

Measurement of EE and substrate oxidation via indirect calorimetry can be undertaken using a number of systems. For short-term measurements, a metabolic cart using a facemask or ventilated hood to collect respiratory gases is often used (Westerterp 2019). However, face masks can only be tolerated for a few hours, limiting measurement time (Westerterp 2019). To overcome this limitation, an open-circuit whole-room calorimeter (or respiration chamber) enables measurement of EE and substrate oxidation under free-living conditions for up to several days. Considered the gold standard for assessing minute-by-minute EE (Brychta et al. 2009), respiration chambers (**Figure 2.5**) are airtight thermally insulated living spaces (typically small), which give the impression of a hotel-type room, equipped with a bed, desk, chair, computer with internet and freezer toilet (Schoffelen et al. 1997, Westerterp 2019). Environmental conditions and consumption of food and drink can be controlled at all times.

Air flow is controlled by either a push or pull system. In a push-type system, air is forced into the chamber to eliminate other sources of in-flow, whereas pull-type systems draw air out to eliminate other sources of outflow (Schoffelen and Plasqui 2018). Both systems have their specific advantages and disadvantages. For accurate results using a push-type system, all expired air must to be collected as the probability of air leaks with these systems with increasing chamber size is high. In contrast, in a pull-type system, even if the chamber is not perfectly sealed, air will not escape as it is pulled from the chamber into one outlet (Schoffelen

and Plasqui 2018). For this reason, pull-type calorimeters are the predominant technique used in today's respiration chambers (Schoffelen and Plasqui 2018). Nevertheless, for both techniques, O_2 consumption and CO_2 production is calculated as the difference in gas composition between incoming and outgoing air (Schoffelen et al. 1997).

Throughout the measurement period, physical activity can be monitored continuously via a radar system working on the Doppler principle (Schoffelen et al. 1997). Through continuous measurement of EE and physical activity, measurements of 24-h sedentary EE, AEE, SPA and DIT can be calculated (Ravussin et al. 1986), in addition to SMR (Schoffelen and Westerterp 2008). Unfortunately, measurement of AEE is limited due to the confined space of a respiration chamber and the subsequent inability to perform many forms of physical activity (Schoffelen and Plasqui 2018).

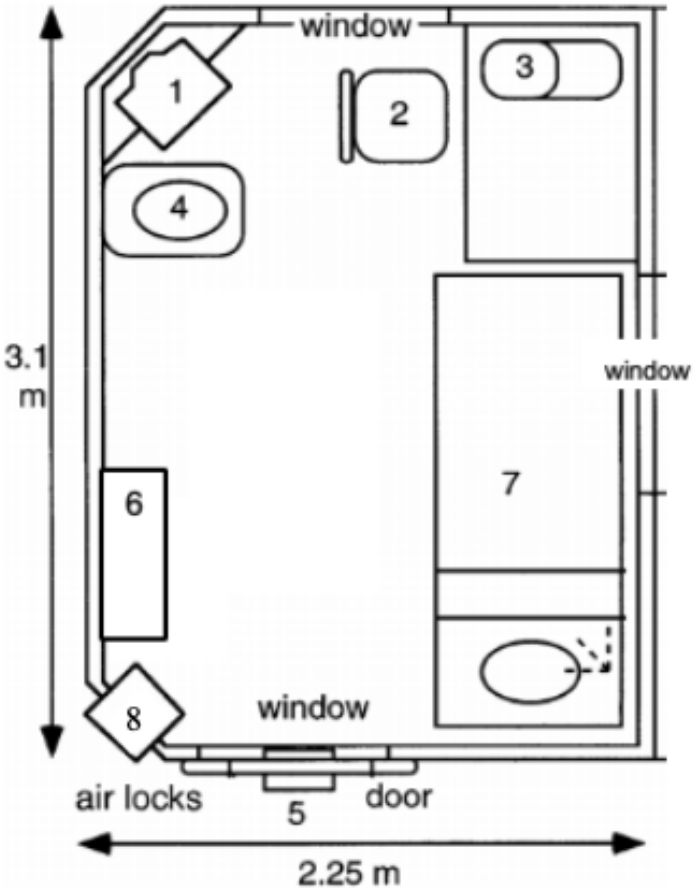

Figure 2.5 Example layout of a respiration chamber similar to that used in the present thesis. 1) TV; 2) chair; 3) sink; 4) deep-freeze toilet; 5; air lock for blood samples; 6) exercise step; 7) bed; 8) air locks (one for food and one for urine/biological samples). Adapted from Schoffelen et al. (1997).

2.3.11 Effects of resistance exercise on fat mass and weight maintenance

Decreases in FM have been reported in numerous studies in older adults following RE training; however, body mass generally remains stable due to a concomitant increase in FFM (Campbell et al. 1994, Englund et al. 2018, Hunter et al. 2000, Pratley et al. 1994, Treuth et al. 1995, Westcott 2012). Regionally, reductions in both visceral (Hunter et al. 2002, Ibañez et al. 2005, Treuth et al. 1994, 1995) and intramuscular adipose tissue have been observed (Moro et al. 2020). A major factor in the loss of FM following RE training is the RE-induced increase in EE, particularly RMR (Westcott 2012). Additionally, changes in appetite may also influence weight maintenance following RE training (Dorling et al. 2018). The effects of RE on EE and appetite will be reviewed in subsequent sections.

2.3.12 Effects of resistance exercise on appetite and satiety

Following an acute bout of RE, several studies have reported a brief suppression (~30-60 min) of subjective appetite (Broom et al. 2009, Freitas et al. 2020, Laan et al. 2010). Broom et al. (2009) also demonstrated a suppression of the orexigenic gut hormone acylated ghrelin. In contrast, others have not reported a suppression of either subjective appetite (Ballard et al. 2009, Larsen et al. 2017, Madzima et al. 2018) or acylated ghrelin (Balaguera-Cortes et al. 2011, Larsen et al. 2017). Disparities may be explained by the RE intensity, as differences in hunger have been reported following an acute bout of high and low intensity exercise (Freitas et al. 2020). Further, acute studies to date report either minimal or no changes in the anorexigenic hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) (Balaguera-Cortes et al. 2011, Broom et al. 2009, Larsen et al. 2017), and no compensatory reduction or significant increase in EI for the remainder of the day in either young (Ballard et al. 2009, Broom et al. 2009, Cadieux et al. 2014, Laan et al. 2010) or older adults (Maltais et al. 2018). The latter of these findings may have important implications for maintaining energy balance in older adults.

Regrettably, few studies have investigated the longitudinal effects of RE on subjective or hormonal appetite. Following 12 weeks of RE, no changes in subjective appetite or concentrations of active ghrelin, PYY or pancreatic polypeptide (PP) in either the fasted or post-prandial state have been reported in middle-aged men who were either overweight or with obesity (Guelfi, Donges, and Duffield 2013). In contrast, a study of male students who were overweight observed decreases and increases in fasting acylated ghrelin and PYY, respectively (Shakiba et al. 2019), and meta-analyses have also reported decreases in the appetite suppressants leptin (Fedewa et al. 2018) and insulin (Ashton et al. 2020). The authors in the study by Shakiba et al. (2019) concluded that changes in appetite hormones might be related to the increase in EE and subsequent loss of body mass following RE training. However, as the abovementioned longitudinal studies were conducted on young and middle-aged adults, these findings may not translate to older adults.

2.3.13 Effects of resistance exercise on energy expenditure

A single bout of RE has been shown to expend ~120-500 kcal/h (Hunter et al. 2013), elevate RMR for up to 48 h post-exercise (Speakman and Selman 2003), and increase DIT (Denzer and Young 2003). Longitudinally, studies of 12-26 weeks in duration have reported ~7-8% increases in RMR (Hunter, McCarthy, and Bamman 2004), with increases of 87 kcal/d (Hunter et al. 2000), 108 kcal/d (Campbell et al. 1994), 109 kcal/d (Treuth et al. 1995) and 119 kcal/d (Pratley et al. 1994) reported. Despite increases in RMR, few studies have assessed the longitudinal effects on TEE in older adults. In the study by Hunter and colleagues (2000), the increase in RMR occurred alongside increases in AEE (120 kcal/d) and TEE (230 kcal/d) as measured by doubly labelled water (DLW). Rises in activity and TEE may be explained by improved metabolic efficiency of spontaneous activities, including walking economy (Valenti, Bonomi, and Westerterp 2016) and ease of movement (Taaffe et al. 1999), leading to an increase in PAL. Indeed, ease of movement positively correlates with SPA (Hunter et al. 2004, 2015) and has been suggested as a mechanism to offset age-related increases in FM in older adults (Hunter et al. 2018).

Whilst improvements in RMR have often been reported, and Hunter et al. (2000) reported an increase in TEE, the majority of studies in the literature have reported either no change or a decrease in TEE following RE training in older adults (Bell et al. 2019, Hunter et al. 2013, Meijer, Westerterp, and Verstappen 1999, Treuth et al. 1995). These findings are often attributed to a reduction in AEE, particularly SPA (Westerterp 2018b). Although the mechanisms are not fully explained, it has been hypothesised that fatigue due to an increase in training frequency results in a compensatory decrease in non-exercise physical activity (Hunter et al. 2018). In support, Hunter et al. (2013) reported a 150 kcal/d decrease in SPA in older women who performed RE three times per week, but an increase of 200 kcal/d in those that trained twice weekly. Other studies that have also reported decreases in AEE likewise employed a three sessions per week training protocol (Bell et al. 2019, Treuth et al. 1995). These findings suggest training thrice weekly during the early phases (0-12 weeks) of RE in older adults may be the breakpoint which negatively impacts non-exercise physical activity. However, challenging this hypothesis, reductions in physical activity have been reported in participants training twice weekly (Meijer, Westerterp, and Verstappen 1999), suggesting additional factors also influence reductions in SPA.

Indeed, SPA is a complex issue, controlled by a number of neuromodulating factors including orexin A (Kotz, Teske, and Billington 2008). Orexin A has been shown to drive activity, control energy balance and regulate adipose tissue (Kotz et al. 2017, Perez-Leighton, Billington, and Kotz 2014, Teske, Billington, and Kotz 2010). Consequently, reductions in SPA reported in older adults following RE may also partly be explained by decreases in orexin A to oppose the increase in RMR and reduction in FM frequently reported (Hunter, McCarthy, and Bamman 2004). This hypothesis requires further investigation.

2.3.14 Effects of resistance exercise on substrate oxidation and balance

The effects of RE on substrate oxidation, particularly in older adults, has not been extensively studied. In young adults, decreased respiratory exchange ratio (RER) (indicating greater fat oxidation) immediately and up to 48-h post exercise has been observed following an acute bout of RE (Allman et al. 2019, Melby et al. 1993, Ormsbee et al. 2007, Schuenke, Mikat, and McBride 2002). Conversely, respiration chamber studies conducted by Melanson and colleagues have not reported similar effects (Melanson et al. 2002, 2005). Differences between chamber and non-chamber studies may be explained by exercise timing (i.e., prior to breakfast in the fasted state vs. following food consumption). Longitudinally, RE has been reported to decrease sleeping and resting RQ in young adults (Kirk et al. 2009), and increase both resting and 24-h fat oxidation in middle- and older-aged adults (Ho et al. 2012, Treuth et al. 1995).

Regarding carbohydrate and glucose oxidation, increased 24-h carbohydrate oxidation in young adults has been observed following an acute bout of RE (Melanson et al. 2002, 2005). However, the longitudinal effects in both young and older adults report conflicting findings with no changes (Kirk et al. 2009, Miller et al. 1994), decreases (Treuth et al. 1995) and increases in carbohydrate/glucose oxidation all reported (Consitt et al. 2016, Sparks et al. 2013). Differences may be explained by measurement of rates of whole-body (Kirk et al. 2009, Miller et al. 1994, Treuth et al. 1995) versus skeletal muscle oxidation (Consitt et al. 2016, Sparks et al. 2013), suggesting the increase in glucose oxidation following RE may be muscle specific.

In both young and older adults, decreases in 24-h nitrogen excretion and protein oxidation (Campbell et al. 1995, Iglay et al. 2007, Moore et al. 2007, Treuth et al. 1995), and improvements in nitrogen balance (Campbell et al. 1995, Moore et al. 2007) have been reported following RE training. These data are consistent with the accretion of FFM frequently reported (Peterson, Sen, and Gordon 2011), and suggest a greater retention of dietary protein in the trained state. Nevertheless, it is important to note that increased nitrogen balance does

not always translate to changes in FFM (Drummen et al. 2018), with studies in older adults reporting no change in nitrogen balance despite significant increases in FFM (Verdijk et al. 2009a, Leenders et al. 2013).

2.3.15 Effect of resistance exercise on metabolic flexibility

At present, data on the effect of RE on metabolic flexibility in older adults is scarce. Meex et al. (2010) established RE once weekly in addition to twice weekly aerobic exercise (AE) improved metabolic flexibility in T2DM patients but not in healthy controls. More recently, Consitt et al. (2016) established impairments in skeletal muscle pyruvate dehydrogenase (PDH) phosphorylation and plasma lactate are indicative of metabolic flexibility, but can be reversed following RE in older adults. Together, these findings suggest RE may be an effective strategy to offset age-related metabolic inflexibility. Although research is in its infancy, increased metabolic flexibility may be explained by improvements in mitochondrial content and function (Goodpaster and Sparks 2017), which have been observed in older adults following RE training (Parry, Roberts, and Kavazis 2020).

2.3.16 Effects of resistance exercise on glucose homeostasis

In elderly individuals, several studies have demonstrated improvements in whole-body insulin sensitivity (~10-30%) and glycaemic control following RE training (Bell et al. 2017, Bucci et al. 2016, Flack et al. 2011, Holwerda et al. 2018, Iglay et al. 2007, Leenders et al. 2013, Miller et al. 1994). These findings are perhaps not surprising considering skeletal muscle is the largest glucose storage depot in the human body (Scott, de Courten, and Ebeling 2016), with increased uptake observed following RE in older adults (Bucci et al. 2016). However, although skeletal muscle hypertrophy following RE is well-documented (Peterson, Sen, and Gordon 2011), increases in SMM alone may not fully explain improvements in insulin sensitivity (Andersen et al. 2003, Holten et al. 2004, Miller et al. 1994). Improvements in skeletal muscle insulin signalling, specifically increased phosphorylation of Akt substrate of 160 kDa (AS160)

and expression of GLUT-4 likely contribute to the remaining unexplained improvements, which may require a reduction in FM in order to be observed (Consitt, Dudley, and Saxena 2019).

2.3.17 Effects of dietary protein on fat mass and weight maintenance

Numerous studies, as reviewed by Drummen and colleagues (2018), have demonstrated high protein diets are effective for weight maintenance and decreasing FM. For instance, meta-analyses have reported a greater long-term reduction in FM following consumption of a high protein diet during weight loss compared to a normal protein diet (Clifton, Condo, and Keogh 2014, Kim et al. 2016b). Additionally, in participants required to maintain a constant body mass for 12 weeks via an energy-controlled diet, Martens et al. (2015a) demonstrated a high protein diet (30% of EI) maintained energy balance and decreased FM by 0.4 kg. In contrast, a group consuming a high carbohydrate, low protein diet (5% of EI) gained 0.4 kg FM due to a resultant increased positive energy balance. These findings suggest a high protein diet may protect against a positive energy balance and increased adiposity whilst maintaining a constant body mass.

2.3.18 Effects of dietary protein on appetite and satiety

Several short-term studies in younger adults using energy balanced diets have demonstrated a greater satiating effect following consumption of a high compared to low protein diet (Bendtsen et al. 2013, Lejeune et al. 2006, Veldhorst, Westerterp-Plantenga, and Westerterp 2009, Veldhorst et al. 2009). Mechanistically, these effects are regulated via several pathways, including increases in anorexigenic (e.g., GLP-1 and PYY) and decreases in orexigenic hormones (e.g., ghrelin and cortisol), rises in DIT and gluconeogenesis, aminostatic theory (i.e., AAs directly serving as satiety signals), and effects on digestion (Drummen et al. 2018). However, although satiating effects of a high protein diet may be beneficial for younger adults, the effect is potentially deleterious for older adults, as substantial declines in both daily energy and protein intake are contributors of age-related declines in SMM (Atalayer and Astbury 2013, Paddon-Jones et al. 2015).

Interestingly, in contrast to data in younger adults, data in the elderly suggests ageing might blunt the suppression of appetite induced by dietary protein. For example, two acute studies conducted by the same research group reported a reduced suppression of appetite following intake of dietary protein in older compared to young adults (Giezenaar et al. 2017, 2020). Butterworth and colleagues (2019) also demonstrated acute ingestion of an EAA gel increased both protein and energy intake. Recently, a meta-analysis by Ben-Harchache et al. (2020) reported either a positive or no effect of longitudinal protein supplementation on total EI. These studies suggest dietary protein could be effective at maintaining energy and protein intake in the elderly, which in turn may aid attenuation of sarcopenia and age-related declines in EE. Furthermore, the ability for older adults to consume ample amounts of dietary protein might prevent the protein leverage hypothesis, that is, continually eating until protein needs are met, regardless of the energy and protein content (Martens, Lemmens, and Westerterp-Plantenga 2013). Prevention of the protein leverage hypothesis may therefore also have implications for prevention of sarcopenic obesity. However, it is important to note that due to limited longitudinal data in older adults, the meta-analysis by Ben-Harchache et al. (2020) only included two studies that assessed the effects of protein supplementation on subjective appetite. These studies provided mixed findings; therefore, further research is needed to examine the long-term effects of dietary protein on subjective appetite ratings in older adults.

2.3.19 Effects of dietary protein on energy expenditure

Increased dietary protein has been promoted to increase EE via increases in resting, sleeping and postprandial metabolism (Leidy et al. 2015). Acutely, 24-h respiration chamber studies have reported increases in DIT (Hochstenbach-Waelen et al. 2009a, Sutton et al. 2016, Westerterp-Plantenga et al. 1999, Westerterp, Wilson, and Rolland 1999), SMR (Hochstenbach-Waelen et al. 2009a, 2009b, Lejeune et al. 2006, Mikkelsen, Toubro, and Astrup 2000), RMR (Mikkelsen, Toubro, and Astrup 2000, Veldhorst, Westerterp-Plantenga, and Westerterp 2009) and TEE (Hochstenbach-Waelen et al. 2009a, 2009b) following consumption of a high protein (25-30% of EI) diet. Longitudinal studies of up to 12 weeks in

duration have also observed increases in SMR and DIT (Bray et al. 2015, Martens et al. 2015a, Sutton et al. 2016), RMR (Bray et al. 2012) and TEE (Bray et al. 2012, 2015). Bray et al. (2015) also observed an adaptive thermogenesis, whereby TEE and SMR remained elevated above baseline values when protein intake was returned back to baseline intake. This higher level of EE could be a mechanism that supports long-term weight maintenance. However, although high protein diets elicit longitudinal increases in RMR and DIT, either no change (Drummen et al. 2020) or decreases (Apolzan et al. 2014, Lejeune et al. 2006, Martens et al. 2015a, Veldhorst, Westerterp-Plantenga, and Westerterp 2009) in AEE has been reported. These findings cannot be explained by changes in physical activity, as activity counts measured inside the respiration chamber in these studies did not change. Therefore, similar to that of RE, the aforementioned findings suggest high protein diets improve the metabolic efficiencies of spontaneous activities, leading to a decline in AEE if physical activity is not increased.

Mechanistically, protein-induced increases in SMR can be explained by increased rates of MPS [which has an energetic cost of 0.86 kcal/g (Hall 2006)] and protein turnover, whilst increases in DIT can be explained by the adenosine-5'-triphosphate (ATP) required for metabolism and oxidation, including urea synthesis (Drummen et al. 2018). To explain the latter, dietary protein uses considerably more of its useable energy (~20-30%) to metabolise compared to carbohydrate (~5-10%) and fat (~0-3%) (Westerterp-Plantenga et al. 2009b). The metabolic efficiency of protein oxidation largely depends on the AA composition of the protein, with higher biological value AA producing a higher thermic effect (Drummen et al. 2018). Gluconeogenesis due to a surplus of dietary protein also contributes (explaining ~42%) to the increases in EE observed (Veldhorst, Westerterp-Plantenga, and Westerterp 2009). Furthermore, although the ability of dietary protein *per se* to increase FFM is limited, gains in skeletal muscle and FFM, which have specific metabolic rates of 12.6 and 24 kcal/kg/d, respectively (Hall 2006, Wang et al. 2010), also partly explain protein-induced increases in EE.

Whilst previously cited studies have demonstrated increases in EE following a high protein diet, all these studies were conducted in young adults. Unfortunately, few studies have assessed the effects in older adults. Acute studies have reported no effects of either 40 g casein prior to sleep on RMR then next morning (Morehen et al. 2020), or 40 g whey protein on DIT (Hawley et al. 2020). The former of these null findings may be attributed to the time difference between protein consumption and RMR measurement [~9 hours (Morehen et al. 2020)], which is longer than the acute pre-sleep protein-induced effect on DIT [~6 hours (Westerterp 2004)]. Additionally, use of an isonitrogenous pea protein supplement as a comparator control supplement might also explain these findings of Hawley et al. (2020). In contrast to these acute studies, a respiration chamber study by Drummen et al. (2020) established 34 months of a high protein diet (25% of EI) increased RMR, induced a negative energy balance and subsequently counteracted weight loss-induced adaptive thermogenesis 34 months following weight loss compared to a typical protein diet (15% of EI). This important mechanistic study provides evidence to support the use of high protein diets to aid EE and oppose weight gain in older adults.

However, whilst Drummen and colleagues (2020) reported a protein-induced increase in RMR, other longitudinal studies in older adults have not observed these effects (Luger et al. 2013, Negro et al. 2019). Insufficient increases in dietary protein (≤0.1 g/kg/d) to stimulate changes in FFM and RMR may clarify these findings. In fact, due to weight loss, participants in the study by Luger et al. (2013) lost 0.8 kg of FFM, likely due to consumption of a suboptimal protein intake to maintain FFM during weight loss (~0.9 g/kg/d). As often cited in this literature review, a protein deviation of ≥0.4 g/kg/d may be required to stimulate gains in SMM in older adults (Park, Choi, and Hwang 2018). As FFM accounts for 50-70% of RMR (Gallagher et al. 2000), of which SMM accounts for ~20% (Geisler and Müller 2017), further research increasing dietary protein intake by ≥0.4 g/kg/d is required to determine the effects on EE in older adults.

2.3.20 Effects of dietary protein on macronutrient oxidation and balance

Intervention studies conducted in both young (Bray et al. 2015, Martens et al. 2015b, Pannemans et al. 1995, Robinson et al. 1990) and middle- to older-aged adults (Campbell et al. 1995, Drummen et al. 2020, Hays et al. 2009, Pannemans, Halliday, and Westerterp 1995, Pannemans et al. 1998) have established high protein diets increase 24-h protein oxidation and improve protein balance. Additionally, others have also demonstrated increases in 24-h fat oxidation (or decreased 24-h RQ) (Drummen et al. 2020, Lejeune et al. 2006, Martens et al. 2015b, Smeets, Janssens, and Westerterp-Plantenga 2013), a greater negative fat balance (Lejeune et al. 2006), and an exercise-induced increase in fat oxidation (Soenen et al. 2010). Protein-induced increase in fat oxidation is likely attributed to increased EE leading to rapid glycogen depletion, principally overnight (Smeets, Janssens, and Westerterp-Plantenga 2013), and due to protein eliciting a negative energy balance which is known to increase fat oxidation (Westerterp 1993). Moreover, some (Drummen et al. 2020, Martens et al. 2015b), but not all of the abovementioned studies that observed increases in fat oxidation (Lejeune et al. 2006, Smeets, Janssens, and Westerterp-Plantenga 2013), also observed reductions in carbohydrate oxidation and balance. Though, these decreases were likely due to the fact carbohydrate was exchanged for dietary protein in these studies. Further, whilst studies have shown a protein-induced effect on fat oxidation, the majority [apart from Drummen et al. (2020)] were conducted on young adults. The findings therefore might not translate to the older population.

2.3.21 Effect of dietary protein on metabolic flexibility

The effect of dietary protein on metabolic flexibility is relatively unknown due to a paucity of published data. In the only study conducted to date to the author's knowledge, Eelderink et al. (2019) reported no effects of high compared to low dairy consumption over 6 weeks in middle- and older-aged adults. These findings may be related to the short duration of the intervention and the lack of difference in protein intake between conditions (~20 g/d). Nevertheless, as dietary protein has been shown to increase SMM and fat oxidation [particularly in the overnight

fasted state (Smeets, Janssens, and Westerterp-Plantenga 2013)], reduce markers of systemic inflammation and decrease FM, all of which contribute to improved metabolic flexibility (Calçada et al. 2014, Smith et al. 2018), it might be hypothesised that dietary protein at a higher daily intake may improve metabolic flexibility in older adults. This hypothesis requires examining in future work.

2.3.22 Effects of dietary protein on glucose homeostasis

The effect of dietary protein on glucose homeostasis in middle- and older-aged adults is controversial (Drummen et al. 2018). Some studies have reported an increase in insulin sensitivity and/or a reduction in fasting glucose (Acheson et al. 2011, Amirani et al. 2020, Luger et al. 2013, Pal, Ellis, and Dhaliwal 2010, Promintzer and Krebs 2006), whereas others have reported either no change (Leenders et al. 2011), or increased whole-body insulin resistance (Linn et al. 2000, Hattersley et al. 2014, Weickert et al. 2011) and an elevated risk of developing T2DM (Tian et al. 2017). Deleterious effects reported in some studies may be related to the source of dietary protein (Drummen et al. 2018). In particular, associations between dietary protein and insulin resistance is most pronounced with high levels of red meat consumption (Sluijs et al. 2010). Additionally, it is thought that dietary protein may induce insulin resistance by activating mTOR and consequent phosphorylation of S6K1 (Weickert 2012). However, these negative effects might be counteracted by reductions in FM, decreases in inflammation and increases in SMM observed following consumption of a high protein diet (Weickert 2012).

2.3.23 Effects of resistance exercise combined with dietary protein on fat mass, energy metabolism, appetite and satiety, and metabolic health

Based on the aforementioned cited evidence, RE combined with dietary protein may synergistically aid reductions in FM, increase components of EE, and improve metabolic health in older adults. Indeed, a meta-analysis of 15 RCTs reported augmented decreases in absolute and %FM following RE combined with protein supplementation (Liao et al. 2017).

More recent RCTs have also observed similar findings, reporting greater reductions in whole-body (Bell et al. 2017) and intermuscular thigh FM (Englund et al. 2018). Of importance, data from Campbell and colleagues (2015) suggests intakes of ≥1.2 g protein/kg/d during RE training leads to greater declines in both absolute and %FM.

From an appetite perspective, in one study, RE combined with whey protein supplementation (which increased daily protein intake from 1.24 g/kg/d to 1.40 g/kg/d) suppressed subjective hunger during the early stages of the intervention (weeks 1-3), but this was reversed at the end of the study (week 11) where hunger was increased (Ridge et al. 2018). However, it is important to note that participants were only supplemented with whey protein on training days, so it is unknown whether daily consumption of this dose would have affected appetite. In a more recent study, 12 weeks of RE combined with whey protein supplementation (which increased dietary protein intake from 1.2 g/kg/d to 1.4 g/kg/d) increased daily and *ad libitum* EI and postprandial subjective appetite in older adults (Johnson et al. 2021). The increase in *ad libitum* EI was associated with an increase in FFM. These findings suggest older adults may adapt to increased dietary protein intake over time. However, it must be noted that the dietary protein intake in these studies was lower than that reported by Morton et al. (2018a) to optimise SMM accretion during RE training (1.6 g/kg/d), and appetite was not compared against either RE or dietary protein alone.

Moreover, despite reported synergistic effects of RE combined with dietary protein on FM, similar effects have not been observed on components of EE in older adults (Amamou et al. 2017, Campbell et al. 1994, Maltais et al. 2016, Weinheimer et al. 2012). These findings may be explained by a lack of sample size (n = 6 per group) and statistical power (Campbell et al. 1994), an inadequate increase in dietary protein (≤0.3 g/kg/d and less than 1.6 g/kg/d) to stimulate additive gains in SMM and EE (Amamou et al. 2017, Maltais et al. 2016), and an adequate (0.97-1.12 g/kg/d) habitual protein intake at baseline (Weinheimer et al. 2012).

Similar to the above, whilst studies have reported improvements in glucose homeostasis following RE training, no additive effects of dietary protein have been observed in the majority of studies (Holwerda et al. 2018, Iglay et al. 2007, Leenders et al. 2013, Maltais et al. 2016, Verdijk et al. 2009a, Weinheimer et al. 2012). Although, Bell et al. (2017) did report an augmented effect of a whey protein-based multi-ingredient supplement on glucose tolerance in healthy older men. In this study, dietary protein was increased from 1.1-1.6 g/kg/d, greater than that of previously cited studies [excluding Weinheimer et al. (2012)]. This synergistic effect was likely attributed to an augmented reduction in FM and decreases in markers of systemic inflammation (Bell et al. 2018). Of importance, the supplement investigated by Bell and colleagues contained additional ingredients such as omega 3 polyunsaturated fatty acids (n-3 PUFA), which has been shown to have insulin sensitising effects (Gao et al. 2017). Consequently, the addition of n-3 PUFA may have influenced the improvement in glucose tolerance. Based on the highlighted limitations of studies investigating the effects of RE combined with dietary protein on EE, appetite and glucose homeostasis, further research with an optimal dietary protein dosing regimen (i.e., 1.6 g/kg/d) is warranted.

In parallel to the limitations of studies examining the synergistic effects of RE and dietary protein on FFM, strength and physical function reported in **section 2.2.10.3.1**, a significant limitation of studies investigating the synergistic effects on energy metabolism and glucose homeostasis was the failure to include a protein only group. The synergistic effects compared to each intervention alone are therefore unknown. Furthermore, studies investigating the effects on energy metabolism only captured RMR; consequently, the effects on 24-h energy metabolism are also unknown. As 24-h EE is not constant, varying based on numerous factors such as time of day and food consumption (Schoffelen and Plasqui 2018), collection of such data is important to further understand the individual and synergistic effects of these interventions on all components of EE (i.e., RMR, SMR, AEE, DIT and TEE). Finally, no study has investigated the synergistic effects on 24-h metabolic flexibility or appetite compared to both RE and dietary protein alone in older adults. The addition of this data would provide the

literature with comprehensive data on the effects of RE and dietary protein on metabolic health and energy balance, which might have important implications for attenuating sarcopenic obesity.

2.4 Part 3: Cognitive function

2.4.1 Introduction

Ageing is associated with degeneration of a number of cognitive capacities, including memory, attention, reaction time and executive function (Hedden and Gabrieli 2004). Such declines can lead to mild cognitive impairment (MCI) and dementia, most commonly Alzheimer's disease (Alzheimer's Society 2014). At present, MCI affects 17.1% of adults aged ≥65 years in the United Kingdom, with reported rates of progression to dementia ranging from ~8-15% per annum (Petersen 2016). Dementia prevalence is currently estimated at ~50 million people worldwide and is projected to affect ~82 million people by 2030 (World Health Organisation 2019). The impact of such conditions, both personally and economically, is extensive (Farina et al. 2017, Wimo et al. 2017). Personally, cognitive impairment is associated with physical impairment (Coppin et al. 2006, Demnitz et al. 2017) and poor quality of life (Woods et al. 2015). Economically, the total cost of dementia in the United Kingdom has been calculated at £26.3 billion per annum (Alzheimer's Society 2014). Identifying safe and effective strategies to prevent cognitive decline is therefore vital for public health.

2.4.2 Age-related declines in cognitive function

Across the human lifespan, increases in cognitive function are observed up until early adulthood, with gradual decreases seen from mid adulthood to older age (Daffner 2010). Generally, cognitive function can be partitioned into either fluid or crystallised intelligence. Fluid intelligence refers to basic processes that depend minimally on prior learning (e.g., problem solving ability), whereas crystallised intelligence refers to learned procedures and knowledge (e.g., general knowledge) (Deary et al. 2009, Salthouse 2009). The core fluid and

crystallised domains include memory, attention, executive function, language, visuospatial abilities and processing speed (Serper et al. 2014). **Figure 2.6** displays estimates of age-related changes in key cognitive domains from a number of studies including the Seattle Longitudinal Study (Hedden and Gabrieli 2004). These estimates suggest declines in all cognitive domains are evident from 55 years of age.

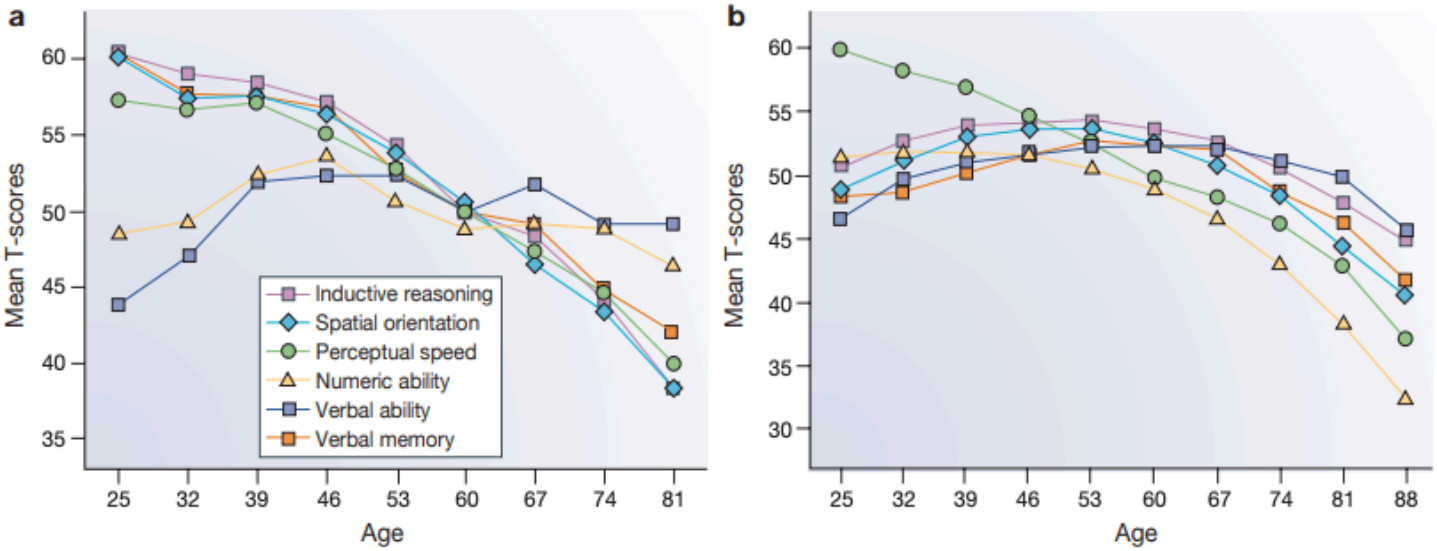

Figure 2.6 Cross-sectional (A) and longitudinal (B) estimates of age-related declines in cognitive domains. Taken from Hedden and Gabrieli (2004).

2.4.3 Mechanisms and risk factors

Several mechanisms and risk factors contribute to age-related cognitive decline. These include structural and neurophysiological changes in the brain, genetic and lifestyle factors, chronic stress and systemic inflammation (Deary et al. 2009, Prince 2014). It is beyond the scope of this thesis to review the literature on the structural and genetic mechanisms of age-related cognitive decline. Therefore, the following sections will briefly review the lifestyle, cardiometabolic, neurobiological and inflammatory mechanisms which are relevant to this thesis.

2.4.3.1 Lifestyle and cardiometabolic factors

Numerous lifestyle and cardiometabolic factors, including obesity, hypertension, insulin resistance and T2DM, and physical inactivity/poor diet have been linked to cognitive decline and dementia (Biessels and Reagan 2015, Cunningham et al. 2020, Nguyen, Killcross, and Jenkins 2014, Stough et al. 2019, Walker, Power, and Gottesman 2017). For example, obesity has been associated with decreased brain volume and atrophy (Raji et al. 2010), and diagnosis of T2DM has been reported to increase the risk of developing dementia by 73% (Biessels et al. 2014). Mechanistically, hippocampal insulin resistance, which has deleterious effects on memory (De Felice, Lourenco, and Ferreira 2014), is understood to be the result of impaired insulin receptor signalling combined with reductions in insulin transport across the blood-brain barrier (Banks, Owen, and Erickson 2012). Hypertension is purported to negatively affect cognitive function by altering brain structure and function via cerebral vessel remodelling, leading to disturbances in cerebral autoregulation, reduced cerebral perfusion and limiting the ability of the brain to clear harmful proteins such as β-amyloid (Walker, Power, and Gottesman 2017). Lastly, data from some (Guure et al. 2017, Sofi et al. 2011), but not all studies (Blondell, Hammersley-Mather, and Veerman 2014), indicates physical inactivity may promote cognitive decline, which may be explained in part by the relationship between inactivity and sarcopenic muscle loss (Peng et al. 2020).

2.4.3.2 Sarcopenia

Several meta-analyses have reported an association between sarcopenia and cognitive impairment (Chang et al. 2016, Cipolli, Yassuda, and Aprahamian 2019, Peng et al. 2020). Common pathologies of both conditions, including chronic systemic inflammation, insulin resistance and reductions in growth factors might explain this relationship (Chang et al. 2016). For instance, associations have been observed between high concentrations of IL-6 and CRP with both parameters of sarcopenia (Alemán et al. 2011, Schaap et al. 2006) and increased risk of dementia (Engelhart et al. 2004). Others have also reported associations between low IGF-1 concentration and insulin resistance with decreased gait speed, low appendicular FFM

and sarcopenia (Gielen et al. 2015, Moon 2014), and cognitive impairment (Al-Delaimy, Von Muhlen, and Barrett-Connor 2009, Kim and Feldman 2015). Lastly, findings from neuroimaging studies suggests sarcopenic muscle loss leads to the presence of white matter and brain atrophy (Burns et al. 2010, Kohara et al. 2017).

Sarcopenia had previously been considered a risk factor for cognitive impairment; however, the relationship may plausibly be bidirectional (Peng et al. 2020). It is also unknown whether the association is purely casual, and which parameter of sarcopenia (SMM, strength or physical function) correlates strongest with cognitive impairment (Cipolli, Yassuda, and Aprahamian 2019). Further longitudinal research is warranted to answer these vital questions.

2.4.3.3 Neurobiological changes

Prior work has established a number of growth and neurotrophic factors, in particular IGF-1 and BDNF, both of which decline with age (Katoh-Semba et al. 2007, Veldhuis et al. 1995), play important roles in maintaining cognitive function. Insulin-like growth factor 1, which is produced both peripherally and locally in the brain (Van Dam et al. 2000), is an important regulator of neurogenesis (Lichtenwalner et al. 2001). Low (<75 ng/mL) and high (>106 ng/mL) peripheral concentrations have been associated with poorer and better cognitive function, respectively (Al-Delaimy, Von Muhlen, and Barrett-Connor 2009, Angelini et al. 2009, Dik et al. 2003).

Brain-derived neurotrophic factor aids the development, preservation and function of neurones, and enhances long-term potentiation, strengthening synapses linked with memory and learning (Alderson et al. 1990, Bramham and Messaoudi 2005, Mattson, Maudsley, and Martin 2004). Declines in BDNF, which have been observed in individuals with MCI and dementia (Laske et al. 2007, Yasutake et al. 2006, Yu et al. 2008), has been associated with impaired memory and general cognitive function (Katoh-Semba et al. 2007, Paulsen et al.

2020). However, others have not observed decreases in individuals with Alzheimer's disease compared to healthy controls (O'Bryant et al. 2011), and some have reported either no association (Ziegenhorn et al. 2007), or an association in the opposite direction (Angelucci et al. 2010), between BDNF and cognitive function. Likewise, longitudinal data is also mixed (Driscoll et al. 2012, Li et al. 2009, Nettiksimmons et al. 2014). Further work, particularly of longitudinal design, is required to determine the effects of BDNF on cognitive decline.

2.4.3.4 Cortisol

Psychological stress and the resulting dysregulation of the HPA axis has been linked with cognitive impairment (Lucassen et al. 2014). Over time, psychological stressors stimulate activity of the HPA axis, leading to alterations in diurnal cortisol secretion (Dickman et al. 2020), which subsequently adversely affects cognitive function (Beluche et al. 2010, Dijckmans et al. 2017, Evans et al. 2011, 2012, Lee et al. 2007). For example, an attenuated decline in diurnal cortisol secretion (Beluche et al. 2010, Dijckmans et al. 2017, Evans et al. 2011), a blunted CAR (Evans et al. 2011), and a high mean cortisol concentration (Lee et al. 2007) have all been associated with poor cognitive function. However, not all studies have reported such associations (Harris et al. 2017, Peavy et al. 2009). Lack of association in these studies might be related to the method of cortisol measurement, such as unsupervised cortisol and cognitive testing (Harris et al. 2017), and inclusion of the CAR in mean daily concentrations (Peavy et al. 2009). Taken together, although the relationship between cognitive function and diurnal cortisol is not fully understood, current evidence suggests a flatter diurnal cortisol profile may contribute to cognitive decline.

2.4.3.5 Inflammation

Epidemiological data suggests systemic inflammation may contribute to brain atrophy (Jefferson et al. 2007, Satizabal et al. 2012, Wersching et al. 2010) and poorer cognitive performance (Marioni et al. 2009, Marsland et al. 2006, Rafnsson et al. 2007). In middle- and

older-aged adults, higher circulating concentrations of IL-6 and CRP have been associated with higher volumes of white matter hyperintensities, lower grey matter and hippocampal volumes, and poorer cognitive performance (Marsland et al. 2006, Satizabal et al. 2012). Mechanistically, systemic inflammation contributes to neurodegeneration and cognitive impairment by crossing the blood-brain barrier, switching primed microglia to an inflammatory phenotype, subsequently increasing the central inflammatory response (Marsland et al. 2015).

2.4.4 Measurement of cognitve function

Several psychological tools have been used in the literature to measure cognitive function (**Table 2.6**). These methods generally assess individual responses of specific cognitive domains in a relevant sample population (Cordell et al. 2013, Pase and Stough 2014). However, no single tool is considered the 'gold standard' for detecting changes in cognitive function (Cordell et al. 2013). Due to the plethora and diversity of tests used within the literature, interpretation and comparison between studies is challenging (Pase and Stough 2014). Inconsistencies in the method of analysis and reporting of data (e.g., reporting raw scores or converting into z-scores) also makes comparison problematic (Pase and Stough 2014). Furthermore, when conducting cognitive testing, several important methodological factors need to be considered. For example, room temperature, nutritional status and timing of cognitive testing may all potentially influence outcomes (Pase and Stough 2014). Adequate control and standardisation of these factors is therefore critical for accurate and reliable results.

At present, the Cambridge Neuropsychological Test Automated Battery (CANTAB) is the most widely publicised testing battery with reports on test-retest reliability and clinical use (Zygouris and Tsolaki 2015). The CANTAB is a touchscreen, tablet-based testing battery, which assesses the cognitive domains memory, executive function, attention and psychomotor speed, and emotional and social cognition (Cambridge Cognition Ltd. 2020). In multiple health and disease states, studies have demonstrated the CANTAB to be sensitive to changes in

cognitive function (Blackwell et al. 2004, Chamberlain et al. 2011, De Luca et al. 2003). Validity and reliability have also been confirmed for detecting age-related cognitive impairment (Rabbitt and Lowe 2000, Wild et al. 2008). The advantages of the CANTAB include the comprehensive assessment of multiple domains of cognitive function, sensitivity for use in older adults, and the touch screen use (Cordell et al. 2013). However, the testing battery does have several limitations, such as difficulty and length of time to administer in addition to lack of normative data for a number of cognitive outcomes (Cordell et al. 2013).

Table 2.6 Summary of cognitive assessment tools used in exercise and/or nutrition trials. Adapted from Cordell et al. (2013) and Pase and Stough (2014)

Test	Advantages	Limitations
7-Minute Screamer	Little/no education bias, primary care validated	Administration difficult, scoring complicated
Abbreviated Mental Test (AMT)	Simple administration, verbal only (no writing/drawing)	Education/ethnicity bias, does not test key cognitive domains (e.g., executive function)
Cambridge Cognitive Examination (CAMCOG)	Tests numerous cognitive domains	Long and difficult administration
Cambridge Neuropsychological Test Automated Battery (CANTAB)	Tests numerous cognitive domains, computer administrated, touch screen	Long duration (depending on tests included), lack of normative data on some tests
Digital span (forwards and backwards)	Reliable and valid, effective method of measuring working memory	More research needed on how the test translates into unfamiliar consequences of real life
General Practitioner Assessment of Cognition (GPCOG)	Primary care validated, no culture/education bias, available in multiple languages, useful informant component	Little data on language/culture bias, informant-based responses only
Mini-Cog	Primary care developed and validated, available in multiple languages, administration time short, no education/culture/race bias	Failure rates may be affected by different word lists
Memory Impairment Screen (MIS	Verbal, little/no education bias	Does not test key cognitive domains (e.g., executive function

Table 2.6 continued

Test	Advantages	Limitations
Mini Mental State Exam (MMSE)	Most widely used test worldwide, used as a criterion method against other methods, quick to administer	Ceiling effect (cognitively able/highly educated individuals score nearly full marks), lacks testing of executive function
Montreal Cognitive Assessment (MoCA)	Available in multiple languages, tests multiple cognitive domains, designed to assess MCI	Education bias (<12 y), limited literature due to recently developed, long administration time
Rey Auditory Verbal Learning Test (RAVLT)	Simple word recall administration	Education bias
Rey-Osterrieth Complex Figure test (ROCF)	Tests numerous domains such as memory, attention, planning, working memory and executive functions	Complex scoring system
Rowland Universal Dementia Assessment (RUDAS)	Little/no education bias, designed for different cultures	Limited published research due to recently developed
Short and Sweet Screening Instrument (SAS-SI)	Effective test for detecting dementia	Memory not tested, lacks data on education/culture biases
Short Blessed Test (SBT)	Verbal only (no writing/drawing)	Executive function not tested, scoring can be burdensome, education/culture bias
St Louis University Mental Status (SLUMS)	Tests numerous cognitive domains, no education bias	Limited published data
Short Portable Mental Status Questionnaire (SPMSQ)	Verbal only (no writing/drawing), quick to administer	Education/culture/race bias, short-term memory not assessed, scoring can be burdensome
Short Test of Mental Status (STMS)	Primary care validated, numerous cognitive domains assessed	Education bias - tested on well-educated individuals, race bias
Stroop test	Multiple variants for repeated measures, simple scoring, quick to administer	Some education bias, potential gender bias
Time and Change Test (T&C)	Brief administration time (<1 min), no education bias	Language/culture bias
Weschler Adult Intelligence Scale-Revised (WAIS-R)	Tests several cognitive domains, reliable and valid	Typically requires trained psychological personnel to conduct, poor assessment of working memory

2.4.5 Effects of resistance exercise on cognitive function

Epidemiological data suggests muscle strengthening exercises may improve executive functioning in older adults (Loprinzi 2016). Additionally, as shown in **Table 2.7**, a number of RCTs of 4-52 weeks in duration have shown improvements in multiple aspects of cognitive function following RE training (Anderson-Hanley, Nimon, and Westen 2010, Best et al. 2015, Cassilhas et al. 2007, Coelho-Júnior et al. 2020, Ikudome et al. 2017, Liu-Ambrose et al. 2010, 2012, Smolarek et al. 2016, Singh et al. 2014, Yoon, Lee, and Song 2018). Results from these studies indicate RE is primarily beneficial for improving the cognitive domains executive function (Anderson-Hanley, Nimon, and Westen 2010, Best et al. 2015, Ikudome et al. 2017, Liu-Ambrose et al. 2010, Yoon, Lee, and Song 2018), memory (Best et al. 2015, Cassilhas et al. 2007, Coelho-Júnior et al. 2020, Ikudome et al. 2017, Marston et al. 2019c), and global cognitive function (Coelho-Júnior et al. 2020, Singh et al. 2014, Smolarek et al. 2016). Furthermore, although not analysed or reported universally, improvements in attention (Liu-Ambrose et al. 2012) and processing speed (Yoon, Lee, and Song 2018) have also been reported. In contrast to these studies, cognitive benefits of RE have not been observed by all (Kimura et al. 2010, Lachman et al. 2006). An insufficient RE intensity (light-moderate) (Lachman et al. 2006) and lack of experimental control (high background noise) (Kimura et al. 2010) might explain these findings.

Whilst recommendations have been set by the ACSM (Chodzko-Zajko et al. 2009) and the NSCA (Fragala et al. 2019) for the optimal frequency (2-3 sessions/week) and intensity (60-80% 1RM) of RE to optimise outcomes such as maintenance of SMM and strength, the optimal intensity to aid cognitive function is of debate. In an acute dose-response study (40-100% 1RM), RE performed at 100% 1RM resulted in the greatest improvement in processing speed, but moderate intensity RE (70% 1RM) elicited superior improvements in executive function (Chang and Etnier 2009). Similarly, Engeroff et al. (2019) reported greater increases in lower cognitive functions, such as attention following high intensity RE (90% 1RM), but higher cognitive functions, including interference control, were improved by RE performed at

moderate intensity (60% 1RM). In contrast to these studies, Chang et al. (2017) did not observe any cognitive benefit of high intensity RE (80% 1RM), reporting worsening effects compared to RE performed at low intensity (30% 1RM). More recently, a meta-analysis conducted by Wilke et al. (2019) reported low (≤50% 1RM) and high intensity RE (75-100% 1RM) elicited similar effects on cognitive function, but RE performed at moderate intensity (50-75%1RM) produced greater improvements compared to both. The reasons for the abovementioned varying effects regarding RE intensity is not clear, but it is thought the dose-response effects may be unique to the cognitive domain assessed (Engeroff et al. 2019). Also, it has been suggested that RE-induced improvements in cognitive function may be highly specific to the participants studied (Chang et al. 2017). Together, these findings suggest low, moderate and high intensity RE may be beneficial for different cognitive domains.

Table 2.7 Summary of intervention studies investigating the effects of resistance exercise on cognitive function in older adults

Study	Participants	Duration and design	Groups	Key findings
Anderson-Hanley, Nimon, and Westen (2010)	32 community-dwelling older adults (age: 55-85 y)	4-week quasi-experimental trial	**RE:** 2-3 times/week (primarily chair and standing exercises)* **Control:** Exercise waiting list *Intensity not defined	RE ↑ executive function but not processing speed compared to control
Best et al. (2015)	155 older women (age: 65-75 y)	12-month RCT with 2-year follow up	**Once weekly:** 2 sets of 6-8 repetitions at 7RM) 2 times/week **Twice weekly:** 2 sets of 6-8 repetitions at 7RM) 2 times/week **Control:** balance and toning (twice weekly)	Both RE frequencies ↑ executive function compared to control. Only twice weekly RE ↑ memory compared to control
Cassilhas et al. (2007)	62 sedentary men (age: 65-75 y)	24-week RCT	**High intensity:** whole-body RE (80%1RM) 3 times/week **Moderate intensity:** whole-body RE (50% 1RM) 3 times/week **Control:** warm-up and stretching 3 times/week	Both moderate and high intensity RE ↑ cognitive performance examining short-term memory, episodic memory and executive/digital span compared to control. No differences occurred between moderate and high intensity groups
Coelho-Júnior et al. (2020)	36 older women (age: >60 y)	22-week RCT (cognitive function assessed after 5 and 14 weeks)	**Traditional RE:** twice weekly RE (5-6/10 RPE, 9 exercises, 8-10 repetitions per set, 3 sets per exercise) **Power training:** twice weekly RE at moderate intensity (RPE 3), as quickly as possible, 8-10 repetitions per set, 3 sets per exercise **Control:** no exercise	Both traditional and power RE ↑ global cognitive function, short-term memory, and dual-task performance. No changes in serum BDNF occurred in any group

Table 2.7 continued

Study	Participants	Duration and design	Groups	Key findings
Ikudome et al. (2017)	170 healthy older adults (age: 52-81 y)	12-week RCT	**RE:** home-based body mass RE (5 exercises, 16 repetitions of each, 2-3 circuits/day) **Control:** maintain normal lifestyle	RE ↑ working memory and inhibitory control (executive function), but not information processing, attention or timing ability compared to control group
Kimura et al. (2010)	119 community-dwelling older adults (age: >65 y)	12-week RCT	**Strength training:** twice weekly RE at 60% 1RM, 2-3 sets of 10 repetitions **Health education:** 1.5 hours of health education twice/month	No differences in reaction time occurred between groups
Lachman et al. (2006)	210 healthy, community-dwelling older adults (age: 60-94 y)	24-week RCT	**RE:** elastic-band (TheraBand) RE (thin to heavy resistance 3 times/week) **Control:** waiting list (continue normal everyday activities)	No differences occurred between groups for memory, but RE level was a significant predictor of memory change
Liu-Ambrose et al. (2010)	155 community-dwelling women (age: 65-75 y)	12-month RCT	**Once weekly:** 2 sets of 6-8 repetitions whole-body RE at 7RM (once weekly) **Twice weekly:** 2 sets of 6-8 repetitions whole-body RE at 7RM (twice weekly) **Control:** balance and toning (twice weekly)	Both RE groups ↑ Stroop test performance (executive function), selective attention and conflict resolution compared to control
Liu-Ambrose et al. (2012)	*Same as above	*Same as above	*Same as above	Twice weekly RE only ↑ selective attention and conflict resolution compared to control. No differences occurred between once and twice weekly RE

Table 2.7 continued

Study	Participants	Duration and design	Groups	Key findings
Marston et al. (2019c)	45 healthy adults (age: 41–69 y)	12-week RCT	**High load, long rest RE:** 5 sets of 5 repetitions at 85% 1RM twice weekly, 3 min between sets **Moderate load, short rest RE:** 3 sets of 10 repetitions at 70% 1RM twice weekly, 60 s between sets **Control:** no exercise	Delayed verbal memory performance ↑ greater in the RE groups when compared to the control group
Singh et al. (2014)	100 MCI participants (age: 70.1 ± 6.7)	6 months RCT with 18 months follow up	**RE:** twice weekly RE (3 sets of 8 repetitions of 5-6 exercises at high intensity) **CT:** computer based cognitive function training **Combined RE+CT:** interventions above combined **Control:** sham cognition + sham exercise (stretching)	RE ↑ global cognitive function compared to control at 6 months. RE maintained executive function and global cognitive function at 18 months follow-up
Smolarek et al. (2016)	37 older women (age: 65.9 ± 5.7y)	12-week RCT	**Strength training:** whole-body RE (60-70% 1RM; 3 sets of 10 exercises) 3 times/week **Control:** maintained habitual physical activity	Strength training ↑ global cognitive function (MoCA points) compared to control group
Yoon, Lee, and Song (2018)	43 cognitively frail older adults (age: 73.9 ± 4.3 y)	16-week RCT	**High-speed whole-body RE:** 2-3 sets of 12-15 repetitions [intensity based on RPE (12-13 - somewhat hard)] 3 times/week **Control:** balance and resistance band stretching (3 times/week)	RE ↑ processing speed and executive function compared to control. No differences in memory or working memory occurred between groups

↑, increased; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; RCT, randomised controlled trial; RE, resistance exercise.

2.4.6 Effects of dietary protein on cognitive function

Epidemiological studies investigating the association between dietary protein and cognitive function have reported mixed findings with some (Lee et al. 2001, Nes et al. 1988, Roberts et al. 2012, Vizuete et al. 2010), but not all studies (Barberger-Gateau et al. 2007, Deschamps et al. 2002, Katsiardanis et al. 2013, Requejo et al. 2003, Velho et al. 2008, Vercambre et al. 2009) reporting positive associations. Similarly, review articles also report inconsistent results (Koh et al. 2015, van de Rest, van der Zwaluw, and De Groot 2013). These disparities may be due to the relatively good health and nutritional status of participants, and differences in the range of dietary protein spread between participants.

Consistent with the above, findings from intervention studies in older adults are also varied (**Table 2.8**). Several studies have reported beneficial effects of dietary protein on aspects of cognitive function (Charlton et al. 2016, Kaplan et al. 2001, Lefferts et al. 2020, van der Zwaluw et al. 2014), whilst others have failed to observe such effects (Bell et al. 2019, Moran et al. 2018, Zajac et al. 2019). Acutely, improvements in memory have been observed following 50.5 g whey protein supplementation (Kaplan et al. 2001). Longitudinal intervention studies of 12-24 weeks have also reported improvements in reaction time (van der Zwaluw et al. 2014), memory (Charlton et al. 2016, Kita et al. 2019), attention (Kita et al. 2019) and emotion identification (Lefferts et al. 2020). Contrary to these studies, Bell et al. (2019) reported no improvement in any aspect of cognitive function in older adults consuming a whey protein-based multi-ingredient supplement twice daily for 6 weeks. Likewise, Moran et al. (2018) also reported no cognitive effects of 6 months consumption of a multi-ingredient supplement containing whey protein. More recently, twice daily consumption of 25 g whey protein for 8 weeks elicited no effects on cognitive function in older adults, but improvements in reasoning speed and reaction time were observed following twice daily consumption of 25 g soy protein in older women only (Zajac et al. 2019). The authors concluded the latter of these findings may have been observed due to the positive impact of soy isoflavones in postmenopausal women (Kritz-Silverstein et al. 2003). It is also worth noting that although two previously cited

studies reported positive effects of protein supplementation on reaction time and emotion identification, respectively (Lefferts et al. 2020, van der Zwaluw et al. 2014), these studies observed no effects on memory or executive functioning, two key cognitive domains associated with ageing (Vaughan and Giovanello 2010, Peters 2006).

The lack of protein-induced effects on cognitive function in several of the abovementioned studies might be related to an insufficient intervention duration and inadequate protein dose. To explain, two of these studies examined the effects over a 6-8-week duration (Bell et al. 2019, Zajac et al. 2019), whereas studies that reported beneficial effects were conducted over at least 12 weeks (Kaplan et al. 2001, Lefferts et al. 2020, van der Zwaluw et al. 2014). Furthermore, while the multi-ingredient supplement investigated by Moran et al. (2018) contained 3000 mg n-3 PUFA, a dose previously reported to attenuate cognitive impairment (Zhang et al. 2016), the supplement only contained 8 g whey protein. As detailed in part 1 of this literature review (**section 2.2.8.3**), Moore et al. (2015) established the breakpoint for maximal stimulation of MPS in older adults to be 0.4 g protein/kg, which has led others to suggest this deviation of dietary protein might be required to stimulate gains in SMM in older adults (Park, Choi, and Hwang 2018). Given the association between sarcopenia and cognitive impairment previously reported (Peng et al. 2020), the findings of Moore et al. (2015) partly support the notion that 8 g whey protein over 6 months was likely insufficient to stimulate changes in SMM and cognitive function. Though, it must be acknowledged that no dose-response study has been conducted to determine the optimal dose of dietary protein to stimulate cognitive benefits in older adults; therefore, this theory is purely speculative.

Table 2.8 Summary of intervention studies investigating the effects of dietary protein on cognitive function in older adults

Study	Participants	Duration and design	Groups	Key findings
Bell et al. (2019)	49 healthy sedentary men (age: 73 ± 6 y)	20-week double-blind RCT split into 2 phases: 6-weeks nutrition only + 12-weeks exercise + nutrition	**SUPP:** 1500 mg n−3 PUFA, 30 g whey protein, 2.5 g creatine, 500 IU vitamin D and 400 mg calcium (twice daily) **CON:** 22 g maltodextrin (twice daily)	No changes in any aspect of cognitive function following phase 1 (6 weeks supplementation) occurred in any group. No changes in plasma BDNF occurred in any group
Charlton et al. (2016)	48 healthy older adults (age: 78.2 ± 6.2 y)	12-week pilot quasi-experimental study	**Pork:** pork-containing meals 4 times/week (average protein per meal 28.1 ± 6.3 g) **Chicken (control):** chicken-containing meals 4 times/week (average protein per meal 25.2 ± 6.2 g)	Chicken group ↑ verbal learning and memory at 6 weeks. Pork had no effect on any cognitive function test
Kaplan et al. (2001)	22 older adults (age: 61-79 y)	Acute repeated-measures crossover design	**Protein:** 260 mL water, 10 mL lemon juice, 50.5 g whey protein isolate **Fat:** 248.9 mL water, 10 mL lemon juice, 41.1 g safflower oil **Carbohydrate:** 260 mL water, 10 mL lemon juice and 50 g glucose **Placebo:** 290 mL water, 10 mL lemon juice and 23.7 mg sodium saccharin	Whey protein ↑ delayed paragraph recall (memory) compared to placebo, but had no effect on attention or executive function
Kita et al. (2019)	114 healthy older adults (age: 50-75 y)	12-week double-blind RCT	**Whey peptide:** 1 g whey peptide tablet [containing 1.6 mg of β-lactopeptide of glycine-thereonine-tryptophan-tyrosine (GTWY)] once daily **Placebo:** 1 g maltodextrin tablet	Whey peptide ↑ associative learning memory and control of attention greater than placebo
Lefferts et al. (2020)	99 healthy older adults (age: 67 ± 6 y)	12-week parallel groups RCT	**Whey protein**: 50 g/d (2 x 25 g) whey protein isolate **Control**: 50 g/d (2 x 25 g) carbohydrate (maltodextrin)	Whey protein ↑ emotion identification compared to control, but no significant differences occurred between groups for working memory, attention, or impulsivity composite scores

Table 2.8 continued

Study	Participants	Duration and design	Groups	Key findings
Moran et al. (2018)	36 community-dwelling older adults (age: 75.1± 3.6 y)	6-month double-blind RCT	**Multi-ingredient nutrition supplement:** 200 mL liquid juice comprising 3000 mg n-3 PUFA [1500 mg docosahexaenoic acid (DHA) and 1500 mg eicosapentaenoic acid (EPA)], 10 µg vitamin D3, 150 mg resveratrol and 8 g whey protein isolate **Placebo:** 200 mL juice only	No significant differences in overall cognitive function or composite cognitive domain scores were observed between groups. Stroop colour-word time ↓ in the multi-ingredient group only compared to the placebo, but this effect was diminished after Bonferroni adjustment
van der Zwaluw et al. (2014)	65 frail or pre-frail elderly individuals (age: 78 ± 8 y in protein group, 81 ± 7 y in placebo group)	24-week double-blind parallel groups RCT	**Protein:** 2 x 15 g protein supplement per day **Control:** 2 x matched placebo (0 g protein) per day	Protein ↓ reaction time greater than the placebo group. No differences occurred between groups for global cognitive function, episodic memory, working memory, information processing or executive function
Zajac et al. (2019)	56 moderately vitamin B12-deficient participants (age: 60 ± 8.5 y)	8-week randomised controlled crossover trial (16 weeks washout)	**Whey protein:** 2 x 25 g whey protein isolate per day **Soy protein:** 2 x 25 g soy protein per day	No effect of whey protein on any cognitive domains. Soy protein ↑ reasoning speed and reaction time in females only

↑, increased; ↓, decreased; PUFA, polyunsaturated fatty acids; RCT, randomised controlled trial.

2.4.7 Effects of resistance exercise combined with dietary protein on cognitive function

At present, only four studies have investigated the effects of RE combined with increased dietary protein intake on cognitive function in older adults (Bell et al. 2019, Formica et al. 2020, Rondanelli et al. 2020, van de Rest et al. 2014). van de Rest and colleagues (2014) demonstrated twice weekly RE performed at 75% 1RM combined with twice daily protein supplementation (15 g/serving) for 24 weeks improved information processing speed greater than protein supplementation alone. In contrast, the opposite effect was observed for verbal fluency, and no differences were observed between RE groups for any cognitive domain. More recently, Formica et al. (2020) demonstrated 24 weeks of multimodal exercise three times per week [including moderate-high intensity (5-8 on the 10-point Borg scale) aerobic + RE] enhanced global cognitive function and executive functioning, but consumption of lean red meat (2 x 80 g cooked weight on training days; ~45 g/d additional protein) did not augment these effects. Paradoxically, multimodal exercise alone improved working memory greater than exercise combined with lean red meat.

Contrary to these studies, Rondanelli et al. (2020) demonstrated physical rehabilitation (including muscle strengthening exercises) combined with twice daily consumption of a whey protein-based nutritional supplement enriched with leucine and vitamin D, improved Trail making test performance (which assesses executive functioning, processing speed, mental flexibility and attention) greater than physical rehabilitation combined with a control supplement in sarcopenic older adults. Additionally, Bell et al. (2019) established multimodal exercise, including twice weekly RE (80% 1RM) and once weekly high intensity interval training (HIIT) [10 x 60 s intervals at 90% max heart rate (HR)] combined with a twice daily whey protein-based multi-ingredient supplement (containing 30 g whey protein), elicited greater effect sizes on reaction time (d = -0.73 vs. -0.44) and MoCA performance (d = 0.53 vs. 0.02) compared to RE combined with a control supplement. A within-group improvement for composite cognitive function was also only observed in the RE plus whey protein-based multi-ingredient supplement group. However, while differences in effect sizes were observed,

it must be noted that no significant between-group differences occurred. The authors attributed the lack of significant effects to a lack of statistical power.

The disparities between the abovementioned studies may be related to the habitual intake, daily dose during the intervention, and deviation of dietary protein. Dietary intake data from participants in the study by van de Rest et al. (2014) published elsewhere (Tieland et al. 2012b), reports dietary protein intake increased by 0.3 g/kg/d (1.0-1.3 g/kg/d). In the study by Formica and colleagues (2020), red meat was only consumed on exercise training days (3 days/week); subsequently, protein intake was increased from 1.26 to 1.40 g/kg/d. In contrast, whilst Rondanelli et al. (2020) increased dietary protein by a similar amount to these studies (0.32 g/kg/d), habitual intake was considerably less (0.79 g/kg/d). Similar to that mentioned previously, the effect of dietary protein on cognitive function may be more profound in protein malnourished individuals. Furthermore, Bell et al. (2019) increased dietary protein by a notably greater amount (0.5 g/kg/d; 1.1-1.6 g/kg/d). Although further research is warranted, it may be that a protein dose of 1.6 g/kg/d (or a deviation of ~0.5 g/kg/d) may be required to stimulate additive effects on cognitive function in older adults habitually consuming adequate amounts of dietary protein. Interestingly, this intake mimics that suggested to maximally increase SMM during RE training (Morton et al. 2018a).

Whilst data from Bell et al. (2019) and Rondanelli et al. (2020) provide preliminary evidence indicating that RE combined with dietary protein may synergistically improve cognitive function in the elderly, several methodological aspects of these studies warrant further discussion. Firstly, the supplements investigated in these studies contained vitamin D, and the supplement examined by Bell et al. (2019) also contained n-3 PUFA. Both of these ingredients have previously been shown to attenuate cognitive decline (Balion et al. 2012, Karr, Alexander, and Winningham 2011). As the isolated effects of dietary protein were not studied, it is unknown whether increased dietary protein *per se* stimulated the additive effects. Moreover, the exercise intervention in the study by Bell and colleagues (2019) included aerobic-based

exercise, which has been shown to aid cognitive function in older adults (Zheng et al. 2016). This was also the case in the study by Formica et al. (2020). Consequently, it is also unknown whether the combination of aerobic plus RE combined with the multi-ingredient supplement yielded the cognitive benefits. The true effects of RE combined with dietary protein on cognitive function in the elderly is therefore limited to that of van de Rest and colleagues (2014).

2.4.8 Mechanisms underlying resistance exercise- and dietary protein-induced improvements in cognitive function

Although previous work has indicated RE and dietary protein may slow or prevent cognitive decline, the mechanisms of action are relatively unknown (Macaulay, Fisher, and Schroeder 2020, van de Rest, van der Zwaluw, and De Groot 2013). At present, changes to brain structure and function, alterations in various neurobiological and inflammatory markers, and improvements in vascular and muscle function, insulin sensitivity, and psychological factors such as mood and sleep quality are all purported to contribute to the cognitive benefits (Kovacevic et al. 2018, Macaulay, Fisher, and Schroeder 2020, van de Rest, van der Zwaluw, and De Groot 2013). The following sections will briefly review these mechanisms.

2.4.1 Skeletal muscle mass and strength

As previously reported in part 1 of this literature review (**section 2.2.10.1.2**), RE is well-known for its effects on FFM (Peterson, Sen, and Gordon 2011), muscular strength (Peterson et al. 2010) and physical function (Arnarson et al. 2013, Oesen et al. 2015). Similarly, although research is mixed, dietary protein has also been shown to increase FFM, muscular strength and physical function in both healthy (Bell et al. 2017, Norton et al. 2016, ten Haaf et al. 2019) and sarcopenic/frail older adults (Bauer et al. 2015, Bo et al. 2019, Shahar et al. 2013, Tieland et al. 2012c). Some studies have also demonstrated synergistic effects of RE combined with dietary protein (Bell et al. 2017, Daly et al. 2014, Kang et al. 2019, Junior et al. 2018, Rondanelli et al. 2016, 2020, Tieland et al. 2012b, Verreijen et al. 2015, Yamada et al. 2019,

Zdzieblik et al. 2015). Therefore, given the previously mentioned association between sarcopenia and cognitive impairment (Chang et al. 2016, Cipolli, Yassuda, and Aprahamian 2019, Peng et al. 2020), RE- and dietary protein-induced improvements in cognitive function may be partially explained by improvements in muscle function. In support of this hypothesis, Liu-Ambrose et al. (2010) reported an association between improvements in selective attention and conflict resolution with increases in gait speed following 12 months of RE. More recently, two studies established improvements in cognitive function following RE were mediated by increases in muscular strength (Forte et al. 2013, Mavros et al. 2017). Unfortunately, no study has examined whether protein-induced effects on SMM, strength or physical function are related to cognitive function in older adults.

In addition, it is also unknown whether the relationship between RE-induced changes in muscle and cognitive function is purely casual or causative due to changes in common pathologies, such as systemic inflammation and insulin sensitivity. Recently, Macaulay, Fisher, and Schroeder (2020) proposed the association might be explained by shared supraspinal neural pathways.

2.4.8.2 Brain structure and function

Several studies have reported improvements in brain structure and function following RE training (Best et al. 2015, Herold et al. 2019, Liu-Ambrose et al. 2012, Suo et al. 2016). Studies employing morphometric imaging have demonstrated expanded grey matter in the posterior cingulate, reversed progression of white matter hyperintensities, and positive functional changes in hemodynamic activity in several areas of the brain (Liu-Ambrose et al. 2012, Suo et al. 2016). Regarding dietary protein, the AA tryptophan has been shown to enhance serotonin receptor activation, which plays a significant role in the regulation of sleep, mood and memory (Camfield et al. 2011, van de Rest, van der Zwaluw, and De Groot 2013). The AA tyrosine is also a precursor for a number of important neurotransmitters, including

norepinephrine, epinephrine and dopamine (Fernstrom and Fernstrom 2007). The latter of which is involved in working memory (Tarn and Roth 1997).

2.4.8.3 Neurobiological markers

2.4.8.3.1 Insulin-like growth factor 1

As reported in **section 2.2.10.4.1**, several studies have reported increased circulating IGF-1 concentration following both RE training (Cassilhas et al. 2010, de Souza Vale et al. 2009, Formica et al. 2020, Jiang et al. 2020, Singh et al. 1999) and increased dietary protein intake (Bauer et al. 2015, Bo et al. 2019, Mehlsen et al. 2017, Zhu et al. 2011). As IGF-1 can cross the blood-brain barrier, it may be that RE- and protein-induced increases in peripheral IGF-1 concentration may influence brain IGF-1 concentration, potentially aiding cognitive function (Van Dam et al. 2000). In part support, Cassilhas and colleagues (2010) reported parallel increases in cognitive function and IGF-1 following RE training; however, no cause-and-effect relationship can be determined from this study. Further, no study has directly assessed the association between protein-induced changes in IGF-1 and cognitive function in older adults. Prospective studies are therefore required to determine whether there is a cause-and-effect relationship between RE- and protein-induced changes in IGF-1 and cognitive function in older adults.

2.4.8.3.2 Brain-derived neurotrophic factor

Increased circulating BDNF concentration following an acute bout of RE has been reported in some (Church et al. 2016, Marston et al. 2017, Vega et al. 2010, Yarrow et al. 2010) but not all studies (Correia et al. 2010, Goekint et al. 2010, Tsai et al. 2018). Discrepant findings might be explained by the intensity of RE, as previous work has demonstrated significant differences between the magnitude of BDNF increase (40% vs. 4%) following RE performed at high and low intensity (Vega et al. 2010). Similar to that of acute studies, findings from longitudinal studies are also equivocal with some (Coelho et al. 2012, Forti et al. 2015, Pereira et al. 2013),

but not all studies (Bell et al. 2019, Formica et al. 2020, Marston et al. 2019a), reporting increases in BDNF. However, in contrast to acute studies, intensity (or frequency) of RE does not seem to explain the disparities between studies as the interventions were all similar and appear adequate based on acute findings (Marston et al. 2019b). It is also unlikely that differences in the health status of participants and baseline BDNF concentration explain these conflicting findings as increases in BDNF have been observed in both frail and healthy elderly individuals (Coelho et al. 2012, Forti et al. 2015), and those with low and high baseline BDNF concentration (Coelho et al. 2012). Further research is warranted to explore the influence of different factors which may affect RE-induced changes in basal BDNF.

The effect of dietary protein on circulating BDNF is relatively unknown due to a lack of published data. Bell et al. (2019) demonstrated 18 weeks of a whey protein-based multi-ingredient supplement had no effect on basal plasma BDNF concentration. In support of these findings, Formica et al. (2020) reported no effect of multimodal exercise combined with consumption of lean red meat three times per week on basal BDNF concentration. Based on these findings, it appears that protein-induced increases in cognitive function are not related to changes in BDNF.

2.4.8.4 Cortisol

As previously noted in **section 2.2.10.4.2**, the effects of RE on the adaptive cortisol response is controversial. Disparities in the literature are likely due to cortisol being measured at only one time point at different times in the morning. Whilst the effect of RE on cortisol is unclear, several studies have reported changes in fasting cortisol (Kraemer et al. 1999, Häkkinen et al. 2002, Izquierdo et al. 2003). Similarly, although findings from studies investigating the effects of dietary protein on cortisol secretion are also mixed, lower cortisol concentration following a high protein compared to a high carbohydrate meal/diet has been reported (Vicennati et al. 2002, Martens et al. 2010). Thoeretically, as altered cortisol secretion contributes to cognitive decline with age (Beluche et al. 2010, Dijckmans et al. 2017, Evans et al. 2011, 2012, Lee et

al. 2007), changes following RE and dietary protein may explain improvements in cognitive function. Though, from a RE perspective, a recent acute study by Wang et al. (2019) disputes this hypothesis, reporting no correlation between RE-induced changes in cognitive function and cortisol secretion. This study was, however, conducted in young adults, which may explain these findings. Further longitudinal work is required to determine whether RE- and protein-induced changes in cognitive function are mediated by changes in cortisol secretion in older adults.

2.4.8.5 Inflammation

As previous reviewed in **section 2.2.10.4.5**, RE may reduce systemic inflammation in older adults if the exercise stimulus elicits increases in SMM and reduces FM (Sardeli et al. 2018). It has been postulated that RE-induced decreases in systemic inflammation may improve cognitive function by supressing the activation of microglia, reducing neuroinflammation (Macaulay, Fisher, and Schroeder 2020). However, limited studies have assessed both cognitive function and inflammation in parallel following RE in older adults. In cognitively impaired older women, 28 weeks of RE significantly increased concentrations of the anti-inflammatory cytokine interleukin-10 (IL-10), which occurred alongside increases in global cognitive function (Chupel et al. 2017). In contrast, Formica et al. (2020) observed improvements in cognitive function following 24 weeks of multimodal exercise incorporating RE, but no changes in inflammatory markers were observed.

At present, no study has examined the relationship between protein-induced changes in inflammation and cognitive function. However, recent work has demonstrated dietary protein may induce anti-inflammatory effects in older adults (Bell et al. 2018, Liberman et al. 2019, Sugawara et al. 2012, Solerte et al. 2008). As systemic inflammation contributes to cognitive decline via increasing central nervous system (CNS) inflammation (Marsland et al. 2015), it may be hypothesised that dietary protein might improve cognitive function through reversing this mechanism. Taken together, it is currently unknown whether RE and protein-induced

improvements in cognitive function are related to a reduction in systemic inflammation. Further research is warranted to examine such association.

2.4.8.6 Vascular function and blood pressure

Both RE (Williams et al. 2007) and dietary protein, specifically whey protein (Camfield et al. 2011, Pal and Ellis 2010), may improve cognitive function via improved cardiovascular health. Following RE training, decreased systolic and diastolic blood pressure (BP) (Ashton et al. 2020), improved endothelial function (Ashor et al. 2015), and a greater resting cerebral perfusion leading to an increase in brain blood flow have been reported (Xu et al. 2014). Whey protein contains lactokinins, which are peptides that inhibit angiotensin converting enzyme (ACE) activity (FitzGerald and Meisel 1999), the key enzyme in the regulation of BP (Pal and Ellis 2010). Acutely, whey protein ingestion has been shown to improve endothelial function (Ballard et al. 2013). Longitudinally, improvements in BP and endothelial function, and reduced aortic stiffness have been observed (Fekete et al. 2016, Pal and Ellis 2010). Whey protein is also comprised of ~20% of its protein from milk, which may partially explain the associations between dairy consumption and reduced risk of hypertension (Hidayat et al. 2017) and arterial stiffness (Crichton et al. 2012a).

2.4.8.7 Glucose homeostasis

As previously discussed in part 2 of this literature review (**section 2.3.16**), RE is an effective stimulus for increasing insulin sensitivity and improving glycaemic control (Westcott 2012). Although the effects of dietary protein on insulin sensitivity are somewhat controversial (Drummen et al. 2018), there is evidence suggesting the BCAA leucine can stimulate insulin production (Yang et al. 2010). Consequently, RE and dietary protein may stimulate the abundance of insulin receptors in the brain (Frazier et al. 2019), improving local brain insulin sensitivity and altering key processes such as cerebral glucose metabolism, which is involved in learning and memory (Doyle et al. 1995).

2.5 Summary from the literature and justification for the research

Based on the review of relevant literature, RE combined with adequate dietary protein may synergistically aid attenuation of sarcopenia and sarcopenic obesity, improve metabolic health and aid cognitive function in older adults. However, several questions remain unanswered, highlighting the need for further research.

Firstly, as previously mentioned in **section 2.3.23**, only four studies have investigated the synergistic effects on components of EE, of which neither examined the effects on 24-h EE. Similarly, no studies have investigated the combined effects on 24-h metabolic flexibility or appetite compared to each intervention alone. Collection of such data is vital to understand whether these interventions elicit augmented effects on aspects of energy metabolism, energy balance and metabolic health in older adults.

Secondly, whilst numerous studies have examined the combined effects of RE and dietary protein on SMM, strength and physical function, the majority of these studies, particularly in healthy older adults, employed an insufficient protein dose to elicit additive effects (**see section 2.2.10.3.1**). Furthermore, only a few studies have employed an experimental design with four intervention groups, of which none measured any hormonal or inflammatory markers related to sarcopenia. The biochemical mechanisms of how RE combined with dietary protein may attenuate sarcopenia is still therefore relatively unknown. Investigation of the synergistic effects employing a daily protein intake of 1.6 g/kg/d, and analysis of hormonal and inflammatory markers, is required to answer this question.

Finally, due to a paucity of data, further research on the combined effects of RE and dietary protein on cognitive function and analysis of mechanisms of action is warranted to determine whether these interventions may synergistically mitigate cognitive decline in the elderly.

CHAPTER 3: General methods

3.1 Chapter overview

This chapter details and justifies the methods used within this thesis. The overall aim is to provide a detailed account of the experimental design, participant recruitment, experimental interventions and outcome measures used in most/all experimental chapters. Study specific methods are detailed in relevant experimental chapters (**Chapters 4, 5 and 6**).

3.2 Study design

The study conducted within this thesis was a 12-week randomised, controlled, double-blind, 4-arm parallel group trial that took place between October 2017 and June 2019. Individual results chapters are outcomes taken from the 12-week clinical trial. Participants are the same group throughout. Following screening (see **section 3.4.2**), eligible participants were randomly assigned to one of four experimental groups: control (CON), whey protein supplementation (PRO), RE + control (EX+CON) or RE + whey protein supplementation (EX+PRO). A coded (A, B, C or D) randomisation scheme was used. Randomisation was performed using the minimization allocation method with stratification for age (dichotomised as 60-63, 64-66, 67-70, 71-74, 75-77 and 78-80 y) and body mass index [(BMI); dichotomised as 18.5-20.4, 20.5-22.4, 22.5-24.4, 24.5-26.4, 26.5-28.4 and 28.5-30 kg/m^2] using free online software (QMinim; http://rct.mui.ac.ir/q/). A key to the randomisation code was held by an investigator who was not directly involved with participant recruitment, exercise training, or testing. At both baseline and 12 weeks, participants completed two testing sessions. The first session involved 24-h metabolic testing where participants resided in respiration chambers, and the second (within 7 days of metabolic testing) involved strength and physical function testing. The research design and sequence of testing is shown diagrammatically in **Figure 3.1**.

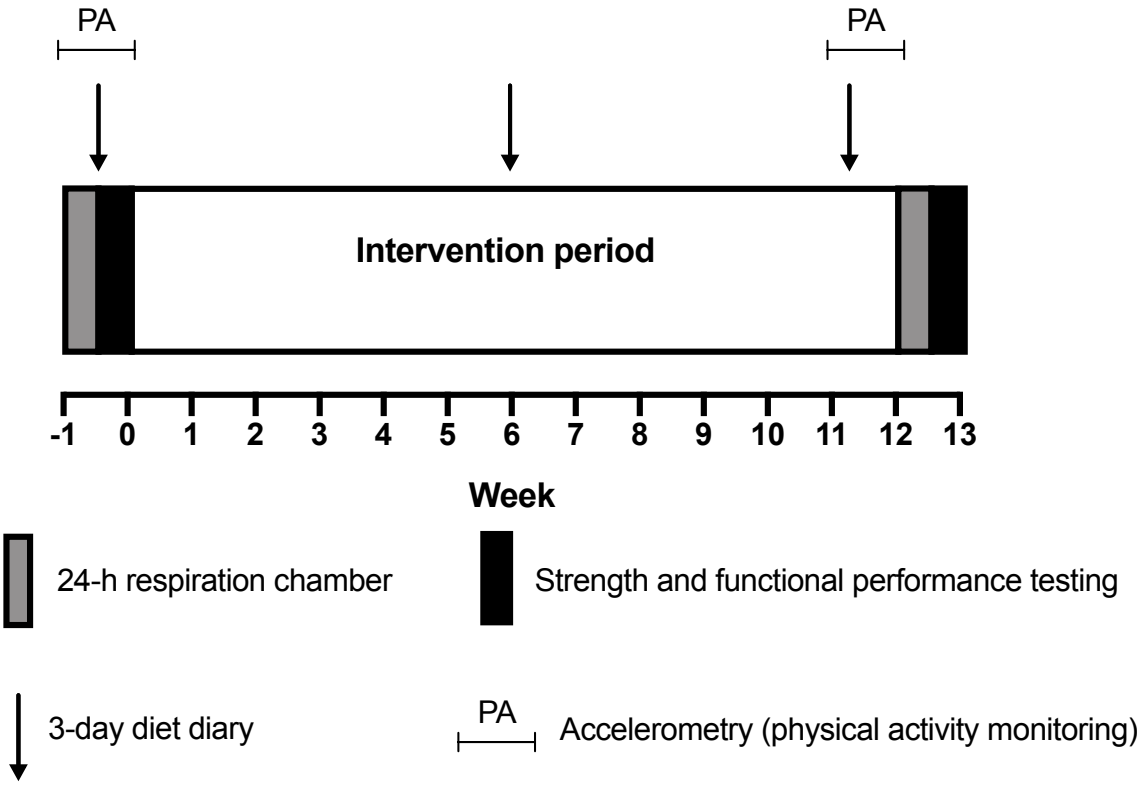

Figure 3.1 Experimental design and sequence of testing.

3.2.1 Justification for study design

Double-blind RCTs are considered the 'gold standard' for intervention-based studies and, when well designed, provide the strongest possible evidence of causation (Misra 2012). In addition, a position paper by the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) also highlight the double-blind, placebo-controlled, parallel-arm design is the mainstay of regulatory design for clinical intervention trials investigating the effectiveness of interventions to treat or prevent sarcopenia (Reginster et al. 2016). Alternative research designs such as cross-over and non-placebo-controlled were considered, however, these designs were not chosen for several reasons. Firstly, the time required to complete a cross-over design with significant wash-out was unfeasible. Secondly, non-placebo-controlled trials significantly increase the risk of bias (i.e., participants in the control groups changing their dietary behaviour, particularly increasing protein intake). Finally, a control product was necessary as differences in body mass may have occurred between

groups purely due to an increase in EI in the experimental groups. Consequently, this may have affected primary endpoints of the study (e.g., 24-h EE). A 4-arm RCT was chosen as few studies (de Carvalho Bastone et al. 2020, Gryson et al. 2014, Kim et al. 2012, Kirk et al. 2020, Kukuljan et al. 2009, Shahar et al. 2013, van de Rest et al. 2014, Verreijen et al. 2017) in the literature have employed such a research design of four experimental groups (control, protein, RE, RE + protein), and only two of these studies included a placebo or energy-matched control product (Gryson et al. 2014, van de Rest et al. 2014). Further, neither of these studies increased dietary protein to 1.6 g/kg/d, the suggested optimal intake to accrete SMM during RE training (Morton et al. 2018a). Thus, additional research utilising a 4-arm randomised controlled design and a protein intake of 1.6 g/kg/d was required to robustly determine both the individual and combined effects of RE and whey protein supplementation.

Minimisation was used to randomise participants to ensure significant imbalances in baseline participant characteristics between groups did not occur (Taves 2010). Age and BMI were chosen *a priori* as stratification variables as previously published data has shown both significantly affect energy metabolism, a primary outcome of this thesis (Geisler and Müller 2017, Manini 2010). Age also affects body composition (Janssen et al. 2000b, Shen et al. 2005, St-Onge and Gallagher 2010), muscle strength (Clark and Manini 2008), cognitive function (Salthouse 2009) and appetite (Akimoto and Miyasaka 2010). Consequently, failure to control for this variable may have significantly affected differences between groups at baseline.

An intervention period of 12 weeks was used as previous studies have reported clinically significant increases in FFM and/or muscle strength (Arnarson et al. 2013, Holwerda et al. 2018, Rondanelli et al. 2016, Verdijk et al. 2009a), components of EE (Campbell et al. 1994), and improvements in cognitive function (Smolarek et al. 2016) following RE training of this duration either with or without additional protein supplementation in older adults. Studies in older adults have also reported significant increases in FFM (Bauer et al. 2013, Mitchell et al.

2017) and cognitive function (Charlton et al. 2016, Kita et al. 2018, Lefferts et al. 2020) following increased protein intake *per se* for 10-12 weeks. Furthermore, Churchward-Venne et al. (2015) established high interindividual variability in the adaptive response to RE training when changes in FFM, muscle fibre size and strength, and physical function were assessed in older adults. The level of responsiveness in this study was strongly influenced by the duration of the RE intervention, with 76.4% of participants displaying improvements in FFM following 12 weeks. Additionally, significant increases in leg press (33 ± 2 kg, $P < 0.001$) and leg extension 1RM (20 ± 1 kg, $P < 0.001$) were observed after 12 weeks. Based on the aforementioned studies, an intervention period of 12 weeks was deemed sufficient to detect significant changes in FFM, muscle strength, and components of EE and cognitive function following RE and/or whey protein supplementation.

3.3 Ethical approval

Ethical approval was granted by Coventry University Ethics Committee (Project reference: P59723, **Appendix A**). In addition, Governance Arrangements for Research Ethics Committees (GafREC) approval was granted by the Research & Development department at University Hospitals Coventry and Warwickshire (UHCW) NHS Trust for use of the respiration chambers within the Human Metabolism Research Unit (HMRU) (**Appendix B**). Following interest in participation, all participants were sent a participant information sheet (PIS) via email or through the post, which explained the research study and were asked to read its entirety (**Appendix C**). Participants were also informed of the nature of the study verbally. Informed consent was obtained in accordance with the Declaration of Helsinki by the chief investigator who had received NHS informed consent and good clinical practice (GCP) training (**Appendix D**). The study was registered on clinicaltrials.gov as NCT03299972.

3.4 Participants

3.4.1 Recruitment

Participants were recruited from Coventry, UK and surrounding areas between October 2017 and February 2019 by several avenues, including newspaper advertisements, contact with local groups and organisations (e.g., parish councils, rambling groups, bridge/bowls clubs), word of mouth, social media, charity shops, libraries, coffee shops and leisure facilities. See **Appendix E** for the study recruitment poster.

3.4.2 Screening and participant flow

A total of 256 individuals responded to advertisements and were sent a PIS for consideration. From these, 85 individuals declined to participate, and 171 individuals were screened for consideration against the inclusion/exclusion criteria (see **sections 3.4.2.1 and 3.4.2.2**). Screening involved two stages. Firstly, participants completed a health and lifestyle questionnaire which screened for age, medical history, medication use and current levels of physical activity (**Appendix F**). Secondly, if participants were eligible following stage 1, at baseline metabolic testing, height and body mass were measured to check BMI, BP was measured, and blood was drawn and analysed for HbA1c, uric acid, brain natriuretic peptide (BNP) and estimated glomerular filtration rate (eGFR) by the pathology department at UHCW to screen for diabetes, susceptibility to gout, heart failure and kidney function. Blood was screened by Professor Martin O. Weickert (Consultant Endocrinologist, UHCW), who provided medical clearance and confirmed participant suitability to begin the intervention. Of the 171 individuals who were screened, 132 did not meet the study eligibility criteria, so were therefore excluded. The remaining 39 individuals were included in the study. No participant had any cognitive impairment, which was assessed by the Mini-Mental State Examination (MMSE) (score >24) (Folstein, Folstein, and McHugh 1975). Of the 39 participants included in the study, three did not participate in respiration chamber assessments (**Chapter 4**) but completed all other aspects of the study (**Chapters 5 and 6**). Three participants also withdrew. As a

result, 33 participants completed metabolic testing and 36 participants completed all other measures. The flow of participants is shown diagrammatically in **Figure 3.2**.

3.4.2.1 Inclusion criteria

- Age >60 and <80 y
- BMI between 18.5-30 kg/m^2
- Not participated in any form of RE within the last 6 months
- Free from musculoskeletal injury

3.4.2.2 Exclusion criteria

- Age <60 or >80 y
- Current smoker, or ex-smoker ceasing <6 months ago
- BMI <18.5 or >30 kg/m^2
- Weight instability (±>3 kg change in body mass in previous 6 months)
- Individuals participating in another research project within the last 6 months involving a dietary and/or exercise intervention
- Existing or past medical history of cancer, diabetes, or vascular, neurological, kidney, pulmonary, digestive, or thyroidal disease, osteoporosis, or a history of falls
- Currently taking protein/amino acid supplements
- Currently prescribed non-steroidal anti-inflammatory medication, hormone replacement therapy (HRT), diabetic medication, beta-blockers, or statins
- Uncontrolled BP (>160/100 mmHg)
- Self-report lactose intolerance, or allergy to any ingredients within study products
- Neuromuscular disorders or injuries or individuals with a pacemaker
- Clinical abnormalities in haematology (assessed by Professor Martin O. Weickert, UHCW)

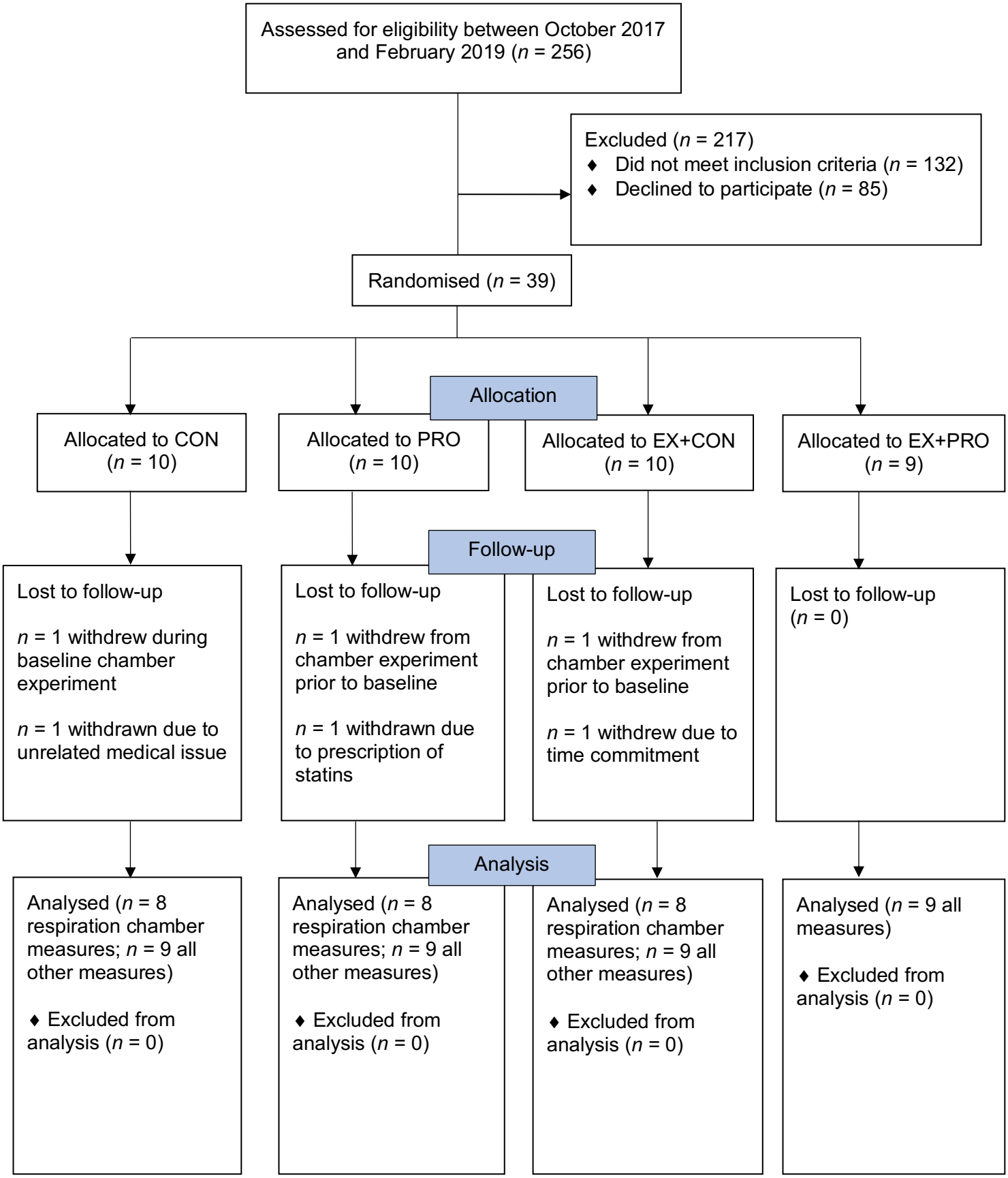

Figure 3.2 Flow of participants throughout the study.

3.4.3 Justification for inclusion/exclusion criteria

Although working groups suggest the target population for clinical trials investigating treatment and preventative interventions to mitigate sarcopenia should be individuals aged ≥65 years (Cruz-Jentoft et al. 2010, Reginster et al. 2016), the age range of 60-80 years was chosen for this preventative study as the onset of age-related declines in SMM, strength, EE and cognitive function are typically noticeable between 40-60 years (Beenakker et al. 2010, Hedden and Gabrieli 2004, Janssen et al. 2000b, Manini 2010). Recently published RE and protein supplementation intervention studies of similar nature have also employed a similar age range (Bell et al. 2017, Junior et al. 2018, Kirk et al. 2019, 2020). Women were excluded from this relatively small study based on data demonstrating sex differences in the age-related changes in body composition and EE, thus likely causing large variability in primary study endpoints (Geisler et al. 2016a, Geisler and Müller 2017). Also, men experience greater losses of FFM per annum (3% vs. 1.7%) and have a short life expectancy than women (Reid et al. 2008). However, as women account for a large proportion of older adults, the exclusion of women is recognised as a limitation of this thesis.

Individuals with obesity (BMI >30 kg/m^2) were also excluded as this study was designed as a preventative study and individuals with sarcopenic obesity display a significantly different metabolic profile to that of individuals without sarcopenic obesity (Perna et al. 2017). Obesity also exacerbates sarcopenia, with the EWGSOP2 advising that sarcopenic obesity should be investigated as a distinct condition (Cruz-Jentoft et al. 2019). Other exclusion criteria were set based upon likely effects on endpoints of the study (i.e., SMM, markers of metabolism) reported in previous studies. For example, intentional weight loss (Gallagher et al. 2000); diabetes mellitus (Ghanassia et al. 2006); current smokers (Rom et al. 2012); prescription of non-steroidal anti-inflammatory medication (Rieu et al. 2009), thyroid medication (Johannsen et al. 2012), or statins (Krishnan and Thompson 2010); RE trained (Ahtiainen et al. 2003); or regular supplementation of protein leading to a high baseline habitual protein intake (Phillips, Chevalier, and Leidy 2016).

3.4.4 Sample size

The primary aim of this thesis was to investigate the individual and combined effects of RE and whey protein supplementation on aspects of 24-h energy metabolism measured in a respiration chamber (**Chapter 4**). As RMR is the largest component of TEE, accounting for 60-80% (Geisler and Müller 2017), sample size was determined based on changes in this variable from a previous respiration chamber study that reported an increase of 109 kcal/d in older adults following 16 weeks of RE (Treuth et al. 1995). Using G*Power (Version 3.1.9.2; Dusseldorf, Germany), a minimum of 48 participants (12/group) were needed to observe a significant group-by-time interaction for a mixed-model analysis of covariance (ANCOVA) with one covariate (α = 0.05; β = 0.8; effect size [Cohen's f^2] = 0.5). However, due to time and budgetary constraints, only 33 participants had data for the primary outcome. A retrospective power analysis of the 33 participants who completed the study and the observed changes in RMR provided a β of 0.60.

3.5 Experimental Interventions

3.5.1 Nutritional Supplements

Participants ingested one sachet of either whey protein isolate [(PRO) Instantized BiPRO; Agropur, Quebec, Canada] or an isocaloric control powder [(CON) Maltodextrin; Myprotein, Northwich, UK] twice daily, consumed directly after breakfast and lunch. The macronutrient and specific AA composition of the experimental supplements related to this thesis per serving can be seen in **Table 3.1**, and the full nutrient and AA composition (per 100 g) can be seen in **Appendix G**. Both supplements were similar in powder weight, unflavoured and were provided in opaque sachets in a double-blinded manner (**Figure 3.3**). Participants prepared their supplement beverages at home by mixing the contents of each sachet with ~200 mL of water combined with a double strength no-added sugar cordial of choice using a handheld shaker (Myprotein, Northwich, UK). Participants were provided with recommendations on cordial flavouring use, which was based on taste testing conducted prior to commencing the study.

However, cordial flavouring was not prescriptive. Compliance was assessed by the number of empty sachets returned by participants at the end of the study and through the use of a supplementation log (**Appendix H**). Empty sachets were counted by a postgraduate researcher not involved in any aspect of the study. To test the success of supplement blinding, participants completed an exit questionnaire on completion of the study. The questionnaire asked, 'Do you know which supplement you were consuming during the intervention?'. The answers were either 'protein', 'control', or 'don't know'. Seven out of 36 participants (19.4%) answered correctly, 3/36 participants (8.4%) answered incorrectly, and 26/36 participants (72.2%) were not sure which supplement they were consuming.

Table 3.1 Nutritional composition of experimental supplements (per serving)[1]

Component	Whey protein isolate (PRO)[1]	Control (CON)
	25 g	23.75 g
Energy, kcal	95	95
Carbohydrate, g	0	23.75
Protein, g	22.8	0
Leucine, g	2.9	0
BCAA, g	5.4	0
EAA, g	11.2	0
Tryptophan, g	0.7	0
Tyrosine, g	0.8	0
Fat, g	0.4	0

[1]Whey protein isolate also contained per serving: vitamin A (<25 IU), vitamin C (<0.5 mg), vitamin D (0.2 mcg), iron (0.25 mg), calcium (21.3 mg), phosphorus (85 mg), magnesium (2.5 mg) chloride (20 mg), sodium (172.5 mg), potassium (17.5 mg). BCAA; branched-chain amino acids (leucine, isoleucine, and valine); EAA, essential amino acids.

Figure 3.3 Coded (A, B, C or D) supplement sachets provided to participants in a double-blinded manner (Flexible Packaging Services Ltd, Wirral, UK).

3.5.1.1 Justification for nutritional interventions

As previously highlighted in **sections 2.2.8.2 and 2.2.10.2.1**, protein ingestion is a potent anabolic stimulus which transiently increases rates of MPS and supresses MPB via the effects of insulin, consequently resulting in a positive net protein balance (Rennie et al. 2004). The stimulation of MPS following protein ingestion is primarily attributed to hyperaminoacidemia of EAA (Volpi et al. 2003), most specifically, leucine (Kimball and Jefferson 2006). Of all dietary proteins, whey protein is considered the highest quality protein due to its high content of EAA, particularly leucine, and its rapid digestibility (Devries and Phillips 2015).

The timing (breakfast and lunch daily) and dose (25 g, including ~3 g leucine) of whey protein used in this study was based on findings of previous work, as highlighted in **section 2.2.10.2.2**, that consistently report that older adults consume their daily protein intake in a skewed pattern (Tieland et al. 2012a, Farsijani et al. 2017, Smeuninx, Greig, and Breen 2020). Based on a hypothesised daily protein distribution similar to that reported by Smeuninx, Greig, and Breen

(2020) (~0.2, ~0.3 and ~0.5 g/kg at breakfast, lunch and dinner, respectively), plus a daily protein intake of ~1 g/kg/d and a mean body mass of ~80 kg of the cohort used in this study to that of others (Bell et al. 2017, Kirk et al. 2019, Smeuninx, Greig, and Breen 2020), the dose of 25 g whey protein (~0.25 g/kg) at breakfast and lunch was postulated to yield a daily protein distribution of ~0.45 g/kg at breakfast, ~0.55 g/kg at lunch, and the evening meal remaining at ~0.5 g/kg. Accordingly, this would ensure all meals met both the per meal protein (0.4 g/kg) and leucine (≥2.5 g) thresholds to maximally stimulate rates of MPS in older adults (Moore et al. 2015). In addition, it was hypothesised that this dose would increase daily dietary protein intake from ~1.0 to 1.6 g/kg/d, the intake recommended to curb sarcopenia (Phillips, Chevalier, and Leidy 2016) and maximise SMM accretion during RE training (Morton et al. 2018a). This deviation of daily dietary protein intake (~0.6 g/kg/d) would also surpass the suggested required deviation of 0.4 g/kg/d to stimulate gains in SMM in healthy older adults (Park, Choi, and Hwang 2018).

Maltodextrin, a polysaccharide produced from grain, starch, corn, potatoes or rice, was used as the control supplement as previous studies have demonstrated carbohydrate intake of 100 g (far greater than that used in this thesis) has a limited effect on rates of MPS (Børsheim et al. 2004). Previous RCTs have also reported no effects of carbohydrate as a control product on FFM in older adults (Bauer et al. 2015, Bell et al. 2017, Norton et al. 2016, Park, Choi, and Hwang 2018). The ingestion of 47.5 g/day of carbohydrate (maltodextrin) used in this study was therefore hypothesised based on previous data to have a minimal effect on SMM over 12 weeks. Furthermore, from a safety perspective, previous RCTs have used similar amounts of maltodextrin (Bell et al. 2017) and glucose (Pal, Ellis, and Dhaliwal 2010) over a comparable intervention period as control products and have reported no significant negative effects on insulin sensitivity or body mass gain. Finally, the supplements were iso-caloric to ensure changes in study endpoints were due to differences in the macronutrient content of the supplements and not due to differences in EI.

Unflavoured products were chosen, and allowance of a no-added sugar cordial of choice for mixture and consumption was provided to participants based on adherence data from previously published RCTs investigating the effects of whey protein alone or combined with RE in older adults (Kirk et al. 2019, 2020, Norton et al. 2016). For example, Kirk et al. (2019) reported only 43% adherence of whey protein supplementation over 16 weeks, which may have significantly affected study endpoints. The authors reported undesirable taste and unpalatability of the supplement, which was reported by participants, was the reason for the poor compliance. Therefore, participants in the present study were provided flexibility in no-added sugar (calorie-free) cordial flavouring to reduce flavour fatigue and increase supplement compliance. Participants were instructed no-added sugar cordial must be used to ensure the calorie content remained at 95 kcal per serving.

The use of returned sachets (both used and unused) and a supplementation log were employed as methods of monitoring compliance as this method has been used by a number of recently published studies of similar nature in older adults (Bell et al. 2017, Holwerda et al. 2018, Kirk et al. 2019, 2020). However, it should be noted that although these methods are easy to implement, inexpensive and are widely used within the literature, their reliability and validity are controversial (Farmer 1999). For example, some researchers have shown that these methods result in overestimation of adherence as participants tend to respond in a manner believed to be socially desirable (Teshome et al. 2018).

3.5.2 Resistance exercise intervention

Supervised, progressive whole-body RE was performed twice weekly at Coventry University Sports Centre during the 12-week intervention by participants in the EX+CON and EX+PRO groups only. All sessions were supervised by a qualified fitness instructor (Corbin Griffen), were 60-75 min in duration, and occurred at least 48 h apart. Each session consisted of a 5-min warm-up on a cycle ergometer, followed by 3 sets of leg press, lateral row, hamstring curl, chest press, leg extension and shoulder press exercises on fixed RE machines (in that order)

(Life Fitness, Rosemont, Illinois, USA). During the first 4 weeks of training, exercise intensity began at 60% 1RM (10-12 repetitions per set) and was increased to 80% 1RM (8 repetitions per set), where it remained until the end of the intervention. The final set of each exercise was performed to volitional muscle failure (typically 8-12 repetitions), which was defined as the inability to perform an additional repetition with the correct form. The time under tension for each repetition was 6 s (3 s concentric, 3 s eccentric). Although not controlled, time under tension was practiced at the beginning of the intervention and was reinforced throughout. Resting periods of 60 s and 2-3 min were allocated between sets and exercises, respectively. Intensity was adjusted according to 1RM tests performed at baseline and during weeks 5 and 9 using the guidelines of Kraemer et al. (2006), and when participants were able to complete >12 repetitions on the third set of each exercise. Sessions concluded with a 5 min cool-down on a cycle ergometer followed by static stretching. Session rate of perceived exertion (RPE) was also obtained (Borg 1998). Compliance and completion of repetitions was monitored by a training log (**Appendix I**).

3.5.2.1 Exercise intervention justification

3.5.2.1.1 Modality

As previously highlighted in **section 2.2.10.1.1**, RE stimulates MPS and elicits a positive net protein balance for up to 48 h post exercise (Phillips et al. 1997, Wilkinson et al. 2014, Brook et al. 2015), leading to muscle mass accretion when performed over a chronic period of time (Phillips 2015). It has also been established that RE stimulates MPS to a greater effect and is the key exercise modality to elicit muscle hypertrophy compared to AE (Grgic et al. 2019). Thus, RE was used for this study aimed at increasing SMM in older adults. All sessions were supervised to facilitate optimal improvements in SMM, strength and physical function, and to maintain participant safety (Fragala et al. 2019). Machine-based exercises were chosen as participants in this study were naive to RE, and it has been suggested that novice lifters benefit

more from this exercise equipment (Fragala et al. 2019). Also, machine-based exercises are more suitable and recommended when training to volitional muscle failure to reduce the likelihood of injury (Schoenfeld and Grgic 2019).

3.5.2.1.2 Intensity

The intensity of RE employed was based upon literature cited in **section 2.2.10.1.3.1**. These data suggest RE performed at a high intensity (~70-80% 1RM) elicits the greatest effects on muscle strength (Borde, Hortobágyi, and Granacher 2015). Whilst RE performed at low and high intensity elicits similar effects on muscle hypertrophy when mechanical work is matched (Borde, Hortobágyi, and Granacher 2015), high intensity RE was chosen on the premise of maximally increasing muscle strength as the EWGSOP2 now consider muscle weakness as the main determinant of sarcopenia (Cruz-Jentoft et al. 2019). The NSCA also state that older adults should perform RE at 70-85% 1RM to optimise strength gains (Fragala et al. 2019). Furthermore, RE performed at high intensity has also been shown to elicit improvements in cognitive function (Wilke et al. 2019) and attenuate metabolic dysfunction (Liu et al. 2019). A familiarisation intensity of 60% 1RM was prescribed for the first four weeks of training to allow for exercise tolerance and to minimise the risk of injury in novice lifters (Fragala et al. 2019).

3.5.2.1.3 Volume

Training volume was chosen based on evidence cited in **section 2.2.10.1.3.2**. Based on the findings of two meta-analyses (Borde, Hortobágyi, and Granacher 2015, Peterson et al. 2010), 2-3 sets per exercise and ~8 repetitions per set appears to elicit the greatest increases in muscle hypertrophy and strength in older adults. The final set was performed to volitional failure to induce a greater volume per exercise (~28 repetitions/exercise); however, failure was not performed for every set as older adults may experience slower post-exercise recovery compared to younger adults (Schoenfeld and Grgic 2019), and failure may not be necessary to achieve increases in SMM and strength in older adults (Fragala et al. 2019).

3.5.2.1.4 Progression

Progressive overload is the gradual increase in stress placed on the body during RE training (Kraemer et al. 2002). The concern when designing RE interventions for elderly individuals with regards to progression is exercise tolerance and optimal recovery (Fragala et al. 2019). 2019). However, progression is essential for continued adaptation (Fragala et al. 2019). Typically, hypertrophy, and changes in muscle fibre type and strength, are evident after a short period of training (~4-8 weeks) (Kraemer and Ratamess 2004); thus, 1RM was measured every 4 weeks and intensity was increased in accordance with each participant.

3.5.2.1.5 Frequency

A frequency of twice weekly RE was chosen based on literature cited in **section 2.2.10.1.3.3**. Based on findings from these studies, 2-3 sessions per week appears to elicit superior increases in muscle strength (Borde, Hortobágyi, and Granacher 2015, Steib, Schoene, and Pfeifer 2010) and hypertrophy (da Silva et al. 2017, Grgic, Schoenfeld, and Latella 2019, Stec et al. 2017). As no significant benefit seems apparent between two and three sessions per week (Borde, Hortobágyi, and Granacher 2015), and 26% of older adults have reported to prefer training twice per week compared to only 1% preferring to train three times per week (Foley, Hillier, and Barnard 2011), twice weekly RE was chosen to aid adherence.

3.5.2.1.6 Exercises and order

It has been recommended that RE interventions for older adults should include exercises that target major muscle groups using multi-joint movements (e.g., leg press, chest press, shoulder press, lateral row/pull down, leg extension) to stimulate whole-body increases in SMM and strength, and improve physical function (Fragala et al. 2019). Large muscle groups were exercised first (e.g., those used in leg press) and upper- and lower-body exercises were alternated to reduce fatigue. The exercise order was kept consistent to ensure this variable did not affect outcomes.

3.5.2.1.7 Rest allocation between sets and time under tension per repetition

In the meta-analysis by Borde, Hortobàgyi, and Granacher (2015), 60 s rest between sets was the most effective at increasing SMM. This meta-analysis also reported the largest effect size for time under tension to increase SMM and strength to be 6 s. Three min was allocated between exercises as numerous studies within the literature, as reviewed by the NSCA (Fragala et al. 2019), have used 2-3 min between exercises, which appears to provide sufficient recovery for symptom-free progression in older adults.

3.6 Outcome measures

This section will outline outcome measures that are reported in all experimental chapters within this thesis. Within individual experimental study chapters, these outcomes will be briefly described and referred to this chapter. Outcome measures only measured in individual study chapters are detailed in those specific chapters. A list of the participant schedule of assessments can be seen in **Table 3.2**.

3.6.1 Body composition

Body composition (FM, kg; FFM, kg; SMM, kg) was assessed in the morning by BIA (BC-418 MA; Tanita Corporation, Tokyo, Japan). Measurement occurred at the same time of day (±1 h) and participants were asked to consume the same breakfast for both visits. Participants did not consume any food or drink in the 1 h before and voided their bladder prior to measurement. Minimal clothing was worn. Skeletal muscle mass (kg) was estimated using the formulae of Janssen et al. (2000a):

$$[(\text{height}^2 / \Omega \times 0.401) + (\text{sex} \times 3.825) + (\text{age} \times -0.071)] + 5.102$$

where height is in cm and sex = 1 for male. Impedance (Ω) was measured at a single frequency of 50 Hz. Whilst BIA has several limitations, as highlighted in **section 2.2.9**, prediction equations have been validated in the elderly and the method has been cross validated against

MRI for measurement of SMM in older adults (Janssen et al. 2000a). Waist and hip circumference were measured by an ergonomic circumference measuring tape (Seca 201; Seca, Malaysia). Waist circumference was measured at the midpoint between the lowest rib margin and the iliac crest. Hip circumference was measured at the widest portion of the hips. Both outcomes were measured to the nearest 0.1 cm.

3.6.2 Dietary analysis

Participants completed 3-day food records [2 weekdays and 1 weekend day (**Appendix J**)] at baseline (prior to commencing the intervention) and during weeks 6 and 12 to monitor changes in habitual dietary intake during the intervention. Prior to completion, participants were instructed on how to record the types and quantities of food and beverages. Participants were asked to use kitchen scales were possible. Following completion, the diaries were checked by the lead investigator for clarity and analysed using Nutritics dietary analysis online software (Version 5.097; Nutritics, Dublin, Ireland). An example analysis can be seen in **Figure 3.4**.

	CARBOHYDRATE	SUGARS	PROTEIN	FAT	SATFAT	ALCOHOL
Intake	187.7g	123.6g	54.9g	63.7g	16.5g	0g
g/kg body-weight	2.8	1.9	0.8	1	0.2	0
Kilocal	751	494	219	574	148	0
Kilocal %	48.6%	32%	14.2%	37.2%	9.6%	0%

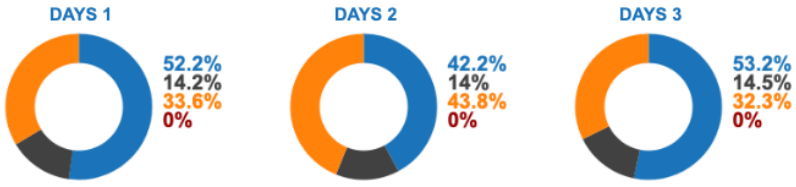

Figure 3.4 Example dietary analysis (ID: 008; age: 72 y).

Many methods are available to measure habitual dietary intake. These include food frequency questionnaires (FFQ), 24-h dietary recalls, technological methods such as ‘snap and send’, and biomarkers such as DLW and urinary nitrogen excretion (Shim, Oh, and Kim 2014).

However, the former methods (FFQ, 24-h dietary recalls) have several limitations, such as recall bias and large measurement error, and the latter methods (biomarkers) are of high cost. Although food records have a number of limitations, including large respondent burden and frequent underreporting, this method also has a number of strengths, including providing detailed information if completed correctly and minimal recall bias (Shim, Oh, and Kim 2014). Dietary records also have acceptable reproducibility [intraclass correlation coefficient (ICC): 0.67-0.84 for total kcal, carbohydrate, fat and protein intake] (Tremblay et al. 1983) and have been used to monitor habitual intake by the majority of studies of similar nature cited in this thesis (e.g., Bell et al. 2017, Kirk et al. 2019, 2020). A recording period of three days was chosen for this study as periods longer than this are unsatisfactory, as reported intakes decrease due to respondent fatigue (Gersovitz, Madden, and Smiciklas-Wright 1978). Two weekdays and one weekend day was chosen as individuals typically modify their diet at weekends (Thompson and Subar 2017); therefore, incorporation of both provides a typical weekly dietary pattern.

3.6.3 Habitual physical activity

Habitual physical activity was measured at baseline and week 12 using a small (3.5 x 3.5 x 1 cm), lightweight (14 g), tri-axial accelerometer (Actigraph GT9X; Actigraph, Pensacola, Florida, USA) sampled at 80 Hz. Participants wore the accelerometer on the dominant wrist for 7 days continuously and were asked to record when the monitor was not worn (e.g., when showering) using a compliance log (**Appendix K**). Time spent sedentary, and in light and moderate-vigorous physical activity (MVPA) was determined during wakening hours based on metabolic equivalent (MET) values using the cut-points of Freedson, Melanson, and Sirard (1998). Data were analysed in 60-s EPOCHs using ActiLife software (Version 6.13.4; Actigraph, Pensacola, Florida, USA). At least 5 d of ≥10 h wear time was required for data to be included in the final analysis (Schrack et al. 2016b). The wrist was chosen for accelerometery placement due to increased compliance reported compared to the traditionally used hip placement in older adults (Schrack et al. 2016a). Wrist placement has also been

validated against established methods for measurement of AEE (van Hees et al. 2011, White et al. 2016), and data from the UK Biobank Study (Doherty et al. 2017), which is the largest accelerometery study to date (including 96,600 participants), has also demonstrated dominant wrist accelerometery to be a valid method for measurement of physical activity.

3.6.4 Blood and saliva collection and analysis

All blood collection reported in this thesis was drawn from participants whilst residing in a respiration chamber for 24 h under highly controlled conditions (see **Chapter 4**) by a research nurse at UHCW. Blood was collected routinely (at 2130, 0815, 0900, 1230, 1355, 1715 and 1835 h) via an indwelling cannula (Vasofix Safety 22G; B. Braun Medical, Sheffield, UK) inserted in an antecubital arm vein. The cannula was flushed after every blood draw with 0.9% sodium chloride (BD Posiflush XS 5 mL; BD, New Jersey, USA) to maintain cannula patency. Individual chapters within this thesis provides data on some, but not all time points, of which are clearly outlined in each experimental chapter. Blood samples were collected using ethylenediaminetetraacetic acid (EDTA), serum separator tube (SST) and heparin vacutainers (BD 3 mL vacutainers; BD, New Jersey, USA). A total of ~70 mL of whole blood (~9 mL per sample apart from the first fasted sample at 0815 h where ~12 mL was drawn) was drawn at both pre- and post-intervention visits. Whole blood was immediately centrifuged (Eppendorf 5702R; Eppendorf UK Ltd, Stevenage, UK) at 1900 relative centrifugal force (x g) for 10 min at 4°C. Aliquots containing plasma and serum were then immediately stored at -80°C until analysis. Saliva samples were collected at 0650 (immediately upon awakening), 0805, 1225, 1700 and 2000 h using a synthetic swab (Salivette; Sarstedt, Nümbrecht, Germany). Samples were immediately centrifuged at 1900 x g for 2 min and stored at -80°C until analysis. For analysis, plasma, serum, and saliva samples were analysed by enzyme-linked immunosorbent assay (ELISA). Specific assays measured in this thesis are detailed in each experimental chapter. The general ELISA methodology is detailed in **section 3.6.5**.

3.6.5 Enzyme-linked immunosorbent assay

For all ELISA experiments, assay-specific protocols were followed according to the manufacturer's procedures. Generally, each assay followed the procedures outlined below.

3.6.5.1 Sample and reagent preparation

All reagents and samples were brought to room temperature prior to use. Plasma and serum samples were thawed for ~1 h and vortexed thoroughly. Several steps were then followed. Firstly, a specific volume (per protocol) of wash buffer concentrate was added to a specific volume (per protocol) of deionized water to prepare a wash buffer. Secondly, a diluted calibrator diluent was created by mixing a specific volume (per protocol) of assay-specific calibrator diluent with a specific volume (per protocol) of deionized water. Thirdly, an assay-specific standard was created by reconstituting the contents of the standard (recombinant human protein) with a specific volume (per protocol) of deionized water. The standard was then rested for a time as per each protocol's procedure prior to use. Fourthly, a standard curve was created by pipetting a set volume (per protocol) of calibrator diluent into polypropylene tubes and using the stock solution to produce a dilution series (see **Figure 3.5**). Each tube was vortexed prior to transfer. Furthermore, a substrate solution was created by mixing two colour reagents (reagent A, stabilized hydrogen peroxide; and reagent B, tetramethylbenzidine) together 15 min prior to use. The substrate solution was protected from light at all times. Lastly, if samples required diluting, a specific dilution factor (per protocol) was applied. Samples were diluted with assay-specific calibrator diluent, if required.

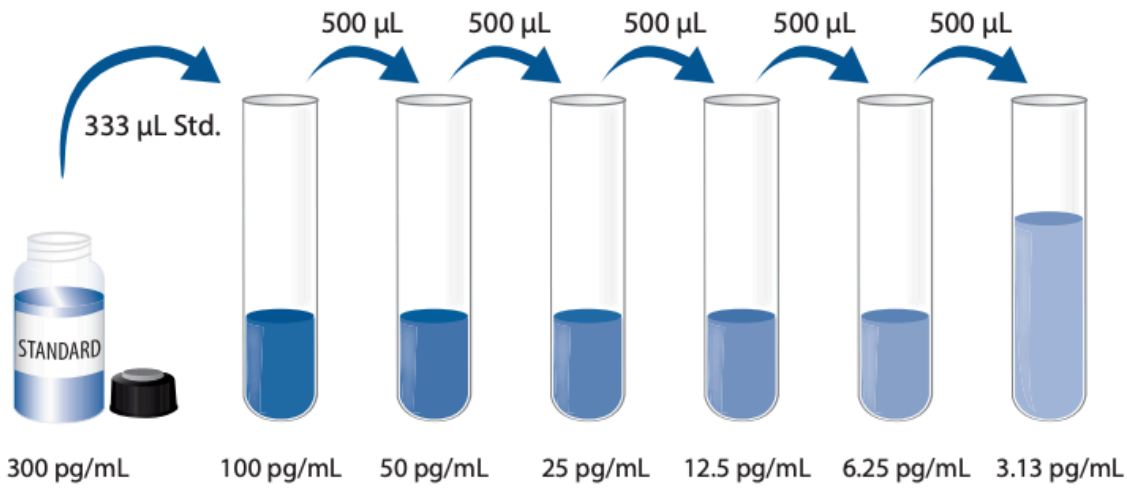

Figure 3.5 Example dilution series for a standard curve (IL-6). Taken from R&D Systems (2018).

3.6.5.2 Assay procedure

To begin, a per protocol volume of assay-specific diluent was added to each well. A per protocol volume of standards, controls, and samples, which were assayed in duplicate, were subsequently added to specific wells outlined by the plate plan. The plate was then incubated at room temperature for a set time (as per protocols procedure). If a horizontal orbital plate shaker was required, the shaker (PHMP-4 Thermoshaker, Grant Bio, Chelmsford, UK) was set to 500 rpm. Following incubation, each cell was aspirated and washed with 400 µL of wash buffer four times using an autowasher (Autura 1000; Mikura, Sussex, UK). After the final wash, the plate was blotted against clean paper towels to ensure removal of wash buffer. A specific volume (per protocol) of assay-specific conjugate was then added to each well and incubated at room temperature for a set time (per protocol), either on the benchtop or using the plate shaker. Following the second incubation, the aforementioned washing procedure was repeated. A set volume of substrate solution (per protocol) was then added to each well and the plate was incubated for 30 min at room temperature with no light exposure. Succeeding the final incubation period, a set volume (per protocol) of stop solution was added to each well. The plate was then inserted into a microplate reader (Biotek, Epoch 2, Vermont, New England,

USA), which determined the optical density of each well using a wavelength of 450 nm and a correction factor of 540 nm.

3.6.5.3 Calculation of results

Duplicate readings were averaged, and the average zero optical density was subtracted from each. A standard curve was created using a curve fit recommended within the ELISA protocol using GraphPad Prism (Version 8.4.3; San Diego, California, USA). Unknown concentrations were interpolated from the standard curve. If samples were diluted, a dilution factor was applied.

3.7 Statistical analysis

Statistical analysis was performed using SPSS version 25 (IBM Corporation, New York, USA). Data are presented as means ± SE and significance was set at $P < 0.05$. All data were checked for normality using the Shapiro-Wilk test. Outliers (±>3 SD from mean) were identified and removed. Non-normally distributed data were transformed by log transformations. When transformation was unsuccessful, non-parametric tests were utilised. Baseline values were analysed by one-way ANOVA for parametric data and using the Mann-Whitney U test for non-parametric data. A mixed-model ANCOVA with time as the within-subjects factor, group as the between-subjects factor, and respective baseline values as covariates was performed on outcome variables. Mauchly's test of sphericity was used to check homogeneity of variance; where necessary, Greenhouse-Geisser adjustment was used to correct any violations of the assumption. Following significant group-by-time interactions, significant within- and between-group differences were identified using post-hoc tests with a Bonferroni correction for multiple comparisons.

For exploratory analyses comparing pooled groups (i.e., exercise and non-exercise groups, and whey protein and control groups), supplement consumed and RE participation were controlled for in the ANCOVA model, respectively. Non-normally distributed data were

analysed using the Scheirer-Ray-Hare two-way ANOVA of ranks test. Post-hoc analysis was conducted using the Mann-Whitney U test. Longitudinal changes within groups were analysed using 2-tailed paired samples *t*-tests for parametric data and the Wilcoxon signed ranks test for non-parametric data. Correlations were analysed using partial correlation (Pearson's for parametric and Spearman's rank-order coefficients for non-parametric data). Participants were pooled for correlation analyses. Age and intervention group were controlled for in baseline and Δbaseline correlations, respectively.

For cognitive test scores only (**Chapter 6**), effect sizes (Cohen's *d*) were calculated for longitudinal changes within groups. Effect sizes were computed as the mean difference between pre- and post-intervention scores divided by the pooled SDs (Cohen 1988). The standard definitions of Cohen's *d* are as follows: very small, 0.01-0.19; small, 0.2-0.49; medium, 0.5-0.79; large, 0.8-1.29; and very large, ≥1.20 (Cohen 1988, Sawilowsky 2009).

Table 3.2 Schedule of assessments

	Metabolic testing (baseline and post-intervention)	Strength and functional performance testing (baseline and post-intervention)	Week 6	Week 12
24-h (2 x 12-h) urine collection	x			
3-d food diary	x (3-d following baseline only)		x	x
7-d physical activity monitoring (accelerometery)	x (for 7-d following baseline only)			x
Blood collection	x			
Blood pressure	x			
Body composition	x	x		
Cognitive function	x			
Continuous glucose monitoring	x			
Functional performance testing (6MWT, SPPB)		x		
Indirect calorimetry	x			
Informed consent	x (baseline only)			
Mini-mental state examination		x (baseline only)		
Saliva collection	x			
Strength testing (1RM, handgrip)		x		
Supplement exit questionnaire		x (post-intervention only)		
VAS appetite scales	x			

1RM, one repetition maximum; 6MWT, 6-minute walk test; SPPB, short physical performance battery; VAS, visual analogue scales. Adverse events were monitored throughout the intervention period.

Thesis map: Study 1

Study	Aims	Key findings
Study 1: Effects of resistance exercise and whey protein supplementation on 24-h energy expenditure, substrate oxidation and metabolic flexibility, body composition, appetite and glucose homeostasis in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on components of 24-h EE, substrate oxidation and metabolic flexibility, body composition, appetite, and glucose homeostasis in healthy older men.	
Study 2: Effects of resistance exercise and whey protein supplementation on skeletal muscle mass, strength, physical function, and hormonal and inflammatory biomarkers in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on SMM, strength, physical function, and hormonal and inflammatory biomarkers in healthy older men. • To determine whether changes in hormonal and inflammatory markers correlate with changes in SMM, strength and physical function.	
Study 3: Effects of resistance exercise and whey protein supplementation on cognitive function in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on cognitive function and neurobiological, inflammatory and insulin sensitivity markers, diurnal salivary cortisol, and BP in healthy older men. • To determine whether changes in neurobiological, inflammatory and insulin sensitivity markers, and changes diurnal salivary cortisol, BP, SMM, strength and physical function are associated with changes in cognitive function.	

CHAPTER 4 (Study 1): Effects of resistance exercise and whey protein supplementation on 24-h energy expenditure, substrate oxidation and metabolic flexibility, body composition, appetite, and glucose homeostasis in healthy older men

4.1 Chapter overview

Ageing is associated with declines in FFM and components of EE, which may result in energy imbalance, increased FM and sarcopenic obesity, and poor metabolic health. Previous studies have shown RE and increased dietary protein intake may mitigate age-related declines in these outcomes. In this chapter, the individual and combined effects of RE and whey protein supplementation on 24-h EE, substrate oxidation and metabolic flexibility, body composition, subjective appetite and glucose homeostasis were investigated in healthy older men.

4.2 Introduction

Increased FM and declines in FFM characterise age-related changes in body composition (St-Onge 2005). Skeletal muscle, which accounts for ~45% of FFM (Geisler and Müller 2017), declines by ~0.5-1% per annum after ~45 years of age (Janssen 2010). When accompanied by concomitant declines in muscle strength and physical function, this gives rise to the phenomenon known as sarcopenia (Cruz-Jentoft et al. 2019). Contrary to SMM, FM has been shown to increase by ~0.2% per annum from 20 years of age (Imboden et al. 2017, Westerterp 2018a). The coexistence of sarcopenia and obesity, termed sarcopenic obesity, is of great concern for the health of older adults as they act synergistically, exacerbating the negative effects of one another (Batsis et al. 2015, Lee et al. 2016, Scott et al. 2018).

Body composition changes with age are ascribed to alterations in energy balance (St-Onge and Gallagher 2010). Regarding the EE component of the energy balance equation (rate of ES = rate of EI – rate of EE), ageing is associated with declines in all three major constituents: RMR, which accounts for 60-80%; AEE, which comprises ~20-50%; and DIT, which uses 5-10% (Manini 2010). The decline in RMR occurs at a rate of ~1-2% per decade from the age of 30 (Elia, Ritz, and Stubbs 2000). Fat-free mass accounts for 50-70% of the variance (Bosy-Westphal et al. 2003, Geisler et al. 2016a), of which SMM accounts for ~25% (Gallagher et al. 1998). Interventions that curb sarcopenia may mitigate age-related declines in components of EE, improve energy balance and attenuate adiposity in older adults.

Resistance exercise is a potent stimulus to mitigate sarcopenia (Phillips and Martinson 2019). Others have also reported increases in TEE (Hunter et al. 2000), RMR (Hunter, McCarthy, and Bamman 2004) and 24-h fat oxidation (Treuth et al. 1995), decreases in the energetic cost of walking (Valenti, Bonomi, and Westerterp 2016) and FM (Westcott 2012), and improvements in metabolic flexibility (Consitt et al. 2016) and glucose homeostasis (Bell et al. 2017, Holwerda et al. 2018, Iglay et al. 2007, Leenders et al. 2013) following RE training in older adults. However, a potential caveat of RE in the elderly is the frequently reported compensatory reduction in AEE, particularly SPA (Westerterp 2018a, 2018b), which has been postulated to occur due to training-related fatigue (Hunter et al. 2018). This effect may be eliminated by performing RE twice as opposed to three times per week (Hunter et al. 2013).

In addition to RE, a high protein diet may also assist in attenuating sarcopenia and age-related reductions in EE, alongside aiding body weight management (Drummen et al. 2018, Phillips and Martinson 2019). Meta-analyses have indicated increased dietary protein may augment decreases in both absolute and %FM (Liao et al. 2017) and amplify the adaptative RE-induced response of skeletal muscle (Cermak et al. 2012, Finger et al. 2015, Liao et al. 2017, Morton et al. 2018a). A high protein diet (25-30% of EI) has also been shown to increase TEE, RMR, SMR and DIT (Bray et al. 2012, Bray et al. 2015, Martens et al. 2015b, Sutton et al. 2016), improve metabolic efficiency of physical activity (Apolzan et al. 2014, Martens et al. 2015a), decrease 24-h RQ and elicit a greater negative fat balance (Lejeune et al. 2006, Martens et al. 2015b, Smeets, Janssens, and Westerterp-Plantenga 2013), and stimulate an adaptative thermogenic increase in TEE and SMR when dietary protein is returned to baseline intake (Bray et al. 2015).

However, whilst the aforementioned meta-analyses suggest increased dietary protein might augment the effect of RE on body composition, the majority of intervention studies in older adults have been unable to replicate supplemental increases in skeletal muscle or FFM (Arnarson et al. 2013, Candow et al. 2006, Chalé et al. 2013, Dulac et al. 2020, Englund et al.

2018, Gryson et al. 2014, Holm et al. 2008, Holwerda et al. 2018, Kukuljan et al. 2009, Leenders et al. 2013, Maltais et al. 2016, Ottestad et al. 2017, Shahar et al. 2013, Thomson et al. 2016, Verdijk et al. 2009a, Verreijen et al. 2017). Similarly, studies investigating the combined effects on components of EE have also observed no additive effects (Amamou et al. 2017, Campbell et al. 1994, Maltais et al. 2016, Weinheimer et al. 2012). Null findings may be attributed to an insufficient increase in dietary protein intake from baseline (<0.4 g/kg/d) and a total dietary protein intake <1.6 g/kg/d, which, might be the breakpoints required to maximally augment increases in SMM and EE (Morton et al. 2018a, Park, Choi, and Hwang 2018). Furthermore, to the authors knowledge, no study has investigated the synergistic effects on 24-h metabolic flexibility or appetite. This data is required to comprehensively determine the combined effects on energy balance and metabolic health in older adults.

4.2.1 Aims and hypotheses

Primary aim

- To investigate the individual and combined effects of RE and whey protein supplementation (aimed to increase dietary protein intake by ≥0.4 g/kg/d to 1.6 g/kg/d) on components of 24-h EE in healthy older men.

Secondary aims

- To investigate the individual and combined effects on body composition, 24-h substrate oxidation and metabolic flexibility, glucose homeostasis, subjective appetite, fasting plasma leptin and diurnal salivary cortisol.

Hypotheses

- Resistance exercise combined with whey protein supplementation will augment increases in skeletal muscle and FFM, elevate rises in components of 24-h EE (e.g., RMR and SMR) and substrate oxidation (i.e., resting and 24-h fat oxidation), and enhance decreases in FM compared to each intervention alone.

- Resistance exercise and whey protein supplementation will synergistically increase 24-h metabolic flexibility, reduce fasting plasma leptin, and have no adverse effects on diurnal salivary cortisol or subjective appetite.

4.3 Methods

4.3.1 Participants and experimental design

Thirty-three healthy, community-dwelling older men [mean ± standard error (SE) age: 66.8 ± 0.7 y] participated in this study. Measurements were taken at baseline and following the 12-week intervention. As previously highlighted in **section 3.2**, participants were randomised to either control (CON, *n* = 8), whey protein (PRO, *n* = 8), RE + control (EX+CON, *n* = 8), or RE + whey protein (EX+PRO, *n* = 9). Full details of the experimental design (**section 3.2**) and eligibility criteria (**sections 3.4.2.1 and 3.4.2.2**) have been previously described. Although not planned in the original study design, exploratory analyses were also conducted between pooled exercise (EX+CON and EX+PRO groups, *n* = 17) and non-exercise groups (CON and PRO groups, *n* = 16), and between pooled whey protein (PRO and EX+PRO groups, *n* = 17) and control groups (CON and EX+CON group, *n* = 16) to determine the effects of RE and whey protein with greater statistical power. All participants provided written informed consent approved by Coventry University Ethics Committee (project code: P59723). The flow of participants throughout the study has been previously presented and can be seen in **Chapter 3** (**Figure 3.2**).

4.3.2 Exercise training and nutritional supplements

Full details of the RE intervention completed by participants in the EX+CON and EX+PRO groups and nutritional supplements consumed by participants in all groups have been previously detailed (**sections 3.5.1 and 3.5.2**). Briefly, the RE intervention consisted of twice-weekly supervised whole-body RE [including 3 sets of 8 repetitions of six exercises (leg press, lateral row, hamstring curl, chest press, leg extension and shoulder press)] performed at 80%

1RM. Participants in the PRO and EX+PRO groups consumed 25 g whey protein containing ~3 g leucine, whereas participants in the CON and EX+CON groups consumed an energy-matched carbohydrate control (maltodextrin). Supplements were consumed twice daily (at breakfast and lunch).

4.3.3 Dietary intake and physical activity

Participants completed 3-day food records (2 weekdays and 1 weekend day) at baseline (prior to commencing the intervention) and during weeks 6 and 12. Dietary records were analysed with the dietary supplements both included and excluded. Full details have been previously described (**section 3.6.2**). Analysis of dietary records exclusive of the dietary supplements was conducted to assess any compensatory effect on participants' habitual dietary intake. To control for acute changes in dietary intake on energy metabolism, participants also completed a food record for the 3 days prior to baseline testing. Participants were provided a copy to replicate their dietary intake on the day of post-intervention metabolic testing. To control for changes in habitual physical activity that may have influenced study endpoints, participants wore a tri-axial accelerometer on the dominant wrist, as described in **section 3.6.3**, for 7 days at baseline and during the final week of the intervention.

4.3.4 Body mass and composition

Body mass and composition (FFM, FM and SMM) was measured in the morning by BIA. Full details have been previously described (**section 3.6.1**). Skeletal muscle mass (kg) was estimated using the formula of Janssen et al. (2000a). Participants did not consume any food or drink in the hour prior and voided their bladder before measurement. Minimal clothing was worn.

4.3.5 Respiration chamber

Participants resided in respiration chambers located at the HMRU, UHCW NHS Trust for measurement of 24-h EE, substrate oxidation and metabolic flexibility, interstitial glucose,

subjective appetite, and diurnal salivary cortisol concentration (**Figure 4.1**). Briefly, the respiration chamber is an airtight and thermally insulated living space (floor dimensions: 2.9 m x 2.1 m) containing a bed, desk, chair, computer and freezer toilet (Schoffelen et al. 1997). Environmental conditions were controlled at all times (relative humidity: 57 ± 5%; temperature: 24 ± 0.5 ˚C). Participants entered the chamber at ~1930 h and left at 2000 h the following evening. The first 30 min acted as a habituation period, and the 24-h measurement period, which is shown diagrammatically in **Figure 4.2**, occurred between 2000-2000 h. A full timeline of the 24-h assessment can be seen in **Appendix L**. Whilst residing in the chamber, participants were fed a study diet designed to provide energy balance. Food intake was partitioned into four separate meals provided at scheduled times (see **Table 4.1** for example). The diet consisted of ~45% of energy from carbohydrate, ~20% of energy from protein, and ~35% of energy from fat. This macronutrient composition mimicked that used in a recent respiration chamber study in older adults (Bush et al. 2018), and is similar to that habitually reported in healthy older men (Farsijani et al. 2016). Energy requirements for each participant were calculated from RMR multiplied by an activity factor of 1.47, obtained from pilot data (**Appendix M**). Participants were prohibited from consuming alcohol or caffeinated drinks inside the chamber, but water and non-caffeinated herbal teas were available ad libitum. Caffeine was prohibited due to its known effect on EE (Hursel et al. 2011).

Participants completed three bouts of 30 min step exercise (Reebok Aerobic Step - height 150 mm; Reebok, Boston, Massachusetts, USA) at a step rate of 75 steps/min. Exercise was performed at 0830 h (EX-1), 1445 h (EX-2) and 1915 h (EX-3). The first bout (EX-1) was performed prior to breakfast in a fasted state. Step rate was paced using a metronome (TempoPerfect, NCH Software, Canberra, Australia) and participants were visually monitored throughout. Step exercise was chosen due to its ease of performing inside a respiration chamber (Westerterp, Wilson, and Rolland 1999), and the exercise volume (~6,750 steps; 75 steps/min x 90 min) was chosen to replicate the step count previously reported in free-living older men (age: 70-74 y; 6,798 steps/d) (Lohne-Seiler et al. 2014). Physical activity within the

chamber was continuously measured by a radar system working on the Doppler principle, and is expressed as a percentage of total time active (Ravussin et al. 1986). During the intervention period, the chambers were validated monthly by alcohol combustion tests. All tests were within specification.

Figure 4.1 Outside (left) and inside (right) of one of the respiration chambers used in this study.

Table 4.1 Example chamber diet (ID: 029_baseline)[1]

Meal/Ingredients	**Contents, g**	**Carbohydrate, g**	**Protein, g**	**Fat, g**	**Energy, kcal**
Evening meal (2000 h)					
Chicken tikka masala	427	56.0	33.7	25.2	587
Breakfast (0915 h)					
Fruit and fibre cereal	85	57.4	7.9	4.5	302
Semi-skimmed milk	156	7.5	5.6	2.8	78
Lunch (1240 h)					
Lasagne	383	39.1	31.1	28.8	539
Garlic bread	57	28.2	2.9	9.5	209
Whole milk yoghurt, Yeo valley strawberry	125	13.1	12.0	4.7	143
Snack (1720 h)					
Wholemeal bread	119	45.0	12.6	3.3	261
Margarine, Lurpack spreadable	17	0.1	0.1	13.3	120
Ham	23	0.3	5.1	0.5	25
Banana (without skin)	136	31.6	1.6	0.4	136
			Total, g		**Total, kcal**
		279	112.6	92.9	2400
			% Energy intake		
		46%	19%	35%	

[1]All products purchased from Tesco, UK (https://www.tesco.com/groceries/en-GB/).

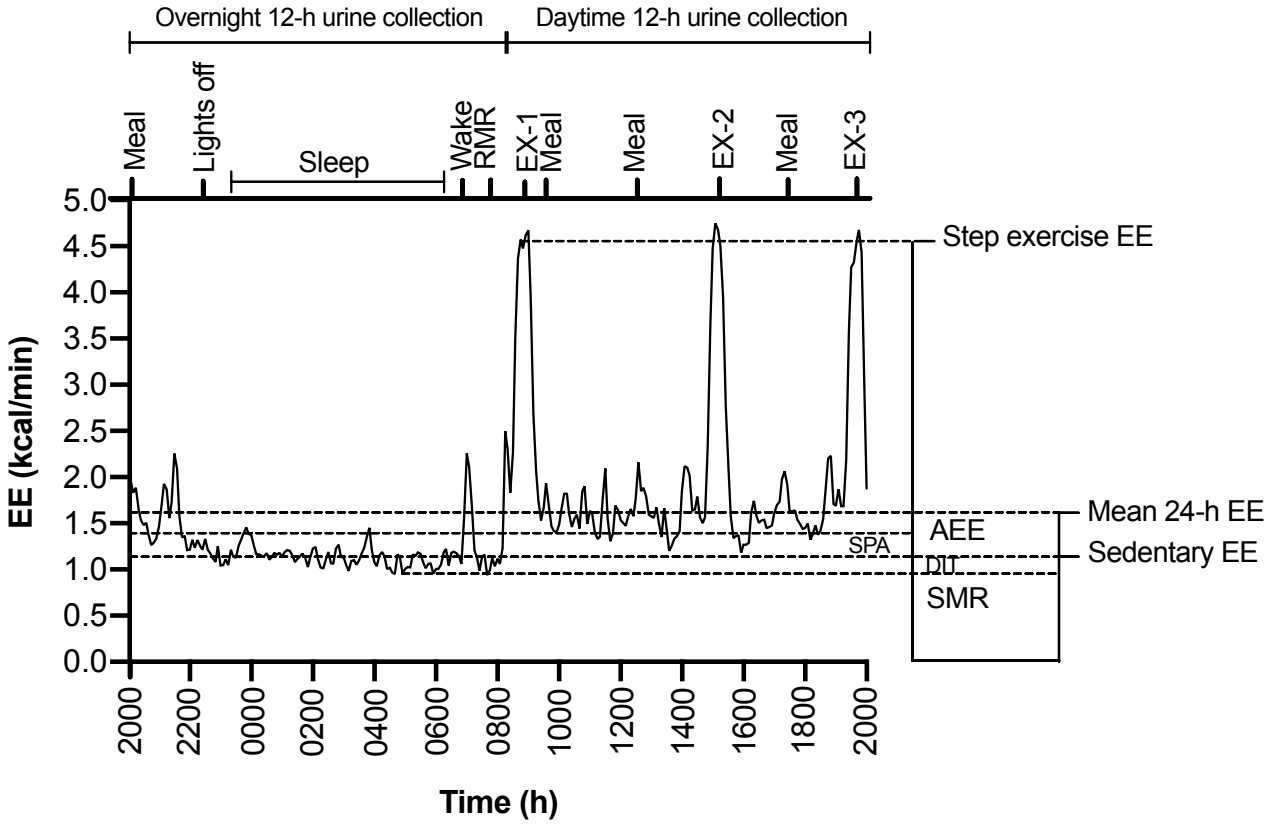

Figure 4.2 Schematic of the 24-h respiration chamber protocol. Energy expenditure (*y*-axis) is plotted against time (*x*-axis bottom) for one participant (ID: 008_baseline; age: 72 y). The protocol is noted on top of the *x*-axis. Components of 24-h EE are illustrated with dashed lines. AEE, activity energy expenditure; DIT, diet-induced thermogenesis; EX-1, step exercise bout 1; EX-2, step exercise bout 2; EX-3, step exercise bout 3; SMR, sleeping metabolic rate; SPA, spontaneous physical activity.

4.3.6 Energy expenditure, substrate oxidation and metabolic flexibility

Energy Expenditure and rates of carbohydrate and fat oxidation were calculated from continuous measurement of $\dot{V}O_2$ and $\dot{V}CO_2$ by indirect calorimetry corrected for protein oxidation using the equation of Brouwer (1957):

$$\text{EE (kcal/min)} = 15.977 \times \dot{V}O_2\,(\text{L}) + 5.515 \times \dot{V}CO_2\,(\text{L}) - 1.352 \times \text{protein oxidation (g/d)} / 4.184$$

$$\text{Carbohydrate oxidation (g/d)} = 4.17 \times \dot{V}CO_2\,(\text{L}) - 2.965 \times \dot{V}O_2\,(\text{L}) - 0.390 \times \text{protein oxidation (g/d)}$$

Fat oxidation (g/d) = 1.718 x $\dot{V}O_2$ – 1.718 x $\dot{V}CO_2$ (L) – 0.315 x protein oxidation (g/d)

Protein oxidation (g/d) = 6.25 x total nitrogen (g/d).

The Brouwer equation was used due to its significant agreement with direct calorimetry (Webb et al. 1988). Protein oxidation was determined from urinary nitrogen excretion measured over 12-h periods (overnight: 2000-0800 h; daytime: 0800-2000 h) (Bingham et al. 1988, Brouwer 1957). Total nitrogen output was calculated by multiplying urinary nitrogen by 1.11 to account for normal losses via faeces and other miscellaneous losses (Bingham et al. 1988). Respiratory quotient, which is an expression of relative fuel utilisation, was calculated by dividing $\dot{V}CO_2$ by $\dot{V}O_2$. Non-protein respiratory quotient (npRQ) was calculated using the following formula:

$\dot{V}CO_2$ (L) - 4.8 x urinary nitrogen (g) / $\dot{V}O_2$ (L) – 6 x urinary nitrogen (g).

Twenty-four-hour metabolic flexibility was defined as the difference between awake npRQ (mean npRQ between 2000-2200 h and 0700-2000 h) and sleeping npRQ (mean npRQ during the defined SMR, as detailed below) (Peterson et al. 2017). Carbohydrate, fat, and protein balances were determined as the difference between intake and oxidation.

Twenty-four-hour EE was partitioned into various components. Total EE represented the total EE between 2000-2000 h; SMR was determined as the lowest EE over a continuous 3 h period between 0000-0600 h (Schoffelen and Westerterp 2008); RMR was measured between 0700-0800 h following awakening at 0650 h, with the participant supine, but awake. The first 20 and last 10 min were discarded, and RMR was calculated during the least restless consecutive 20 min period between 0720-0750 h (Adriaens, Schoffelen, and Westerterp 2003). The energy cost of step exercise was determined as the mean EE whilst participants were in steady state, defined when EE reached a plateau and was maintained for a minimum of 15 min (McClave et al. 2003, Reeves et al. 2004). Other components of EE, including sedentary EE, AEE, SPA

and DIT were calculated using the intercept method, as shown in **Figure 4.3** (Hall et al. 2016, Ravussin et al. 1986, Westerterp, Wilson, and Rolland 1999). This approach involved plotting EE against physical activity, both averaged over 30 min, over the non-exercise intervals of the 24-h measurement period. The *y*-intercept (*c*) of the regression line represents EE in the inactive state, defined as sedentary EE, which consists of SMR and DIT. Activity EE was calculated by subtracting sedentary EE from TEE, and the difference between sedentary EE and SMR represented DIT. Spontaneous physical activity was determined as the slope (*m*) of the regression line and was extrapolated to 24-h values by multiplying by the mean chamber activity of the total non-exercise period (Ravussin et al. 1986). Physical activity level was determined by dividing TEE by RMR. Throughout this study, components of EE and substrate oxidation are expressed as raw values, as well as normalised to body mass and composition (FFM, SMM and FM), where appropriate.

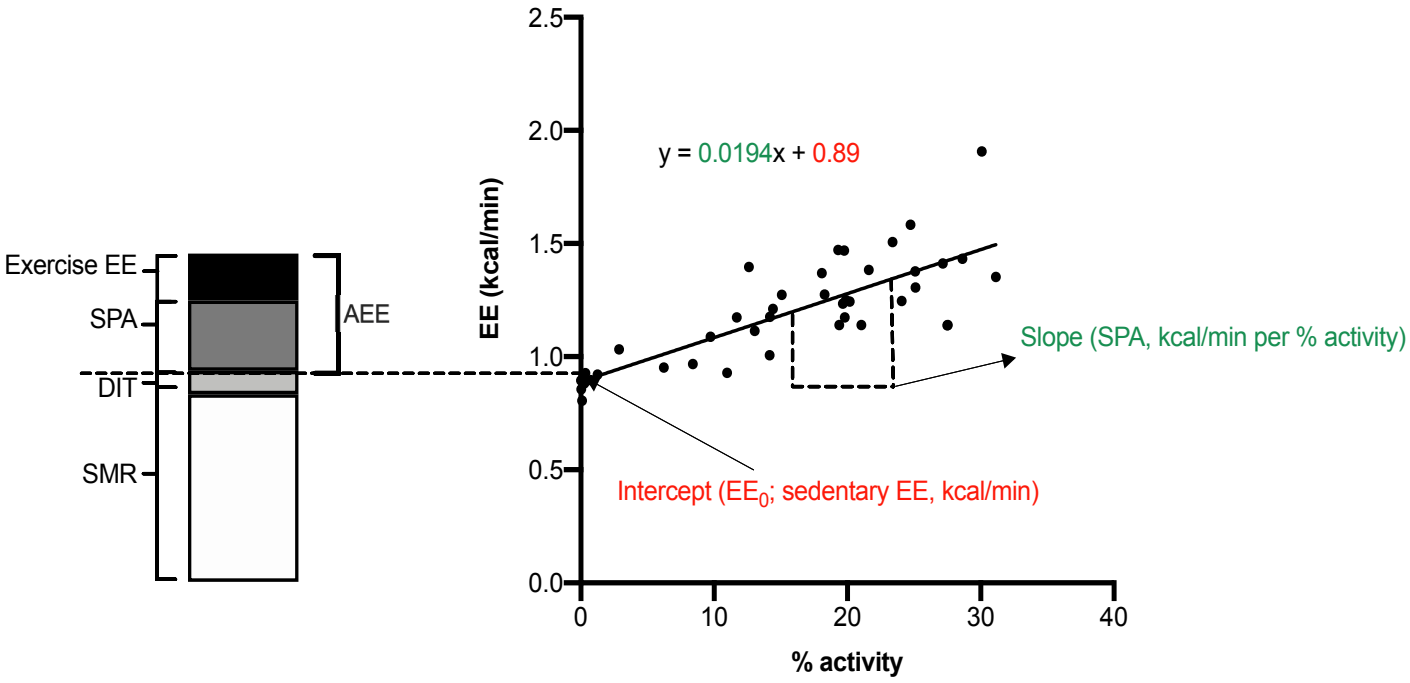

Figure 4.3 Calculation of sedentary EE, AEE, SPA and DIT using the intercept method for one participant (ID: 028_baseline) (Hall et al. 2016, Ravussin et al. 1986, Westerterp, Wilson, and Rolland 1999). Energy expenditure was plotted against physical activity, both averaged over 30 min intervals, over the non-exercise intervals (21.5 h) of the 24-h measurement period. The *y*-intercept (*c*) represents EE at zero activity (EE_0), defined as sedentary EE (kcal/min), which consists of SMR and DIT. The slope (*m*) of the regression line represents SPA (kcal/min per % activity).

For measurement of the abovementioned variables, the non-exercise periods of EE and activity (21.5 h) were used and extrapolated to 24-h values due to previous studies that have demonstrated physical activity within the chamber significantly affects the *y*-intercept (*c*) and the slope (*x*) of the regression line, leading to underestimation of DIT (Ravussin et al. 1986, Tataranni et al. 1995, Usui et al. 2015). Indeed, when including exercise periods within the analysis in this study, 8/33 (24.2%) and 9/33 (27.2%) of participants' DIT was negative at baseline and 12 weeks, respectively. In contrast, 5/33 (15.2%) and 2/33 (6.1%) of participants' DIT was negative at baseline and 12 weeks, respectively, when only the non-exercise data, as employed by Hall et al. (2016), was analysed. Furthermore, when both methods of DIT were statistically compared, significant differences were observed at 12 weeks ($P = 0.001$).

An example of how inclusion of exercise may affect the calculation of DIT is shown in **Figure 4.4**.

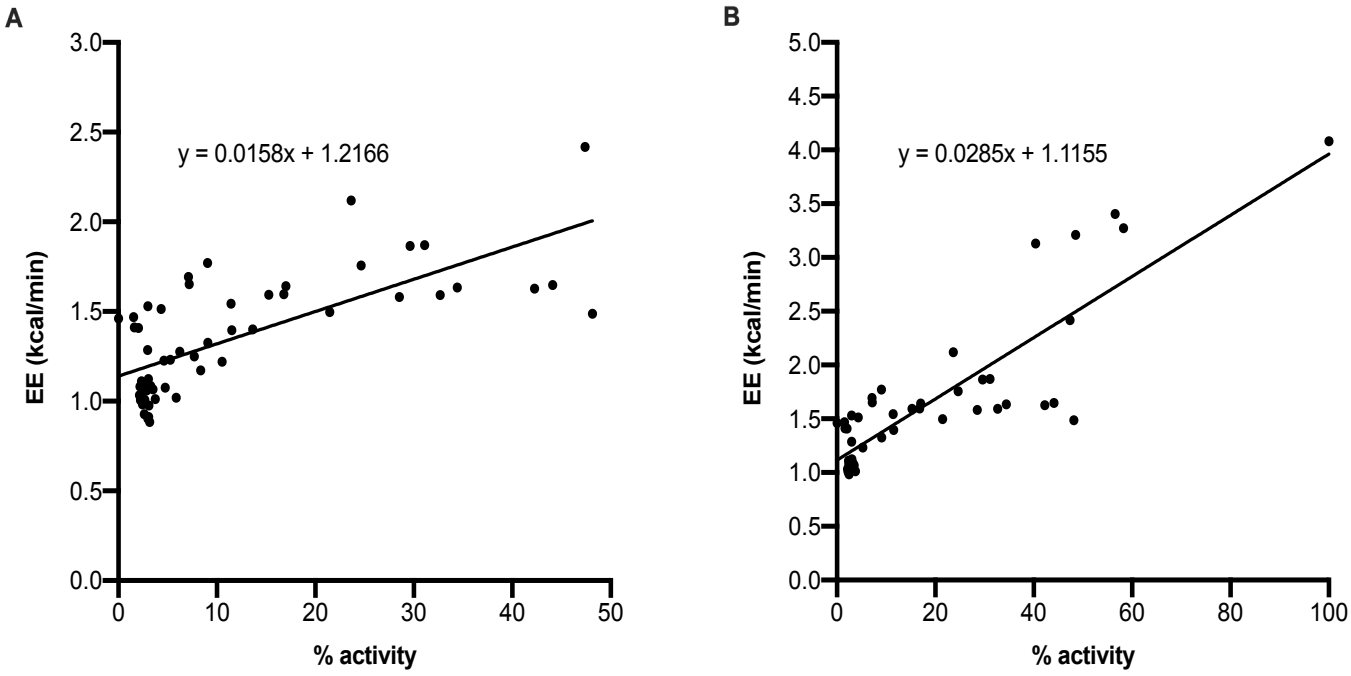

Figure 4.4 Differences in the calculation of DIT for one participant (ID: 018_12 weeks) using only data in the non-exercise intervals (A) compared to inclusion of exercise (B). As shown in panel B, the extreme data point from the step exercise influenced the determination of the *y*-intercept. As a result, DIT was underestimated compared to calculation without exercise (120 vs. 240 kcal/d).

4.3.7 Continuous glucose monitoring

To measure 24-h interstitial glucose, participants wore a flash glucose monitor (FreeStyle Libre Flash; Abbott Diabetes Care, Witney, UK) whilst residing in the respiration chamber. The device was inserted subcutaneously in the posterior compartment of the upper arm and captured glucose from interstitial fluid every 15 min, which was transmitted to a receiver. Twenty-four-hour average interstitial glucose was calculated as the mean of all 15 min samples and 24-h interstitial glucose variability represented the CV (Hall et al. 2019).

4.3.8 Appetite profile

Appetite profile was measured by means of visual analogue scales [(VAS), **Appendix N**] (Flint et al. 2000). These are paper based 100 mm scales with 'not at all' or 'extremely' anchored on either side, combined with questions on hunger, satiety, fullness, and desire to eat. Participants were asked to draw a line along the 100 mm scale relating to their perception of the question asked. The lines were then measured (in mm) and a sub-sample (*n* = 100) were measured by Professor Derek Renshaw. Validity and reproducibility of VAS to measure appetite have been previously confirmed (Flint et al. 2000). Participants completed the VAS nine times (at 1955 and 2130 h on day 1, and at 0805, 0900, 1225, 1355, 1720, 1835 and 1955 h on day 2). Research staff within the laboratory did not consume food in sight of participants to eliminate a food cue, but participants did have access to the time of day during the 24-h period. The area under the curve (AUC) for appetite ratings was measured over the 24-h measurement period using the trapezoidal method.

4.3.9 Urine, fasting plasma and saliva measurements

Urine samples were analysed enzymatically for urinary urea (UREA kit; Roche, Mannheim, Germany) and creatinine (CREA kit; Roche, Mannheim, Germany) using an automated clinical chemistry analyser (Cobas c702 Analyser; Roche, Mannheim, Germany). Venous blood was collected at 0815 h following the observed overnight fasting period (>10 h) via an indwelling cannula inserted in an antecubital arm vein. Whole blood was collected into EDTA coated vacutainers then centrifuged at 1900 x g for 10 min at 4°C (see **section 3.6.4** for detailed blood collection methodology). Aliquots containing plasma were stored at -80°C until analysis. Due to difficulty in blood collection, blood was unable to be drawn from two participants (*n* = 1 participant in both the CON and EX+CON groups). Therefore, *n* = 31 participants had full blood data. Samples were analysed for plasma glucose using a glucose analyser (Biosen C-Line Glucose and Lactate Analyser; EKF Diagnostics, Cardiff, UK). Plasma insulin (EIA-2935; DRG Instruments GmbH, Marburg, Germany) and leptin (DLP00; R&D Systems Inc., Abbington, UK) were analysed by ELISA. Insulin resistance (homeostatic model assessment of insulin

resistance; HOMA-IR), insulin sensitivity (quantitative insulin sensitivity check index; QUICKI) and beta-cell function (HOMA-beta) were calculated from fasting glucose and insulin concentrations using standard equations (Katz et al. 2000, Matthews et al. 1985):

$$\text{HOMA-IR} = \text{Fasting insulin } (\mu U/mL) \times \text{fasting glucose } (mmol/L) / 22.5$$

$$\text{QUICKI} = 1/ [\log(\text{fasting insulin } (\mu U/mL)) + \log(\text{fasting glucose } (mmol/L))]$$

$$\text{HOMA-beta} = 20 \times \text{fasting insulin } (\mu U/mL) / \text{fasting glucose } (mmol/L) – 3.5.$$

Saliva samples were collected immediately upon awakening at 0650 h, and at 0805, 1225, 1700 and 2000 h using a synthetic swab (see **section 3.6.4** for detailed methodology). Participants were asked to rinse their mouth with water prior to each collection. Samples were centrifuged at 1900 x g for 2 min and stored at -80°C. Samples were analysed for salivary cortisol by ELISA (Item No. 1-3002; Salimetrics, Pennsylvania, USA). Salivary cortisol AUC (nmol/L x 790 min) was calculated using the trapezoidal method. The CV for plasma glucose was 0.5% and the intra-assay CV was 9.5%, 8.3% and 9.0% for plasma insulin and leptin, and salivary cortisol, respectively.

4.3.10 Statistical analysis

Statistical analysis performed in this chapter has previously been detailed and can be seen in **Section 3.7**.

4.4 Results

4.4.1 Participants and compliance

Thirty-nine older men were randomised: 33 completed the study and 6 withdrew. Reasons for withdrawal have been previously displayed and can be seen in **Figure 3.2**. Baseline characteristics of the 33 participants who completed the study are shown in **Table 4.2**. The mean attendance to the RE sessions was 98.0 ± 1.0% in the EX+CON group and 98.2 ± 1.2%

in the EX+PRO group, with no differences between groups (P = 0.98). All participants completed their prescribed repetitions for sets 1-2 of each exercise; during the final set (to volitional failure) the mean number of completed repetitions was 9.1 ± 0.3 in the EX+CON group and 9.1 ± 0.2 in the EX+PRO group, with no differences between groups (P = 0.94). The mean compliance with experimental supplements was 95.8 ± 0.6% and did not differ between groups (CON: 94.4 ± 1.3%; PRO: 96.9 ± 1.1%; EX+CON: 96.0 ± 1.1%; EX+PRO: 96.1 ± 1.3%, P = 0.64). The majority of participants (80.6%) were unable to judge treatment allocation.

Table 4.2 Baseline characteristics of participants[1]

	CON	PRO	EX+CON	EX+PRO	*P* value[3]	Overall
n	8	8	8	9	-	33
Age, y	66.6 ± 1.8	66.3 ± 1.7	66.5 ± 1.2	67.8 ± 1.2	0.89	66.8 ± 0.7
Height, m	1.78 ± 0.02	1.78 ± 0.02	1.77 ± 0.02	1.74 ± 0.03	0.63	1.76 ± 0.01
Body mass, kg	80.5 ± 3.4	79.7 ± 3.0	76.1 ± 3.8	80.9 ± 4.0	0.77	79.3 ± 1.8
BMI, kg/m^2	25.5 ± 1.0	25.2 ± 0.6	24.4 ± 0.8	26.6 ± 0.8	0.31	25.4 ± 0.4
FFM, kg	60.2 ± 1.6	60.9 ± 1.6	57.8 ± 2.8	60.5 ± 2.9	0.80	59.9 ± 1.9
SMM, kg	26.8 ± 0.7	27.5 ± 0.6	25.6 ± 1.2	26.9 ± 1.3	0.62	26.7 ± 0.7
FM, kg	20.3 ± 2.4	18.6 ± 1.8	18.2 ± 1.7	20.4 ± 1.5	0.78	19.4 ± 0.9
FM, %	24.7 ± 2.1	23.2 ± 1.7	23.8 ± 1.6	25.1 ± 1.2	0.84	24.2 ± 0.8
TEE, kcal/d	2417 ± 101	2529 ± 74	2460 ± 86	2454 ± 86	0.84	2465 ± 42
RMR, kcal/d	1602 ± 80	1661 ± 63	1631 ± 66	1609 ± 68	0.94	1625 ± 33
PAL	1.51 ± 0.03	1.53 ± 0.04	1.51 ± 0.05	1.53 ± 0.03	0.97	1.52 ± 0.02
Fasting plasma glucose, mmol/L	5.6 ± 0.2	6.0 ± 0.3	5.9 ± 0.3	5.7 ± 0.2	0.72	5.8 ± 0.1
HOMA-IR	2.4 ± 0.5	2.7 ± 0.5	2.3 ± 0.4	2.2 ± 0.4	0.85	2.4 ± 0.2
Step count, steps/d	10,840 ± 668	12,396 ± 1,398	12,171 ± 1,153	11,346 ± 907	0.71	11,678 ± 516
Time spent sedentary, min/d	275 ± 14	277 ± 41	285 ± 30	289 ± 20	0.98	282 ± 13
Time spent in MVPA, min/d	109 ± 8	119 ± 14	126 ± 16	135 ± 10	0.52	123 ± 6

[1]Values are means ± SE. [3]*P* value refers to differences between groups analysed by one-way ANOVA. No significant differences in baseline characteristics occurred between either pooled exercise and non-exercise groups, or between pooled whey protein and control groups (data not shown). BMI, body mass index; FFM, fat-free mass; FM, fat mass; HOMA-IR, homeostatic model assessment of insulin resistance; MVPA, moderate-vigorous physical activity; PAL, physical activity level; RMR, resting metabolic rate; SMM, skeletal muscle mass; TEE, total energy expenditure.

4.4.2 Dietary intake

At baseline, no differences in carbohydrate, fat, protein or total EI occurred between groups. At weeks 6 and 12, protein intake increased in the PRO and EX+PRO groups greater than the CON and EX+CON groups ($P < 0.001$, **Table 4.3**). Carbohydrate intake increased in the EX+CON group greater than the EX+PRO group at weeks 6 and 12 ($P < 0.05$), and greater than the PRO group at week 12 only ($P = 0.01$). Total EI increased over time in the EX+PRO group at week 6 ($P = 0.03$) but not at week 12 ($P = 0.75$) and increased in the CON group at week 12 only ($P = 0.001$). When participants' dietary intake was analysed excluding experimental supplements, total EI decreased over time in the PRO group at week 12 ($P = 0.02$). Total EI also decreased to a greater extent over time when whey protein groups were pooled and compared to control supplement groups pooled ($P = 0.01$). Habitual carbohydrate intake decreased over time in the CON group at week 6 ($P = 0.04$), but not at week 12 ($P = 0.68$). Fat intake decreased greater in the PRO compared to the CON group at week 12 ($P = 0.02$). When whey protein supplement groups were pooled, fat intake decreased greater than control supplement groups pooled between weeks 6 and 12 ($P = 0.004$).

Table 4.3 Dietary intake (including and excluding experimental supplements) during the intervention period[1]

	Pooled	CON		PRO		EX+CON		EX+PRO		
	Baseline	6 weeks	12 weeks	6 weeks	12 weeks	6 weeks	12 weeks	6 weeks	12 weeks	*P* value[2]
Energy, kcal/d										
Diet[3]	1999 ± 59	1822 ± 104	1920 ± 84	1878 ± 147	1741 ± 159$^{\#}$	1972 ± 93	2021 ± 128	2048 ± 97	1969 ± 141	0.08
Total[3]	-	1989 ± 116	2086 ± 89$^{\#}$	2069 ±147	1932 ± 159	2163 ± 93	2211 ± 128	2238 ± 97$^{\#}$	2159 ± 141	0.09
Protein, g/d										
Diet[3]	82 ± 2	77 ± 6	75 ± 4	82 ± 4	81 ± 5	86 ± 5	84 ± 5	85 ± 6	79 ± 3	0.87
Total[3]	-	77 ± 6	75 ± 4	128 ± 4$^{*¥\#}$	126 ± 5$^{*¥\#}$	86 ± 5	84 ± 5	131 ± 6$^{*¥\#}$	125 ± 3$^{*¥\#}$	< 0.001
Protein, g/kg/d										
Diet[3]	1.0 ± 0.02	1.0 ± 0.1	0.9 ± 0.04	1.0 ± 0.03	1.0 ± 0.04	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.05	1.0 ± 0.05	0.37
Total	-	1.0 ± 0.1	0.9 ± 0.04	1.6 ± 0.04$^{*¥\#}$	1.5 ± 0.1$^{*¥\#}$	1.1 ± 0.1	1.1 ± 0.1	1.6 ± 0.1$^{*¥\#}$	1.6 ± 0.1$^{*¥\#}$	< 0.001
Protein, %										
Diet	17 ± 0.4	17 ± 1	16 ± 1	18 ± 1	19 ± 1	18 ± 1	17 ± 1	17 ± 1	17 ± 1	0.47
Total[3]	-	16 ± 1	15 ± 1	25 ± 1$^{*¥\#}$	27 ± 2$^{*¥\#}$	16 ± 1	15 ± 1	24 ± 1$^{*¥\#}$	24 ± 1$^{*¥\#}$	< 0.001
Carbohydrate, g/d										
Diet	234 ± 8	197 ± 9$^{\#}$	211 ± 18	196 ± 23	204 ± 18	224 ± 16	231 ± 12	221 ± 11	238 ± 16	0.87
Total[3]	-	239 ± 12	253 ± 17	196 ± 23	204 ± 18	272 ± 16$^{\$\#}$	279 ± 12$^{‡\$\#}$	221 ± 11	238 ± 16	0.02
Carbohydrate, %										
Diet[3]	48 ± 12	44 ± 2$^{\#}$	44 ± 3	42 ± 4	47 ± 2	46 ± 2	46 ± 2	44 ± 2	49 ± 2	0.69
Total	-	48 ± 2‡	48 ± 2	38 ± 4	42 ± 1	50 ± 2$^{\$}$	51 ± 2$^{‡\#}$	40 ± 2	44 ± 2	0.001
Fat, g/d										
Diet	70 ± 3	69 ± 6	75 ± 4‡	73 ±11	55 ± 8	71 ± 6	70 ± 9	77 ± 5	61 ± 8	0.02
Total	-	69 ± 6	75 ± 4‡	74 ±11	56 ± 9	71 ± 6	70 ± 9	78 ± 5	62 ± 8	0.03
Fat, %										
Diet	32 ± 1	34 ± 2	35 ± 1	35 ± 4	28 ± 2	32 ± 2	31 ± 2	34 ± 1	27 ± 2	0.21
Total[3]	-	31 ± 2	32 ± 1	32 ± 4	25 ± 2	29 ± 2	28 ± 2	31 ± 1	25 ± 2	0.08

[1]Values are means ± SE. Baseline values for individual groups are not shown but no significant differences occurred between groups for any dietary marker. [2]*P* value refers to respective group-by-time interaction.[3]Significant main effect of time, $P < 0.05$. *Significantly ($P < 0.05$) greater than CON group at respective time point. ‡Significantly greater than PRO group at respective time point. ¥Significantly greater than EX+CON group at respective time point. $Significantly greater than EX+PRO group at respective time point. #$P < 0.05$ from baseline value. ¢$P < 0.05$ from week 6. – indicates same value as diet. Diet = intake from habitual intake (excluding supplements). Total = intake from habitual diet plus experimental supplements.

4.4.3 Habitual physical activity

No differences in any marker of habitual physical activity occurred between groups at baseline (**Table 4.2**). Following the intervention, daily step count (P = 0.52), time spent sedentary (P = 0.48), and in light (P = 0.92) and MVPA (P = 0.80) did not differ between groups over time. No significant within-group changes occurred.

4.4.4 Body mass and composition

Body mass did not change over time in either the PRO or EX+PRO groups, whereas participants gained 0.9 ± 0.3 kg (P = 0.007) in the CON group and 1.3 ± 0.2 kg (P < 0.001) in the EX+CON group. When control supplement groups were pooled, body mass increased greater than whey protein groups pooled (P = 0.04, **Figure 4.5A**). Fat-free mass and SMM increased in only the EX+CON (FFM: 1.0 ± 0.4 kg, P = 0.06; SMM: 0.6 ± 0.2 kg, P = 0.042) and EX+PRO groups (FFM: 1.0 ± 0.4 kg, P = 0.05; SMM: 0.6 ± 0.3 kg, P = 0.06), but no differences occurred between groups. When exercise groups were pooled, FFM increased greater than non-exercise groups pooled (P = 0.04, **Figure 4.5B**). Fat mass significantly increased over time in the CON group (0.8 ± 0.3 kg, P = 0.02) and decreased, but not significantly, in the EX+PRO group (-0.9 ± 0.5 kg, P = 0.09). When whey protein supplement groups were pooled, both absolute (P = 0.03, **Figure 4.5C**) and %FM (P = 0.04) decreased greater than control supplement groups pooled. Body mass index increased greater in the pooled control supplement group compared to the pooled whey protein supplement group (P = 0.04).

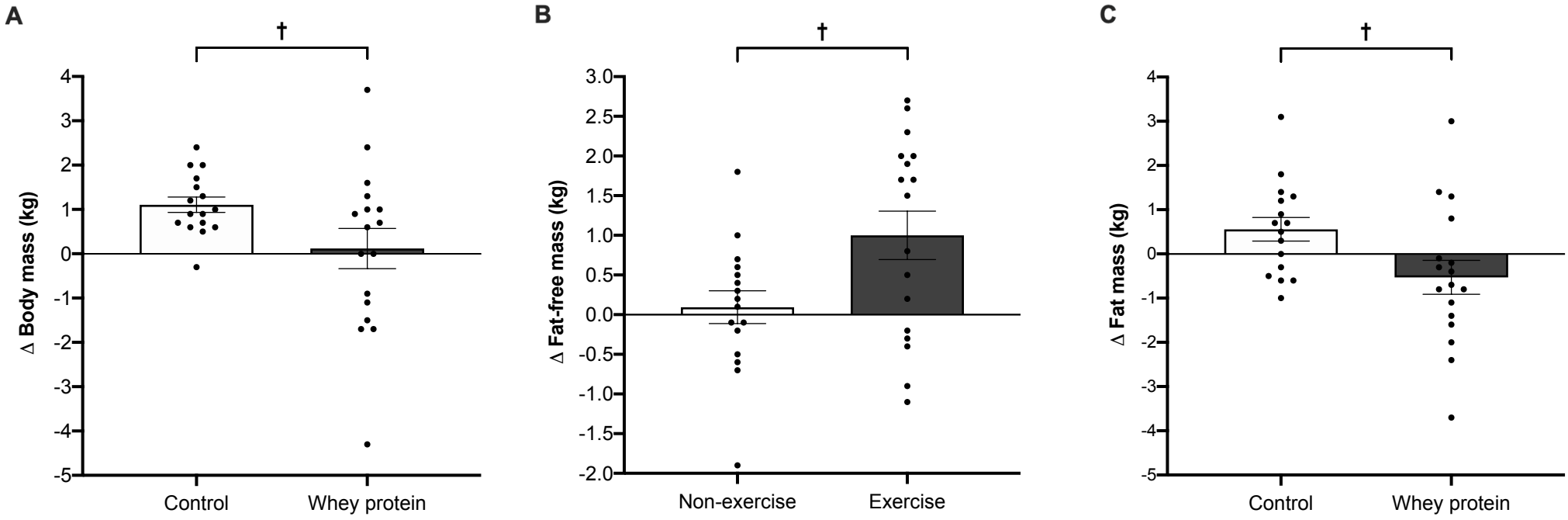

Figure 4.5 Changes in (A) body mass between pooled whey protein (n = 17) and control supplement groups (n = 16); (B) fat-free mass between pooled exercise (n = 17) and non-exercise groups (n = 16); and (C) fat mass between pooled whey protein and control supplement groups over the intervention period (means ± SE). Circles represent individual data points. Data were analysed using a mixed-model ANCOVA with baseline values and either exercise or non-exercise (for pooled whey protein vs. control groups, panels A and C), and supplement consumed (whey protein or control; panel B) included as covariates. $^{\dagger}P < 0.05$ between groups.

4.4.5 Energy expenditure and substrate oxidation

4.4.5.1 Total EE

Total EE decreased in all groups from baseline to 12 weeks, but only significantly in the PRO group (-78 ± 26 kcal/d, P = 0.02, **Table 4.4**). When normalised to both FFM and SMM, decreases in TEE over time in the CON, PRO and EX+PRO groups became significant (P < 0.05). No differences were observed between groups (P = 0.26).

4.4.5.2 24-h substrate oxidation

Twenty-four-hour protein oxidation increased over time in the PRO and EX+PRO groups greater than the CON and EX+CON groups (P < 0.05, **Figure 4.6A**). The increase was driven by rises in overnight protein oxidation, which increased in the PRO (29 ± 6 g/d) and EX+PRO groups (30 ± 10 g/d) greater than the CON and EX+CON groups (P < 0.05, **Figure 4.6B**). No within- or between-group differences occurred for daytime protein oxidation. Protein balance was positive in all groups at baseline but decreased in the PRO and EX+PRO groups greater than the EX+CON group when protein intake was returned to the chamber diet at week 12 (P < 0.05, **Figure 4.6C**). No within- or between-group differences in 24-h oxidation or balance of carbohydrate or fat, or RQ, was observed over the course of the study (**Table 4.4**).

Table 4.4 Energy expenditure, 24-h macronutrient oxidation and balances, and appetite profile for each treatment group at baseline and 12 weeks[1]

	CON		PRO		EX+CON		EX+PRO		*P* value	
	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Time	Group x time
TEE, kcal/d	2417 ± 101	2372 ± 95	2529 ± 74	2451 ± 85[#]	2460 ± 86	2413 ± 96	2455 ± 86	2439 ± 88	0.54	0.26
Sedentary EE, kcal/d	1761 ± 81	1708 ± 80	1727 ± 78	1724 ± 77	1711 ± 47	1750 ± 75	1720 ± 75	1798 ± 64	0.19	0.17
RMR, kcal/d	1602 ± 80	1586 ± 66	1661 ± 63	1598 ± 57	1631 ± 66	1670 ± 64	1609 ± 68	1643 ± 65	0.25	0.11
SMR,[2] kcal/d	1561 ± 73	1554 ± 68	1600 ± 56	1597 ± 62	1562 ± 79	1607 ± 78[#]	1598 ± 64	1643 ± 60[#]	0.40	0.15
AEE, kcal/d	656 ± 38	664 ± 45	803 ± 42	727 ± 42[#]	748 ± 59	663 ± 30	735 ± 47	642 ± 41	0.008	0.41
SPA, kcal/d	358 ± 30	346 ± 37	461 ± 41	384 ± 37[#]	421 ± 43	345 ± 16	407 ± 46	297 ± 33[#]	0.004	0.27
DIT, kcal/d	143 ± 20	134 ± 27	126 ± 47	128 ± 47	153 ± 31	170 ± 20	122 ± 38	155 ± 25	0.001	0.79
DIT, % of EI	6.1 ± 0.9	5.7 ± 1.1	4.9 ± 1.9	5.1 ± 1.9	6.7 ± 1.4	7.1 ± 0.9	4.9 ± 1.5	6.4 ± 1.1	0.001	0.80
Protein oxidation, g/d	86 ± 8	80 ± 6	89 ± 5	108 ± 6[*¥#]	84 ± 6	80 ± 6	83 ± 6	102 ± 7[*¥#]	0.005	0.001
Carbohydrate oxidation, g/d	267 ± 14	255 ± 12	243 ± 18	238 ± 23	240 ± 14	240 ± 14	253 ± 16	245 ± 7	0.008	0.95
Fat oxidation, g/d	92 ± 9	96 ± 10	113 ± 52	99 ± 4	110 ± 7	106 ± 5	105 ± 6	97 ± 7	0.08	0.47
RQ	0.86 ± 0.01	0.85 ± 0.01	0.84 ± 0.01	0.84 ± 0.01	0.84 ± 0.01	0.84 ± 0.01	0.84 ± 0.01	0.84 ± 0.01	0.01	0.99
Protein balance, g/d	28 ± 8	24 ± 5	26 ± 8	5 ± 9[¥#]	31 ± 6	36 ± 4	28 ± 4	9 ± 5[¥#]	0.12	0.001
Carbohydrate balance, g/d	37 ± 13	34 ± 15	52 ± 12	56 ± 14	56 ± 11	48 ± 9	41 ± 11	55 ± 12	0.001	0.63
Fat balance, g/d	0 ± 7	-6 ± 8	-15 ± 7	-1 ± 7	-15 ± 9	-9 ± 6	-9 ± 9	-1 ± 7	0.55	0.28
Energy balance, kcal/d	80 ± 70	30 ± 56	-12 ± 79	35 ± 62	44 ± 98	73 ± 74	37 ± 61	61 ± 37	0.57	0.82
Hunger AUC, mm x 24 h	60,446±7521	61,418±6556	60,184±6703	62,553±6848	57,811±4103	69,608±2892	51,673±5803	57,880±5371	0.001	0.48
Satiety AUC, mm x 24 h	75,625±5467	77,649±6341	72,501±6597	66,871±3779	69,285±3928	64,790±3779	79,107±6505	75,065±4101	0.07	0.42
Fullness AUC, mm x 24 h	74,435±6782	78,329±6918	60,019±10201	59,579±9220	65,514±3363	63,951±5523	77,723±5719	71,901±3548	0.02	0.49
Desire to eat AUC, mm x 24 h	69,471±8387	69,382±7112	80,207±10,688	71,250±7045	71,553±3876	77,956±3809	58,871±6325	64,534±4680	0.001	0.39

[1]Values are means ± SE. Baseline values were not significantly different between groups. [2]CON and EX+CON, $n = 7$ ($n = 1$ outlier, >3SD from mean removed from each group). AEE, activity energy expenditure; AUC, area under the curve; DIT, diet-induced thermogenesis; EI, energy intake; PAL, physical activity level; RMR, resting metabolic rate; RQ, respiratory quotient; SMR, sleeping metabolic rate; SPA, spontaneous physical activity. *Significantly ($P < 0.05$) greater than CON group. ¥Significantly greater than EX+CON group. #$P < 0.05$ from baseline value.

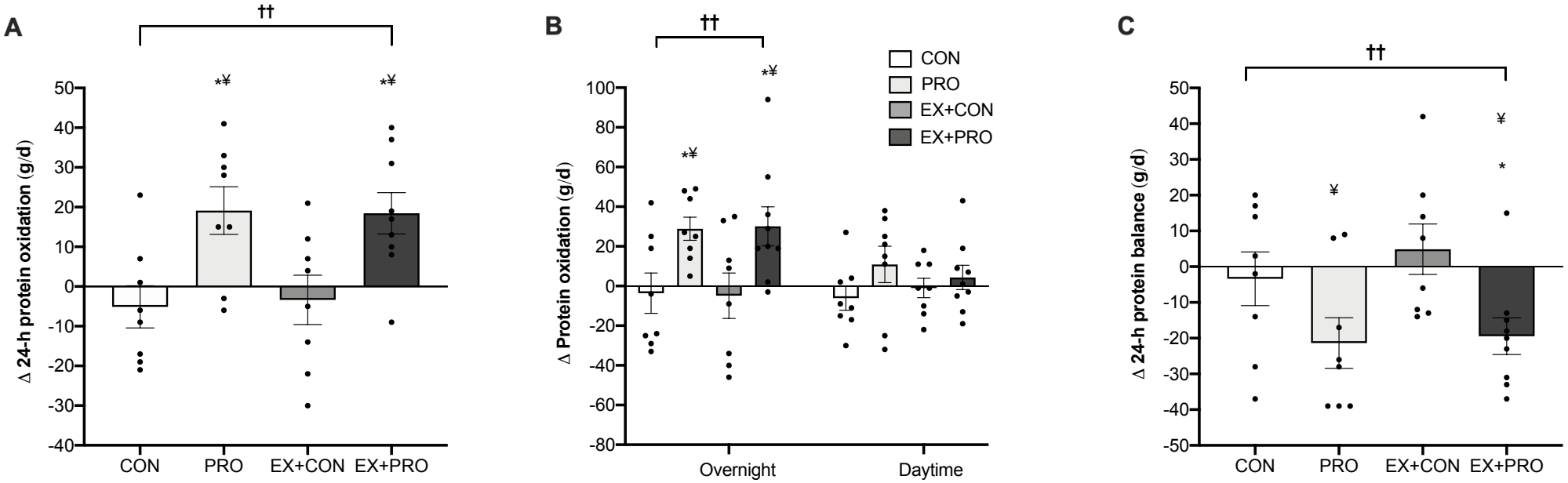

Figure 4.6 Changes in (A) 24-h protein oxidation; (B) overnight and daytime protein oxidation; and (C) 24-h protein balance between groups (means ± SE). Circles represent individual data points. Data were analysed using a mixed-model ANCOVA with baseline values included as a covariate. ††Significant group-by-time interaction, $P < 0.01$. *Significantly greater than the CON group. ¥Significantly greater than EX+CON group.

4.4.5.3 Resting, sedentary and sleeping EE and substrate oxidation

No differences in RMR (P = 0.11) or sedentary EE (P = 0.17) occurred between groups over the course of the study. When exercise groups were pooled, both RMR (36 ± 14 kcal/d) and sedentary EE (60 ± 30 kcal/d) increased over time greater than non-exercise groups pooled (P < 0.05, **Figure 4.7**), but not when normalised to skeletal muscle or FFM. Resting fat oxidation decreased over time in the PRO group greater than the EX+CON group (P = 0.02), which remained significant when normalised to body composition variables (P < 0.05). No within- or between-group differences in resting carbohydrate oxidation or RQ were observed.

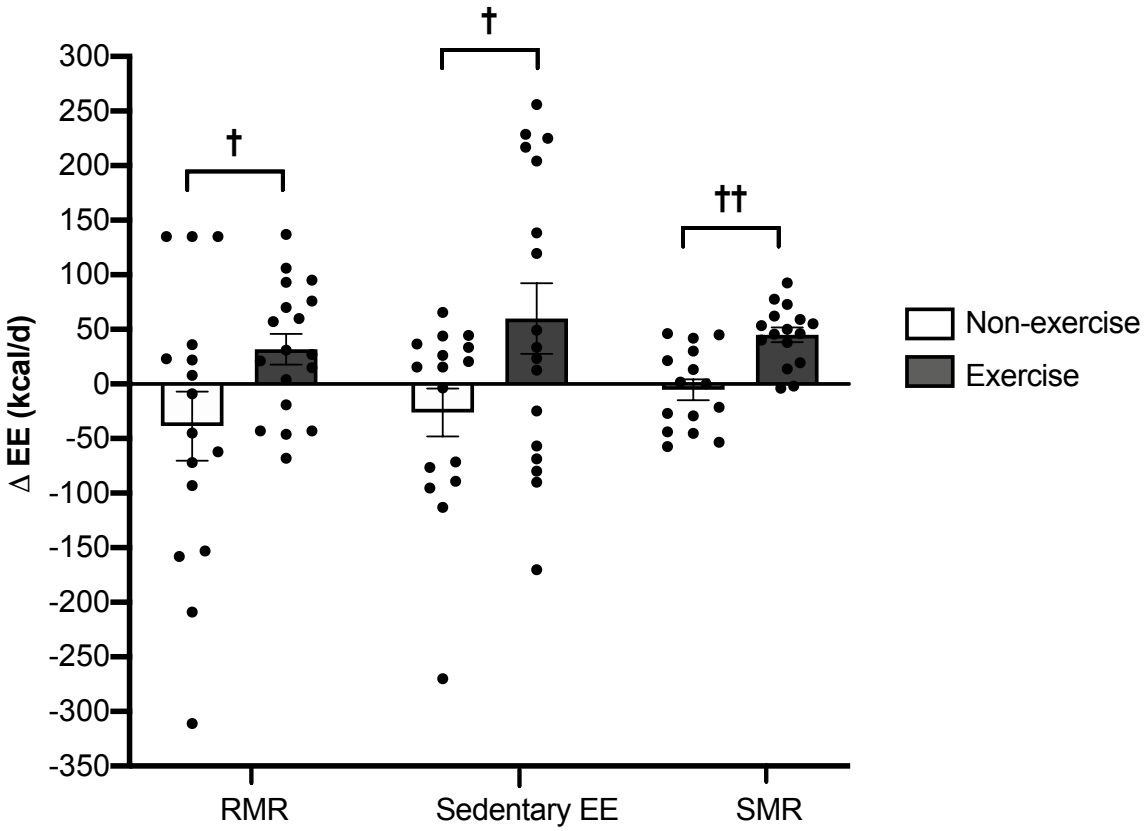

Figure 4.7 Changes in resting metabolic rate (RMR), sedentary EE and sleeping metabolic rate (SMR) between pooled exercise (EX+CON and EX+PRO; n = 17 for RMR and sedentary EE; n = 16 for SMR) and non-exercise groups (CON and PRO; n = 16 for RMR and sedentary EE; n = 15 for SMR) over the intervention period (means ± SE). Circles represent individual data points. Data were analysed using a mixed-model ANCOVA with baseline values and supplement consumed (whey protein or control) included as covariates. $^{\dagger}P$ < 0.05 between groups. $^{\dagger\dagger}P$ < 0.01 between groups.

Sleeping metabolic rate significantly increased over time in the EX+CON (45 ± 12 kcal/d, P = 0.008) and EX+PRO groups (45 ± 9 kcal/d, P = 0.04), but not when normalised to FFM or SMM. No differences occurred between groups (P = 0.15). When exercise groups were pooled, SMR increased greater than non-exercise groups pooled (P < 0.001, **Figure 4.7**). No within- or between-group differences occurred for sleeping carbohydrate or fat oxidation.

4.4.5.4 Activity EE and physical activity

Activity EE significantly decreased over time in the PRO group (-76 ± 26 kcal/d, P = 0.02), and tended to decrease in the EX+CON (-85 ± 40 kcal/d, P = 0.07) and EX+PRO groups (-93 ± 43 kcal/d, P = 0.06) (**Figure 4.8A**). When normalised to SMM, AEE significantly decreased in these groups (P < 0.05). Spontaneous physical activity decreased over time in the PRO (-77 ± 22 kcal/d, P = 0.01) and EX+PRO groups (-110 ± kcal/d, P = 0.045), and decreased, but not significantly, in the EX+CON group (-76 ± 38 kcal/d, P = 0.09) (**Figure 4.8B**). When normalised to body composition variables, SPA decreased over time in the PRO and EX+CON groups (P < 0.05), whereas in the EX+PRO group, SPA was no longer significantly decreased when normalised to FM only (P = 0.17).

In the PRO and EX+CON groups, the decrease in SPA over the course of the study occurred due to a reduction in the energy cost of SPA per unit of activity, which significantly decreased in the PRO group (P = 0.03, **Figure 4.8C**). In contrast, the decline in SPA in the EX+PRO group occurred due to a notable reduction in chamber activity during the non-exercise periods, which decreased greater than the PRO group (P = 0.049, **Figure 4.8D**). Change in chamber activity negatively correlated with ΔRMR (r = -0.41, P = 0.02, **Figure 4.8E**). Physical activity level decreased over time in the EX+CON group (-0.06 ± 0.02, P = 0.02) and decreased, but not significantly, in the EX+PRO group (-0.04 ± 0.02, P = 0.09). No differences occurred between groups (P = 0.20); however, there was a trend towards a greater decrease in PAL when pooled exercise groups were compared to pooled non-exercise groups (P = 0.06, **Figure 4.8F**).

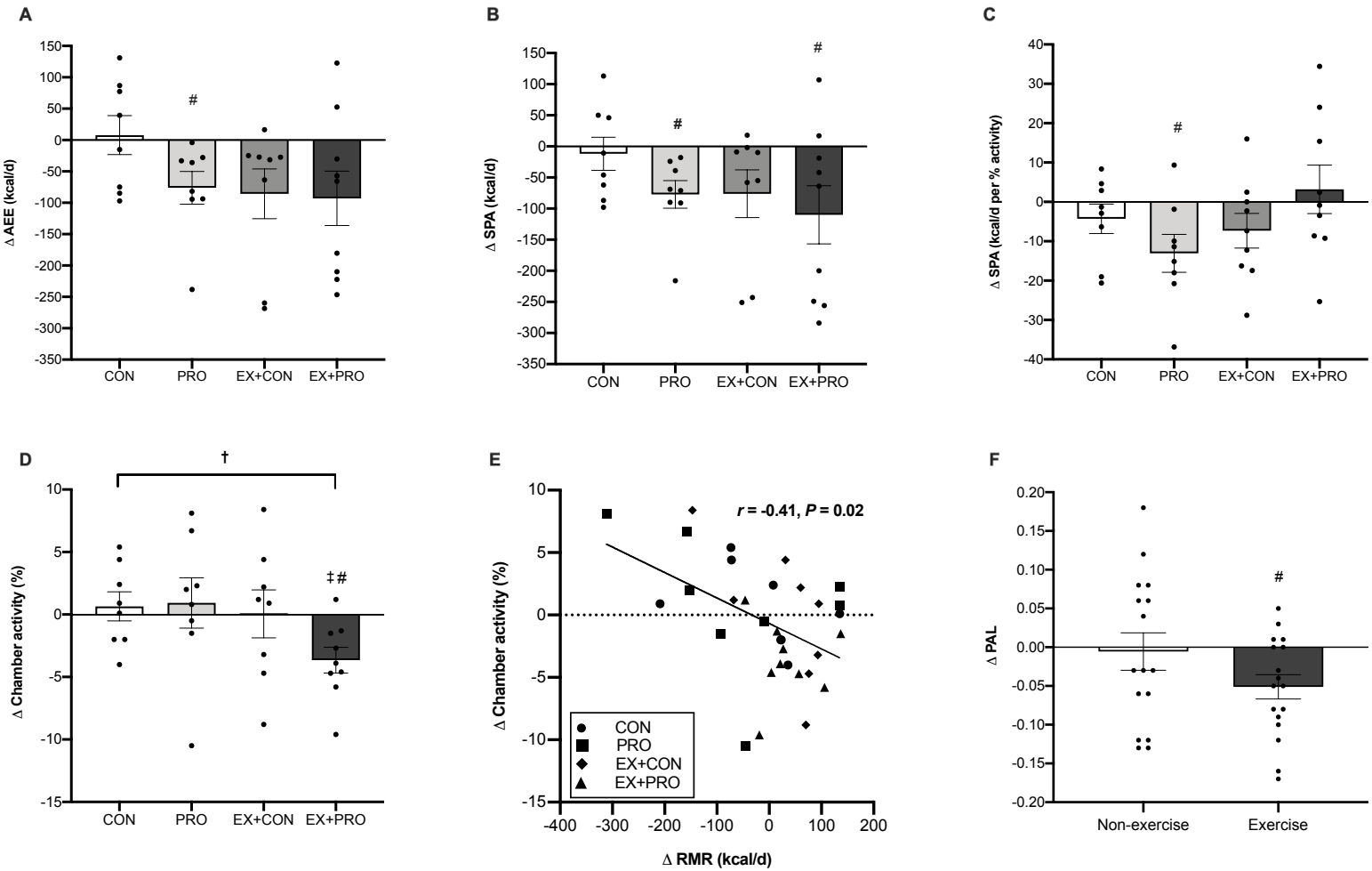

Figure 4.8 Changes in (A) AEE; (B) SPA (kcal/d); (C) SPA (kcal/d per % activity); and (D) chamber activity during the non-exercise periods for each treatment group over the intervention period (means ± SE); (E) correlation between ΔRMR and Δchamber activity; and (F) change in PAL between exercise and non-exercise groups (means ± SE). Individual data points are shown with circles (apart from panel E where different shapes represent participants in different experimental groups). AEE; activity energy expenditure; PAL, physical activity level; RMR, resting metabolic rate; SPA, spontaneous physical activity. [†]Significant group-by-time interaction, $P < 0.05$. [‡]Significantly ($P < 0.05$) less than PRO group. [#]$P < 0.05$ from baseline.

4.4.5.5 Exercise EE and substrate oxidation

At baseline and 12 weeks, fat oxidation was significantly greater and carbohydrate oxidation was significantly less during the fasted step exercise bout (EX-1) than during both EX-2 (P < 0.001) and EX-3 (P < 0.001, **Figure 4.9**). Energy expenditure did not differ between step exercise bouts at baseline. In contrast, at 12 weeks, EE during EX-1 (4.7 ± 0.1 kcal/min) was significantly less than during both EX-2 (4.9 ± 0.1 kcal/min, P < 0.001) and EX-3 (4.8 ± 0.1 kcal/min, P < 0.001).

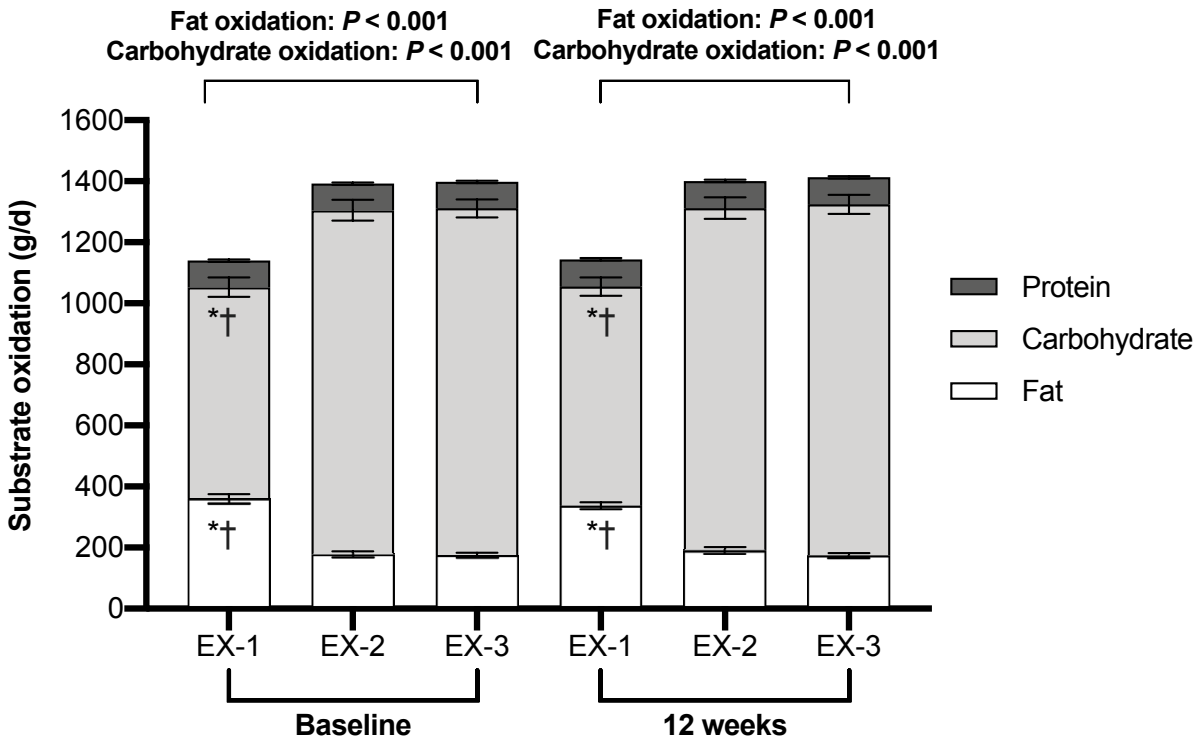

Figure 4.9 Carbohydrate, fat and protein oxidation during the three step exercise bouts performed at 75 steps/min at baseline and 12 weeks (n = 33; means ± SE). EX-1, step exercise bout 1 (0830 h in the fasted state); EX-2, step exercise bout 2 (1445 h); EX-3, step exercise bout 3 (1915 h). Baseline and 12-week data were analysed by repeated measures ANOVA. *P < 0.001 compared to EX-2. $^{\dagger}P$ < 0.001 compared to EX-3.

Following the intervention, EE tended to decrease over time during EX-1 in the PRO group (-0.3 ± 0.2 kcal/min, P = 0.06, **Table 4.5**), which became significant when normalised to SMM

(P = 0.04). Energy expenditure also decreased over time during EX-1 in the EX+CON group when normalised to FFM (P = 0.047). Fat oxidation decreased over time during EX-1 in the PRO group (-71 ± 23 g/d, P = 0.02), which remained decreased when normalised to body composition variables (P < 0.05). During EX-2 (-0.1 ± 0.1 kcal/min, P = 0.049) and EX-3 (-0.2 ± 0.1 kcal/min, P = 0.02), modest, but significant decreases in EE occurred over the course of the study in the EX+CON group, which remained significant when normalised to body composition variables (P < 0.05). Neither EE, nor substrate oxidation changed over time during any step exercise bout in the CON or EX+PRO groups, and no differences occurred between groups.

Table 4.5 Step exercise energy expenditure and substrate oxidation for each treatment group at baseline and 12 weeks[1]

	CON		PRO		EX+CON		EX+PRO		*P* value	
	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Time	Group x time
EX-1										
EE, kcal/min	4.5 ± 0.3	4.6 ± 0.2	5.0 ± 0.2	4.7 ± 0.2	4.7 ± 0.2	4.6 ± 0.2	4.7 ± 0.2	4.8 ± 0.2	0.76	0.08
Carbohydrate oxidation, g/d	649 ± 58	705 ± 47	661 ± 86	695 ± 95	693 ± 68	688 ± 54	760 ± 47	776 ± 47	0.02	0.96
Fat oxidation, g/d	348 ± 38	335 ± 25	416 ± 24	344 ± 23[#]	351 ± 24	342 ± 22	328 ± 25	326 ± 26	0.009	0.50
Protein oxidation, g/d	90 ± 8	84 ± 7	89 ± 5	100 ± 5	85 ± 9	85 ± 8	88 ± 7	92 ± 9	0.009	0.27
RQ	0.84 ± 0.01	0.84 ± 0.01	0.82 ± 0.01	0.84 ± 0.01	0.84 ± 0.01	0.84 ± 0.01	0.85 ± 0.01	0.85 ± 0.01	0.02	0.79
EX-2										
EE,[2] kcal/min	4.6 ± 0.2	4.8 ± 0.2	5.0 ± 0.2	5.0 ± 0.2	4.8 ± 0.2	4.7 ± 0.3[#]	4.9 ± 0.2	4.9 ± 0.2	0.29	0.35
Carbohydrate oxidation, g/d	1047 ± 71	1056 ± 70	1165 ± 69	1162 ± 93	1143 ± 80	1091 ± 68	1154 ± 58	1168 ± 54	0.19	0.71
Fat oxidation, g/d	174 ± 33	203 ± 24	185 ± 21	179 ± 22	174 ± 20	191 ± 17	173 ± 12	190 ± 24	< 0.001	0.86
Protein oxidation, g/d	90 ± 8	84 ± 7	89 ± 5	100 ± 5	85 ± 9	85 ± 8	88 ± 7	92 ± 9	0.009	0.27
RQ	0.91 ± 0.01	0.90 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.92 ± 0.01	0.91 ± 0.01	0.92 ± 0.01	0.91 ± 0.01	0.003	0.77
EX-3										
EE,[2] kcal/min	4.6 ± 0.2	4.7 ± 0.2	5.0 ± 0.2	4.9 ± 0.2	4.9 ± 0.2	4.7 ± 0.3[#]	4.9 ± 0.2	4.9 ± 0.2	0.09	0.26
Carbohydrate oxidation, g/d	1075 ± 56	1067 ± 50	1164 ± 69	1159 ± 77	1133 ± 71	1152 ± 64	1172 ± 53	1219 ± 56	0.05	0.59
Fat oxidation, g/d	166 ± 21	185 ± 23	184 ± 17	180 ± 19	179 ± 24	162 ± 19	170 ± 13	168 ± 14	0.02	0.47
Protein oxidation, g/d	90 ± 8	84 ± 7	89 ± 5	100 ± 5	85 ± 9	85 ± 8	88 ± 7	92 ± 9	0.009	0.27
RQ	0.92 ± 0.01	0.92 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.92 ± 0.01	0.92 ± 0.01	0.91 ± 0.01	0.92 ± 0.01	0.04	0.47

[1]Values are means ± SE. Baseline values were not significantly different between groups. [2]EX+PRO, n = 8 (1 outlier removed, >3SD from mean). Protein oxidation data has been added for completeness but was obtained from one urine sample between 0800-2000 h. EE, energy expenditure; EX-1, step exercise bout 1 (0830 h in the fasted state); EX-2, step exercise bout 2 (1445 h); EX-3, step exercise bout 3 (1915 h); RQ, respiratory quotient. [#]$P < 0.05$ from baseline value.

4.4.5.6 Diet-induced thermogenesis

Diet-induced thermogenesis was similar between groups at baseline (CON: 6.1 ± 0.9%; PRO: 4.9 ± 1.9%; EX+CON: 6.7 ± 1.4%; EX+PRO: 4.9 ± 1.5%, P = 0.78). A high inter-individual variability was observed (range -3.1-12.5%). At 12 weeks when protein intake was returned to the baseline chamber diet, no within- or between-group differences in DIT (expressed as both kcal/d and % EI) occurred (**Table 4.4**).

4.4.5.7 Metabolic flexibility

Twenty-four-hour metabolic flexibility increased over time in only the EX+CON (P = 0.007) and EX+PRO groups (P = 0.01), but no significant differences occurred between groups (P = 0.06). When exercise groups were pooled, 24-h metabolic flexibility increased greater than non-exercise groups pooled (P = 0.01, **Figure 4.10**). The increase in 24-h metabolic flexibility when exercise groups were pooled was driven by decreases in sleeping npRQ (-0.019 ± 0.007, P = 0.02) rather than changes in awake npRQ (0.003 ± 0.005, P = 0.48).

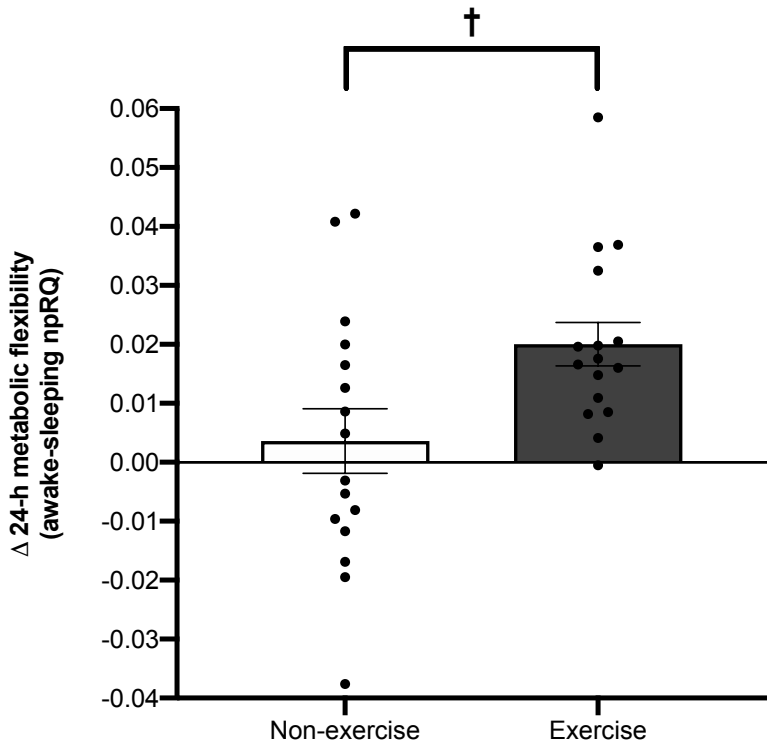

Figure 4.10 Change in 24-h metabolic flexibility between pooled exercise (*n* = 17) and non-exercise groups (*n* = 16) (means ± SE). Circles represent individual data points. Data were analysed using a mixed-model ANCOVA with baseline values and supplement consumed (whey protein or control) included as covariates. npRQ, non-protein respiratory quotient. $^{\dagger}P <$ 0.05 between groups.

4.4.6 Appetite profile

No differences in hunger ($P = 0.71$), satiety ($P = 0.66$), fullness ($P = 0.26$) or desire to eat VAS AUC ($P = 0.28$) occurred between groups at baseline (**Table 4.4**). Following the intervention, increases in hunger AUC occurred in the EX+CON (11,798 mm x 24 h, $P = 0.053$) and EX+PRO groups (6,207 mm x 24 h, $P = 0.052$), which trended towards statistical significance. The increase became significant when exercise groups were pooled ($P = 0.006$); however, no significant differences in any appetite rating occurred between groups. When whey protein groups were pooled, no within- or between-group differences for any marker of subjective appetite occurred.

4.4.7 Continuous glucose monitoring

At baseline, 24-h average interstitial glucose concentration was 5.5 ± 0.4 mmol/L in the CON group, 5.1 ± 0.2 mmol/L in the PRO group, 5.5 ± 0.2 mmol/L in the EX+CON group, and 5.7 ± 0.2 mmol/L in the EX+PRO group with no differences between groups (P = 0.36). After 12 weeks, no differences in 24-h average interstitial glucose (P = 0.58) or interstitial glucose variability (P = 0.31) occurred between groups, and no significant within-group differences were observed for either variable (**Table 4.6**).

4.4.8 Fasting plasma measurements and diurnal salivary cortisol

Fasting plasma leptin (-1.2 ± 0.5 ng/mL, P = 0.045) and plasma glucose concentration (-0.3 ± 0.1 mmol/L, P = 0.04) decreased over time in only the EX+PRO group, and fasting plasma insulin tended to decrease in both the EX+CON (-1.6 ± 0.7 mU/L, P = 0.06) and EX+PRO groups (-2.1 ± 1.1 mU/L, P = 0.09, **Table 4.6**). In the EX+PRO group, HOMA-IR significantly decreased (-0.6 ± 0.3, P = 0.04) and QUICKI increased over the duration of the study (0.02 ± 0.01, P = 0.02). In the PRO and EX+CON groups, HOMA-IR tended to decrease (PRO: -0.6 ± 0.3, P = 0.07; EX+CON: -0.5 ± 0.2, P = 0.06), and QUICKI significantly increased in the PRO group (0.03 ± 0.01, P = 0.048) and tended to increase in the EX+CON group (0.02 ± 0.01, P = 0.05). No differences occurred between groups for either HOMA-IR (P = 0.25) or QUICKI (P = 0.23). When protein supplement groups were pooled, QUICKI tended to increase greater than control groups pooled (P = 0.06). No within- or between-group differences occurred for HOMA-beta over the course of the study.

At baseline, no group-by-timepoint interaction (P = 0.79) or differences in the AUC (P = 0.28) for salivary cortisol occurred between groups. Following the intervention, no significant group-by-time-by-time point interaction occurred for salivary cortisol (P = 0.57); however, awakening cortisol concentration (0650 h) increased, but not significantly, in the PRO (5.5 ± 2.9 nmol/L, P = 0.09) and EX+PRO groups (7.2 ± 4.1 nmol/L, P = 0.06). When these groups were pooled, the increase in awakening cortisol concentration was significantly greater than control

supplement groups pooled (P = 0.049, **Figure 4.11**). No within- or between-group changes occurred for salivary cortisol AUC.

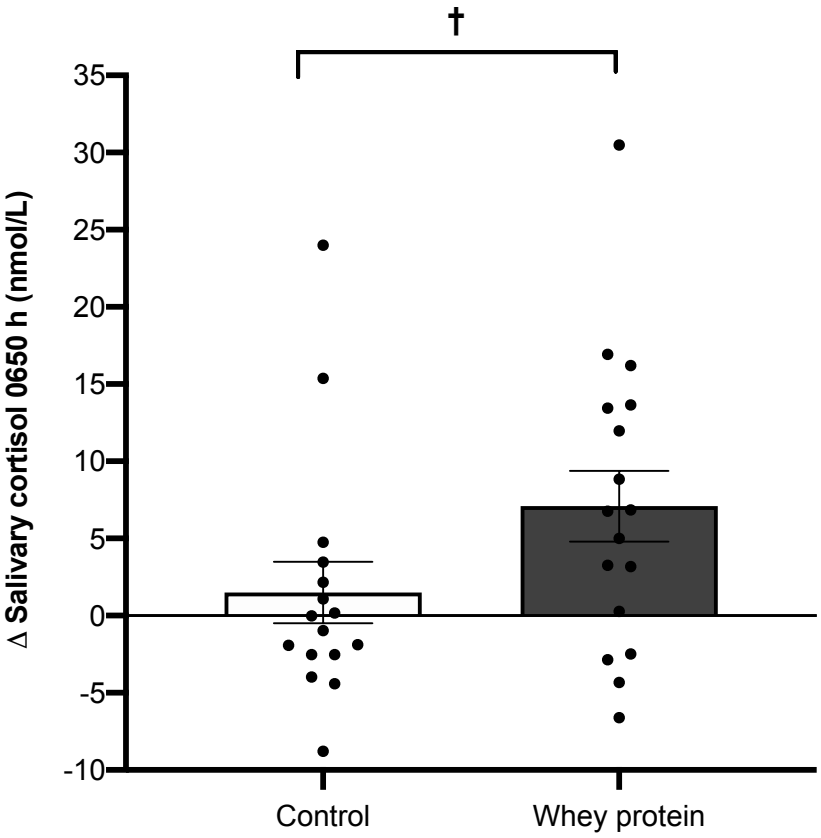

Figure 4.11 Change in awakening (0650 h) salivary cortisol concentration between pooled whey protein (n = 17) and control supplement groups (n = 16) (means ± SE). Circles represent individual data points. Data were analysed using the Scheirer-Ray-Hare two-way ANOVA of ranks test. $^{\dagger}P < 0.05$ between groups.

Table 4.6 Fasting plasma, continuous glucose monitoring and salivary cortisol markers at baseline and 12 weeks for each treatment group[1]

	CON		PRO		EX+CON		EX+PRO		*P* value	
	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Time	Group x time
Plasma glucose,[2] mmol/L	5.6 ± 0.2	5.7 ± 0.3	6.0 ± 0.3	5.7 ± 0.1	5.9 ± 0.3	5.6 ± 0.3	5.7 ± 0.2	5.4 ± 0.1[#]	0.09	0.64
Plasma insulin,[2] mU/L	10.6 ± 1.9	10.6 ± 2.3	10.2 ± 1.6	8.4 ± 2.3	9.0 ± 1.6	7.4 ± 1.4	8.8 ± 1.8	6.7 ± 1.3	0.20	0.16
HOMA-IR[2]	2.6 ± 0.5	2.6 ± 0.5	2.7 ± 0.5	2.2 ± 0.6	2.3 ± 0.4	1.8 ± 0.4	2.2 ± 0.4	1.6 ± 0.3[#]	0.92	0.25
HOMA-beta[2]	111 ± 21	111 ± 25	87 ± 16	74 ± 18	81 ± 15	72 ± 15	83 ± 18	76 ± 21	0.27	0.61
QUICKI[2]	0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.37 ± 0.02[#]	0.34 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.37 ± 0.01[#]	0.24	0.23
24-h average interstitial glucose,[3] mmol/L	5.5 ± 0.4	5.2 ± 0.3	5.1 ± 0.2	5.1 ± 0.2	5.5 ± 0.2	5.7 ± 0.2	5.7 ± 0.2	5.8 ± 0.2	0.64	0.58
24-h interstitial glucose variability,[3] %	22.3 ± 2.3	28.3 ± 2.2	22.2 ± 2.4	24.5 ± 2.8	28.9 ± 2.6	28.8 ± 2.5	21.1 ± 2.4	22.8 ± 2.8	0.06	0.31
Plasma leptin,[2] ng/mL	5.5 ± 1.0	5.5 ± 1.0	5.0 ± 1.4	5.1 ± 1.3	4.2 ± 1.1	4.2 ± 1.2	5.4 ± 1.0	4.2 ± 0.6[#]	0.07	0.43
Salivary cortisol 0650 h, nmol/L	8.2 ± 1.6	9.4 ± 1.3	9.3 ± 1.5	14.8 ± 2.7	11.1 ± 1.7	13.0 ± 3.2	9.3 ± 1.9	17.2 ± 4.0	< 0.001	0.21
Salivary cortisol AUC, nmol/L x 790 min	4067 ± 551	4349 ± 528	4088 ± 196	45450 ± 425	5588 ± 969	5326 ± 778	4127 ± 587	4530 ± 414	0.001	0.99

[1]Values are means ± SE. Baseline values were not significantly different between groups. [2]CON, *n* = 7; PRO, *n* = 8; EX+CON, *n* = 7; EX+PRO, *n* = 9. [3]CON, *n* = 7; PRO, *n* = 8; EX+CON, *n* = 7; EX+PRO, *n* = 8. HOMA-beta, homeostatic model assessment of beta-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, quantitative insulin-sensitivity check index. [#]*P* < 0.05 from baseline value.

4.5 Discussion

This study is unique in examining both the individual and combined effects of RE and whey protein supplementation on 24-h EE, substrate oxidation and metabolic flexibility, body composition, appetite, and glucose homeostasis in healthy older men. The main findings were:

i) Resistance exercise significantly increased FFM, RMR, SMR and metabolic flexibility compared to non-exercise. Resistance exercise also elicited within-group increases in subjective hunger and insulin sensitivity.

ii) Whey protein supplementation improved body weight maintenance, reduced FM, and increased insulin sensitivity compared to an isocaloric carbohydrate control; however, increased overnight protein oxidation and awakening cortisol secretion, and reduced 24-h protein balance.

iii) Whey protein supplementation resulted in a compensatory decrease in habitual EI via a reduction in fat intake but had no adverse effect on total protein or EI, or 24-h subjective appetite.

iv) Resistance exercise alone, but not combined with whey protein supplementation, reduced the energy cost of step exercise and spontaneous activity.

v) Whey protein supplementation alone reduced the energy cost of spontaneous activity and fasted step exercise.

vi) Resistance exercise combined with whey protein supplementation did not significantly augment changes in body composition, 24-h EE, substrate oxidation or metabolic flexibility, or glucose homeostasis compared to either RE or whey protein supplementation alone.

4.5.1 Effects on energy expenditure and physical activity

Twelve weeks of RE resulted in combined mean increases in RMR (36 ± 14 kcal/d), sedentary EE (60 ± 30 kcal/d) and SMR (45 ± 7 kcal/d). It is likely muscle hypertrophy largely explained these increases. The increase in SMR is in line with those reported in elderly women (Treuth

et al. 1995); however, the rise in RMR in the present study was considerably less than others that have reported increases of ~7-9% (Hunter, McCarthy, and Bamman 2004). For example, increases of 87 kcal/d (Hunter et al. 2000), 108 kcal/d (Campbell et al. 1994), 109 kcal/d (Treuth et al. 1995), and 119 kcal/d (Pratley et al. 1994) have been reported following RE training in older adults. With the exception of the study by Hunter et al. (2000), whereby participants gained 2 kg of FFM following the RE intervention, it is unlikely that RE-induced changes in FFM explain the discrepancies between studies. Fat-free mass increased by 1.0 ± 0.3 kg in the present study, similar or greater than that reported by the remaining of these studies (0.5-1.4 kg) (Campbell et al. 1994, Pratley et al. 1994, Treuth et al. 1995). Also, when normalised to FFM, RMR remained significantly elevated in two of these studies (Pratley et al. 1994, Treuth et al. 1995), contradictory to the findings of this study. Inconsistencies between studies are therefore likely due to alternative factors, including differences in participant training status, sex, rates of resting post-intervention MPS and sympathetic nervous system activity, and differences in the time of post-intervention RMR measurement relative to termination of the final RE session (Geisler et al. 2016b, Geisler and Müller 2017, Schutz 2011, Speakman and Selman 2003). Consequently, although RE significantly increased and may offset age-related declines in RMR, the results from this study suggest the clinical significance in active healthy men is relatively small.

The present study examined whether twice daily ingestion of a leucine-rich whey protein supplement would augment RE-induced increases in components of EE in healthy older men. Despite rises in RMR, SMR and sedentary EE following RE, the addition of whey protein supplementation did not further increase these EE components. Protein supplementation alone also had no effect on these EE constituents. These findings are in agreement with studies that demonstrated no synergistic effects of combined RE and increased dietary protein intake on RMR (Amamou et al. 2017, Campbell et al. 1994, Maltais et al. 2016, Weinheimer et al. 2012), and no effect of a high protein diet on either RMR (Luger et al. 2013, Negro et al. 2019) or SMR in older adults (Drummen et al. 2020). However, the findings of the present

study are in contrast with studies in younger adults that demonstrated protein-induced increases in SMR (Bray et al. 2015, Martens et al. 2015b), RMR (Bray et al. 2012) and TEE (Bray et al. 2012, 2015). Further, the findings of this study also contradict those of Drummen et al. (2020), who reported an increase in RMR following 34 months of a high compared to a moderate protein diet in older adults. This study did, however, only measure RMR post-intervention; therefore, it is unknown whether there was a group-by-time interaction.

The lack of individual and augmented increase in EE following increased dietary protein intake in the present study and those previously mentioned (Amamou et al. 2017, Campbell et al. 1994, Maltais et al. 2016, Weinheimer et al. 2012) is likely due to a lack of protein-induced increase in FFM. These findings are consistent with others that demonstrated no individual (Björkman et al. 2020, Kim et al. 2012, Kirk et al. 2020, Verreijen et al. 2017, Zhu et al. 2015) or synergistic effects (Arnarson et al. 2013, Candow et al. 2006, Dulac et al. 2020, Holm et al. 2008, Holwerda et al. 2018, Kirk et al. 2020, Kukuljan et al. 2009, Leenders et al. 2013, Thomson et al. 2016, Verdijk et al. 2009a) of increased dietary protein on FFM in healthy older adults habitually consuming sufficient amounts (~1.0-1.2 g/kg/d) of dietary protein according to consensus groups (Bauer et al. 2013, Deutz et al. 2014). In contrast, studies conducted in older adults habitually consuming insufficient (≤1.0 g/kg/d) amounts of dietary protein have observed both individual (Bauer et al. 2015, Bo et al. 2019, Kang et al. 2020, Park, Choi, and Hwang 2018, ten Haaf et al. 2019) and augmented effects (Kang et al. 2019, Rondanelli et al. 2016, 2020, Tieland et al. 2012b, Yamada et al. 2019, Zdzieblik et al. 2015). These findings suggest synergistic effects on EE may have been observed in the present study if participants were consuming insufficient (<1.0 g/kg/d) amounts of dietary protein.

However, this study hypothesised, based on prior work that demonstrated increased dietary protein alone, or combined with RE, may increase FFM in older adults habitually consuming ~1.0-1.2 g/kg/d of dietary protein if the deviation in dietary protein from baseline is sufficient (≥0.4 g/kg/d), and total daily protein intake reaches ~1.6 g/kg/d (Bell et al. 2017, Daly et al.

2014, Junior et al. 2018, Norton et al. 2016), additive effects on EE may still be observed. As the present study failed to detect individual and synergistic effects following consumption of 1.6 g/kg/d (0.6 g/kg/d deviation from baseline), the findings suggest habitual protein intake may be the most important factor in determining the effectiveness of increased dietary protein intake on FFM and subsequent effects on EE in healthy older men.

In the present study, although RE increased several components of EE, TEE decreased by 30 ± 15 kcal/d. Resistance exercise also decreased PAL, an observation frequently seen in older adults (Westerterp 2018c). Furthermore, SPA decreased in both RE groups in addition to the PRO group. In the PRO and EX+CON groups, the decrease in SPA was the result of a reduction in the energetic cost of spontaneous activity. This was accompanied by modest decreases in the energetic cost of step exercise, but only in the fasted state in the PRO group. The improvement in metabolic efficiency in the EX+CON group is consistent with others that have demonstrated improvements in metabolic efficiency of locomotion and functional tasks in older adults following RE (Hunter et al. 1995, Valenti, Bonomi, and Westerterp 2016, Wang et al. 2017a). The enhancement in the PRO group is consistent with high protein intervention studies in younger adults (Apolzan et al. 2014, Martens et al. 2015a). Together, these findings are of clinical importance as less energy required for activity may prolong independence and aid quality of life (QOL) (Hunter et al. 2018).

Unlike previous studies as reviewed by Hunter and colleagues (2018), the increase in metabolic efficiency in the PRO and EX+CON groups did not increase non-exercise activity, neither inside the respiration chamber, nor in free-living during week 12 as measured by accelerometry. As prior work has shown increasing AEE is an effective strategy in the defense against adiposity (Kotz et al. 2017), these findings somewhat question the use of these interventions to increase habitual physical activity. Nonetheless, it is important to note that the lack of increase in non-exercise activity may be due to participants being confined to respiration chambers, and due to the fact accelerometry was measured during week 12 as a

control measure when participants were instructed not to change their habitual activity. Future work should therefore explore the effects of RE and increased dietary protein on post-intervention non-exercise physical activity in free-living conditions in this population.

Contrary to the PRO and EX+CON groups, the decrease in SPA observed in the EX+PRO group was due to a reduction in non-exercise activity within the respiratory chamber, which decreased by 3.7 ± 1.0%. This equated to a 53 min (1440 min x 0.037) reduction in non-exercise activity. This finding is not unique, as several studies have observed reductions in non-exercise activity in older adults following exercise interventions (Hunter et al. 2018, Melanson 2017). It has been suggested that fatigue due to an increase in training frequency may explain these findings (Hunter et al. 2018); however, this theory is unlikely in the present study as chamber activity in the EX+CON group, of whom completed the identical exercise intervention and had similar baseline characteristics, did not change. An alternative explanation may be a compensatory reduction in activity to maintain energy balance and oppose the reduction in FM, which was observed predominantly in the EX+PRO group (Hall et al. 2012). In support, spontaneous activity is regulated by neuromodulating factors including orexin A, which drives activity, controls energy balance and regulates adipose tissue (Kotz et al. 2017, Perez-Leighton, Billington, and Kotz 2014, Teske, Billington, and Kotz 2010).

Diet-induced thermogenesis of participants in this study was similar to that reported in previous studies in older adults (Du et al. 2014, Morgan and York 1983). Likewise, consistent with others (Sutton et al. 2016, Tataranni et al. 1995, Watanabe et al. 2006), DIT when fed the same diet whilst residing in the same controlled environment was highly variable (range 15.6%). This variability may be explained in part by the methodology used to calculate DIT in respiration chamber studies (Kumahara, Tanaka, and Schutz 2011). Additionally, although the acute and longitudinal effects of dietary protein on DIT are well known in younger adults (Westerterp-Plantenga et al. 2009b), a recently published study reported no effect in older adults (Drummen et al. 2020). Previous data in young adults has also established no metabolic

adaptation in DIT following a high protein diet when protein intake was returned back to baseline (Sutton et al. 2016). The findings of this study coincide with both these studies.

4.5.2 Effects on body mass and composition

Whilst whey protein supplementation had no effect on components of EE, maintenance of body mass and BMI, and reductions in absolute and %FM were observed compared to an isocaloric carbohydrate control. These findings coincide with others that have shown high protein diets effectively maintain energy balance and body mass, and decrease FM (Clifton, Condo, and Keogh 2014, Drummen et al. 2018, Kim et al. 2016b, Martens et al. 2015b). Compensatory reductions in habitual fat and EI observed in the whey protein supplementation groups might partially explain these results. Furthermore, although no significant augmented reduction in FM was observed compared to RE or dietary protein alone, a favourable decrease was observed compared to RE alone (-0.9 vs. 0.4 kg), a finding also reported by others (Bell et al. 2017, Liao et al. 2017). Taken together, these findings suggest increased dietary protein intake may protect against energy imbalance, and age-related increases in adiposity and sarcopenic obesity.

4.5.3 Effects on appetite

Although whey protein supplementation resulted in a compensatory reduction in fat and EI of the habitual diet, which was observed particularly in the PRO group between weeks 6-12, no adverse reduction in overall protein or EI occurred when dietary supplements were included in the analysis. Additionally, no adverse effect on 24-h subjective appetite was observed whilst residing in the respiration chamber under highly controlled conditions. These findings are in agreement with previous acute (Giezenaar et al. 2017, 2020) and longitudinal studies in older adults (Ben-Harchache et al. 2020, Johnson et al. 2021, Ridge et al. 2018, Tieland et al. 2012c). In contrast, studies in younger adults have established a greater acute (Lejeune et al. 2006, Veldhorst et al. 2009, Veldhorst, Westerterp-Plantenga, and Westerterp 2009) and chronic satiating effect of increased dietary protein (Martens et al. 2015a). Although the

mechanisms are currently unclear (Giezenaar et al. 2020), data from this study supports a blunted protein-induced suppression of appetite in older adults. However, it must be noted that participants in the present study were not fed a high protein diet in the respiratory chamber during post-intervention testing, which may confound these findings. This methodology was chosen as the primary aim of this study was to determine the combined effects of RE and whey protein supplementation on SMM and subsequent effects on energy metabolism. Nevertheless, as reductions in appetite and dietary protein intake may contribute to sarcopenia (Atalayer and Astbury 2013, Paddon-Jones et al. 2015), the blunted protein-induced suppression of appetite in older adults may be of clinical importance and supports the role of protein supplementation to increase total protein intake without adverse effects on total EI.

To add to the above, the present study also observed an increase in 24-h subjective hunger following RE. Regrettably, few studies have investigated the longitudinal effects of RE on appetite, and the present study, to the authors knowledge, is the first to assess the effects in older adults. In partial agreement with the findings of this study, meta-analyses have reported RE-induced decreases in two tonic appetite suppressants, leptin (Fedewa et al. 2018) and insulin (Ashton et al. 2020). However, others have reported no changes in subjective appetite (Guelfi, Donges, and Duffield 2013), and decreases in orexigenic and increases in obesogenic hormones (Shakiba et al. 2019). Inconsistent findings may be related to participant characteristics (i.e., normal BMI vs. obesity, age), changes in body composition, and due to differences in the RE intervention. As previously highlighted, since reduced appetite with age contributes to sarcopenia, the findings of this study suggest RE might be a beneficial strategy to indirectly mitigate sarcopenia by increasing perceived hunger. Though, only subjective appetite was measured; therefore, the longitudinal effects of RE and increased dietary protein intake on appetite-related hormones are unknown and should be examined in future work.

4.5.4 Effects on substrate oxidation

The present study observed greater rates of fat oxidation during the fasted step exercise bout compared to step exercise performed postprandially at 1445 h and 1915 h at both baseline and 12 weeks. These findings are similar to that of Edinburgh et al. (2020), who observed higher rates of fat oxidation following an acute bout of fasted exercise compared to exercise performed following consumption of breakfast. Edinburgh et al. (2020) also observed a greater sustained increase in fat oxidation when exercise was performed in the fasted state for 6 weeks alongside improvements in insulin sensitivity. Based on these data, research investigating the longitudinal effects of fasted exercise in the elderly, of whom are at greater risk of metabolic dysfunction (Hunter et al. 2019), is warranted to determine the safety and effectiveness on mitigating age-related metabolic disease.

The increase in 24-h protein oxidation following 12 weeks whey protein supplementation concurs with studies in both young (Bray et al. 2015, Martens et al. 2015b, Robinson et al. 1990, Pannemans et al. 1995) and older adults (Drummen et al. 2020, Pannemans, Halliday, and Westerterp 1995, Pannemans et al. 1998). In the present study, raised overnight as opposed to daytime protein oxidation stimulated the 24-h increase, consistent with prior work that demonstrated diurnal differences in nitrogen excretion cycling following adaptation to a high protein diet (Price et al. 1994). Moreover, the present study also established when dietary protein intake (~25% of EI at 6 and 12 weeks in the PRO and EX+PRO groups) was returned to that of the chamber diet (~20% of EI), 24-h protein balance significantly reduced. This outcome is in agreement with others that established a significantly lower net protein balance in elderly men following habituation to a high protein diet (Højfeldt et al. 2020), and a reduced nitrogen balance following termination of a high protein diet until nitrogen output met the new level of intake (Waterlow 1999). These findings indicate a potential drawback of habitually ingesting high amounts of dietary protein in the elderly. Further, as a higher protein turnover increases protein requirements to maintain nitrogen balance (Gaine et al. 2006), the findings of the present study highlight that elderly individuals consuming high amounts of dietary

protein (~1.6 g/kg/d) should refrain from drastically reducing protein intake to minimise transient periods of reduced protein balance.

4.5.5 Effects on metabolic flexibility and insulin sensitivity

Growing evidence demonstrates metabolic flexibility - the ability to adjust rates of substrate oxidation to changes in fuel availability (Kelley and Mandarino 2000) - is impaired in the elderly (Smith et al. 2018). Accumulating evidence also links impairments in metabolic flexibility to obesity, insulin resistance and T2DM (Goodpaster and Sparks 2017). In the present study, 24-h metabolic flexibility increased greater following RE compared to non-exercise, a finding which might be clinically relevant to the metabolic heath of older adults. In agreement, others have also reported increases in metabolic flexibility following RE in the elderly (Consitt et al. 2016, Meex et al. 2010). Although not assessed in this study, several plausible mechanisms, including increased skeletal muscle mitochondrial biogenesis, content and function, and improved glucose transport via increased activation of the insulin signalling cascade, may explain these findings (Consitt, Dudley, and Saxena 2019, Goodpaster and Sparks 2017). Furthermore, both RE alone, and combined with whey protein supplementation, also increased insulin sensitivity, consistent with prior work in older adults (Bell et al. 2017, Bucci et al. 2016, Flack et al. 2011, Holwerda et al. 2018, Iglay et al. 2007, Leenders et al. 2013, Miller et al. 1994). Also, in agreement with others (Andersen et al. 2003, Holten et al. 2004, Miller et al. 1994), changes in SMM following RE did not explain improvements in insulin sensitivity. Therefore, the insulin sensitising effects of RE in this study might be explained by improvements in skeletal muscle insulin signalling, specifically phosphorylation of AS160 and expression of GLUT-4 (Consitt, Dudley, and Saxena 2019).

Consistent with recent work that reported no effects of high compared to low dairy consumption over 6 weeks in middle- and older-aged adults (Eelderink et al. 2019), whey protein supplementation *per se* did not notably increase 24-h metabolic flexibility. However, when protein supplement groups were pooled, a significant increase in insulin sensitivity was

observed, coherent with previous studies (Acheson et al. 2011, Luger et al. 2013, Pal, Ellis, and Dhaliwal 2010). The protein-induced increase in insulin sensitivity but not metabolic flexibility may be due to changes in insulin sensitivity appearing before changes in metabolic flexibility, an observation also seen in participants exposed to 8 weeks of overfeeding (Peterson et al. 2017).

4.5.6 Effects on salivary cortisol

The present study investigated the effects of RE and whey protein supplementation on diurnal salivary cortisol concentration based on prior work that reported a relationship between stress and increased prevalence of obesity (Hewagalamulage et al. 2016), and increased cortisol and appetite (Horner et al. 2020). Others have also demonstrated dysfunction of the HPA axis in the elderly, that is, an attenuated diurnal decline in cortisol resulting in a higher evening nadir (Piazza et al. 2010). Previous studies have indicated the macronutrient composition of a meal might influence the magnitude of cortisol response. Two studies demonstrated elevated cortisol concentration following a high carbohydrate compared to a high protein/fat meal (Vicennati et al. 2002, Martens et al. 2010). In contrast, others have reported a protein-induced increase in cortisol concentration (Slag et al. 1981, Gibson et al. 1999). More recently, no differences were observed between a high protein and high carbohydrate diet (Lemmens et al. 2011). In the present study, awakening salivary cortisol concentration (0650 h) increased following 12 weeks ingestion of whey protein, but no differences occurred at any other time point. This increase may have occurred due to an increase in nocturnal ureagenesis and gluconeogenesis, confirming dietary protein might have gone beyond a critical threshold for the nitrogen demands of these participants (Schutz 2011).

4.5.7 Strengths and limitations

To date, only a few studies of ≥4 weeks in duration have assessed the effects of either a dietary or exercise intervention on components of EE and substrate oxidation using respiration chambers in older adults (Bush et al. 2018, Drummen et al. 2020, Morio et al. 1998, Treuth et

al. 1995). Also, no study, to the authors knowledge, has investigated the individual and combined effects of RE and whey protein supplementation on 24-h metabolic flexibility and appetite in older adults. The investigation of both the individual and combined effects of a dietary and exercise intervention using a 4-arm experimental design over 12-weeks on these outcomes are key strengths and novel aspects of this study. Furthermore, unlike previous studies that have investigated the combined effects of these interventions (Amamou et al. 2017, Campbell et al. 1994, Maltais et al. 2016, Weinheimer et al. 2012), multiple components of EE and substrate oxidation were measured over a 24-h period using gold standard methodology under highly controlled conditions. A limitation of this study is the relatively small number of participants; however, such a limitation is inherent in work of this nature. The sample size is also congruent with previous respiration chamber studies (Apolzan et al. 2014, Bray et al. 2015, Melanson et al. 2007, Westerterp-Plantenga et al. 2009a). Nevertheless, it is recognised that the sample achieved was underpowered to detect between-group differences on several outcome variables. Effect sizes (Cohen's f^2) obtained from changes in RMR (f^2 = 0.50) and SMR (f^2 = 0.47) between groups indicated a sample size of 48-56 participants (12-14/group) would have been required to detect between-group differences in these variables. A second limitation is the inclusion of only men who were healthy and had a normal-to-overweight BMI. Women were excluded based on sex differences in the age-related decline in RMR (Geisler et al. 2016b). Individuals with obesity were excluded due to differences in metabolic profile (Perna et al. 2017), and due to the preventative nature of this study. Investigation of these groups, in addition to older adults with insufficient habitual protein intakes (<1 g/kg/d), should form future work.

4.5.8 Conclusion

Twelve weeks of RE significantly increased FFM and elicited modest, but significant increases in RMR, SMR, sedentary EE and 24-h metabolic flexibility in healthy older men compared to non-exercise. In addition, RE also resulted in within-group increases in the metabolic efficiency of step exercise and spontaneous activity, subjective hunger, and insulin sensitivity.

Based on these findings, RE should be promoted in older adults to aid metabolic health. Whey protein supplementation aided body weight maintenance, reduced FM, and improved insulin sensitivity, supporting the role of increased dietary protein intake to mitigate sarcopenic obesity. However, the results from this study suggest a potential caveat of older individuals habitually consuming a high protein diet is an increase in protein turnover in the overnight fasting period, which may have deleterious effects on protein balance. Resistance exercise combined with whey protein supplementation elicited no significant additive effects on any markers compared to either RE or dietary protein alone; though, a favourable decrease in FM was observed compared to RE alone.

Thesis map: Study 2

Study	Aims	Key findings
Study 1: Effects of resistance exercise and whey protein supplementation on 24-h energy expenditure, substrate oxidation and metabolic flexibility, body composition, appetite, and glucose homeostasis in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on components of 24-h EE, substrate oxidation and metabolic flexibility, body composition, appetite, and glucose homeostasis in healthy older men.	• Resistance exercise significantly increased FFM, RMR, SMR, sedentary EE and 24-h metabolic flexibility compared to non-exercise. RE also resulted in within-group increases in subjective hunger and insulin sensitivity, and within-group decreases in the energy cost of step exercise and spontaneous activity. • Whey protein supplementation improved body weight maintenance and reduced FM, and increased insulin sensitivity; however, resulted in an increase in overnight protein oxidation and awakening cortisol secretion, and reduced 24-h protein balance. • Whey protein supplementation had no adverse effects on total protein or EI, or 24-h subjective appetite. • Resistance exercise combined with whey protein supplementation did not significantly augment changes in body composition, 24-h EE, substrate oxidation or metabolic flexibility, or markers of glucose homeostasis compared to either RE or whey protein supplementation alone.
Study 2: Effects of resistance exercise and whey protein supplementation on skeletal muscle mass, strength, physical function, and hormonal and inflammatory biomarkers in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on SMM, strength, physical function, and hormonal and inflammatory biomarkers in healthy older men. • To determine whether changes in hormonal and inflammatory markers correlate with changes in SMM, strength and physical function.	

Thesis map: Study 2 continued

Study	Aims	Key findings
Study 3: Effects of resistance exercise and whey protein supplementation on cognitive function in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on cognitive function and neurobiological, inflammatory and insulin sensitivity markers, diurnal salivary cortisol, and BP in healthy older men. • To determine whether changes in neurobiological, inflammatory and insulin sensitivity markers, and changes diurnal salivary cortisol, BP, SMM, strength and physical function are associated with changes in cognitive function.	

CHAPTER 5 (Study 2): Effects of resistance exercise and whey protein supplementation on skeletal muscle mass, strength, physical function, and hormonal and inflammatory biomarkers in healthy older men

5.1 Chapter Overview

Ageing is associated with declines in SMM, strength and physical function (sarcopenia). Previous studies have shown RE and increased dietary protein intake alone may mitigate age-related sarcopenia. Meta-analyses also suggest increased dietary protein intake may augment the RE-induced response of skeletal muscle; however, most individual studies in older adults have failed to observe such effects. Null findings may be explained by an inadequate deviation of dietary protein from baseline (<0.4 g/kg/d) and an insufficient daily protein intake (<1.6 g/kg/d) to augment the effects of RE. Furthermore, the biochemical mechanisms of how these interventions may synergistically mitigate sarcopenia are relatively unknown. In this chapter, the individual and combined effects of RE and whey protein supplementation (aimed to increase daily protein intake to 1.6 g/kg/d) on SMM, strength, physical function and multiple biochemical pathways related to sarcopenia were investigated in healthy older men.

5.2 Introduction

Age-related declines in SMM and strength, termed sarcopenia (Cruz-Jentoft et al. 2019), progress at rates of ~0.5-1% and ~1-3% per annum, respectively, manifesting around the fifth decade of life (Clark and Manini 2008, Janssen 2010). Sarcopenia is associated with various negative health outcomes, including an increased risk of falls and fractures, impairments in the ability to perform ADL (Beaudart et al. 2017), a greater risk of cardiovascular (Bahat and Ilhan 2016) and metabolic disease (Hunter et al. 2019), and an elevated mortality risk (de Buyser et al. 2016). In economic terms, in the United Kingdom alone, the annual cost associated with muscle weakness is estimated at £2.5 billion (Pinedo-Villanueva et al. 2019). Hence, interventions that attenuate sarcopenia are imperative.

Resistance exercise is a potent stimulus to curb sarcopenia (Phillips and Martinson 2019). Meta-analyses have reported increases in FFM (Peterson, Sen, and Gordon 2011), muscular strength (Peterson et al. 2010) and physical function (Yoshimura et al. 2017) following RE training in older adults. Regarding the optimal RE intensity, meta-analyses suggest for

optimisation of muscle strength, which is considered the primary index of sarcopenia by the EWGSOP2 (Cruz-Jentoft et al. 2019), RE performed at high intensity (~70-80% 1RM) elicits the largest effects (Borde, Hortobágyi, and Granacher 2015, Steib, Schoene, and Pfeifer 2010). Concerning RE frequency, comparable increases in parameters of sarcopenia have been observed between 2-3 sessions per week (da Silva et al. 2017, Grgic, Schoenfeld, and Latella 2019, Kneffel et al. 2020, Stec et al. 2017). As previous work has reported older adults prefer training twice compared to three times per week (Foley, Hillier, and Barnard 2011), RE performed twice weekly may be most beneficial to aid long-term adherence and mitigation of sarcopenia in this population.

In addition to RE, increased dietary protein intake is also recommended to curb sarcopenia (Phillips and Martinson 2019). Observational data over a 3-year period has shown higher intakes of dietary protein is associated with a greater retention of FFM (Houston et al. 2008). Others have also presented data indicating higher quantities of dietary protein (>1 g/kg/d) may preserve muscle strength (McLean et al. 2016) and physical function (Mustafa et al. 2018). Furthermore, meta-analyses suggest increased dietary protein intake may augment the adaptive response of skeletal muscle to RE (Cermak et al. 2012, Finger et al. 2015, Liao et al. 2017, Morton et al. 2018a). However, whilst several individual studies in older adults have demonstrated greater increases in skeletal muscle and/or FFM, muscle strength, and physical function following combined RE and increased dietary protein intake compared to RE alone (Bell et al. 2017, Daly et al. 2014, Kang et al. 2019, Junior et al. 2018, Rondanelli et al. 2016, 2020, Tieland et al. 2012b, Verreijen et al. 2015, Yamada et al. 2019, Zdzieblik et al. 2015), the majority of studies have not observed additive effects (Arnarson et al. 2013, Candow et al. 2006, Chalé et al. 2013, de Carvalho Bastone et al. 2020, Dulac et al. 2020, Englund et al. 2018, Fielding et al. 2017, Gryson et al. 2014, Hofmann et al. 2016, Holm et al. 2008, Holwerda et al. 2018, Kim et al. 2012, Kirk et al. 2019, 2020, Kukuljan et al. 2009, Leenders et al. 2013, Maltais, Ladouceur, and Dionne 2016, Oesen et al. 2015, Ottestad et al. 2017, Shahar et al. 2013, Thomson et al. 2016, Verdijk et al. 2009a, Verreijen et al. 2017).

Inconsistent findings may be explained by the population studied, habitual protein intake of participants, and characteristics of the protein intervention. Of note, studies in healthy older adults that have observed synergistic effects deviated dietary protein intake by 0.5-0.6 g/kg/d (Bell et al. 2017, Junior et al. 2018), which exceeds the proposed deviation required to elicit gains in SMM (≥0.4 g/kg/d) (Moore et al. 2015, Park, Choi, and Hwang 2018). In contrast, several studies that have failed to observe additive effects deviated dietary protein intake by ≤0.3 g/kg/d (Arnarson et al. 2013, Dulac et al. 2020, Gryson et al. 2014, Hofmann et al. 2016, Holwerda et al. 2018, Kirk et al. 2019, 2020, Kukuljan et al. 2009, Leenders et al. 2013, Maltais, Ladouceur, and Dionne 2016, Verdijk et al. 2009a). Additionally, a meta-regression conducted by Morton et al. (2018a) also suggests ~1.6 g protein/kg/d might be required to maximally augment RE-induced gains in FFM. This level of dietary protein was only achieved by one of the aforementioned studies (Bell et al. 2017). Taken together, current evidence suggests a dietary modification of ≥0.4 g protein/kg/d and a total protein intake of 1.6 g/kg/d may be required to amplify RE-induced effects on SMM, strength and physical function in healthy older adults.

A limitation of many of the aforementioned studies investigating the synergistic effects of RE and increased dietary protein intake in older adults was the failure to include a protein only group. In fact, only 7/33 studies cited examined the combined effects compared to both RE and dietary protein alone (de Carvalho Bastone et al. 2020, Gryson et al. 2014, Kim et al. 2012, Kirk et al. 2020, Kukuljan et al. 2009, Shahar et al. 2013, Verreijen et al. 2017). In these studies, dietary protein intake was increased by ≤0.3 g/kg/d and total protein intake was <1.6 g/kg/d. Consequently, the synergistic effects utilising the proposed optimal protein dose of 1.6 g/kg/d compared to both interventions alone is currently unknown. As not all older adults are able or willing to perform RE (Dismore et al. 2020), collection of such data is vital to determine the effectiveness of this nutrient compared to both RE alone and combined with increased dietary protein.

Furthermore, several biochemical pathways are involved in the pathogenesis of sarcopenia, including chronic systemic inflammation (e.g., increases in IL-6, CRP and TNF-α) and changes in the hormonal milieu (e.g., reduced IGF-1, flattened diurnal cortisol secretion, and increased myostatin) (Beyer, Mets, and Bautmans 2012, McKee and Morley 2019, White and Lebrasseur 2014). However, there is currently limited evidence on the synergistic effects of RE and increased dietary protein intake on these biomarkers, and no study has examined all of these biomarkers following these interventions alone and combined.

5.2.1 Aims and hypotheses

Primary aim

- To investigate the individual and combined effects of RE and whey protein supplementation (aimed to increase dietary protein intake by ≥0.4 g/kg/d to ~1.6 g/kg/d) on SMM, strength and physical function in healthy older men.

Secondary aims

- To investigate the effects of these interventions on multiple hormonal and inflammatory biomarkers associated with sarcopenia, and to determine whether individual participant changes in these markers correlate with changes in SMM, strength and physical function.

Hypotheses

- Combined RE and whey protein supplementation will augment the effects on SMM, strength and physical function compared each intervention alone.
- Resistance exercise and whey protein supplementation will synergistically increase IGF-1, will augment decreases in myostatin and markers of systemic inflammation, and will amplify the salivary cortisol slope (i.e., increased awakening and decreased evening cortisol secretion).
- Changes in SMM, strength and physical function will correlate with changes in hormonal and inflammatory biomarkers.

5.3 Methods

5.3.1 Participants and experimental design

Thirty-six healthy, community-dwelling older men (mean ± SE age: 66.9 ± 0.7 y) participated in this 4-arm, double-blind RCT. All measurements were assessed at baseline and following the 12-week intervention. As previously highlighted in **section 3.2**, participants were randomised to either control (CON, *n* = 9), whey protein (PRO, *n* = 9), RE + control (EX+CON, *n* = 9), or RE + whey protein (EX+PRO, *n* = 9). Full details of the experimental design (**section 3.2**) and eligibility criteria (**sections 3.4.2.1 and 3.4.2.2**) have been previously described. To minimise diurnal variation, body composition, muscular strength and physical function measures were performed at the same time of day (± 1 h) at baseline and following the 12-week intervention. Although not planned in the original study design, exploratory analyses were also conducted between pooled exercise (EX+CON and EX+PRO groups, *n* = 18) and non-exercise groups (CON and PRO groups, *n* = 18), and between pooled whey protein (PRO and EX+PRO groups, *n* = 18) and control groups (CON and EX+CON group, *n* = 18). All participants provided written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by Coventry University Ethics Committee (project code: P59723). The flow of participants throughout the study has been previously presented and can be seen in **Chapter 3** (**Figure 3.1**).

5.3.2 Exercise training and nutritional supplements

Participants in the EX+CON and EX+PRO groups participated in supervised whole-body RE twice weekly, which consisted of 3 sets of 8 repetitions on six exercise machines (leg press, lateral row, hamstring curl, chest press, leg extension and shoulder press) performed at 80% 1RM (see **section 3.5.2** for a detailed description of the RE intervention). Participants in the PRO and EX+PRO groups consumed 25 g whey protein supplementation containing ~3 g leucine, whereas participants in the CON and EX+CON groups consumed an energy-matched carbohydrate control supplement (maltodextrin). Supplements were consumed twice daily (at

breakfast and lunch). A full description of the nutritional interventions can be seen in **section 3.5.1**.

5.3.3 Dietary intake

Participants completed 3-day food records (2 weekdays and 1 weekend day) at baseline (prior to commencing the intervention) and during weeks 6 and 12. Dietary records were analysed using Nutritics dietary analysis software (see **section 3.6.2** for a detailed description).

5.3.4 Habitual physical activity

To control for changes in habitual physical activity levels that may have influenced outcome measures, participants wore a tri-axial accelerometer on the dominant wrist for 7 days at baseline and during the final week of the intervention (see **section 3.6.3** for a detailed description). Time spent sedentary, and in light and MVPA was determined during waking hours (Freedson, Melanson, and Sirard 1998).

5.3.5 Body composition

Body composition (SMM, FFM and FM) was measured in the morning by BIA. Participants were asked to void their bladder prior to measurement. Full details have been previously described (see **section 3.6.1**). Skeletal muscle index (SMI, kg/m^2) and fat mass index (FMI, kg/m^2) were calculated by dividing SMM and FM by height squared, respectively. Waist circumference, which is a predictive measure of visceral adipose tissue (Janssen et al. 2002), was measured at the midpoint between the lowest rib margin and the iliac crest. Hip circumference was measured at the widest portion of the hips. Both outcomes were measured to the nearest 0.1 cm using a measuring tape (Seca 201; Seca GmbH, Hamburg, Germany).

5.3.6 Muscle strength

Maximal strength was assessed by 1RM tests on the leg press and leg extension machines (Life Fitness, Rosemont, Illinois, USA) using the guidelines of Kraemer et al. (2006). Proper

lifting technique was demonstrated and practiced by participants prior to baseline 1RM testing to minimise a potential learning effect (Levinger et al. 2009, Phillips et al. 2004). For 1RM testing, participants first completed 5-10 repetitions at 40-60% perceived 1RM followed by 3-5 repetitions at 60-80% perceived 1RM. The load was then gradually increased, and participants performed one repetition at each increased load until they were unable to achieve a complete repetition. One repetition maximum was determined as the last successful lift prior to failure. Three min rest was allocated between each maximal lift. Test-retest reliability of leg press 1RM has been previously confirmed in healthy older men [ICC = 0.95, 95% confidence interval (CI) (0.88–0.98)] (LeBrasseur et al. 2008). Strong correlations have also been reported in elderly individuals between leg press (r = 0.60, 95% CI 0.24-0.82) and leg extension 1RM (r = 0.75, 95% CI 0.49-0.89) and isokinetic peak torque (Verdijk et al. 2009b). Leg press and extension exercises were chosen for 1RM tests as lower extremity strength is strongly associated with functional limitations and disability (Puthoff and Nielsen 2007), and many combined RE and protein supplementation RCTs in older adults have assessed these outcomes (Bell et al. 2017, Holwerda et al. 2018, Leenders et al. 2013, Verdijk et al. 2009a); therefore, enabling comparison with previous work.

Handgrip strength was measured using a JAMAR hydraulic handgrip dynamometer (Jamar 5030J1; Sammons Preston, Bolingbrook, Illinois, USA) using standardised procedures (Roberts et al. 2011). Previous studies have shown handgrip dynamometry is a valid and reliable instrument for measurement of muscle strength in older adults (Mijnarends et al. 2013, Wang, Olson, and Protas 2002). A strong correlation with measurement of muscle strength by isokinetic dynamometry has also been reported (Reed et al. 1993). Handgrip strength was assessed as previous studies have shown low grip strength is a powerful predictor of increased functional limitations, poor quality of life and mortality (Ibrahim et al. 2016, Leong et al. 2015).

5.3.7 Physical function

Physical function was assessed by the SPPB and the 6MWT. The SPPB was conducted using standard procedures, which consisted of three timed tests: 4 m gait speed, time to perform five chair raises, and standing balance (feet together, semi-tandem and tandem) (Guralnik et al. 2000). Each test was scored equally between 0 and 4. The total score between 0 and 12 was used for analysis. A recent meta-analysis showed poor performance (<10 points) on the SPPB is predictive of all-cause mortality (Pessini, Barbosa, and Trindade 2016). Reliability and validity has also been confirmed by numerous studies highlighted in a systematic review (Mijnarends et al. 2013). The 6MWT was performed adhering to guidelines set by the American Thoracic Society (Crapo et al. 2002). A 30 m indoor track was marked out with cones at either end. Participants were informed the aim of the test was to cover as much distance as possible in six min. Shorter distance on the 6MWT has been associated with an impaired ability to perform ADL, systemic inflammation and increased risk of CVD, and T2DM (Enright et al. 2003). A strong test-retest reliability (ICC = 0.88) for the 6MWT has been previously reported in elderly men (Rikli and Jones 1998).

5.3.8 Blood and saliva collection and analysis

Venous blood was collected following a >10 h overnight fast. Whole blood was collected into EDTA, heparin and SST vacutainers, then centrifuged at 1900 x g for 10 min at 4°C. Serum samples were rested for 30 min prior to centrifuging to allow for sufficient clotting. Aliquots containing plasma and serum were stored at -80°C until analysis (see **section 3.6.4** for detailed blood collection methodology). Due to difficulty in blood collection, blood was unable to be drawn from two participants (*n* = 1 participant in both the CON and EX+CON groups). Therefore, 34 participants had full blood data. Commercially available ELISA's were used to analyse plasma IL-6 [Item No. D6050 and HS600C (the latter for high sensitivity IL-6)], high sensitivity TNF-α (Item No. HSTA00E) and CRP (Item No. DCRP00), and serum IGF-1 (Item No. DB100B) and myostatin (Item No. DGDF80) (R&D Systems Inc., Abbington, UK). Estimated glomerular filtration rate was also analysed by the pathology department at UHCW.

Blood samples were collected >72 h following the final RE session to allow for biomarkers to return to basal concentrations (Schoenfeld 2012).

Saliva samples were collected whilst participants (*n* = 33) resided in respiration chambers for 24 h under highly controlled conditions, as previously described (see **Chapter 4**). Samples were collected immediately upon awakening at 0650 h, and at 0805, 1225, 1700 and 2000 h using a synthetic swab. Samples were centrifuged at 1900 x g for 2 min and stored at -80°C until analysis. Saliva samples were analysed for cortisol by ELISA (Item No. 1-3002; Salimetrics, Pennsylvania, USA). Salivary cortisol data (samples 1-5; 0650-200 h) was used to calculate two indices. Firstly, all five samples were used to calculate salivary cortisol AUC (nmol/L x 790 min) using the trapezoidal method. Secondly, cortisol slope (peak-to-evening) was calculated as the rate of salivary cortisol change from peak morning (0650 or 0805 h, depending on the highest concentration) to evening (2000 h) (Adam et al. 2017). The intra-assay CV was 9.8%, 11.8%, 9.9%, 9.9%, 9.1%, 7.7% and 9% for plasma IL-6, high sensitivity IL-6, high sensitivity TNF-α and CRP, serum IGF-1 and myostatin, and salivary cortisol, respectively.

5.3.9 Statistical analysis

Statistical analysis performed in this chapter has previously been detailed and can be seen in **Section 3.7**.

5.4 Results

5.4.1 Participants and safety

Thirty-nine older men were randomised: 36 completed the study and 3 withdrew. Reasons for withdrawal have been previously detailed and can be seen in **Chapter 3** (**Figure 3.1**). Baseline characteristics of the 36 participants who completed the study are shown in **Table 5.1**. No

significant differences were observed for any outcome variable at baseline (P > 0.05). Resistance exercise participation was well tolerated with only one participant missing a single session due to muscle soreness. No other adverse events were reported following RE. Following whey protein supplementation, renal function was not adversely affected, confirmed by an eGFR of >60 mL/min/1.73m^2 in all participants following the intervention. Two participants in the PRO group did, however, experience episodes of gout.

5.4.2 Exercise and supplement adherence

Participants in the EX+CON and EX+PRO groups attended 98.2 ± 1.0% and 98.2 ± 1.2% of their prescribed RE sessions, respectively. All participants completed their prescribed repetitions for sets 1 and 2 of each exercise. During the final set (to volitional failure), the mean number of completed repetitions was 9.1 ± 0.3 in the EX+CON group and 9.1 ± 0.2 in the EX+PRO group. No differences occurred between groups for either RE compliance (P = 0.63) or the mean number of repetitions completed during the final set (P = 0.97). Compliance with the dietary supplements was 94.1 ± 1.2%, 96.8 ± 1.0%, 96.1 ± 1.3% and 96.1 ± 1.3% in the CON, PRO, EX+CON and EX+PRO groups, respectively, with no differences between groups (P = 0.50). Eighty percent of participants were unable to judge treatment allocation based on the supplement exit questionnaire (see section **3.5.1**).

Table 5.1 Baseline characteristics of participants[1]

	CON	PRO	EX+CON	EX+PRO	*P* value[3]	Overall
n	9	9	9	9	-	36
Age, y	67.2 ± 1.7	65.6 ± 1.7	67.1 ± 1.3	67.8 ± 1.3	0.75	66.9 ± 0.7
Height, m	1.77 ± 0.01	1.76 ± 0.03	1.77 ± 0.02	1.74 ± 0.03	0.71	1.76 ± 0.01
Body mass, kg	79.0 ± 3.4	78.0 ± 3.1	78.2 ± 3.9	80.9 ± 4.0	0.94	79.0 ± 1.8
BMI, kg/m^2	25.1 ± 1.0	25.0 ± 0.6	25.1 ± 0.9	26.6 ± 0.8	0.50	25.5 ± 0.4
FFM, kg	59.8 ± 1.5	60.0 ± 1.7	58.5 ± 2.6	60.5 ± 2.9	0.94	59.7 ± 1.1
SMM, kg	26.7 ± 0.6	27.2 ± 0.7	25.9 ± 1.1	26.9 ± 1.3	0.79	26.7 ± 0.5
SMI, kg/m^2	8.5 ± 0.2	8.8 ± 0.2	8.3 ± 0.2	8.9 ± 0.3	0.19	8.6 ± 0.1
FM, kg	19.2 ± 2.4	18.0 ± 1.7	19.6 ± 2.0	20.4 ± 1.5	0.85	19.3 ± 0.3
FM, %	23.8 ± 2.0	22.7 ± 1.5	24.8 ± 1.7	25.1 ± 1.2	0.74	24.1 ± 0.8
Handgrip strength, kg	41.8 ± 1.8	36.5 ± 2.5	39.9 ± 4.1	41.8 ± 2.1	0.37	40.1 ± 1.3
Leg extension 1RM, kg	63 ± 6	58 ± 3	52 ± 5	59 ± 4	0.52	58 ± 2
Leg press 1RM, kg	116 ± 9	107 ± 7	107 ± 9	118 ± 7	0.66	112 ± 4
SPPB, points	11.7 ± 0.2	11.4 ± 0.2	11.2 ± 0.3	11.8 ± 0.1	0.38	11.5 ± 0.1
Fasting plasma glucose, mmol/L	5.7 ± 0.2	5.9 ± 0.3	5.8 ± 0.3	5.7 ± 0.2	0.80	5.7 ± 0.1
HOMA-IR[4]	2.6 ± 0.4	2.7 ± 0.4	2.9 ± 0.7	2.2 ± 0.4	0.85	2.6 ± 0.2
Systolic BP, mmHg	127 ± 6	133 ± 6	133 ± 7	136 ± 4	0.57	132 ± 3
Diastolic BP, mmHg	78 ± 2	76 ± 3	77 ± 4	77 ± 4	0.98	77 ± 1
Step count, steps/d	10,766 ± 594	12,670 ± 1263	12,061 ± 1023	11,346 ± 907	0.55	11,710 ± 483
MVPA, min/d	110 ± 8	127 ± 15	137 ± 18	135 ± 10	0.50	127 ± 7

[1]Values are means ± SE. [3]*P* value refers to differences between groups analysed by one-way ANOVA. No significant differences in baseline characteristics occurred between pooled exercise and non-exercise groups, or between pooled whey protein and control groups (data not shown). [4]CON and EX+CON groups, *n* = 8. 1RM, one repetition maximum; BMI, body mass index; BP, blood pressure; FFM, fat-free mass; FM, fat mass; FMI, fat mass index; MVPA, moderate-vigorous physical activity; SMI, skeletal muscle index; SMM, skeletal muscle mass; SPPB, short physical performance battery.

5.4.3 Dietary intake

Significant group-by-time interactions were observed for dietary protein (expressed as g/d, g/kg/d, and % energy; $P < 0.001$) and carbohydrate intake (expressed as g/d and % energy; $P < 0.05$, **Table 5.2**). Protein intake increased over time in the PRO and EX+PRO groups greater than both the CON and EX+CON groups at weeks 6 ($P < 0.001$) and 12 ($P < 0.001$). Carbohydrate intake increased over time in the EX+CON group greater than the CON and EX+PRO groups at week 6 ($P < 0.05$), and greater than the PRO and EX+PRO groups at week 12 ($P < 0.05$). Total EI increased over time in the EX+PRO group at week 6 ($P = 0.03$) and increased in the CON group at weeks 6 and 12 ($P < 0.05$).

Table 5.2 Dietary intake during the intervention period (including supplements)[1]

	Pooled	CON		PRO		EX+CON		EX+PRO		
	Baseline	6 weeks	12 weeks	6 weeks	12 weeks	6 weeks	12 weeks	6 weeks	12 weeks	*P* value[2]
Energy,[3] kcal/d	1964 ± 59	1944 ± 111[#]	2013 ± 107[#]	2055 ± 130	1937 ± 140	2177 ± 83	2176 ± 118	2238 ± 97[#]	2159 ± 141	0.30
Protein										
g/d[3]	81 ± 2	77 ± 5	74 ± 4	129 ± 4[*¥#]	125 ± 5[*¥#]	86 ± 4	82 ± 5	131 ± 6[*¥#]	125 ± 3[*¥#]	< 0.001
g/kg/d[3]	1.0 ± 0.02	1.0 ± 0.05	0.9 ± 0.03	1.6 ± 0.1[*¥#]	1.6 ± 0.1[*¥#]	1.1 ± 0.1	1.0 ± 0.1	1.6 ± 0.1[*¥#]	1.6 ± 0.1[*¥#]	< 0.001
%	17 ± 0.4	16 ± 1	15 ± 1	26 ± 1[*¥#]	26 ± 1[*¥#]	16 ± 1	15 ± 1	24 ± 1[*¥#]	24 ± 1[*¥#]	< 0.001
Carbohydrate										
g/d[3]	232 ± 8	207 ± 23	250 ± 15	200 ± 21	209 ± 16	279 ± 16[*$#]	280 ± 11[‡$#]	221 ± 11	238 ± 16	0.007
%[3]	48 ± 1	42 ± 4	50 ± 2	39 ± 4	43 ± 2	51 ± 2[‡$]	52 ± 2[‡$#]	40 ± 2	44 ± 2	0.03
Fat										
g/d[3]	68 ± 3	68 ± 5	71 ± 6	71 ± 10	55 ± 8	70 ± 5	68 ± 8	78 ± 5	62 ± 8	0.06
%[3]	31 ± 1	31 ± 2	31 ± 1[$]	31 ± 3	25 ± 2	29 ± 1	28 ± 2	31 ± 1	25 ± 2	0.08

[1]Values are means ± SE. Baseline values for individual groups are not shown but no significant differences occurred between groups for any dietary marker. [2]*P* value refers to respective group-by-time interaction.[3]Significant main effect of time, $P < 0.05$. *Significantly ($P < 0.05$) greater than CON group at respective time point. ‡Significantly greater than PRO group at respective time point. ¥Significantly greater than EX+CON group at respective time point. $Significantly greater than EX+PRO group at respective time point. #$P < 0.05$ from baseline value.

5.4.4 Habitual physical activity

At baseline, markers of habitual physical activity were similar between groups (**Table 5.1**). Following the intervention, no significant differences in daily step count (P = 0.61), or time spent sedentary (P = 0.45), or in light (P = 0.67) or in MVPA (P = 0.80) occurred between groups over time. No significant within-group differences occurred for any marker of habitual physical activity.

5.4.5 Body composition

No significant within- or between-group differences occurred over time for FFM; however, when RE groups were pooled, FFM increased over time greater than non-exercise groups pooled (0.9 ± 0.3 vs. 0.0 ± 0.2 kg, P = 0.045, **Figure 5.1**). Skeletal muscle mass increased by 0.5 ± 0.2 kg and 0.6 ± 0.3 kg in the EX+CON and EX+PRO groups, respectively, both of which trended towards statistical significance (P = 0.06, **Table 5.3**). No differences occurred between groups (P = 0.35). Fat mass and BMI significantly increased over time in the CON group (P < 0.05), and FM decreased, but not significantly, by -0.9 ± 0.5 kg (P = 0.09) in the EX+PRO group. Also, in only the EX+PRO group, waist circumference significantly decreased over time (P = 0.01). No significant differences in FM, FMI, BMI or waist circumference occurred between groups; although, %FM decreased greater in the EX+PRO group compared to the CON group (P = 0.03).

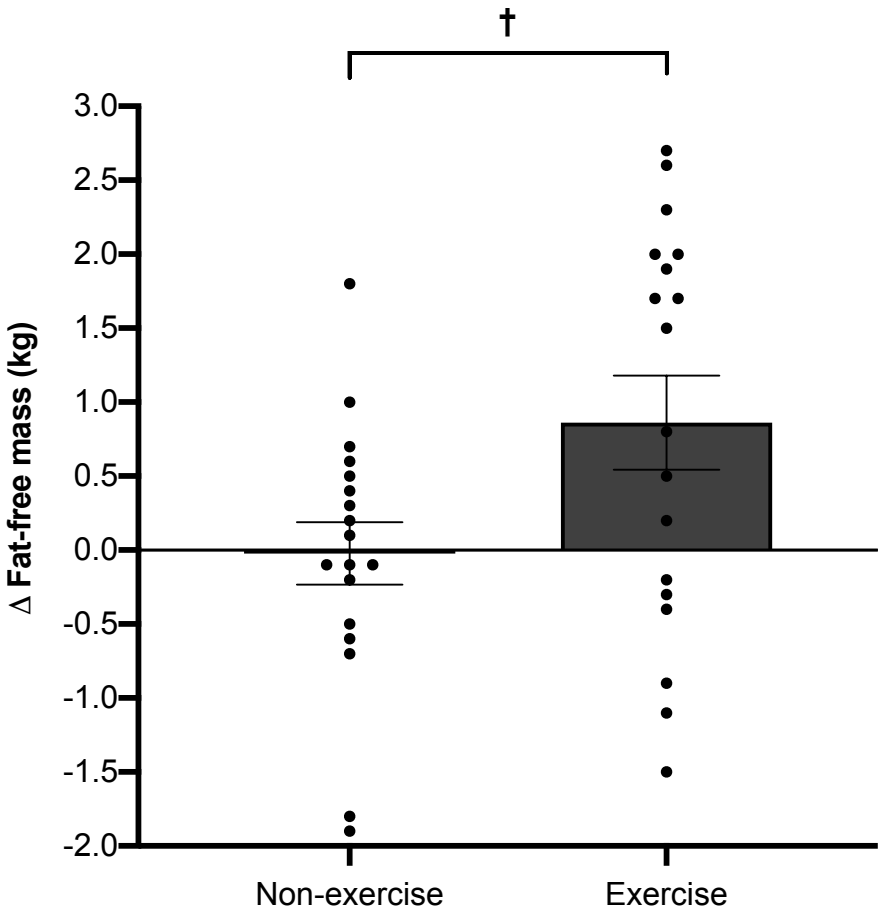

Figure 5.1 Change in fat-free mass between pooled exercise (*n* = 18) and non-exercise groups (*n* = 18) (means ± SE). Circles represent individual participant changes. Data were analysed by mixed-model ANOVA with baseline values and supplement consumed (whey protein or control) included as covariates. $^{\dagger}P < 0.05$ between groups.

Table 5.3 Body composition, muscle strength and physical function measures for each treatment group at baseline and 12 weeks[1]

	CON		PRO		EX+CON		EX+PRO		*P* value	
	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Time	Group x time
Body composition										
Body mass, kg	79.0 ± 3.4	79.8 ± 3.3$^{\#}$	78.0 ± 3.1	78.3 ± 3.3	78.2 ± 3.9	79.0 ± 3.8	80.9 ± 4.0	81.0 ± 4.1	0.48	0.74
BMI, kg/m^2	25.1 ± 1.0	25.3 ± 0.9$^{\#}$	25.0 ± 0.6	25.1 ± 0.7	25.1 ± 0.9	25.3 ± 0.9	26.6 ± 0.8	26.6 ± 0.8	0.45	0.80
FFM, kg	59.8 ± 1.5	59.8 ± 1.6	60.0 ± 1.7	60.0 ± 1.9	58.5 ± 2.6	59.2 ± 2.5	60.5 ± 2.9	61.5 ± 2.9	0.92	0.23
SMM, kg	26.7 ± 0.6	26.7 ± 0.7	27.2 ± 0.7	27.5 ± 0.8	25.9 ± 1.1	26.4 ± 1.1	26.9 ± 1.3	27.5 ± 1.3	0.99	0.35
SMI, kg/m^2	8.5 ± 0.2	8.5 ± 0.2	8.8 ± 0.2	8.9 ± 0.2	8.3 ± 0.2	8.4 ± 0.2	8.9 ± 0.3	9.1 ± 0.2	0.25	0.23
FM, kg	19.2 ± 2.4	20.1 ± 2.2$^{\#}$	18.0 ± 1.7	18.3 ± 1.9	19.6 ± 2.0	19.8 ± 2.1	20.4 ± 1.5	19.5 ± 1.7	0.97	0.08
FM, %	23.8 ± 2.0	24.7 ± 1.9$^{\#\$}$	22.7 ± 1.5	22.9 ± 1.7	24.8 ± 1.7	24.6 ± 2.0	25.1 ± 1.2	23.8 ± 1.5	0.72	0.04
FMI, kg/m^2	6.1 ± 0.7	6.4 ± 0.7$^{\#}$	5.7 ± 0.5	5.9 ± 0.6	6.3 ± 0.6	6.3 ± 0.6	6.7 ± 0.5	6.4 ± 0.5	0.89	0.07
Waist circumference, cm	92.5 ± 2.6	92.8 ± 2.4	92.8 ± 3.0	93.1 ± 3.1	91.3 ± 3.5	91.7 ± 3.7	98.1 ± 3.4	97.0 ± 3.3$^{\#}$	0.82	0.44
Waist:hip ratio	0.93 ± 0.02	0.92 ± 0.02	0.91 ± 0.02	0.91 ± 0.02	0.91 ± 0.02	0.91 ± 0.02	0.97 ± 0.02	0.96 ± 0.02	0.53	0.65
Muscle strength										
Leg extension 1RM, kg	63 ± 6	65 ± 6	58 ± 3	64 ± 4	52 ± 5	72 ± 6$^{\#*\ddagger}$	59 ± 4	80 ± 4$^{\#*\ddagger}$	0.17	< 0.001
Leg press 1RM, kg	116 ± 9	119 ± 9	107 ± 7	113 ± 6	107 ± 9	137 ± 8$^{\#*\ddagger}$	118 ± 7	157 ± 7$^{\#*\ddagger}$	< 0.001	< 0.001
Handgrip strength, kg	41.8 ± 1.7	43.0 ± 1.5	36.2 ± 2.2	38.7 ± 2.1	40.7 ± 3.7	42.0 ± 3.8	41.8 ± 2.1	43.4 ± 1.8	0.38	0.58
Physical function										
6MWT, m	639 ± 21	636 ± 20	616 ± 18	626 ± 17	627 ± 30	648 ± 30$^{\#}$	591 ± 26	612 ± 24$^{\#}$	0.55	0.53
SPPB, points	11.7 ± 0.2	11.8 ± 0.1	11.4 ± 0.2	11.8 ± 0.2	11.2 ± 0.3	11.6 ± 0.3	11.8 ± 0.1	12.0 ± 0	< 0.001	0.84
4 m gait speed, m/s	1.09 ± 0.08	1.08 ± 0.06	1.13 ± 0.04	1.24 ± 0.06	1.14 ± 0.04	1.15 ± 0.04	1.22 ± 0.05	1.26 ± 0.03	0.001	0.043

[1]Values are means ± SE. 1RM, one repetition maximum; 6MWT, 6-minute walk test; BMI, body mass index; FFM, fat-free mass; FM, fat mass; FMI, fat mass index; SMI, skeletal muscle index; SMM, skeletal muscle mass; SPPB, short physical performance battery. *Significantly ($P < 0.05$) greater than CON group. ‡Significantly greater than PRO group. $^{\$}$Significantly greater than EX+PRO group. $^{\#}P < 0.05$ from baseline.

5.4.6 Muscle strength

Significant group-by-time interactions were observed for both leg extension ($P < 0.001$, **Figure 5.2A**) and leg press 1RM ($P < 0.001$, **Figure 5.2B**). Leg extension 1RM increased over time by 38% (52 ± 5 to 72 ± 6 kg, $P < 0.001$) and 36% (59 ± 4 to 80 ± 4 kg, $P < 0.001$) in the EX+CON and EX+PRO groups, respectively. Leg press 1RM increased by 28% (107 ± 9 to 137 ± 8 kg, $P < 0.001$) and 33% (118 ± 7 to 157 ± 7 kg, $P < 0.001$) in the EX+CON and EX+PRO groups, respectively. Post-hoc analysis revealed both variables in the EX+CON and EX+PRO groups increased over time greater than the CON and PRO groups ($P < 0.001$). No differences were observed between either the CON and PRO groups, or the EX+CON and EX+PRO groups. When whey protein supplement groups were pooled, there was a trend towards a greater increase in leg press 1RM compared to control supplements pooled ($P = 0.06$). No significant within- or between-group differences in handgrip strength were observed (**Table 5.3**).

5.4.7 Physical function

A significant group-by-time interaction was observed for 4 m gait speed ($P = 0.043$, **Table 5.3**) but not SPPB ($P = 0.84$) or 6MWT distance ($P = 0.53$). In the PRO group, gait speed increased by 0.11 ± 0.06 m/s, which tended to increase over time greater than the CON group ($P = 0.06$). When whey protein supplement groups were pooled, 4 m gait speed increased greater than control groups pooled ($P = 0.007$, **Figure 5.2C**). Although no significant differences in 6MWT distance occurred between groups, significant within-group increases occurred in both the EX+CON (21 ± 7 m, $P = 0.02$) and EX+PRO groups (21 ± 6 m, $P = 0.007$). When RE groups were pooled, 6MWT increased greater than non-exercise groups pooled ($P = 0.04$, **Figure 5.2D**). No significant within-group differences were observed for SPPB.

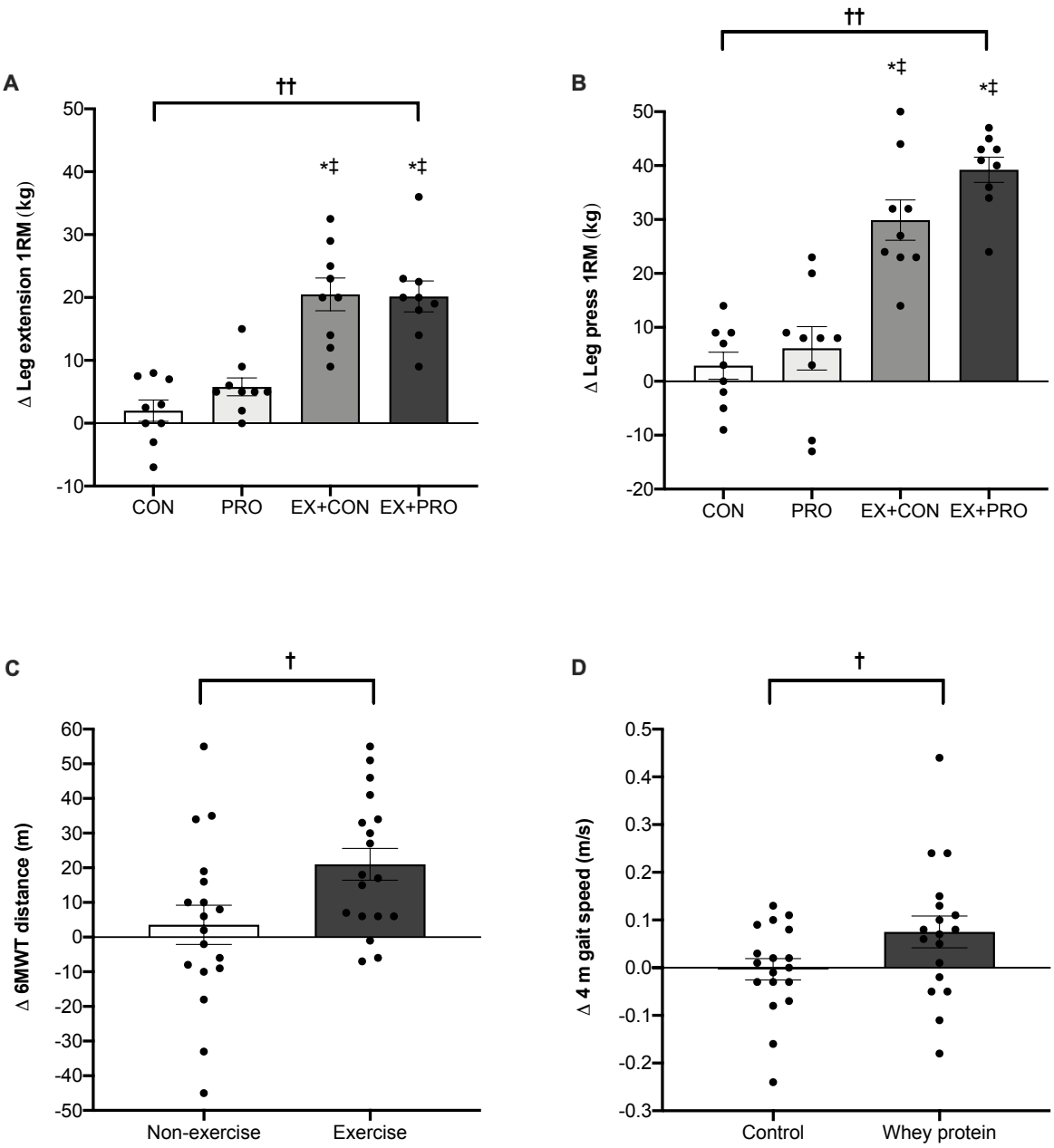

Figure 5.2 Changes in (A) leg extension 1RM and (B) leg press 1RM between intervention groups; change in (C) 6MWT distance between pooled exercise and non-exercise groups; and (D) change in 4 m gait speed between pooled whey protein and control supplement groups (means ± SE). Circles represent individual data points. Analyses were performed using a mixed-model ANCOVA with baseline values only included as a covariate (for panels A and B), baseline values and supplement consumed (whey protein or control) included as covariates (for panel C only), and baseline values and exercise/non-exercise included as covariates (for panel D only). 1RM, one repetition maximum; 6MWT, 6 min walk test. $^{\dagger}P < 0.05$ between groups. $^{\dagger\dagger}P < 0.01$ between groups. *Significantly ($P < 0.05$) greater than CON group. ‡Significantly greater than PRO group.

5.4.8 Hormonal and inflammatory biomarkers

At baseline, salivary cortisol AUC was significantly inversely correlated with SMI (ρ = -0.54, *P* = 0.001), handgrip strength (ρ = -0.36, *P* = 0.046), 6MWT distance (ρ = -0.46, *P* = 0.08) and leg press 1RM (ρ = -0.53, *P* = 0.002) (**Figure 5.3**). When each salivary cortisol time point was analysed separately, no significant correlations were observed with any of the abovementioned variables; however, the 2000 h time point was significantly correlated with leg press 1RM (ρ = -0.39, *P* = 0.03), and SPPB (ρ = 0.40, *P* = 0.03). No significant correlations were observed between baseline concentrations of plasma IL-6, TNF-α, or CRP, serum IGF-1 or myostatin, or salivary cortisol slope and any sarcopenia parameter.

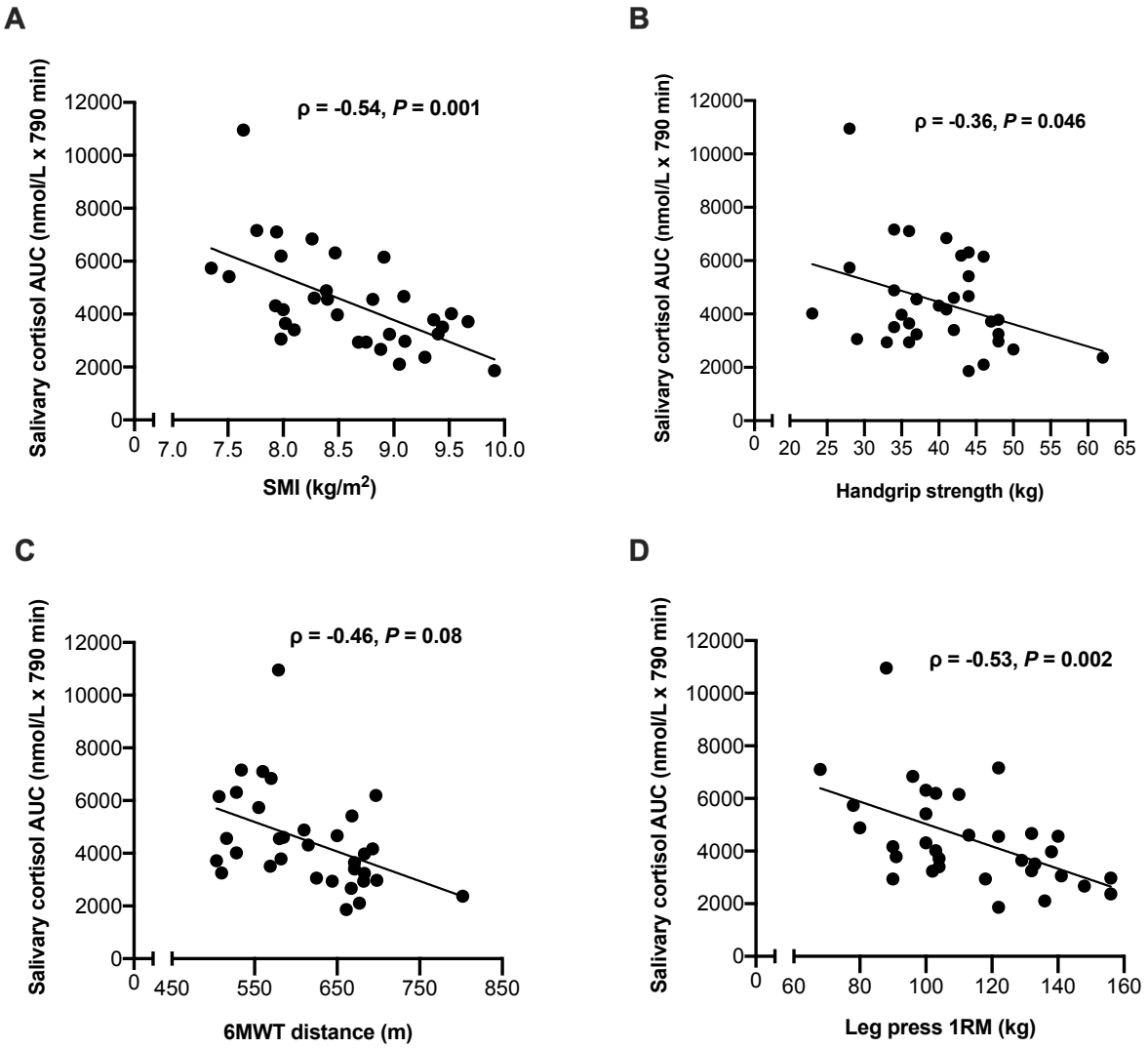

Figure 5.3 Correlation between salivary cortisol AUC and (A) SMI; (B) handgrip strength; (C) 6MWT distance; and (D) leg press 1RM at baseline (*n* = 33). Correlations were analysed by partial correlation (Spearman's rank-order coefficients) controlled for age. 6MWT, 6-minute walk test; AUC, area under the curve; SMI, skeletal muscle index; SPPB, short physical performance battery.

Changes in fasting hormonal and inflammatory biomarkers, and diurnal salivary cortisol indices over the intervention period can be seen in **Table 5.4**. Both plasma IL-6 and TNF-α significantly decreased over time in the EX+PRO group by 21% (-1.2 ± 0.6 pg/mL, *P* = 0.01) and 20% (-0.6 ± 0.2 pg/mL, *P* = 0.03), respectively. Similar decreases were also observed in the EX+CON group (plasma IL6: -25%, -0.8 ± 0.5 pg/mL; plasma TNF-α: -20.6%, -0.7 ± 0.5 pg/mL), but values did not significantly differ from baseline (*P* = 0.15; *P* = 0.21, respectively).

No differences occurred between groups for either variable (P = 0.13; P = 0.11, respectively). When RE groups were pooled, both IL-6 (P = 0.048) and TNF-α (P = 0.02) significantly decreased over time greater than non-exercise groups pooled (**Figure 5.4**). Salivary cortisol slope (peak morning to 2000 h) increased in the EX+PRO group by 92.1% (8.1 ± 2.8 nmol/L, P = 0.02), but no differences occurred between groups (P = 0.15). No significant within- or between-group differences were observed for plasma CRP, serum IGF-1 or myostatin, or any index of salivary cortisol. When whey protein groups were pooled, serum myostatin tended to increase greater than control supplement groups pooled (P = 0.05). No correlations were observed between changes in SMM, FM, muscle strength or physical function and changes in any hormonal or inflammatory biomarker.

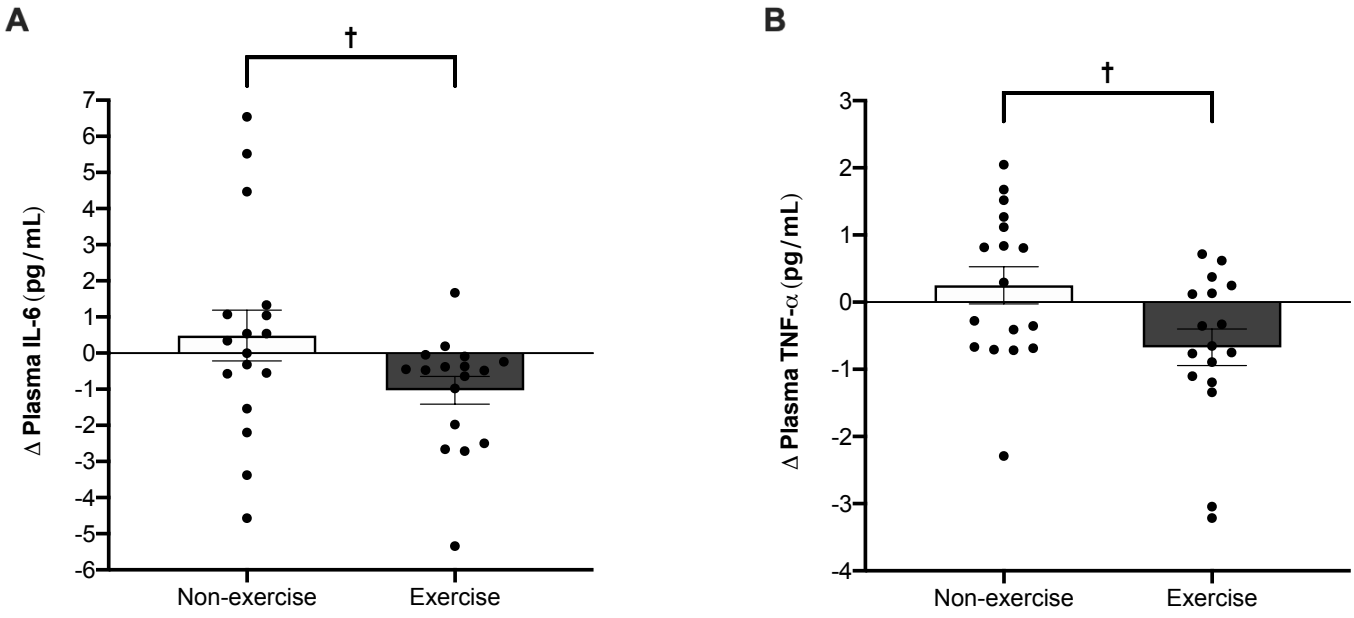

Figure 5.4 Changes in (A) plasma IL-6 and (B) TNF-α between pooled exercise (n = 17) and non-exercise groups (n = 17) (means ± SE). Circles represent individual participant changes. Data were analysed by mixed-model ANCOVA with baseline values and supplement consumed (whey protein or control) included as covariates. $^{\dagger}P < 0.05$ between groups. IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.

Table 5.4 Fasted hormonal and inflammatory biomarkers, and salivary cortisol indices for each treatment group at baseline and 12 weeks[1]

	CON		PRO		EX+CON		EX+PRO		*P* value	
	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Time	Group x time
Serum IGF-1,[2] ng/mL	152 ± 34	130 ± 29	119 ± 17	110 ± 14	137 ± 16	119 ± 12	118 ± 15	100 ± 10	0.07	0.86
Serum myostatin,[3] ng/mL	2.2 ± 0.5	2.0 ± 0.4	1.9 ± 0.3	2.2 ± 0.3	1.8 ± 0.3	1.6 ± 0.1	2.3 ± 0.2	2.4 ± 0.3	0.06	0.15
Plasma IL-6,[2] pg/mL	4.9 ± 1.2	5.8 ± 1.3	4.0 ± 1.0	4.0 ± 1.2	3.2 ± 0.9	2.4 ± 0.6	5.8 ± 1.8	4.6 ± 1.2[#]	0.09	0.13
Plasma TNF-α,[2] pg/mL	3.2 ± 0.3	3.0 ± 0.3	2.4 ± 0.3	3.0 ± 0.3	3.4 ± 0.6	2.7 ± 0.3	3.0 ± 0.4	2.4 ± 0.2[#]	< 0.001	0.11
Plasma CRP,[2] ng/mL	2.4 ± 0.6	1.7 ± 0.3	1.6 ± 0.4	1.1 ± 0.5	0.8 ± 0.2	0.8 ± 0.2	2.0 ± 0.4	1.8 ± 0.2	0.18	0.18
Salivary cortisol AUC,[3] nmol/L x 790 min	4067 ± 551	4349 ± 528	4088 ± 196	45450 ± 425	5588 ± 969	5326 ± 778	4127 ± 587	4530 ± 414	0.001	0.99
Salivary cortisol slope,[3] nmol/L	9.6 ± 1.1	9.1 ± 1.4	10.6 ± 0.8	13.6 ± 2.4	12.5 ± 2.5	15.7 ± 2.7	8.8 ± 1.4	16.9 ± 3.5[#]	0.004	0.15
Salivary cortisol (2000 h),[3] nmol/L	2.1 ± 0.5	1.9 ± 0.5	1.9 ± 0.4	2.2 ± 0.6	2.2 ± 0.6	1.8 ± 0.7	2.1 ± 0.7	2.6 ± 0.6	0.009	0.64

[1]Values are means ± SE. [2]*n* = 34 (CON, *n* = 8; PRO, *n* = 9; EX+CON, *n* = 8; EX+PRO, *n* = 9). [3]*n* = 33 (CON, *n* = 8; PRO, *n* = 8; EX+CON, *n* = 8; EX+PRO, *n* = 9). AUC, area under the curve; CRP, C-reactive protein; IGF-1, insulin-like growth factor 1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha. [#]*P* < 0.05 from baseline value.

5.5 Discussion

This study investigated the individual and combined effects of RE and whey protein supplementation on skeletal muscle and FFM, muscle strength, physical function, and hormonal and inflammatory biomarkers associated with sarcopenia in healthy older men. The main findings were:

i) Resistance exercise significantly increased FFM, muscle strength and physical function and decreased markers of systemic inflammation.
ii) Whey protein supplementation alone did not increase skeletal muscle or FFM and tended to increase serum myostatin. When whey protein supplement groups were pooled, physical function (4 m gait speed) significantly increased, and muscle strength (leg press 1RM) tended to increase greater than consuming an isocaloric carbohydrate control.
iii) No synergistic effects of RE and whey protein supplementation occurred for any parameter of sarcopenia compared to RE or whey protein supplementation alone.
iv) Changes in hormonal and inflammatory markers did not correlate with changes in SMM, muscle strength or physical function, but diurnal salivary cortisol secretion did significantly correlate with multiple parameters of sarcopenia at baseline.

5.5.1 Effects on skeletal muscle and FFM, muscle strength and physical function

Twelve weeks of progressive whole-body RE resulted in a combined mean increase of 0.9 ± 0.3 kg FFM, of which 0.6 ± 0.2 kg was an increase in SMM. This corresponds to increases of 1.2% and 2.3%, respectively. The magnitude of FFM increase in the present study is in line with previous studies that reported increases of ~1 kg following 12 weeks of RE in older adults (Campbell et al. 1995, Holwerda et al. 2018, Leenders et al. 2013, Verdijk et al. 2009a). The increase in FFM and SMM in the present study was accompanied by 36%, 31% and 3.4% increases in leg extension and leg press 1RM, and 6MWT distance, respectively. These findings add to the current body of literature that have reported similar increases in muscle strength (Arnarson et al. 2013, Bell et al. 2017, Holwerda et al. 2018, Kirk et al. 2019, Leenders

et al. 2013, Verdijk et al. 2009a) and physical function as measured by 6MWT distance following ≥12 weeks of RE training alone (Arnarson et al. 2013, Oesen et al. 2015), or combined with endurance-based training elements in older adults (Bell et al. 2017, Kirk et al. 2019). Taken together, considering SMM, strength and physical function decline at rates of ~0.5-1%, ~1-3%, and ~0.5% per annum around mid-life, respectively (Clark and Manini 2008, Janssen 2010, Daly et al. 2013), which are associated with numerous adverse effects on human health (Cruz-Jentoft et al. 2019), and increases the economic burden to healthcare services (Goates et al. 2019, Pinedo-Villanueva et al. 2019), the findings of the present study are both clinically and economically relevant.

The lack of effect of whey protein supplementation on skeletal muscle and FFM is in agreement with some (Björkman et al. 2020, Cramer et al. 2016, de Carvalho Bastone et al. 2020, Kim et al. 2012, Kirk et al. 2020, Kukuljan et al. 2009, Verreijen et al. 2017, Zhu et al. 2015) but not all previous studies (Bauer et al. 2015, Bell et al. 2017, Bo et al. 2018, Kang et al. 2020, Mitchell et al. 2017, Negro et al. 2018, Norton et al. 2016, ten Haaf et al. 2019). It was hypothesised that disparities between previous studies may be partially explained by differences in the deviation of dietary protein from baseline (≥0.4 vs. <0.4 g/kg/d). Although, the findings of this study oppose this hypothesis as dietary protein was increased by 0.6 g/kg/d and observed no effect. This data is in contrast to others that increased dietary protein intake by 0.4-0.6 g/kg/d (Bell et al. 2017, Norton et al. 2016). Of note, the whey protein-based multi-ingredient supplement consumed by participants in the study by Bell and colleagues (2017) contained vitamin D and n-3 PUFA. Both of these ingredients have been shown to aid muscle hypertrophy and function (Rosendahl-Riise et al. 2017, Smith et al. 2015). Also, the study by Norton and colleagues (2016) was double the duration of the present study, which might have provided a greater timeframe for protein-induced increases in FFM. These methodological differences may explain the inconsistent findings. Additionally, the decrease in protein balance, particularly in the overnight fasting period as reported in **Chapter 4**, might also

explain the lack of protein-induced increase in skeletal muscle and FFM following whey protein supplementation in the present study.

Whilst whey protein supplementation elicited no effect on skeletal muscle or FFM, the present study observed a significantly greater increase in physical performance (4 m gait speed) and a trend towards a greater increase muscle strength (leg press 1RM) when whey protein supplement groups were pooled and compared to control supplement groups pooled. These outcomes are in agreement with others that have reported increases in muscle strength and/or physical function following increased dietary protein intake in older adults (Bauer et al. 2015, Bell et al. 2017, Kang et al. 2020, ten Haaf et al. 2019, Tieland et al. 2012b). As not all older adults are able or willing to perform RE (Dismore et al. 2020), these findings suggest higher intakes of dietary protein may be of clinical importance to attenuate declines in muscle function in these groups.

This study tested the hypothesis that twice daily supplementation of a high leucine whey protein supplement would augment the effects of RE on SMM, strength and physical function. Despite gains in these outcomes following RE training alone, and an increase in physical function and a trend for an increase in muscle strength following whey protein supplementation, no synergistic effects were observed. These findings are consistent with the majority of previously published studies (Arnarson et al. 2013, Candow et al. 2006, Chalé et al. 2013, de Carvalho Bastone et al. 2020, Dulac et al. 2020, Englund et al. 2018, Fielding et al. 2017, Gryson et al. 2014, Hofmann et al. 2016, Holm et al. 2008, Holwerda et al. 2018, Kim et al. 2012, Kirk et al. 2019, 2020, Kukuljan et al. 2009, Leenders et al. 2013, Maltais, Ladouceur, and Dionne 2016, Oesen et al. 2015, Ottestad et al. 2017, Shahar et al. 2013, Thomson et al. 2016, Verdijk et al. 2009a, Verreijen et al. 2017). Similar to that of many of these studies, the population used in this study were non-frail, i.e., displayed high baseline physical function scores (SPPB: 11.5 ± 0.1; 6MWT: 618 ± 12 m), were already physically active (MVPA: 127 ± 7 min/d), and consumed sufficient but not optimal amounts of dietary

protein (1.0 ± 0.02 g/kg/d) at baseline according to the PROT-AGE and ESPEN consensus groups (Bauer et al. 2013, Deutz et al. 2014).

In contrast to the present and abovementioned studies, other studies have reported synergistic effects of RE and increased dietary protein on parameters of sarcopenia (Bell et al. 2017, Daly et al. 2014, Kang et al. 2019, Junior et al. 2018, Rondanelli et al. 2016, 2020, Tieland et al. 2012b, Verreijen et al. 2015, Yamada et al. 2019, Zdzieblik et al. 2015). Participants in the majority of these studies were sarcopenic or frail older adults with habitual protein intakes ≤1 g/kg/d (Kang et al. 2019, Rondanelli et al. 2016, Tieland et al. 2012b, Yamada et al. 2019, Zdzieblik et al. 2015). Based on these comparisons, the findings of the present and previous studies imply a relatively good health status, and an adequate habitual dietary protein intake may mask the supplemental effects in older adults performing RE. However, it is important to note that augmented effects have been observed in healthy older adults habitually consuming adequate (1.0-1.1 g/kg/d) amounts of dietary protein (Bell et al. 2017, Daly et al. 2014, Junior et al. 2018). Therefore, as the present study observed an effect of whey protein supplementation on muscle function when whey protein groups were pooled, it may be that the sample size of this study was insufficient to detect post-hoc differences between the EX+CON and EX+PRO groups. Additional RCTs including a substantially larger sample size to that of the present study are required to determine whether increased dietary protein (at a dose of ~1.6 g/kg/d) elicits additive effects during RE in healthy active older adults habitually consuming adequate amounts of dietary protein at baseline.

5.5.2 Effects on hormonal and growth factors

At baseline, no significant correlations were observed between SMM, strength or physical function and serum IGF-1 or myostatin. In contrast, significant inverse correlations were observed between diurnal salivary cortisol AUC [from 5 measurements across waking hours (0650, 0805, 1225, 1700 and 2000 h) under highly controlled conditions] and multiple parameters of sarcopenia [SMI, muscle strength (handgrip strength and leg press 1RM), and

physical function (6MWT distance)]. In agreement, previous studies have also observed elevated cortisol concentration in sarcopenic compared to non-sarcopenic elderly individuals (Waters et al. 2008), and inverse associations between atypical diurnal cortisol secretion (attenuated morning and elevated pre-sleep cortisol concentration) and poor physical performance in older adults (Gardner et al. 2011, Heaney, Phillips, and Carroll 2012, Sousa et al. 2017). However, although baseline data from this study, albeit in a small sample size, suggests measurement of diurnal salivary cortisol may be an effective method to identify sarcopenic individuals, the clinical application is somewhat limited due to both the cost and required participant compliance to gain such data. Unfortunately, not one single time point of salivary cortisol significantly inversely correlated with all components of sarcopenia similar to that of the diurnal profile; although, measurement at 2000 h did show some promise. Consistent with this observation, a recent study also reported an association between high salivary cortisol secretion at 2000 h and sarcopenia prevalence (Gonzalez et al. 2018).

In addition to the above, no changes in salivary cortisol indices occurred following RE, despite improvements in parameters of sarcopenia being observed, further questioning the use of salivary cortisol as a potential biomarker of sarcopenia. Consistent with the present study, studies that have analysed the effects of RE on basal cortisol in both serum (Häkkinen et al. 2001, 2002, Izquierdo et al. 2003) and saliva in older adults (Ahn and Kim 2018) have also reported no changes. It should be noted that two of these studies did report significant decreases in basal cortisol secretion at some point during the intervention, just not following (Häkkinen et al. 2002, Izquierdo et al. 2003). However, a significant limitation of these studies was the singular measurement of cortisol in the morning between 0800 and 1000 h. It is well known that cortisol secretion follows a diurnal rhythm, that is, high upon waking, surging 50-60% 30-40 min after waking, decreasing rapidly in the subsequent hours following waking and then slowly declining until reaching a nadir in the late evening (Adam et al. 2017). The novel findings of present study are that RE does not decrease diurnal cortisol secretion, and RE-

induced changes in SMM and strength are not associated with changes in diurnal salivary cortisol in healthy older men.

The lack of decrease in serum myostatin following RE training is consistent with some (Hofmann et al. 2016, Kim et al. 2007, Planella-Farrugia et al. 2019) but not all studies (Bagheri et al. 2020, Binns et al. 2017, Negaresh et al. 2019, Shabani and Izaddoust 2018). It has been hypothesised that an insufficient RE intensity and high pre-post test myostatin variability amongst participants might explain the above null findings. In the present study, RE intensity was high (80% 1RM), and SMM increased by 0.6 ± 0.2 kg, similar to that of Bagheri and colleagues (2020) who reported a decrease in myostatin following RE in elderly women. This suggests that the intensity of RE and/or change in SMM in the present study does not explain the descrepancies with previous stuies. Instead, similar to that of Kim et al. (2007), the pre-post variability in serum myostatin was large (-1.7-1.1 ng/mL), which most likely explains the lack of decrease in the present study.

The increase in serum myostatin following whey protein supplementation, which tended to increase greater than control supplement groups pooled, is in agreement with longitudinal data in younger adults (Paoli et al. 2015). Furthermore, an acute study in middle- and older-aged men demonstrated dietary protein hindered the decrease in myostatin mRNA following RE (Hulmi et al. 2008). As reported in **Chapter 4**, whey protein supplementation led to a significant rise in nocturnal protein oxidation and a decrease in nocturnal protein balance. Consequently, the increase in fasting serum myostatin may have reflected an increase in protein breakdown in the overnight fasting period following 12 weeks of whey protein supplementation.

However, it must be noted that the validity of myostatin as both a biomarker of sarcopenia and a method to monitor response to treatments has been questioned, making interpretation of data and comparisons with the literature difficult (Peng et al. 2018). For example, whilst the

muscle-wasting effect of myostatin is well-known (Elliott et al. 2012), evidence from studies investigating the relationship between myostatin and SMM is far from congruent. Some studies have reported an inverse relationship between myostatin and age-related declines in SMM (Ju and Chen 2012, Yarasheski et al. 2002), whilst others have reported associations between reduced SMM and low myostatin concentration (Christensen et al. 2013, Furihata et al. 2016, Lenk et al. 2012, Peng et al. 2018). Clearly, further research to clarify the potential of myostatin as a biomarker of sarcopenia is warranted.

Previous studies have claimed that circulating hormones, including IGF-1, are mechanistically and directly related to RE-induced increases in SMM (Kraemer, Ratamess, and Nindl 2017, Mangine et al. 2017). Indeed, in older adults, intervention studies have reported accompanying elevated basal IGF-1 concentration with increased SMM following RE interventions (Chen et al. 2017, Cunha et al. 2020, Rondanelli et al. 2016). However, there is also substantial contrary evidence that suggests circulating systemic hormones at rest both in young (Mobley et al. 2018, Morton et al. 2016, 2018b) and older adults (Arnarson et al. 2015, Häkkinen et al. 2000, 2001, Holwerda et al. 2018, Nascimento et al. 2019, Shahar et al. 2013, Walsh et al. 2015) are not related to RE-induced increases in SMM. In agreement, although RE increased SMM in the present study, serum IGF-1 did not increase, and change in concentration did not correlate with change in SMM. Furthermore, in the previously cited study by Morton et al. (2018b), although neither circulating nor intramuscular hormones influenced or were related to skeletal muscle hypertrophy following RE in young men, intramuscular androgen receptor content was. These findings are supported by other studies that have also demonstrated increases in skeletal muscle androgen receptor content following RE training (Ahtiainen et al. 2011, Mitchell et al. 2013) and suggest that local intramuscular factors as opposed to changes in systemic hormonal concentrations might underpin increases in SMM following RE. Although, it should be noted that two recent studies have not observed an increase in intramuscular androgen receptor content following RE (Haun et al. 2019, Mobley et al. 2018). Whilst further research on the effects of RE on SMM and intramuscular androgen

receptor content is warranted, particularly in older adults, it is possible, although speculative, that the RE training programme in this study induced changes at the hormone receptor level, which in turn elicited increases in SMM.

5.5.3 Effects on systemic inflammation

A key finding of this study was the significant reduction in markers of systemic inflammation following RE training. Age-related, low-grade systemic inflammation, termed inflammaging (Franceschi et al. 2006), is associated with numerous adverse health outcomes, including CVD, insulin resistance and a higher risk of mortality (Calder et al. 2017). Inflammation is also often cited in the aetiology of sarcopenia (Beyer, Mets, and Bautmans 2012). In the present study, the pro-inflammatory cytokines IL-6 and TNF-α significantly reduced by ~20% in the EX+PRO group and decreased, but not significantly, by a similar magnitude in the EX+CON group. When RE groups were pooled, both IL-6 and TNF-α significantly decreased greater than non-exercise groups pooled. These reductions are consistent, but greater than the ~11-12% declines in IL-6 and TNF-α following RE and HIIT combined with a whey protein-based multi-ingredient supplement in healthy older men (Bell et al. 2018). Additional studies in older adults have also reported reductions in markers of systemic inflammation following RE alone (Sardeli et al. 2018) and combined with whey protein supplementation (Rondanelli et al. 2016). Given that IL-6 in particular is strongly associated with advancing age, morbidity and mortality (Beavers et al. 2010, Ershler 1993), these findings illustrate the importance of performing RE in older age to delay degeneration of health in older adults. Furthermore, previous work has also shown IL-6 and TNF-α have important roles in the pathogenesis of insulin resistance (Kim et al. 2004, Plomgaard et al. 2005). These reductions may therefore also help explain the improvement in insulin sensitivity observed in both the EX+CON and EX+PRO groups reported in **Chapter 4**.

Contrary to the findings of the present and abovementioned studies, others have failed to report reductions in inflammatory markers following RE training alone (Hangelbroek et al. 2018), or combined with either AE (Beavers et al. 2010, Grosicki et al. 2020) or protein supplementation (Shahar et al. 2013). Conflicting results may in part be related to the health status of the population studied. Participants in studies that have not reported a reduction in markers of systemic inflammation were sarcopenic/frail with reduced physical function (SPPB score of ≤10) (Hangelbroek et al. 2018, Grosicki et al. 2020, Shahar et al. 2013), whereas healthy, well-functioning older adults were used in the present study and that of Bell et al. (2018). Although, it is important to note that Rondanelli et al. (2016) reported a between-group difference in CRP between whey protein and placebo groups following 12 weeks physical activity in sarcopenic elderly; however, this finding was driven more by a within-group increase in the placebo group (0.44, 95% CI -0.02-0.90, P = 0.06) rather than a significant decrease following whey protein supplementation (-0.19, 95% CI -0.57-0.19, P = 0.33). Together, data from these studies suggests RE is not effective at reducing systemic inflammation in sarcopenic/frail older adults who are prone to systemic inflammation. In support, in a sub analysis of TNF-α in a meta-analysis conducted by Sardeli et al. (2018), only those studies that investigated healthy older adults demonstrated reduced TNF-α following RE interventions. Furthermore, it has been suggested that modulating inflammation is likely most effective at the early stage of health decline when the compensatory capacity is not completely exhausted (Ferrucci and Fabbri 2018), further supporting this hypothesis.

In addition to the above, contradictory results of the aforementioned studies might also be related to changes in FM following RE training. In particular, changes in central (predominantly visceral) adiposity, which is a well-known causative factor of inflammaging (Beyer, Mets, and Bautmans 2012). In the combined RE and whey protein supplement groups in the present study and that of Bell et al. (2018), reductions in whole-body FM as well as proxy markers of visceral adipose tissue (waist circumference and trunk FM, respectively) were observed. In

contrast, no changes in FM occurred in studies that reported no changes in pro-inflammatory markers (Beavers et al. 2010, Hangelbroek et al. 2018). Furthermore, in the previously mentioned meta-analysis by Sardeli and colleagues (2018), only studies that demonstrated reductions in FM also reported reductions in TNF-α. However, it must be noted that no changes in FM or waist circumference were observed in the EX+CON group, suggesting change in FM cannot fully explain the decrease in systemic inflammation in the present study.

Previous research has consistently reported inverse relationships between IL-6 and SMM, strength and physical function in older adults (Cesari et al. 2005, Grosicki et al. 2020, Hangelbroek et al. 2018, Visser et al. 2002). Conversely, the results from baseline data in the present study do not support these findings. The relatively good health status of participants in this study may explain this discrepancy. Furthermore, the present study also did not report any correlations between changes in markers of systemic inflammation and changes in parameters of sarcopenia. These findings are in contrast with Grosicki et al. (2020), who demonstrated an association between changes in IL-6 and physical function (gait speed) following a 6-month physical activity programme that included RE. Conversely, the findings of the present study are in agreement with Hangelbroek and colleagues (2018) who reported no significant correlations between changes in IL-6 and TNF-α, and changes in FFM and markers of muscle strength following 24 weeks RE in frail older adults. Inconsistencies might in part be related to the sample sizes between studies. Though, due to limited data, further research examining the relationship between changes systemic inflammation and parameters of sarcopenia is warranted to determine its role in curbing the disease.

5.5.4 Safety and compliance

In agreement with previous work as reviewed by Fragala et al. (2019), twice weekly RE was well tolerated in this population, confirmed by the high compliance (~98%) and no serious adverse events reported. Similarly, 12 weeks of whey protein supplementation was also well

accepted (compliance ~96%). It is postulated that the supervised nature of the RE programme (1-to-1 or 2-to-1 basis with a qualified exercise professional) was a key factor in the high compliance observed. Indeed, a recent meta-analysis reported greater effects of supervised compared to non-supervised RE in older adults (Lacroix et al. 2017). Furthermore, although not reported, it is believed that in addition to the supervision, the strong rapport built between the exercise professional and the participants was a key factor in the compliance seen. This hypothesis is based on verbal feedback from participants throughout the research study. Therefore, future research should investigate the role of the exercise professional and relationship with the exerciser on RE compliance in older adults.

Consistent with the findings of a meta-analysis conducted by Devries and colleagues (2018), the high protein diet (1.6 g/kg/d) had no adverse effect on renal function as confirmed by eGFR (>60 mL/min/1.73m^2 in all participants following the intervention). Based on these findings, a high protein diet does not adversely affect renal function in healthy older men. However, of note, two participants in the PRO group did experience episodes of gout during the intervention period. In agreement, although not often reported, increased uric acid, which is a causative factor of gout (Zhang et al. 2012), has been reported in elderly males consuming high amounts of dietary protein (Durainayagam et al. 2019). A recent animal model study has also reported similar findings (Hong et al. 2020). Therefore, although the present study could not directly determine the causality, further research into the effects of high protein diets and incidence of gout is warranted due to its known causative effects on hypertension, CVD and T2DM risk (Hong et al. 2020).

5.5.5 Strengths and limitations

The present study is only one of few that has investigated both the independent and combined effects of RE and increased dietary protein intake over a ≥10 week period on SMM, strength and physical function in older adults (de Carvalho Bastone et al. 2020, Gryson et al. 2014, Kim et al. 2012, Kirk et al. 2020, Kukuljan et al. 2009, Shahar et al. 2013, Verreijen et al.

2017). As such, the current research extends our understanding of this topic. In contrast to the above cited studies, the present study employed a double-blind, randomised, controlled design, increased dietary protein to the optimal dose (1.6 g/kg/d) to maximally augment RE-induced increases in SMM (Morton et al. 2018a), and comprehensively measured multiple hormonal (serum IGF-1 and myostatin, and salivary cortisol) and inflammatory (plasma IL-6, TNF-α and CRP) biomarkers related to sarcopenia. These are novel aspects of this study.

Limitations of this study include the small sample size per group and the inclusion of only men. The sample size in this study affected statistical power to determine between-group differences between the four intervention groups for SMM, physical function and markers of systemic inflammation. Retrospective power analysis revealed a sample size of 104 (26/group), 72 (18/group) and 60 (15/group) participants would have been required to determine between-group differences in these outcomes, respectively. Further, due to the small effect observed between the CON and PRO, and EX+CON and EX+PRO groups, the sample size of this study was significantly underpowered to detect post-hoc differences between these groups. Although, it should be noted that the primary outcome of the overall trial was a change in RMR as detailed in **Chapter 3**, and this study was a secondary analysis of outcomes of interest related to sarcopenia. Women were excluded from this relatively small study to reduce the likelihood of high variability in outcome variables. Nevertheless, as women account for a large proportion of older adults, this is a major limitation of the study.

5.5.6 Conclusion

Twelve weeks of RE significantly increased FFM, muscle strength and physical function, tended to increase SMM and decreased circulation concentrations of markers of systemic inflammation in healthy older men. Whey protein supplementation alone was ineffective at increasing skeletal muscle or FFM, and tended to increase serum myostatin; however, a greater increase in physical function (gait speed) and a trend towards a greater increase in muscle strength (leg press 1RM) was observed when whey protein supplements were pooled

and compared to control supplements pooled. Despite these increases following RE and whey protein supplementation independently, no synergistic effects were observed for any parameter of sarcopenia compared to RE or whey protein supplementation alone. Data from this study also suggests changes in parameters of sarcopenia are not related to changes in hormonal or inflammatory markers, but diurnal salivary cortisol concentration did correlate with parameters of sarcopenia at baseline.

Thesis map: Study 3

Study	Aims	Key findings
Study 1: Effects of resistance exercise and whey protein supplementation on 24-h energy expenditure, substrate oxidation and metabolic flexibility, body composition, appetite and glucose homeostasis in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on components of 24-h EE, substrate oxidation and metabolic flexibility, body composition, appetite, and glucose homeostasis in healthy older men.	• Resistance exercise significantly increased FFM, RMR, SMR, sedentary EE and 24-h metabolic flexibility compared to non-exercise. RE also resulted in within-group increases in subjective hunger and insulin sensitivity, and within-group decreases in the energy cost of step exercise and spontaneous activity. • Whey protein supplementation improved body weight maintenance and reduced FM, and increased insulin sensitivity; however, resulted in an increase in overnight protein oxidation and awakening cortisol secretion, and reduced 24-h protein balance. • Whey protein supplementation had no adverse effects on total protein or EI, or 24-h subjective appetite. • Resistance exercise combined with whey protein supplementation did not significantly augment changes in body composition, 24-h EE, substrate oxidation or metabolic flexibility, or markers of glucose homeostasis compared to either RE or whey protein supplementation alone.
Study 2: Effects of resistance exercise and whey protein supplementation on skeletal muscle mass, strength, physical function, and hormonal and inflammatory biomarkers in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on SMM, strength, physical function, and hormonal and inflammatory biomarkers in healthy older men. • To determine whether changes in hormonal and inflammatory biomarkers correlate with changes in SMM, strength and physical function.	• Resistance exercise significantly increased FFM, muscle strength and physical function, and decreased markers of systemic inflammation. • Whey protein supplementation alone increased physical function (4 m gait speed) and muscle strength (leg press 1RM). • No synergistic effects occurred for any parameter of sarcopenia compared to RE or whey protein alone. • Changes in hormonal and inflammatory biomarkers did not correlate with changes in parameters of sarcopenia, but diurnal salivary cortisol and sarcopenia indices did at baseline.

Thesis map: Study 3 continued

Study	Aims	Key findings
Study 3: Effects of resistance exercise and whey protein supplementation on cognitive function in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on cognitive function and neurobiological, inflammatory and insulin sensitivity markers, diurnal salivary cortisol, and BP in healthy older men. • To determine whether changes in neurobiological, inflammatory and insulin sensitivity markers, and changes diurnal salivary cortisol, BP, SMM, strength and physical function are associated with changes in cognitive function.	

CHAPTER 6 (Study 3): Effects of resistance exercise and whey protein supplementation on cognitive function in healthy older men

6.1 Chapter overview

Ageing is associated with declines in cognitive function. Previous studies have shown RE and increased dietary protein intake may mitigate age-related cognitive impairment. However, little work has investigated the synergistic effects of these interventions. In this chapter, the individual and combined effects of RE and whey protein supplementation on cognitive function were investigated in healthy older men. Blood and salivary markers, and measurement of BP, SMM, strength and physical function were also analysed to identify potential mechanisms of action.

6.2 Introduction

Ageing is associated with an increased risk of chronic conditions such as cognitive impairment and dementia (Daviglus et al. 2010). Currently, the worldwide prevalence of dementia is estimated at ~50 million people, which is expected to increase to ~82 million people by 2030 (World Health Organisation 2019). The impact of such conditions, both personally and economically, is extensive (Farina et al. 2017, Wimo et al. 2017). Cognitive impairment decreases the ability of affected individuals to perform ADL (Giebel, Sutcliffe, and Challis 2015) and increases the financial and psychological burden on families and caregivers (Kasper et al. 2015). Recently, meta-analyses have reported the age-related decline in SMM, strength and physical function, termed sarcopenia (Cruz-Jentoft et al. 2019), is associated with cognitive impairment (Chang et al. 2016, Cipolli, Yassuda, and Aprahamian 2019, Peng et al. 2020). Common pathologies of both conditions, including chronic systemic inflammation, insulin resistance and reductions in growth factors may explain this relationship (Chang et al. 2016). Although the mechanisms require further investigation, it may be speculated that strategies that curb sarcopenia may also assist in attenuating age-related declines in cognitive function.

Resistance exercise is well known for its attenuating sarcopenic effects (Phillips and Martinson 2019). Additionally, several studies have also reported enhanced cognitive function following

RE, including improvements in executive function (Anderson-Hanley, Nimon, and Westen 2010, Best et al. 2015, Ikudome et al. 2017, Liu-Ambrose et al. 2010, Yoon, Lee, and Song 2018), memory (Best et al. 2015, Cassilhas et al. 2007, Coelho-Júnior et al. 2020, Ikudome et al. 2017, Marston et al. 2019c), and global cognitive function (Coelho-Júnior et al. 2020, Singh et al. 2014, Smolarek et al. 2016). Exercise-induced improvements in cognitive function are purported to be related to several factors, including structural changes in the brain, increases in circulating concentrations of various neurobiological markers such as BDNF and IGF-1, and decreases in cortisol secretion and inflammation (Ahlskog et al. 2011, Cassilhas et al. 2007, Liu-Ambrose et al. 2012, Tsai et al. 2014, 2015, Walsh et al. 2015).

In addition to RE, it is recommended that older adults should consume higher intakes of dietary protein to attenuate sarcopenia (Phillips and Martinson 2019). Evidence also suggests increased dietary protein intake may play a role in delaying age-related cognitive impairment (van de Rest, van der Zwaluw, and De Groot 2013). For instance, previous studies have demonstrated acute improvements in memory (Kaplan et al. 2001) and longitudinal improvements in reaction time, memory and emotion identification following increased dietary protein intake in older adults (Charlton et al. 2016, Kita et al. 2019, Lefferts et al. 2020, van der Zwaluw et al. 2014). These improvements are purported to be due to protein-induced increases in IGF-1 and decreases in inflammation (Bordoni et al. 2017, Journel et al. 2012), amplified brain insulin receptor signalling stimulation (Frazier et al. 2019), and increased brain neurotransmitter availability (van de Rest, van der Zwaluw, and De Groot 2013).

In addition to the singular effects of RE and whey protein supplementation on cognitive function, recent evidence also suggests these interventions may synergistically curb cognitive decline. Two studies have reported synergistic improvements in reaction time, executive function, memory, and processing speed (Bell et al. 2019, Rondanelli et al. 2020). However, it must be noted that others have not observed augmented effects (Formica et al. 2020, van de Rest et al. 2014); although, van de Rest and colleagues (2014) did report an additive effect

on information processing speed compared to protein supplementation alone. Inconsistencies might be related to the habitual protein intake of participants, and due to differences in the daily dose and deviation of dietary protein intake from baseline. To add, in both studies that reported null findings (Formica et al. 2020, van de Rest et al. 2014), habitual protein intake was sufficient (1.0-1.26 g/kg/d) according to consensus groups (Bauer et al. 2013, Deutz et al. 2014), and was increased by ≤0.3 g/kg/d during the intervention. In contrast, whilst Rondanelli et al. (2020) increased dietary protein intake by a similar amount to these studies (0.32 g/kg/d), habitual intake was considerably less (0.79 g/kg/d). Bell et al. (2019) also increased dietary protein intake by a notably greater amount to the aforementioned studies (0.5 g/kg/d; 1.1-1.6 g/kg/d). Although further research is required, it may be that in healthy older adults habitually consuming sufficient amounts of dietary protein (~1.0-1.2 g/kg/d), a dose of 1.6 g/kg/d (and a deviation of ~0.5 g/kg/d) might be required to stimulate additive effects on cognitive function.

6.2.1 Aims and hypotheses

Primary aim

- To investigate the individual and combined effects of RE and whey protein supplementation (aimed to increase dietary protein intake to ~1.6 g/kg/d) on cognitive function in healthy older men.

Secondary aims

- To investigate the individual and synergistic effects on neurobiological, inflammatory and insulin sensitivity markers, diurnal salivary cortisol, and BP.
- To determine whether changes in these markers in addition to changes in SMM, strength and physical function are associated with changes in cognitive function.

Hypotheses

- Combined RE and whey protein supplementation will enhance cognitive function to a greater extent than RE and whey protein supplementation alone.

- Greater increases in circulating neurobiological biomarkers, and greater decreases in inflammatory biomarkers, diurnal salivary cortisol and BP will be observed following RE plus whey protein supplementation compared to each intervention alone.
- Changes in neurobiological, inflammatory and insulin sensitivity markers, diurnal salivary cortisol in addition to changes in SMM, strength and physical function will be associated with changes in cognitive function.

6.3 Methods

6.3.1 Participants and experimental design

Thirty-six healthy older men [(mean ± SE) age: 66.9 ± 0.7 y] participated in this 12-week, 4-arm, double-blind RCT. All measurements were taken at baseline and following the 12-week intervention**.** Participants were randomised to either control (CON, *n* = 9), whey protein (PRO, *n* = 9), RE + control (EX+CON, *n* = 9), or RE + whey protein (EX+PRO, *n* = 9). Full details of the experimental design and eligibility criteria have been detailed previously in **Chapter 3** (**sections 3.2, 3.4.2.1 and 3.4.2.2**). Although not planned in the original study design, exploratory analyses were also conducted between pooled exercise (EX+CON and EX+PRO groups, *n* = 18) and non-exercise groups (CON and PRO groups, *n* = 18), and between pooled whey protein (PRO and EX+PRO groups, *n* = 18) and control supplement groups (CON and EX+CON groups, *n* = 18). All participants provided written informed consent in accordance with the Declaration of Helsinki. Ethical approval was granted by Coventry University Ethics Committee (project code: P59723). The flow of participants throughout the study has been previously presented and can be seen in **Chapter 3** (**Figure 3.1**).

6.3.2 Exercise training and nutritional supplements

Full details of the RE intervention completed by participants in the EX+CON and EX+PRO groups, and nutritional supplements consumed by all participants have been previously detailed (see **Chapter 3 - sections 3.5.1 and 3.5.2**). Briefly, participants in the PRO and

EX+PRO groups consumed 25 g whey protein containing ~3 g leucine, 0.7 g tryptophan and 0.8 tyrosine, whereas participants in the CON and EX+CON groups consumed an energy-matched carbohydrate control. Supplements were consumed twice daily (at breakfast and lunch). The RE intervention consisted of twice-weekly supervised whole-body RE. During each session, participants completed two sets of eight repetitions followed by one set to volitional failure on six exercise machines (leg press, lateral row, hamstring curl, chest press, leg extension and shoulder press). Resistance exercise was performed at 80% 1RM.

6.3.3 Dietary intake and habitual physical activity

Participants completed 3-day food records (2 weekdays and 1 weekend day) at baseline (prior to commencing the intervention), and during weeks 6 and 12. Prior to baseline measurements, participants also completed a 3-day dietary recall and were provided a copy to replicate their intake on the day before post-intervention cognitive testing. Habitual physical activity was assessed using a tri-axial accelerometer (Actigraph GT9X; Actigraph, Pensacola, Florida, USA) on the dominant wrist for 7 days at baseline and during the final week of the intervention. Full details of the dietary and accelerometery analyses have been previously described (see **Chapter 3 - sections 3.6.2 and 3.6.3**).

6.3.4 Skeletal muscle mass, maximal strength and physical function

Full details of the methods used to measure SMM, maximal strength and physical function have been previously described (see **Chapter 3 – section 3.6.1** for SMM measurement, and **Chapter 5 – sections 5.3.6 and 5.3.7** for maximal strength and functional performance assessments). Briefly, SMM was measured by BIA using the equation of Janssen et al. (2000a). Skeletal muscle index (SMI, kg/m^2) was calculated by dividing SMM by height squared. Maximal strength (1RM) was assessed on the leg press and leg extension machines. Physical function was assessed by the SPPB and the 6MWT. The SPPB included 3 timed tests [4 m gait speed, time to perform five chair raises, and standing balance (feet together,

semi-tandem and tandem)]. Each test was scored equally (0-4), and the total score between 0 and 12 was used for analysis.

6.3.5 Cognitive function

Cognitive function was assessed whilst participants resided in respiration chambers as previously detailed in **Chapter 4**. Testing was performed under highly controlled standardised environmental conditions (relative humidity: 57 ± 5%; temperature: 24 ± 0.5 ˚C). Participants were free from distractions at all times. To minimise diurnal variation, cognitive function was assessed at 1000 h at baseline and following the 12-week intervention. Prior to testing, participants were prohibited from consuming alcohol or caffeine for 24 h and consumed a standardised breakfast at 0900 h (Tesco fruit and fibre with semi-skimmed milk; Tesco PLC, Hertfordshire, UK). The quantity of breakfast was scaled for each participant, based on energy requirements to achieve energy balance within the respiration chamber. Three participants in this study (*n* = 1 participant in the CON, PRO and EX+CON groups) did not participate in respiration chamber experiments; however, testing procedures (i.e., testing environment, timing, breakfast consumption and alcohol/caffeine restrictions) remained consistent for these participants.

At baseline only (outside of the respiration chamber), the Mini-Mental State Examination (MSSE) was performed to determine general cognitive status (Folstein, Folstein, and McHugh 1975). At baseline and 12 weeks, participants completed a battery of neuropsychological tests (£10.46 per test) (CANTAB; Cambridge Cognition, Cambridge, UK) on a tablet computer (iPad Air 2; Apple Inc., California, USA). The CANTAB has previously been established as a valid and reliable method for detecting age-related cognitive impairments (Rabbitt and Lowe 2000, Wild et al. 2008). The testing battery assessed the cognitive domains working memory, episodic memory, executive function, recognition and mental response speed. These were analysed as previous work has demonstrated ageing typically affects these cognitive domains (Hedden and Gabrieli 2004). The age-related decline in these cognitive functions has also

been associated with reduced physical function (Coppin et al. 2006, Demnitz et al. 2017) and poor quality of life (Woods et al. 2015). The testing battery consisted of the following tests: Motor Screening Task (MOT), Reaction Time (RTI), Spatial Working Memory (SWM), Paired Associates Learning Test (PALT), Multitasking Test (MTT), and Delayed Matching to Sample (DMS). The order of cognitive tests was consistent for both visits. Full details of each test are detailed below. To eliminate a potential learning effect, participants were familiarised with the CANTAB interface prior to testing. A short familiarisation trial of each test was also performed immediately prior to the final assessment trial at both visits. The tablet computer was kept on a computer stand throughout cognitive testing to ensure the computer tablet height was consistent for both visits. Participants were asked to sit a comfortable distance from the screen; finger distance from the screen was recommended at enough room to easily touch the entire screen as quickly as possible.

6.3.5.1 Motor Screening Task (MOT) (Familiarisation test) – 2 min

The MOT was conducted to relax participants and familiarise them with the CANTAB interface. Participants were required to touch a flashing cross shown at different locations on the screen (**Figure 6.1**). The test measured the mean latency (ms) to correctly respond and touch the stimulus. The MOT provided a general assay of whether sensorimotor or comprehension difficulties limited collection of valid data from participants (Cambridge Cognition Ltd. 2020).

Figure 6.1 Visual display of the Motor Screening Task. © Copyright 2018 Cambridge Cognition Limited. All rights reserved.

6.3.5.2 Reaction Time (RTI) – 4 min

The RTI test assessed motor and mental response speed, and movement and reaction time (Cambridge Cognition Ltd. 2020). Participants were instructed to hold down a touchscreen button at the bottom of the screen (**Figure 6.2**). A yellow spot then appeared inside one of the five circles; participants were required to release the touchscreen button and touch the yellow spot as quickly as possible with the same finger. This was repeated 30 times. The following outcomes were analysed:

- Reaction time (ms) – the median time taken to release the response button after presentation of the target stimulus (for correct trials only).
- Movement time (ms) – the median time taken to release the response button and select the target stimulus (for correct trials only).

Figure 6.2 Visual display of the Reaction Time test. © Copyright 2018 Cambridge Cognition Limited. All rights reserved.

6.3.5.3 Spatial Working Memory (SWM) – 6 min

Spatial working memory assessed participants ability to retain and manipulate spatial information in working memory (Cambridge Cognition Ltd. 2020). The test also assessed notable executive function demands, including strategy. Participants were instructed to find a token hidden inside boxes and move the token into the column on the right-hand side of the screen (**Figure 6.3**). A token was never hidden in the same box twice; therefore, participants should not have returned to a box which had already contained a token. The test had three stages (6, 8 and 12 boxes). Outcomes analysed were:

- Between errors (*n*) - the number of times a box was reopened in which a token had previously been found. This indicated a failure to recall (calculated for 6, 8 and 12 box stages, and all stages combined).
- Strategy (*n*) - the number of times a new search began using the same box started with previously. A lower score indicates a high strategy use.

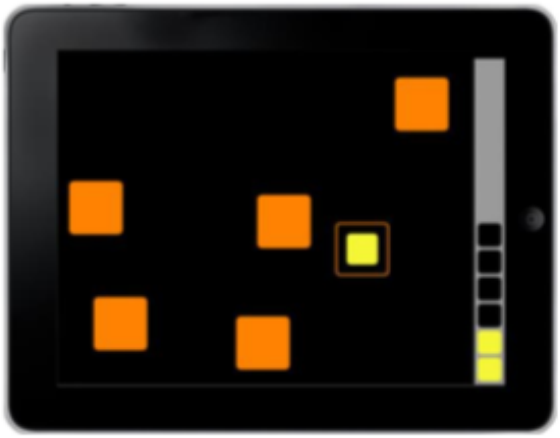

Figure 6.3 Visual display of the Spatial Working Memory test. © Copyright 2018 Cambridge Cognition Limited. All rights reserved.

6.3.5.4 Paired Associates Learning Test (PALT) – 12 min

The PALT assessed visuospatial episodic memory (Cambridge Cognition Ltd. 2020). Boxes were displayed on the screen and were opened one-by-one in a randomised order to reveal patterns hidden inside (**Figure 6.4**). Patterns were then displayed in the middle of the screen one at a time. Participants were required to touch the box where the pattern was located. If the participant made an error, the patterns were re-presented to remind them of their locations. The following outcomes were analysed:

- Total errors (*n*) – the number of times an incorrect box was chosen.
- First attempt memory score (*n*) - the number of times the correct box was chosen on the first attempt.

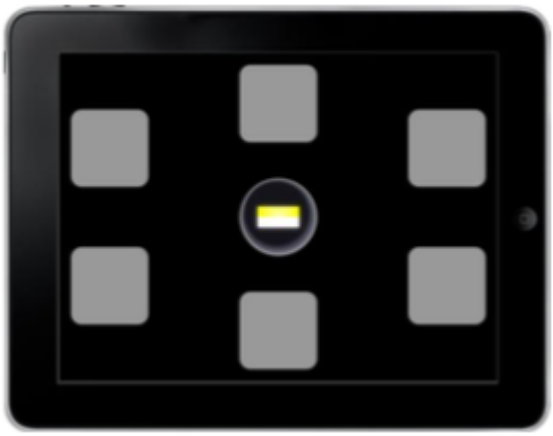

Figure 6.4 Visual display of the Paired Associates Learning Test. © Copyright 2018 Cambridge Cognition Limited. All rights reserved.

6.3.5.5 Multitasking Test (MTT) – 8 min

The MTT assessed executive functioning. The test measured participants ability to use multiple sources of potentially conflicting information to guide behaviour (Cambridge Cognition Ltd. 2020). An arrow was displayed on either the right- or left-hand side of the screen and participants were required to make a right or left response. Participants learnt whether to respond either according to the arrow direction, or according to the side of the screen the arrow appeared, no matter what the direction was. During the assessment stage, a cue was presented prior to the presentation of the arrow indicating whether the participant should respond according to the arrow direction *or* side (**Figure 6.5**). In some sections of the test, the same rule was applied consistently, whereas in the final phase, the rules were intermixed in a random order. The direction and side of the arrow was also incongruent for some trials. Outcomes analysed were:

- Total incorrect (*n*) – the number of trials the incorrect button within the response window was pressed.
- Reaction latency (ms) – the median latency of response (from stimulus appearance to pressing the response button - calculated across all correct trials).

- Incongruency cost (ms) – the difference between the reaction latency on trials that were congruent versus trials that were incongruent. A higher incongruency cost indicates processing conflicting information takes a longer.
- Multitasking cost (ms) – the difference between the reaction latency during trials in which both rules (direction *and* side) were used versus trials in which only a single rule (direction *or* side) was used. A positive score signifies a slower response during multitasking trials, indicating a higher cost of managing multiple sources of information.

Figure 6.5 Visual display of the Multitasking Test. © Copyright 2018 Cambridge Cognition Limited. All rights reserved.

6.3.5.6 Delayed Matching to Sample (DMS) – 7 min

Delayed matching to sample measured short-term visual recognition memory (Cambridge Cognition Ltd. 2020). The test measured participants' ability to match a complex visual pattern to four samples, both simultaneously, or after a brief delay (0, 4 or 12 s) (**Figure 6.6**). Participants were instructed to touch the pattern that matched the sample. The percentage (%) of correct trials (for 0, 4 and 12 s delays individually, and all delays combined) were used as outcome measures.

Figure 6.6 Visual display of the Delayed Matching to Sample test. © Copyright 2018 Cambridge Cognition Limited. All rights reserved.

6.3.5.7 Domain-specific z-scores

In addition to analysis of individual cognitive function test scores, individual scores were transformed into z-scores [(value-mean)/SD] at baseline and 12 weeks, using the mean and SD of the total sample at baseline as the reference population (Formica et al. 2020). To remain consistent with other cognitive outcomes (i.e., a higher z-score indicating better cognitive performance), z-scores relating to reaction time and errors, where a lower score indicates better performance, were reversed (value x -1). Individual z-scores were clustered into compound scores for the following five cognitive domains:

- Psychomotor/attention = $(Z_{\text{MOT mean latency}} + Z_{\text{RTI reaction time}} + Z_{\text{RTI movement time}})/3$
- Executive function = $(Z_{\text{MTT total incorrect}} + Z_{\text{MTT reaction latency}} + Z_{\text{MTT incongruency cost}} + Z_{\text{MTT multitasking cost}} + Z_{\text{SWM strategy score}})/5$
- Episodic memory = $(Z_{\text{PALT total errors}} + Z_{\text{PALT first attempt memory score}})/2$
- Working memory = $[Z_{\text{SWM between errors (all boxes)}} + Z_{\text{SWM between errors (6 boxes)}} + Z_{\text{SWM between errors (8 boxes)}} + Z_{\text{SWM between errors (12 boxes)}}]/4$
- Global cognitive function = $(Z_{\text{composite psychomotor/attention}} + Z_{\text{composite executive function}} + Z_{\text{composite episodic memory}} + Z_{\text{composite working memory}})/4$

6.3.6 Blood pressure

Blood pressure was measured at multiple times (at 2130, 0805, 0900, 1225, 1355, 1715, 1835 and 1955 h) whilst participants (*n* = 33) resided in the respiration chamber for 24 h under highly controlled conditions (see **Chapter 4**). Measurements were taken whilst participants were rested in a seated position using a British and Irish Hypertension Society (BIHS) validated automated BP monitor (Omron M6; Omron Health Care Ltd., Milton Keynes, UK). Prior to entering the chamber, participants were trained how to correctly self-administer BP measurement. Systolic and diastolic BP measured in the morning (0805 h), and the average systolic and diastolic BP over the 8 measurements (2130-1955 h) were used as outcome measures. Blood pressure was assessed as previous studies have shown an association between hypertension and cognitive impairment in older adults (Kritz-Silverstein et al. 2017, McDonald et al. 2017). It has been suggested that this relationship might be mediated by vascular reserve impairment and microvascular disease (Novak and Hajjar 2010).

6.3.7 Blood and saliva collection and analysis

Venous blood was collected at 0815 h following a >10 h overnight fast. Whole blood was collected into EDTA, heparin and SST vacutainers then centrifuged at 1900 x g for 10 min at 4°C. Serum samples were rested for 30 min prior to centrifuging to allow for sufficient clotting. Aliquots containing plasma and serum were stored at -80°C until analysis (see **section 3.6.4** for detailed blood collection methodology). Due to difficulty in blood collection, blood was unable to be drawn from two participants (*n* = 1 participant in the CON and EX+CON groups). Therefore, *n* = 34 participants had full blood data. Plasma glucose was analysed using a glucose analyser (Biosen C-Line Glucose and Lactate Analyser; EKF Diagnostics, Cardiff, UK). Commercially available ELISA kits were used to analyse serum total BDNF (Item No. DNBT00) and IGF-1 (Item No. DB100B), and plasma IL-6 [Item No. D6050 and HS600C (the latter for high sensitivity)], TNF-α (Item No. HSTA00E), CRP (Item No. DCRP00) (R&D Systems Inc., Abbington, UK), and insulin (EIA-2935; DRG Instruments GmbH, Marburg, Germany). Insulin resistance (HOMA-IR) and sensitivity (QUICKI) were calculated from fasting

glucose and insulin concentrations using standard equations (Katz et al. 2000, Matthews et al. 1985):

HOMA-IR: Fasting insulin (μU/mL) x fasting glucose (mmol/L) / 22.5

QUICKI: 1 / [log(fasting insulin (μU/mL)) + log(fasting glucose (mmol/L))].

The CV for plasma glucose was 0.5%, and the intra-assay CV was 9.5% for plasma insulin, 8.5% for serum BDNF, 9.1% for serum IGF-1, 9.8% and 11.8% for plasma IL-6 and IL-6 high sensitivity, respectively, 9.9% for high sensitivity plasma TNF-α, and 9.9% for plasma CRP.

Saliva samples were collected whilst participants resided in the respiration chamber. As previously mentioned, three participants (*n* = 1 participant from CON, PRO and EX+CON groups) in this study did not participate in respiration chamber measurements; therefore, saliva was collected from *n* = 33 participants. Samples were collected immediately upon waking at 0650 h, and at 0805, 1225, 1700 and 2000 h using a synthetic swab (Salivette; Sarstedt Nümbrecht, Germany). Samples were centrifuged at 1900 x g for 2 min and stored at -80°C until analysis. Samples were analysed for salivary cortisol by ELISA (Item No. 1-3002; Salimetrics, Pennsylvania, USA). The intra-assay CV was 9%. Salivary cortisol data (samples 1-5; 0650-200 h) was used to calculate multiple indices. Firstly, all five samples were used to calculate salivary cortisol AUC (nmol/L x 790 min) using the trapezoidal method. Secondly, cortisol slope (peak-to-evening) was calculated as the rate of salivary cortisol change from the peak morning (0650 or 0805 h, whichever concentration was highest) to the evening (2000 h) (Adam et al. 2017). Thirdly, salivary cortisol at 2000 h was also analysed individually as elevated evening cortisol has been associated with poorer cognitive functioning (Geerlings et al. 2015).

6.3.8 Statistical analysis

Statistical analysis performed in this chapter has previously been detailed and can be seen in **Section 3.7**.

6.4 Results

6.4.1 Participants and compliance

Thirty-nine older men were randomised: 36 completed the study and 3 withdrew. Reasons for withdrawal have been previously detailed in **Chapter 3** (see **Figure 3.2**). Participants were 66.9 ± 0.7 years of age, had no cognitive impairments [assessed by the MMSE (29.4 ± 0.1 points)], had 14.4 ± 0.5 years of full-time education and were slightly overweight according to BMI (25.5 ± 0.4 kg/m^2). Baseline characteristics of the 36 participants who completed the study are shown in **Table 6.1**. Compliance was high for both consumption of the nutritional supplements (CON: 94.1 ± 1.2%; PRO: 96.8 ± 1.0%; EX+CON: 96.1 ± 1.3%; EX+PRO: 96.1 ± 1.3%) and attendance to the RE training programme (EX+CON: 98.2 ± 1.0%; EX+PRO: 98.2 ± 1.2%). During the final set (to volitional failure) the mean number of completed repetitions was 9.1 ± 0.3 in the EX+CON group and 9.1 ± 0.2 in the EX+PRO group. No differences occurred between groups for supplement compliance (P = 0.50), or between RE groups for exercise compliance (P = 0.63) or the mean number of repetitions completed on the final set of each exercise (P = 0.97).

Table 6.1 Baseline characteristics of participants[1]

	CON	PRO	EX+CON	EX+PRO	*P* value[3]	Overall
n	9	9	9	9	-	36
Age, y	67.2 ± 1.7	65.6 ± 1.7	67.1 ± 1.3	67.8 ± 1.3	0.75	66.9 ± 0.7
MSSE, points	29.6 ± 0.3	29.3 ± 0.3	29.4 ± 0.3	29.4 ± 0.3	0.88	29.4 ± 0.1
Education, y	14.4 ± 0.9	14.0 ± 1.0	14.7 ± 1.2	14.4 ± 0.9	0.96	14.4 ± 0.5
Height, m	1.77 ± 0.01	1.76 ± 0.03	1.77 ± 0.02	1.74 ± 0.03	0.71	1.76 ± 0.01
Body mass, kg	79.0 ± 3.4	78.0 ± 3.1	78.2 ± 3.9	80.9 ± 4.0	0.94	79.0 ± 1.8
BMI, kg/m^2	25.1 ± 1.0	25.0 ± 0.6	25.1 ± 0.9	26.6 ± 0.8	0.50	25.5 ± 0.4
SMM, kg	26.7 ± 0.6	27.2 ± 0.7	25.9 ± 1.1	26.9 ± 1.3	0.79	26.7 ± 0.5
SMI, kg/m^2	8.5 ± 0.2	8.8 ± 0.2	8.3 ± 0.2	8.9 ± 0.3	0.19	8.6 ± 0.1
FM, kg	19.2 ± 2.4	18.0 ± 1.7	19.6 ± 2.0	20.4 ± 1.5	0.85	19.3 ± 0.3
FM, %	23.8 ± 2.0	22.7 ± 1.5	24.8 ± 1.7	25.1 ± 1.2	0.74	24.1 ± 0.8
Leg extension 1RM, kg	63 ± 6	58 ± 3	52 ± 5	59 ± 4	0.52	58 ± 2
Leg press 1RM, kg	116 ± 9	107 ± 7	107 ± 9	118 ± 7	0.66	112 ± 4
SPPB, points	11.7 ± 0.2	11.4 ± 0.2	11.2 ± 0.3	11.8 ± 0.1	0.38	11.5 ± 0.1
6MWT, m	639 ± 21	616 ± 18	627 ± 30	591 ± 26	0.54	618 ± 12
Gait speed, m/s	1.09 ± 0.08	1.13 ± 0.04	1.14 ± 0.04	1.22 ± 0.05	0.51	1.15 ± 0.02
Systolic BP, mmHg	127 ± 6	133 ± 6	133 ± 7	136 ± 4	0.57	132 ± 3
Diastolic BP, mmHg	78 ± 2	76 ± 3	77 ± 4	77 ± 4	0.98	77 ± 1

[1]Values are means ± SE. [3]*P* value refers to differences between groups analysed by one-way ANOVA. No significant differences in baseline characteristics occurred between pooled exercise and non-exercise groups, or between pooled whey protein and control supplement groups (data not shown). 1RM, one repetition maximum; BMI, body mass index; BP, blood pressure; FM, fat mass; MMSE, mini-mental state examination; SMM, skeletal muscle mass; SPPB, short physical performance battery. Education (y) refers to the total amount of full-time education attended from primary school up to postgraduate university level.

6.4.2 Habitual dietary intake and physical activity

Dietary intake and habitual physical activity data during the intervention period has been previously reported in **Chapter 5** (**sections 5.4.3 and 5.4.4**). Briefly, protein intake increased over time in the PRO and EX+PRO groups greater than both the CON and EX+CON groups at weeks 6 ($P < 0.001$) and 12 ($P < 0.001$). Carbohydrate intake increased over time in the EX+CON group greater than the CON and EX+PRO groups at week 6 ($P < 0.05$), and greater than the PRO and EX+PRO groups at week 12 ($P < 0.05$). Total EI increased over time in the EX+PRO group at week 6 ($P = 0.03$) and increased in the CON group at weeks 6 and 12 ($P < 0.05$). No within- or between-group differences occurred for time spent sedentary, or in light or MVPA.

6.4.3 Skeletal muscle mass, maximal strength, and physical function

Skeletal muscle mass, maximal strength, and physical function changes over the intervention period for each intervention group have previously been reported in detail in **Chapter 5**. Briefly, SMM increased over time in the EX+CON (0.5 ± 0.2 kg) and EX+PRO groups (0.6 ± 0.3 kg), both of which trended towards statistical significance ($P = 0.06$). When RE groups were pooled, increases in SMM over time became significant (0.6 ± 0.2 kg, $P = 0.006$) and SMI also significantly increased (0.2 ± 0.1 kg/m^2, $P = 0.007$). Leg extension and leg press 1RM significantly increased in only the EX+CON (leg extension: +38%; 52 ± 5 to 72 ± 6 kg, $P < 0.001$; leg press: +28%, 107 ± 9 to 137 ± 8 kg, $P < 0.001$) and EX+PRO groups (leg extension: +36%; 59 ± 4 to 80 ± 4 kg, $P < 0.001$; leg press: +33%, 118 ± 7 to 157 ± 7 kg, $P < 0.001$). Increases in both leg press and leg extension 1RM in the EX+CON and EX+PRO groups were greater than both the CON and PRO groups ($P < 0.001$). Six minute walk test distance also increased over time in only these groups (EX+CON: 21 ± 7 m, $P = 0.02$; EX+PRO: 21 ± 6 m, $P = 0.007$). When RE groups were pooled, 6MWT distance increased over time greater than non-exercise groups pooled ($P = 0.04$). Gait speed increased by 0.11 ± 0.06 m/s in the PRO group, which tended to increase over time greater than the CON group ($P = 0.06$).

6.4.4 Cognitive function

6.4.4.1 Motor Screening Task (MOT)

All participants completed the MOT to ensure familiarisation with the CANTAB interface. No participants displayed any sensorimotor or comprehension difficulties during the test at either baseline or 12 weeks. Data collected at both visits was therefore deemed valid for all participants. At baseline, mean latency was comparable between groups (P = 0.40, **Table 6.2**). Mean latency significantly decreased over time in the PRO (-101.3 ± 32.6 ms, P = 0.02, d = -1.10), EX+CON (-80.3 ± 29.2 ms, P = 0.02, d = -1.22) and EX+PRO groups (-80.6 ± 24.2 ms, P = 0.02, d = -1.42). No changes were observed in the CON group (27.0 ± 48.4 ms, P = 0.47, d = 0.24). When whey protein supplement groups were pooled, mean latency decreased over time greater than control supplement groups pooled (P = 0.03). The same effect was not observed between pooled exercise and non-exercise groups (P = 0.12).

6.4.4.2 Reaction Time (RTI)

Both reaction (P = 0.36) and movement time (P = 0.52) were similar between groups at baseline and no within- or between-group differences occurred for either variable over the course of the study. No differences occurred between pooled exercise and non-exercise groups (P = 0.91; P = 0.93, respectively), or between pooled whey protein and control supplement groups (P = 0.34; P = 0.61, respectively).

6.4.4.3 Spatial Working Memory (SWM)

Spatial working memory test scores were comparable between groups at baseline (P > 0.61). Following the intervention, between errors (12 boxes) decreased in only the PRO group by 38% (-14.7 ± 4.9, P = 0.02, d = -1.0, **Figure 6.7**). Differences between groups were not significant (P = 0.09). No within- or between-group differences were observed for any other

SWM test score, and no differences occurred between pooled exercise and non-exercise groups, or between pooled whey protein and control supplement groups.

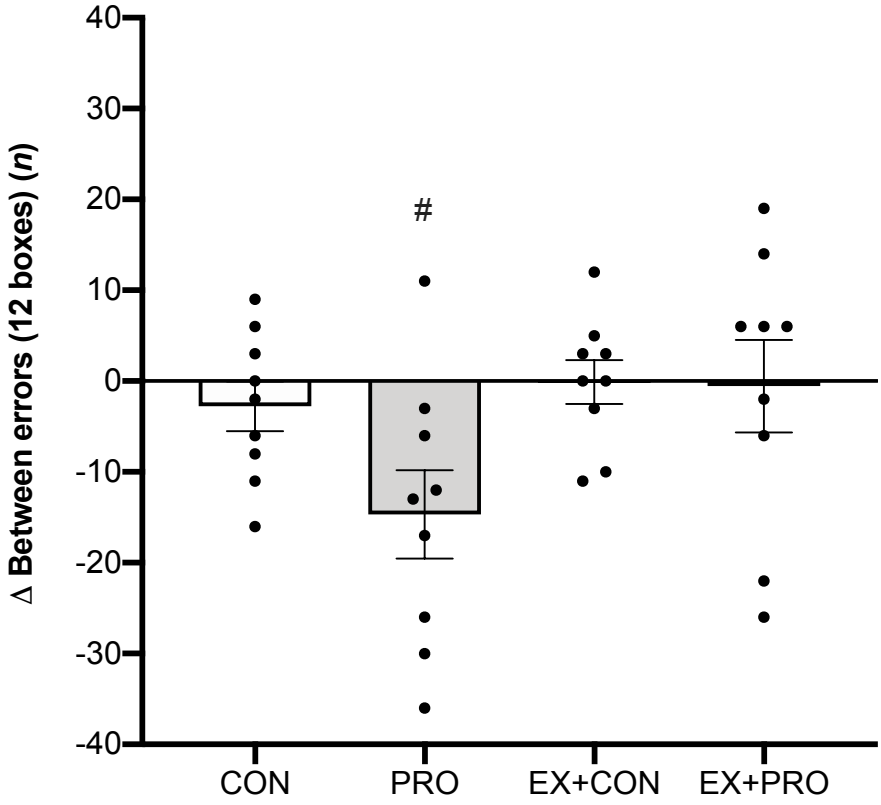

Figure 6.7 Change in between errors (12 box stage) on the Spatial Working Memory test for each treatment group over the intervention period (means ± SE). Circles represent individual data points. $^{\#}P < 0.05$ from baseline.

Table 6.2 Cognitive function test scores for each treatment group at baseline and 12 weeks[1]

	CON		PRO		EX+CON		EX+PRO		*P* value	
	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Time	Group x time
Motor Screening Task										
Mean latency, ms	801.6 ± 25.1	827.8 ± 29.4	873.6 ± 43.6	772.3 ± 39.7#	883.2 ± 69.8	789.1 ± 63.1#	800.8 ± 49.0	720.2 ± 52.4#	0.05	0.06
Reaction time										
5-choice reaction time, ms	364.1 ± 12.7	371.1 ± 10.1	386.8 ± 15.4	395.2 ± 17.0	400.0 ± 15.0	402.4 ± 19.6	388.3 ± 9.2	392.9 ± 9.5	0.36	0.98
5-choice movement time, ms	303.7 ± 22.4	284.9 ± 21.3	322.9 ± 20.8	321.4 ± 18.9	282.3 ± 17.0	308.7 ± 14.2	293.5 ± 17.7	279.1 ± 10.5	< 0.001	0.30
Spatial Working Memory										
Between errors (All boxes), *n*	13.5 ± 2.6	9.1 ± 3.0	15.6 ± 3.3	11.4 ± 3.0	16.3 ± 3.3	12.9 ± 2.6	14.2 ± 2.6	13.4 ± 3.4	0.21	0.75
Between errors (6 boxes), *n*	3.2 ± 1.0	2.1 ± 0.9	4.4 ± 1.2	2.3 ± 0.9	4.8 ± 1.0	4.0 ± 1.0	3.3 ± 1.0	2.8 ± 1.2	0.02	0.87
Between errors (8 boxes), *n*	10.8 ± 1.9	6.2 ± 1.9	10.0 ± 2.3	8.6 ± 2.1	10.8 ± 1.6	8.0 ± 1.7	10.2 ± 2.2	9.6 ± 2.3	0.007	0.72
Between errors (12 boxes), *n*	35.4 ± 3.1	32.7 ± 3.0	38.7 ± 4.7	24.0 ± 3.9#	31.8 ± 2.4	31.7 ± 3.0	32.7 ± 5.1	32.1 ± 4.7	0.009	0.09
Strategy score	8.1 ± 0.6	6.6 ± 1.1	7.4 ± 1.1	6.3 ± 1.1	8.6 ± 1.1	8.3 ± 0.8	8.7 ± 1.0	7.1 ± 1.1	0.01	0.61
Paired Associates Learning										
Total errors, *n*	15.8 ± 4.2	15.9 ± 4.4	16.0 ± 3.5	17.1 ± 4.5	24.9 ± 3.9	21.1 ± 5.5	24.9 ± 5.9	11.9 ± 2.5#	0.77	0.37
First attempt memory score, *n*	13.2 ± 1.1	11.8 ± 1.1	12.8 ± 1.0	12.6 ± 1.6	9.9 ± 1.1	11 .3 ± 1.6	10.7 ± 1.7	12.8 ± 1.4	0.03	0.54
Multitasking Test										
Total incorrect,[2] *n*	2.4 ± 0.8	1.6 ± 0.6	4.2 ± 1.5	2.1 ± 0.7	3.9 ± 1.8	2.3 ± 0.6	4.1 ± 1.9	1.8 ± 0.6	0.02	0.90
Reaction latency, ms	724.2 ± 20.9	723.6 ± 20.7	741.9 ± 38.8	735.4 ± 32.0	698.2 ± 47.1	737.5 ± 38.2	715.4 ± 39.0	713.1 ± 39.8	0.002	0.71
Incongruency cost,[3] ms	87.2 ± 29.9	86.1 ± 20.8	81.3 ± 19.5	63.9 ± 16.6	82.6 ± 13.9	116.3 ± 17.6#	92.1 ± 14.8	98.6 ± 18.6	0.01	0.10
Multitasking cost,[3,4] ms	205.9 ± 40.0	202.5 ± 29.4	337.7 ± 64.4	219.9 ± 45.0#	324.8 ± 43.1	218.3 ± 34.9#	270.9 ± 64.3	233.5 ± 38.7	0.02	0.41
Delayed Matching to Sample										
% correct (All delays)	85.1 ± 2.4	88.9 ± 2.1	81.4 ± 3.5	84.6 ± 3.3	86.6 ± 2.9	80.7 ± 4.5	84.4 ± 3.1	84.4 ± 3.7	0.001	0.77
% correct (0 s delay)	84.4 ± 4.4	91.1 ± 3.5	82.2 ± 5.2	88.9 ± 6.8	91.1 ± 3.5	82.2 ± 7.0	95.6 ± 2.9	84.4 ± 6.5	< 0.001	0.98
% correct (4 s delay)	86.7 ± 5.8	84.4 ± 5.6	80.0 ± 6.7	86.7 ± 5.8	84.4 ± 5.6	82.2 ± 4.0	80.0 ± 8.8	88.9 ± 4.8	< 0.001	0.67
% correct (12 s delay)	84.4 ± 4.4	91.1 ± 4.8	82.2 ± 5.2	77.8 ± 6.2	84.4 ± 2.9	77.8 ± 6.2	77.8 ± 6.2	80.0 ± 5.8	0.001	0.33
Domain specific z-scores										
Psychomotor/attention	0.23 ± 0.17	0.20 ± 0.19	-0.21 ± 0.22	-0.05 ± 0.25	-0.14 ± 0.24	-0.11 ± 0.28	0.09 ± 0.24	0.33 ± 0.16	0.12	0.50
Executive function	0.15 ± 0.18	0.29 ± 0.15	-0.08 ± 0.26	0.30 ± 0.18#	0.03 ± 0.13	-0.07 ± 0.16	-0.24 ± 0.31	0.06 ± 0.27#	0.08	0.32
Episodic memory	0.34 ± 0.29	0.15 ± 0.29	0.27 ± 0.24	0.21 ± 0.36	-0.41 ± 0.27	-0.09 ± 0.39	-0.32 ± 0.42	0.43 ± 0.26	0.047	0.46
Working memory	-0.10 ± 0.63	0.79 ± 0.55	-0.60 ± 0.86	1.80 ± 0.68#	0.29 ± 0.50	0.58 ± 0.48	0.37 ± 0.82	0.54 ± 0.82	0.001	0.10
Global cognitive function	0.16 ± 0.27	0.36 ± 0.22	-0.15 ± 0.28	0.58 ± 0.24#	-0.06 ± 0.22	0.08 ± 0.22	-0.03 ± 0.34	0.33 ± 0.31	< 0.001	0.15

[1]Values are means ± SE. [2]CON (*n* = 8), *n* = 1 participants data removed (>3SD from mean). [3]EX+PRO (*n* = 8), *n* = 1 participants data removed (>3 SD from mean). [4]EX+CON (*n* = 8), *n* = 1 participants data removed (>3 SD from mean). #*P* < 0.05 from baseline.

6.4.4.4 Paired Associates Learning Test (PALT)

At baseline, no differences in total errors (P = 0.25) or first attempt memory score (P = 0.35) occurred between groups. At 12 weeks, total errors decreased in the EX+PRO group by 52.2% (-13.0 ± 5.5, P = 0.045, d = -0.79, **Figure 6.8A**), but no differences occurred between groups (P = 0.37). No within- or between-group differences were observed for first attempt memory score.

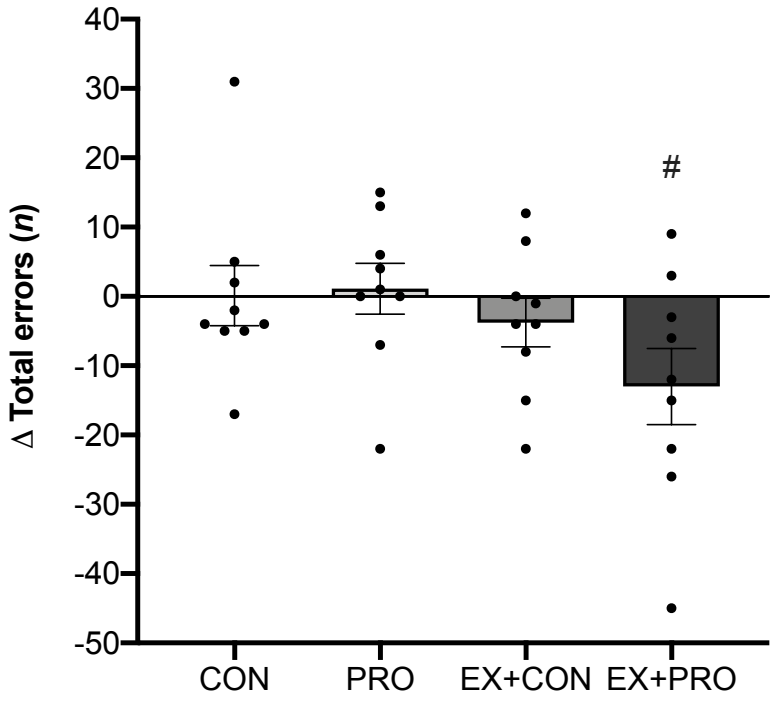

Figure 6.8 Change in total errors on the Paired Associates Learning Test between groups and (means ± SE). Circles represent individual data points. $^{\#}P < 0.05$ from baseline.

6.4.4.5 Multitasking Test (MTT)

Multitasking test scores were similar between groups at baseline (P > 0.25). Following the intervention, incongruency cost increased in the EX+CON group by 40.7% (33.8 ± 10.1 ms, P = 0.03, d = 1.17), but no significant differences occurred between groups (P = 0.10). When exercise groups were pooled, incongruency cost increased compared to non-exercise groups pooled (P = 0.046, **Figure 6.9A**). **Figure 6.9B** shows multitasking cost decreased by 34.9%

in the PRO group (-117.8 ± 25.8 ms, P = 0.002, d = -1.50), and by 32.8% in the EX+CON (-106.5 ± 32.9 ms, P = 0.01, d = -1.14). No differences occurred between groups (P = 0.41).

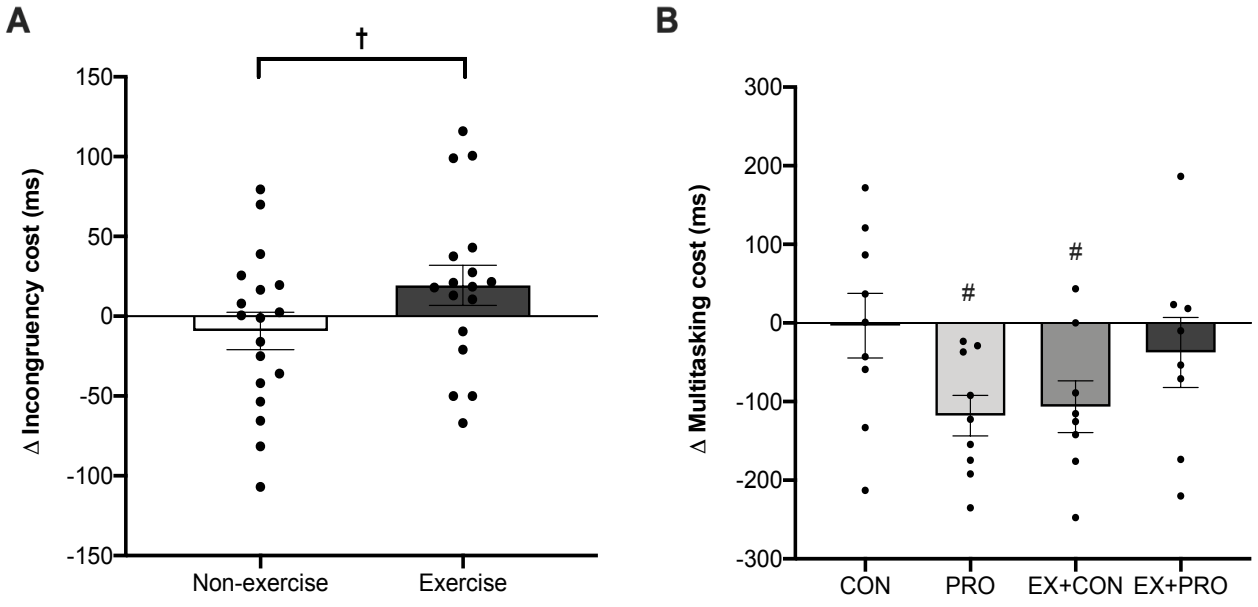

Figure 6.9 Changes in (A) incongruency cost between pooled exercise (n = 17) and non-exercise (n = 18) groups, and (B) multitasking cost between groups over the intervention period on the Multitasking Test (means ± SE). Circles represent individual data points. n = 1 participant's data removed from the pooled exercise group for incongruency cost, and n = 1 participant's data removed from the EX+CON and EX+PRO groups data for multitasking cost (>3 SD from mean). †Significant group x time interaction, P = 0.046. #P < 0.05 from baseline.

6.4.4.6 Delated Matching to Samples (DMS)

At baseline, no differences were observed between groups for any DMS test score and no within- or between-group differences occurred over the intervention period. No differences occurred between pooled exercise and non-exercise groups, or between pooled whey protein and control supplement groups for any DMS test score.

6.4.4.7 Domain-specific composite z-scores

Composite domain-specific z-scores were similar between groups at baseline (P > 0.22). For changes from baseline to 12 weeks (**Table 6.2**), the PRO group experienced increases in

executive function (0.38 ± 0.12 SD, *P* = 0.01, *d* = 1.05), working memory (2.4 ± 0.76 SD, *P* = 0.01, *d* = 1.05) and global cognitive function (0.72 ± 0.24 SD, *P* = 0.02, *d* = 0.98). In the EX+PRO group, composite executive function z-score significantly increased over time by 0.30 ± 0.12 SD (*P* = 0.04, *d* = 0.78). No significant changes over time for any domain-specific z-score was observed in either the CON or EX+CON groups, and no differences occurred between groups. When exercise groups were pooled, episodic memory z-score increased by 0.53 ± 0.23 SD (*P* = 0.03, *d* = 0.54, **Figure 6.10A**), but the increase was not significantly greater than non-exercise groups pooled (*P* = 0.14). When whey protein groups were pooled, global cognitive function tended to increase greater than control supplement groups pooled (*P* = 0.06, **Figure 6.10B**).

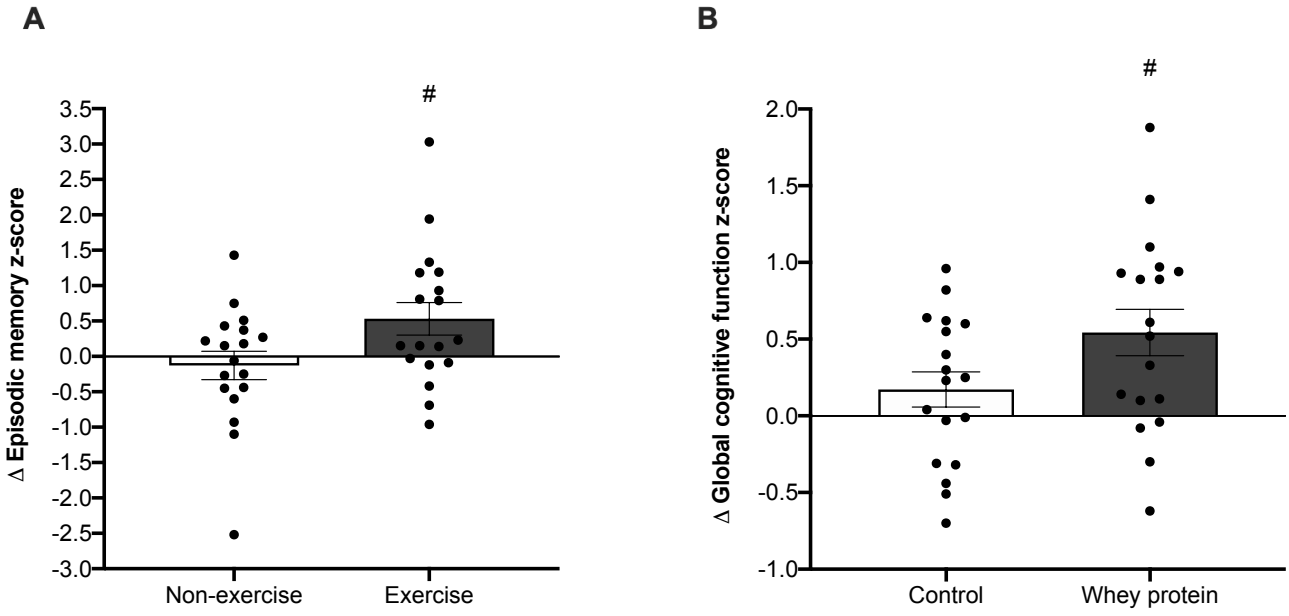

Figure 6.10 Changes in domain-specific z-scores for (A) episodic memory between pooled exercise (*n* = 18) and non-exercise groups (*n* = 18), and (B) global cognitive function between pooled whey protein (*n* = 18) and control supplement (*n* = 18) groups (means ± SE). Circles represent individual data points. $^{\#}P < 0.05$ from baseline.

6.4.5 Blood pressure

Morning (0805 h) and average systolic and diastolic BP (8 readings between 2130-1955 h) were similar between groups at baseline ($P > 0.47$, **Table 6.1**). At 12 weeks, morning systolic and diastolic BP decreased in only the PRO group by 6.4% (-8 ± 2 mmHg, $P = 0.001$) and 7% (-4 ± 2 mmHg, $P = 0.02$), respectively. No differences occurred between groups for any BP variable.

6.4.6 Neurobiological, inflammatory, salivary cortisol and insulin sensitivity markers

At baseline, no differences occurred between groups for any of the above markers ($P > 0.08$). Following the intervention, both plasma IL-6 and TNF-α significantly decreased in the EX+PRO group by 21% (-1.2 ± 0.6 pg/mL, $P = 0.01$) and 20% (-0.6 ± 0.2 pg/mL, $P = 0.03$), respectively (**Table 6.3**). No differences occurred between groups for either variable ($P = 0.13$; $P = 0.11$, respectively). As previously reported in **Chapter 5** (see **Figure 5.4**), when RE groups were pooled, both IL-6 ($P = 0.048$) and TNF-α ($P = 0.02$) decreased greater than non-exercise groups pooled. Insulin resistance (HOMA-IR) decreased significantly over time in only the EX+PRO group (-0.5 ± 0.2, $P = 0.04$), and insulin sensitivity (QUICKI) increased in both the PRO (0.02 ± 0.01, $P = 0.04$) and EX+PRO groups (0.02 ± 0.01, $P = 0.02$). No differences occurred between groups for either variable ($P = 0.23$; $P = 0.22$, respectively). The slope of salivary cortisol from peak morning to evening increased in the EX+PRO group by 92.1% (8.1 ± 2.8 nmol/L, $P = 0.02$), but no differences occurred between groups ($P = 0.15$). No within- or between-group differences were observed for plasma CRP, serum total BDNF or IGF-1, or salivary cortisol AUC or 2000 h salivary cortisol.

Table 6.3 Fasting neurobiological, inflammatory, insulin sensitivity and salivary cortisol markers for each treatment group at baseline and 12 weeks[1]

	CON		PRO		EX+CON		EX+PRO		*P* value	
	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Time	Group x time
Serum total BDNF,[2] pg/mL	27.1 ± 3.2	19.8 ± 2.9	22.8 ± 1.4	25.7 ± 3.2	22.5 ± 2.9	28.6 ± 4.4	26.6 ± 1.1	25.2 ± 1.9	0.01	0.22
Serum IGF-1,[2] ng/mL	152 ± 34	130 ± 29	119 ± 17	110 ±14	137 ± 16	119 ± 12	118 ± 15	100 ± 10	0.07	0.86
Plasma IL-6,[2] pg/mL	4.9 ± 1.2	5.8 ± 1.3	4.0 ± 1.0	4.0 ± 1.2	3.2 ± 0.9	2.4 ± 0.6	5.8 ± 1.8	4.6 ± 1.2$^{\#}$	0.09	0.13
Plasma TNF-α,[2] pg/mL	3.2 ± 0.3	3.0 ± 0.3	2.4 ± 0.3	3.0 ± 0.3	3.4 ± 0.6	2.7 ± 0.3	3.0 ± 0.4	2.4 ± 0.2$^{\#}$	< 0.001	0.11
Plasma CRP,[2] ng/mL	2.4 ± 0.6	1.7 ± 0.3	1.6 ± 0.4	1.1 ± 0.5	0.8 ± 0.2	0.8 ± 0.2	2.0 ± 0.4	1.8 ± 0.2	0.18	0.18
Salivary cortisol AUC,[3] nmol/L x 790 min	4067 ± 551	4349 ± 528	4088 ± 196	45450 ± 425	5588 ± 969	5326 ± 778	4127 ± 587	4530 ± 414	0.001	0.99
Salivary cortisol (2000 h),[3] nmol/L	2.1 ± 0.5	1.9 ± 0.5	1.9 ± 0.4	2.2 ± 0.6	2.2 ± 0.6	1.8 ± 0.7	2.1 ± 0.7	2.6 ± 0.6	0.009	0.64
Salivary cortisol slope,[3] nmol/L	9.6 ± 1.1	9.1 ± 1.4	10.6 ± 0.8	13.6 ± 2.4	12.5 ± 2.5	15.7 ± 2.7	8.8 ± 1.4	16.9 ± 3.5$^{\#}$	0.004	0.15
HOMA-IR[2]	2.6 ± 0.4	2.6 ± 0.5	2.7 ± 0.4	2.3 ± 0.6	2.9 ± 0.7	2.4 ± 0.6	2.2 ± 0.4	1.6 ± 0.3$^{\#}$	0.15	0.23
QUICKI[2]	0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.36 ± 0.02$^{\#}$	0.33 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.37 ± 0.01$^{\#}$	0.18	0.22

[1]Values are means ± SE. [2]*n* = 34; CON (*n* = 8), PRO (*n* = 9), EX+CON (*n* = 8), EX+PRO (*n* = 9). [3]*n* = 33; CON (*n* = 8), PRO (*n* = 8), EX+CON (*n* = 8), EX+PRO (*n* = 9). AUC, area under the curve; BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor 1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; QUICKI, quantitative insulin-sensitivity check index. $^{\#}P <$ 0.05 from baseline.

6.4.7 Correlation analysis

No significant correlations (baseline or Δbaseline) were observed between any neurobiological, inflammatory, salivary cortisol or insulin sensitivity marker and any domain specific cognitive function z-score. Similarly, no correlations (baseline or Δbaseline) were observed for morning or average systolic or diastolic BP and any cognitive domain z-score. In contrast, at baseline, leg extension 1RM positively correlated with global cognitive function z-score (r = 0.34, P = 0.045, **Figure 6.11A**). Significant positive correlations were also observed between SPPB score and psychomotor/attention (ρ = 0.51, P = 0.002, **Figure 6.11B**) and episodic memory z-scores (ρ = 0.35, P = 0.04, **Figure 6.11C**), and between 6MWT distance and psychomotor/attention z-score (r = 0.39, P = 0.02, **Figure 6.11D**). Following the intervention, ΔSMI positively correlated with Δepisodic memory z-score (r = 0.34, P = 0.046, **Figure 6.11E**). No correlations were observed for changes in maximal strength or physical function tests and any domain-specific z-score.

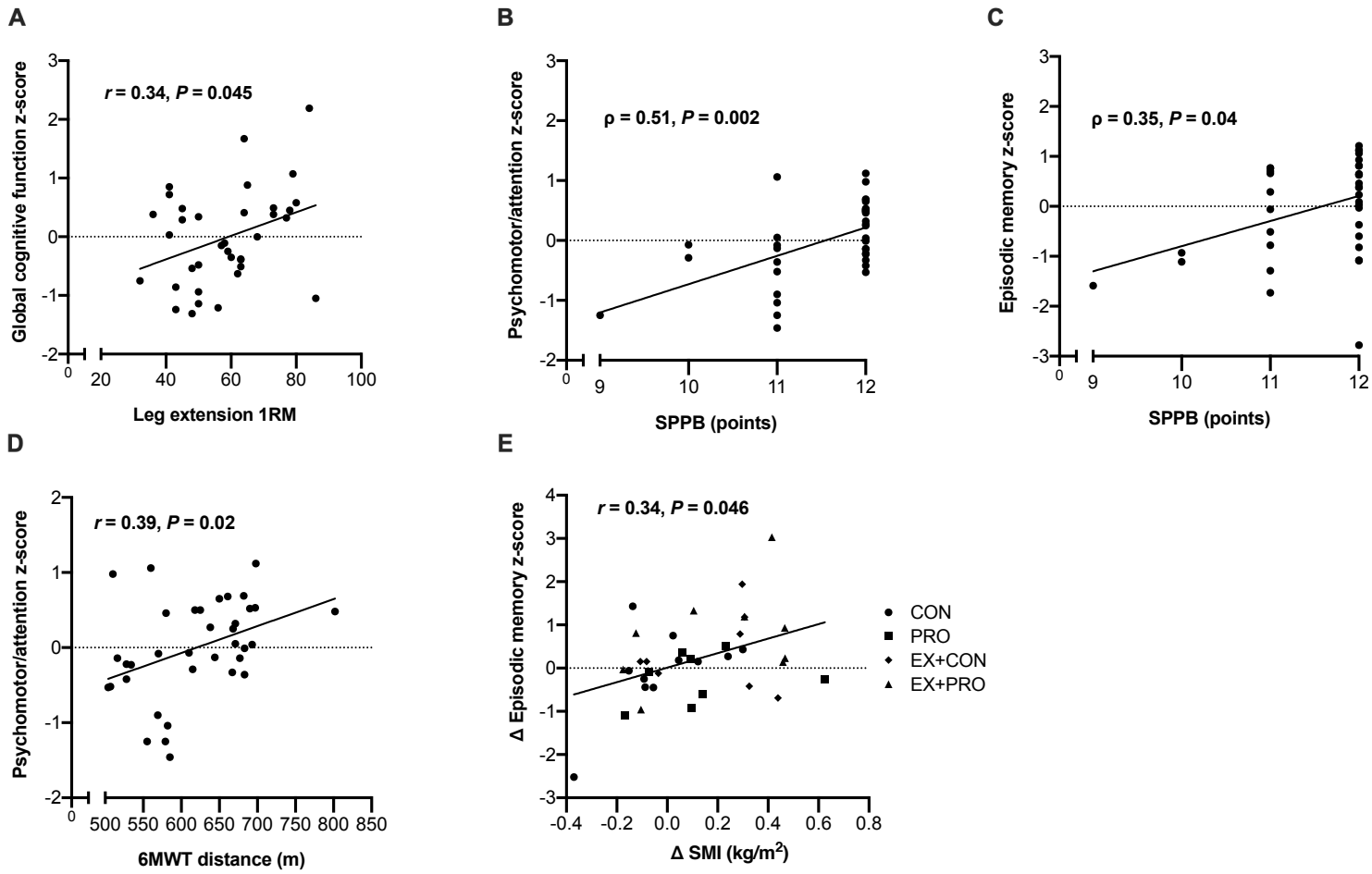

Figure 6.11 Baseline correlations between (A) leg extension 1RM and global cognitive function z-score; (B) SPPB score and psychomotor/attention z-score; (C) SPPB score and episodic memory z-score; and (D) 6MWT distance and psychomotor/attention z-score. Panel E shows the correlation between ΔSMI and Δepisodic memory z-score following the intervention. 1RM, one repetition maximum; 6MWT, 6 min walk test; SMI, skeletal muscle index; SPPB, short physical performance battery.

6.5 Discussion

This 4-arm, double-blind RCT investigated the individual and combined effects of 12 weeks RE and whey protein supplementation on cognitive function in healthy older men. The present study also sought to determine the individual and combined effects of these interventions on various neurobiological, inflammatory, insulin sensitivity and diurnal salivary cortisol markers, and SMM, maximal strength and physical function to identify mechanisms of action. The main findings were:

i) In both the main and exploratory analyses, no significant between-group differences occurred for any outcome variable.
ii) Whey protein supplementation *per se* elicited a within-group improvement in global cognitive function, working memory and executive function. When whey protein supplement groups were pooled, there was also a trend towards a greater increase in global cognitive function compared to control supplement groups pooled.
iii) Resistance exercise alone demonstrated a within-group improvement in multitasking efficiency but worsened processing speed. When RE groups were pooled, a within-group increase in episodic memory was observed.
iv) Resistance exercise plus whey protein supplementation elicited a within-group enhancement in executive functioning and tended to improve episodic memory and global cognitive function. However, no significant additive effects were observed compared to RE or whey protein supplementation alone.
v) At baseline, parameters of sarcopenia (muscle strength and physical performance) positively correlated with cognitive function domains.
vi) Changes in domain-specific z-scores did not correlate with changes in any neurobiological, inflammatory or insulin sensitivity marker, nor BP or salivary cortisol indices. However, an association was observed between changes in SMI and episodic memory.

6.5.1 Effects of whey protein supplementation on cognitive function

Protein supplementation *per se* elicited large within-group effects on composite scores for the cognitive domains executive function, working memory and global cognitive function. Previous work has suggested a 0.5 SD improvement in cognitive function may be considered clinically meaningful in cognitively healthy adults (Crichton et al. 2012). In the present study, whey protein supplementation induced larger improvements than 0.5 SD in working memory and global cognitive function, suggesting these findings are of clinical importance. The improvement in working memory coincides with previous acute (Kaplan et al. 2001) and longitudinal studies (Charlton et al. 2016). Additionally, although not observed in the present study, longitudinal improvements in reaction time, memory and emotion identification have also been observed by others (Charlton et al. 2016, Kita et al. 2019, Lefferts et al. 2020, van der Zwaluw et al. 2014). Importantly, as not all older adults are able or willing to perform RE (Dismore et al. 2020), these findings are of particular importance and suggest that increased dietary protein alone may attenuate age-related declines in cognitive function in these individuals. However, contrary to these findings, cognitive benefits of increased dietary protein have not been observed in all studies (Bell et al. 2019, Moran et al. 2018, Zajac et al. 2019). Six weeks consumption of a whey protein-based multi-ingredient supplement did not elicit improvements in any domains of cognitive function in healthy older men (Bell et al. 2019). Similarly, others have also reported no effects of 8 weeks supplementation of whey protein (Zajac et al. 2019), or 6 months consumption of a multi-ingredient supplement containing whey protein (Moran et al. 2018). Furthermore, although two previously cited studies have reported improvements in either reaction time or emotion identification following protein supplementation (Lefferts et al. 2020, van der Zwaluw et al. 2014), these studies observed no improvements in memory or executive functioning, which is in contrast to the findings of this study.

The disparities in findings between studies investigating the effects of increased dietary protein on cognitive function in the elderly might be due to differences in the duration of

intervention and the protein dose employed. In support, two previously cited studies that did not report positive benefits of increased dietary protein investigated the effects over a 6-8 week duration (Bell et al. 2019, Zajac et al. 2019), which, based on prior research, was likely insufficient to stimulate longitudinal changes in cognitive function (Vellas et al. 2008). Moreover, the multi-ingredient supplement investigated by Moran and colleagues (2018) contained only 8 g whey protein, and dietary data from participants in the study by van der Zwaluw et al. (2014), published elsewhere (Tieland et al. 2012c), reported that protein intake increased by 0.4 g/kg/d (1.0-1.4 g/kg/kg/d). These deviations in protein intake were less than that of the present study (0.6 g/kg/d; 1.0-1.6 g/kg/d). The dose of protein supplemented in these studies may therefore have been insufficient to yield changes in cognitive function. However, at present, no dose-response study has been conducted to determine the optimal daily intake of protein to improve cognitive function in older adults. Therefore, this dosing hypothesis is purely speculative and should be examined in future work. It is difficult to explain why Lefferts and colleagues (2020) did not report improvements in memory or executive functioning following protein supplementation considering the dose (50 g/d) and the duration of study (12 weeks) was identical to that of the present study. A significant limitation of this study, however, was the failure to control for dietary intake; therefore, whether the daily dose of protein (in g/kg/d) before and the deviation from baseline during the study whilst consuming the protein supplement was similar to that of the present study is unknown.

6.5.2 Effects of resistance exercise on cognitive function

Consistent with previous research in older adults (Anderson-Hanley, Nimon, and Westen 2010, Best et al. 2015, Cassilhas et al. 2007, Coelho-Júnior et al. 2020, Ikudome et al. 2017, Liu-Ambrose et al. 2010, Marston et al. 2019c, Yoon, Lee, and Song 2018), this study observed within-group improvements in memory and aspects of executive function following RE training. Specifically, the present study observed a large within-group effect on multitasking efficiency. When RE groups were pooled, a medium effect was also observed for the reduction in total errors on the PALT and an increase in composite episodic memory z-

score. However, it must be noted that these increases were driven more by the EX+PRO as opposed to the EX+CON group. Nevertheless, these findings suggest RE with or without additional protein supplementation may improve the QOL of elderly individuals and delay age-related cognitive impairment. For instance, episodic memory, which supports remembering past events [i.e., what, where and when certain events happened (Tulving 2002)], is considered the most age sensitive (Nyberg 2017) and is a common characteristic of the memory loss seen in individuals with Alzheimer's disease (Peters 2006). Multitasking is the ability to complete several tasks within a limited time by efficiently switching between tasks (Kosowicz and MacPherson 2017). In everyday context, this translates to activities such as preparing a meal, shopping and driving (Phillips, Kliegel, and Martin 2006).

Although RE elicited within-group improvements in certain aspects of executive functioning, other aspects, such as incongruency cost, which is the time required to process conflicting information, worsened. Furthermore, whilst episodic memory improved when RE groups were pooled, no effect was observed for working memory. These findings are in contrast with previous studies that observed improvements in these cognitive aspects following RE in older adults (Cassilhas et al. 2007, Ikudome et al. 2017, van de Rest et al. 2014, Yoon, Lee, and Song 2018). Other studies are, however, in agreement with the findings observed in this study, reporting no improvements in either processing speed (Anderson-Hanley, Nimon, and Westen 2010, Bell et al. 2019) or working memory (Liu-Ambrose et al. 2010). The disparities between studies in the literature may be explained by the intensity of RE employed. To add, studies that have demonstrated improvements in working memory (Ikudome et al. 2017) and processing speed (van de Rest et al. 2014, Yoon, Lee, and Song 2018) employed an intervention of moderate intensity RE (≤70% 1RM). Contrastingly, the present study and others that did not observe improvements employed a higher RE intensity (Bell et al. 2019, Liu-Ambrose et al. 2010). Additionally, a recent systematic review by Herold and colleagues (2019) concluded that moderate compared to high intensity RE was more beneficial for improving working memory and processing speed. This conclusion is supported by a meta-

analysis that reported greater effect sizes following moderate compared to high intensity RE in healthy adults (Wilke et al. 2019). However, it must be noted that the intensity of RE in the present study (80% 1RM) was chosen for the primary purpose of increasing skeletal muscle strength, which is the main determinant of sarcopenia and most predominantly associated with health-related outcomes (Cruz-Jentoft et al. 2019, Menant et al. 2017), and not speciifcally for the aim of improving cognitive function.

6.5.3 Effects of combined resistance exercise and whey protein supplementation on cognitive function

The present study reported no statistically significant augmented effects of RE plus whey protein supplementation on domains of cognitive function compared to RE or whey protein supplementation alone. The lack of significant synergistic effects compared to RE alone is in agreement with recently published data in older adults (Bell et al. 2019, Formica et al. 2020, van de Rest et al. 2014). In frail elderly individuals, protein supplementation combined with RE did not stimulate additive effects on episodic memory, attention and working memory, information processing speed, or executive functioning compared to RE alone (van de Rest et al. 2014). van de Rest et al. (2014) did, however, observe an additive effect on information processing speed compared to protein alone, but the opposite was reported for verbal fluency. Interestingly, the present study observed similar findings, whereby whey protein supplementation alone improved cognitive function to a greater extent on certain cognitive tests than performing RE with or without whey protein supplementation.

More recently, Formica et al. (2020) established multimodal exercise (aerobic + RE) improved global cognitive function and executive function in community-dwelling older adults, but consumption of lean red meat (2 x 80 g cooked weight on training days; ~45 g/d additional protein) did not enhance these cognitive domains. Contrary to this, the present study observed within-group improvements in these domains in the EX+PRO but not the EX+CON group. Also, the EX+PRO group observed a significant within-group decrease in total errors on the

PALT, which assessed episodic memory. The effect size for the reduction in total errors was also considerably greater in the EX+PRO compared to the EX+CON group (*d* = -0.79 vs -0.37). The lack of synergistic effects in the study by Formica and colleagues (2020) was likely due to red meat being consumed, and dietary intake increased, on only 3 days per week.

Bell et al. (2019) established no significant synergistic effects of multimodal exercise (RE + HIIT) combined with twice daily consumption of a whey protein-based multi-ingredient supplement on cognitive function in healthy older men. Nevertheless, similar to the present study, within-group improvements in composite cognitive function in only the exercise + whey protein group and greater effect sizes on a number of individual cognitive function tests compared to exercise alone were reported. In agreement, Rondanelli et al. (2020) demonstrated physical rehabilitation (including muscle strengthening, balance and gait exercises) combined with twice daily consumption of a leucine- and vitamin D-enriched whey protein supplement improved Trail Making Test performance, which examines executive functioning, processing speed, visual search speed and mental flexibility. However, it must be noted that the whey protein supplements in both of the above studies contained vitamin D, and in the study by Bell et al. (2019), the supplement also contained n−3 PUFA. These ingredients have previously been shown to attenuate cognitive decline (Balion et al. 2012, Karr, Alexander, and Winningham 2011), questioning the sole synergistic effects. Further research with a larger sample size is required to confirm whether increased dietary protein augments RE-induced improvements in cognitive function.

6.5.4 Mechanisms of action

The mechanisms through which exercise and dietary protein are purported to improve cognitive function is via increases in various growth and neurotrophic factors, decreases in inflammation and cortisol secretion, and reductions in cardiovascular risk factors (Ahlskog et al. 2011, Camfield et al. 2011, Liu-Ambrose et al. 2012, Tsai et al. 2015, Walsh et al. 2015). However, in the present study, no changes in serum total BDNF or IGF-1 occurred in any

group. These findings coincide with recently published studies that also demonstrated no increases in peripheral concentrations of IGF-1 or BDNF following 12 weeks RE training in healthy adults (Bell et al. 2019, Marston et al. 2019a). Furthermore, a meta-analysis conducted by Dinoff and colleagues (2016) reported RE was not effective at increasing circulating concentrations of BDNF, but AE was. Consequently, research to date indicates that participation in regular AE may be required alongside RE to stimulate increases in BDNF.

In parallel with the within-group improvements in cognitive function, the present study reported significant reductions in concentrations of markers of systemic inflammation and an increase in diurnal salivary cortisol slope in the EX+PRO group, improved insulin sensitivity in the PRO and EX+PRO groups, and a reduction in morning systolic and diastolic BP in only the PRO group. An important finding from this study is that although RE and whey protein supplementation produced favourable changes that support the purported mechanisms of improved cognitive function, changes were not associated with alterations in composite cognitive domain scores. Contrary to these findings, a significant positive correlation was observed between changes in SMI and episodic memory. Likewise, at baseline, associations were also observed between numerous parameters of sarcopenia (i.e., muscle strength and physical function) and domain-specific cognitive function z-scores. These findings are consistent with previous studies that reported associations between lower extremity strength and cognitive functioning (Frith and Loprinzi 2018), and changes in muscle strength and cognitive function following RE training (Mavros et al. 2017).

6.5.5 Relationship between sarcopenia and cognitive function

Data from the present study coincides with recent meta-analyses that reported associations between sarcopenia and cognitive impairment (Chang et al. 2016, Cipolli, Yassuda, and Aprahamian 2019, Peng et al. 2020). Collectively, these findings suggest that attenuating age-related declines in SMM, strength and physical function may play a key role in mitigating cognitive decline in older adults. However, at present, whether this association is purely casual

is unknown (Cipolli, Yassuda, and Aprahamian 2019). Investigation into the mechanisms that govern this relationship should be prioritised in future work, as should mechanisms that explain improvements in cognitive function following RE and/or increased dietary protein.

6.5.6 Strengths and limitations

The strengths of this study include the 4-arm, double-blind, randomised controlled design and the comprehensive assessment of multiple domains of cognitive function, indices of sarcopenia, BP, and analysis of numerous neurobiological, inflammatory and insulin sensitivity markers. Also, this study assessed diurnal salivary cortisol secretion pre- and post-intervention under highly controlled conditions. Collectively, this enabled investigation into the individual and combined effects of RE and whey protein supplementation on cognitive function in healthy older men and allowed exploration of potential mechanisms of action. In addition, compliance to both the RE intervention (98.2 ± 0.7%) and the protein supplementation (96.4 ± 0.8%) was high, indicating these interventions are feasible strategies to curb cognitive decline in healthy older adults.

This study also has several limitations that warrant further discussion. Firstly, only healthy volunteers who had high levels of physical functioning (SPPB: 11.5 ± 0.1; 6MWT: 618 ± 12 m), habitually consumed sufficient amounts of dietary protein (1.0 ± 0.02 g/kg/d) (Bauer et al. 2013, Deutz et al. 2014), and were generally well educated (14.4 ± 0.5 y) participated in this study. Inclusion of solely these participants may have limited the ability to detect greater exercise- and protein-induced improvements in cognitive function. A second limitation was the measurement of only basal concentrations of growth and neurotrophic factors >72 h following the final RE session and not also immediately following an acute bout of exercise pre- and post-intervention. Consequently, any longitudinal effects of RE and whey protein supplementation on acute exercise-induced changes in growth and neurotrophic factors were missed. Thirdly, this study was relatively short in duration (12 weeks), which although was sufficient to detect within-group RE- and protein-induced changes in cognitive function

domains, greater effects may have been observed over a longer intervention period (Vellas et al. 2008). Finally, although within-group differences in individual and composite cognitive function tests were observed, this study was underpowered to detect between-group differences. The results presented in this study should therefore be interpreted with caution. Post-hoc sample size calculations revealed the following number of participants would have been required to detect between-group differences in key domain-specific cognitive scores: executive function, 88 (22/group); episodic memory, 120 (30/group); working memory, 56 (14/group); and global cognitive function, 64 (16/group).

6.5.7 Conclusion

The primary findings from this study demonstrate that RE and whey protein supplementation alone and combined do not elicit significant between-group differences compared to control. Nevertheless, this study does show twice daily ingestion of whey protein supplementation for 12 weeks is an effective stimulus to elicit within-group improvements in multiple domains of cognitive function, including executive function, working memory and global cognitive function. Resistance exercise alone, and combined with whey protein supplementation, may also elicit within-group improvements in executive function and episodic memory, but whey protein does not stimulate significant additive effects on these outcomes. Nevertheless, as a 0.5 SD improvement in cognitive function has previously been deemed clinically meaningful (Crichton et al. 2012), which was observed particularly following whey protein supplementation, these findings may be of clinical importance to curb age-related declines in cognitive function. Lastly, change in cognitive function domains were not associated with changes in neurobiological or inflammatory markers, but change in SMI was associated with change in episodic memory. This finding highlights the importance of attenuating age-related declines in SMM to preserve cognitive function in older adults.

Thesis map: Key findings

Study	Aims	Key findings
Study 1: Effects of resistance exercise and whey protein supplementation on 24-h energy expenditure, substrate oxidation and metabolic flexibility, body composition, appetite and glucose homeostasis in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on components of 24-h EE, substrate oxidation and metabolic flexibility, body composition, appetite, and glucose homeostasis in healthy older men.	• Resistance exercise significantly increased FFM, RMR, SMR, sedentary EE and 24-h metabolic flexibility compared to non-exercise. RE also resulted in within-group increases in subjective hunger and insulin sensitivity, and within-group decreases in the energy cost of step exercise and spontaneous activity. • Whey protein supplementation improved body weight maintenance and reduced FM, and increased insulin sensitivity; however, resulted in an increase in overnight protein oxidation and awakening cortisol secretion, and reduced 24-h protein balance. • Whey protein supplementation had no adverses effect on total protein or EI, or 24-h subjective appetite. • Resistance exercise combined with whey protein supplementation did not significantly augment changes in body composition, 24-h EE, substrate oxidation or metabolic flexibility, or markers of glucose homeostasis compared to either RE or whey protein supplementation alone.
Study 2: Effects of resistance exercise and whey protein supplementation on skeletal muscle mass, strength, physical function, and hormonal and inflammatory biomarkers in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on SMM, strength, physical function, and hormonal and inflammatory biomarkers in healthy older men. • To determine whether changes in hormonal and inflammatory biomarkers correlate with changes in SMM, strength and physical function.	• Resistance exercise significantly increased FFM, muscle strength and physical function, and decreased markers of systemic inflammation. • Whey protein supplementation alone increased physical function (4 m gait speed) and muscle strength (leg press 1RM). • No synergistic effects occurred for any parameter of sarcopenia compared to RE or whey protein alone. • Changes in hormonal and inflammatory biomarkers did not correlate with changes in parameters of sarcopenia, but diurnal salivary cortisol and sarcopenia indices did at baseline.

Thesis map: Key findings continued

Study	Aims	Key findings
Study 3: Effects of resistance exercise and whey protein supplementation on cognitive function in healthy older men	• To investigate the individual and combined effects of RE and whey protein supplementation on cognitive function and neurobiological, inflammatory and insulin sensitivity markers, diurnal salivary cortisol, and BP in healthy older men. • To determine whether changes in neurobiological, inflammatory and insulin sensitivity markers, and changes diurnal salivary cortisol, BP, SMM, strength and physical function are associated with changes in cognitive function.	• Resistance exercise and whey protein supplementation alone and combined did not elicit significant between-group differences compared to control. • Whey protein supplementation *per se* elicited within-group improvements in global cognitive function, working memory and executive function. When whey protein supplement groups were pooled, there was a trend towards a greater increase in global cognitive function compared control supplement groups pooled. • Resistance exercise alone elicited within-group improvements in multitasking efficiency but worsened processing speed. When RE groups were pooled, episodic memory significantly improved. • Resistance exercise plus whey protein supplementation elicited within-group enhancements in executive functioning and tended to improve episodic memory and global cognitive function. However, no significant additive effects were observed compared to RE or whey protein supplementation alone. • At baseline, parameters of sarcopenia (muscle strength and physical performance) positively correlated with several cognitive function domains. • Changes in domain-specific z-scores did not correlate with changes in any neurobiological, inflammatory or insulin sensitivity marker, nor BP or salivary cortisol indices. However, an association was observed between changes in SMI and episodic memory.

CHAPTER 7: General discussion

7.1 Introduction

This thesis investigated the individual and combined effects of RE and whey protein supplementation in healthy older men. To achieve this, a 12-week, 4-arm, double-blind RCT was conducted between October 2017 and May 2019. Participants ingested either 25 g whey protein supplementation (or an energy-matched control) twice daily with or without twice weekly supervised RE. Numerous outcome variables were assessed pre- and post-intervention, including 24-h energy metabolism, parameters of sarcopenia, cognitive function and biomarkers relating to sarcopenia, metabolic health and cognitive decline. The following chapter will present a summary of the key findings presented in this thesis. Additionally, clinical and practical applications besides strengths and limitations of the thesis will be presented, and directions for future research will be proposed.

7.2 Summary of key findings

This thesis demonstrates as little as 12 weeks of RE in healthy older men is an effective stimulus to improve multiple health-related outcomes. Specifically, RE increased FFM, muscle strength and physical function, augmented multiple components of EE (i.e., RMR, SMR and sedentary EE) as well as 24-h metabolic flexibility and decreased markers of systemic inflammation compared to non-exercise. Additionally, RE also elicited within-group improvements in subjective hunger and insulin sensitivity, reduced the energetic cost of step exercise and stimulated within-group improvements in the cognitive functions multitasking efficiency and episodic memory. Whilst several of these outcomes have been reported previously in the literature, the original contribution of data in this thesis is the longitudinal effects of RE on 24-h metabolic flexibility and subjective hunger, and the effects on numerous biomarkers in older healthy older men. However, whilst RE improved many outcomes, PAL was in fact decreased and a within-group decrease in processing speed was observed. Although, the former of these may have been due to participants being confined to respiration chambers and not measured post-intervention in free-living.

In addition to RE, data presented in this thesis suggests whey protein supplementation may also be an effective stimulus to offset declines in health-related outcomes. For instance, twice daily whey protein supplementation for 12 weeks aided maintenance of body mass, reduced FM, decreased the energetic cost of spontaneous activities and step exercise in the fasted state, and increased insulin sensitivity compared to an isocaloric carbohydrate control. Furthermore, whey protein supplementation also increased gait speed, tended to increase muscle strength, and stimulated multiple within-group improvements in multiple components of cognitive function, including working memory, executive function, and global cognitive function. However, results from this thesis highlight a potential caveat of a longitudinal high protein diet via whey protein supplementation in the elderly. That is, an increase in protein oxidation in the overnight fasted state and subsequent decrease in overnight protein balance. Whilst this effect has been seen following a shorter duration in older adults by others (Højfeldt et al. 2020), data presented in this thesis adds original contribution to the literature that this effect is also observed after 12 weeks.

Based on the findings of published meta-analyses (Cermak et al. 2012, Finger et al. 2015, Liao et al. 2017, Morton et al. 2018a) and RCTs (Bell et al. 2017, 2019, Daly et al. 2014, Junior et al. 2018, Rondanelli et al. 2016, 2020, Tieland et al. 2012b, Verreijen et al. 2015, Yamada et al. 2019, Zdzieblik et al. 2015), it was hypothesised that twice daily whey protein supplementation would augment the effects of RE on components of EE and metabolic health, and parameters of sarcopenia and cognitive function in healthy older men. However, the results of this thesis suggest that combined intervention does not significantly amplify the effects of any of the aforementioned markers compared to either RE or whey protein supplementation alone. Furthermore, only combined RE and whey protein supplementation and not either intervention individually had an adverse effect on spontaneous activity. It is hypothesised this decrease may be a compensatory mechanism to offset declines in FM (Hall et al. 2012).

Two interesting findings were observed from the baseline data within this thesis. Firstly, diurnal salivary cortisol secretion significantly inversely correlated with multiple parameters of sarcopenia; secondly, several components of cognitive function correlated with sarcopenia indices. For example, significant inverse correlations were observed between diurnal salivary cortisol secretion and SMI, muscle strength (handgrip strength and leg press 1RM) and physical function (6MWT distance). Whilst these findings suggest measurement of salivary cortisol may be a promising biomarker of sarcopenia, no changes in diurnal salivary cortisol occurred following RE, despite increases in parameters of sarcopenia observed, which questions its clinical application as a biomarker. Additionally, several cognitive domains (i.e., global cognitive function, psychomotor/attention, episodic memory) correlated with parameters of sarcopenia at baseline [i.e., muscle strength (leg extension 1RM) and physical performance (SPPB and 6MWT distance)]. These findings emphasise the importance of maintaining SMM and function in older age to offset age-related declines in cognitive function.

An observation seen on numerous outcomes reported in this thesis is high interindividual variability. In particular, large interindividual variability occurred in bodyweight management outcomes. For example, following whey protein supplementation, change in body mass varied between -4.5 kg and 3.5 kg. This variance may be partially explained by interindividual differences in the effect of the high protein diet on EE outcomes, EI and physical activity. In support, Bray et al. (2012) observed interindividual variability in RMR (ranging from ~0-260 kcal/d) following 8 weeks of overeating dietary protein. Thus, although not observed post-intervention inside the respiration chamber when protein intake was returned to baseline levels, differences in EE may have occurred in free-living between participants randomised to whey protein supplementation groups whilst they were consuming the supplementation. Additionally, observed differences in EI and physical activity (step count) from baseline to 12 weeks (-230 to 501 kcal/d for EI and -2307 to 5356 steps/d) was seen following whey protein supplementation. Together, as all the above outcomes influence body mass, it is likely that

interindividual differences in body mass following whey protein supplementation may be explained by physiological and environmental factors.

In addition to change in body mass following whey protein supplementation, high interindividual variability (ranging from -1.5 to 2.7 kg) was seen in change in FFM following RE training. As FFM is strongly associated with EE (Manini 2010), this variability also likely contributed to the variability also seen in EE outcomes following RE. It has been proposed that differential responses in ribosome biogenesis and consequent MPS rate during RE explain some of the interindividual variance (Roberts et al. 2018). Additionally, high responders to RE have also been shown to experience greater satellite cell proliferation, higher intramuscular androgen receptor content, and express a muscle microRNA profile that enhanced IGF-1 during RE than low responders (Roberts et al. 2018). However, as the present study did not investigate these mechanistic outcomes and limited data is available in older adults examining the effects of high vs. low responders (Roberts et al. 2018), future research should investigate these mechanisms to aid identification of extrinsic factors (e.g., exercise dose) to aid maximal response in low responders.

Following the intervention, no correlations were observed between changes in parameters of sarcopenia and changes in hormonal or inflammatory biomarkers. Similarly, changes in cognitive function were not correlated with any markers purported to be mechanistically associated with cognitive decline (i.e., neurobiological, inflammatory or insulin sensitivity markers, BP or cortisol secretion). Together, these findings suggest that changes in SMM, strength and physical function following RE and/or increased dietary protein intake are not explained by changes in hormonal or inflammatory pathways, and that changes in cognitive function are likely not explained by changes in inflammation, growth factors, insulin sensitivity, the HPA axis or BP.

7.3 Clinical and practical applications

The findings of this thesis have a number of important applications for both the health of elderly individuals and also to healthcare services globally. Firstly, as age-related obesity, which has risen by ~35-60% in middle- and older-aged adults since 1980 (Flegal et al. 2012, Gutiérrez-Fisac et al. 2012, Howel 2012, Oreopoulos et al. 2009), is heavily linked to metabolic disease (Wannamethee and Atkins 2015), the increase in components of EE, metabolic flexibility and insulin sensitivity following RE, and the maintenance of body mass, reduction in FM and enhanced insulin sensitivity following whey protein supplementation reported in study 1 (**Chapter 4**), may offset sarcopenic obesity and age-related metabolic dysfunction. As a result, long-term use of these interventions may allow older adults to live their later years with improved weight maintenance and metabolic function, thereby mitigating their risk of metabolic disease. Also, these findings indicate RE and whey protein supplementation may also reduce the demand of healthcare services to provide clinical care and reduce the economic burden of age-related metabolic disease (König et al. 2015).

Secondly, as RE elicited a within-group increase in 24-h subjective hunger and whey protein supplementation did not adversely affect appetite, the findings of **Chapter 4** may have clinical implications for reducing the anorexia of ageing, a condition characterised by reductions in appetite and increased satiety (Wysokiński et al. 2015), which affects ~20% of older adults (Atalayer and Astbury 2013). As reductions in energy and protein intake are two key contributors of sarcopenic muscle loss and frailty (Atalayer and Astbury 2013, Paddon-Jones et al. 2015), the increase in subjective hunger following RE may indirectly offset sarcopenic muscle loss via effects on appetite (i.e., consuming more energy and potentially protein intake). Also, as whey protein supplementation did not adversely affect appetite or overall EI, and may offer small but significant benefits over an isocaloric control for muscle strength, physical function and components of cognitive function (as reported in **Chapter's 5 and 6**), these findings might also have significant implications as a strategy to offset age-related

declines in sarcopenia and cognitive function in those who are unable or unwilling to perform RE (Dismore et al. 2020).

A third noteworthy implication of the present thesis is the clinically significant increases in SMM (2.3%), muscle strength (31-36%) and physical function (3.4%) following RE training as reported in **Chapter 5**. Previous work has demonstrated from ~45 years of age, SMM, strength and physical function decline at rates of ~0.5-1%, ~1-3%, and ~0.5% per annum, respectively (Clark and Manini 2008, Daly et al. 2013, Janssen 2010). The increases in these parameters following as little as 12 weeks of RE may theoretically offset 5-10 years of age-related declines in these parameters. Furthermore, age-related sarcopenia has been associated with an increased risk of falls, fractures and impairments in the ability to perform ADL (Beaudart et al. 2017), cardiovascular (Bahat and Ilhan 2016) and metabolic disease (Hunter et al. 2019), and mortality (de Buyser et al. 2016). The notable improvements in sarcopenic parameters following RE training may therefore curb age-related prevalence of these outcomes.

Additionally, if the above findings were replicated in a sarcopenic cohort, they might also offer practical implications in the everyday lives of older adults, such as improved physical function and QOL. Indeed, an increase in muscle strength has been associated with a reduced likelihood of impairments in the ability to perform ADL, increased functional independence, and a decreased need for family/friend support (Gopinath et al. 2017). Furthermore, similar to that as highlighted above regarding the logistical and economic burden of age-related metabolic disease, data published from the NHS has reported the incidence of falls in 2015/16 was 204,269 (NHS 2017), with a financial burden estimated at £2.5 billion (Pinedo-Villanueva et al. 2019). As such, as improvements in muscle strength and physical function are associated with a reduction in the incidence of falls in older adults (Smee et al. 2012, Yang et al. 2018), improvements in these parameters may also have significant logistical and economic implications to healthcare services globally.

Alongside improvements in parameters of sarcopenia, the reduction in systemic inflammation following RE also offers numerous clinical and practical applications. As previously highlighted in this thesis (**section 2.2.8.5**), inflammaging is a condition characterised by low-grade systemic inflammation with age (Franceschi et al. 2006) and is associated with reduced physical function, muscle strength, CVD, insulin resistance and T2DM, cognitive decline and a higher risk of mortality (Calder et al. 2017). The decrease in systemic inflammation reported in **Chapter 5** may therefore have significant clinical implications for attenuating these outcomes. Furthermore, reducing the incidence of the abovementioned conditions may also have financial implications. As examples, at present, CVD (NHS 2019) and T2DM (Diabetes UK 2014) are estimated to cost the NHS £7 billion and £8 billion per annum, respectively. Whilst the causes of these diseases are multifactorial (Colpani et al. 2018, Rawshani et al. 2018), even a slight reduction in the prevalence via a reduction in inflammatory pathways may have a significant impact on disease incidence and progression.

In addition to the effects of RE on parameters of sarcopenia, **Chapter 5** also shows whey protein supplementation provided a significant greater effect on physical function and a trend for a greater increase in muscle strength compared to an isocaloric carbohydrate control. Whilst the effect was considerably less than that of RE, which has also been acknowledged by others (Phillips and Martinson 2019), these findings may have significant implications for older adults who are unable or unwilling to perform RE (Dismore et al. 2020). Indeed, as highlighted above, sarcopenia has both financial and health-related adverse effects. Whilst whey protein supplementation elicited no effect on SMM, recently published revised diagnostic criteria of sarcopenia by the EWGSOP2 emphasise muscle strength as the primary parameter due to research demonstrating a stronger association with adverse health outcomes (Cruz-Jentoft et al. 2019). Therefore, even a small attenuation of muscle strength and physical function decline with age may aid QOL and reduce the incidence of associated adverse health outcomes.

Lastly, the within-group improvements in cognitive function following whey protein supplementation and RE reported in study 3 (**Chapter 6**) also offers clinical and practical applications. Whey protein supplementation resulted in improvements in working memory, executive functioning, and global cognitive function. Resistance exercise alone increased episodic memory, and RE combined with whey protein supplementation increased executive functioning. Working memory is the temporary storage of information prior to entering long-term memory (Harada, Love, and Triebel 2013), and episodic memory is a person's unique memory of a specific event (Peters 2006). Executive functioning includes planning, organising and problem-solving skills (Harada, Love, and Triebel 2013), and global cognitive function encompasses aspects of all cognitive domains. In older adults, a 0.5 SD improvement in cognitive function has been suggested to be clinically meaningful (Crichton et al. 2012). The magnitude of increase in working memory and global cognitive function following whey protein supplementation (2.4 ± 0.76 SD and 0.72 ± 0.24 SD, respectively), the increase in episodic memory following RE (0.74 ± 0.37 SD), and the increase in executive functioning following combined RE and whey protein supplementation (0.53 ± 0.23 SD), were all above this clinically meaningful change and can therefore be deemed significant findings.

In practical terms, the improvements in cognitive function might have implications for the everyday lives of older adults, such as improved ability to follow and remember instructions, enhanced medication adherence, and superior capability to plan social activities, prepare meals and manage finances. Overall, this may aid autonomy, enhance inclusion in society, and improve overall QOL (Baars and Dohmen 2013). In clinical terms, MCI affects 17.1% of adults aged ≥65 years in the United Kingdom, with global prevalence of dementia estimated at ~50 million people (Petersen 2016, World Health Organisation 2019). Attenuation of cognitive decline either via RE or increased dietary protein intake may consequently reduce the overwhelming patient clinical need from healthcare professionals. On an economic level, a recent study in the United Kingdom reported a 30-50% reduction in cognitive decline would result in a total societal saving of £670-£1100 per person over 18 months (Lenox-Smith et al.

2018). Taking into account the high prevalence of dementia coupled with the economic cost in the United Kingdom (£26.3 billion per annum) (Alzheimer's Society 2014), the findings from **Chapter 6** might have significant economic implications to healthcare providers.

7.4 Strengths of the thesis

The series of studies presented in this thesis have numerous strengths that enhance the novelty of this work. These will be presented in subsequent sections.

7.4.1 Study design

The present thesis adopted a 4-arm, double-blind, randomised controlled experimental design. This contrasts with most previous studies cited in this thesis that investigated the synergistic effects of RE and whey protein supplementation on parameters of sarcopenia, metabolic health and cognitive function. Of note, many of these studies failed to include a protein only group and compared the synergistic effects against just RE alone. In fact, only 8/39 studies cited in this thesis that investigated the combined effects of RE and increased dietary protein employed a 4-arm design which compared the synergistic effects to both interventions alone (de Carvalho Bastone et al. 2020, Gryson et al. 2014, Kim et al. 2012, Kirk et al. 2020, Kukuljan et al. 2009, Shahar et al. 2013, van de Rest et al. 2014, Verreijen et al. 2017). As previously highlighted in this chapter, not all older adults are willing or able to perform RE (Dismore et al. 2020); therefore, analysis of the effects of whey protein supplementation alone also allowed for comparison with both RE alone, and RE combined with whey protein supplementation. Subsequently, this enabled investigation into the efficacy of this nutrient in those participants unable to perform RE.

7.4.2 Use of an energy-matched control product

Only two studies cited in this thesis that investigated the individual and combined effects of RE and dietary protein including four experimental groups supplemented participants in the control groups with a placebo or energy-matched control supplement (Gryson et al. 2014, van

de Rest et al. 2014). The non-placebo/energy-matched methodological approach in the remaining six 4-arm studies cited in this thesis may have increased the risk of bias (i.e., participants in non-protein groups increasing their protein intake) (de Carvalho Bastone et al. 2020, Kim et al. 2012, Kirk et al. 2020, Kukuljan et al. 2009, Shahar et al. 2013, Verreijen et al. 2017). For this reason, an expert working group has recommended the double-blind RCT as the mainstay of experimental design for trials investigating the effectiveness of interventions to treat or prevent sarcopenia (Reginster et al. 2016). As participants in control groups in the present thesis were supplemented with an isocaloric control product, it can be hypothesised with a higher degree of certainty than the majority of previous work that the findings presented are as a result of differences in macronutrient content and not due to differences in EI provided by the intervention feed.

7.4.3 Optimal dietary protein intake

It has been hypothesised that the lack of effect of increased dietary protein in many previous studies cited in this thesis was due to dietary protein being insufficiently increased during the intervention period (<0.4 g/kg/d) and total dietary protein intake being <1.6 g/kg/d, the reported intake required to maximally accrete SMM during RE (Park, Choi, and Hwang 2018, Morton et al. 2018a). In the present thesis, dietary protein intake of participants was increased via whey protein supplementation by >0.4 g/kg/d (from ~1.1 to 1.6 g/kg/d). Therefore, individual studies in the present thesis are novel in the fact they are only one of a few studies (Bell et al. 2017, 2018, 2019, Norton et al. 2016, Weinheimer et al. 2012) that have investigated the individual and combined effects of RE and increased dietary protein intake in older adults employing the optimal dose of dietary protein.

7.4.4 Multidisciplinary investigation and resistance exercise supervision

The multidisciplinary nature of the present thesis and the supervision of RE sessions are also noteworthy strengths of this work. As ageing is a multifactorial concept, with many pathologies interlinking with one another (Lloyd-Sherlock et al. 2012), the multidisciplinary investigation

allowed for analysis of a wide range of topical areas observed during the ageing process. Consequently, the findings may aid in the holistic attenuation of age-related disease. Regarding the exercise supervision, each session was supervised on a 1-to-1 or 2-to-1 basis with a qualified exercise professional, ensuring the exercise intervention was conducted in a safe manner. Potentially, although speculative, the close supervision of the exercise intervention might have resulted in greater effects on primary outcomes measured. In support, a recent meta-analysis reported augmented effects of supervised compared to non-supervised RE in older adults (Lacroix et al. 2017). Furthermore, the supervised nature of the RE intervention may have also contributed to the high compliance reported (98.2 ± 0.7%). Importantly, high compliance to RE is likely a key strategy to mitigate sarcopenic muscle loss; however, such supervision is not feasible in the long-term, highlighting the need for follow-up data in this population.

7.4.5 Monitoring of dietary intake and habitual physical activity

Both habitual dietary intake and physical activity were monitored throughout the intervention to control for potential changes which may have influenced key outcomes. Whilst most previous studies in the literature controlled for changes in dietary intake, few studies have controlled for changes in physical activity using accelerometery. This level of experimental control subsequently allowed for examination of the true individual and combined effects of RE and whey protein supplementation without questioning external factors which may have influenced findings.

7.4.6 Use of gold standard methodology to measure 24-h energy metabolism

A key strength of study 1 (**Chapter 4**) was the use of respiration chambers to measure 24-h energy metabolism. Respiration chambers allow for continuous measurement of EE and substrate utilisation under highly controlled conditions (e.g., controlling for dietary intake and physical activity) for 24 h up to serval days (Schoffelen and Plasqui 2018). As a result, they are also considered the gold standard instrument for measurement of minute-by-minute EE

(Brychta et al. 2009). Furthermore, whilst the number of respiration chambers globally is growing, there are still only a limited amount of research centres with such facilities (Schutz 2018). Use of this instrumentation is a novel aspect of this thesis as it allowed for measurement of multiple components of 24-h EE (i.e., RMR, SMR, AEE, SPA, sedentary EE, TEE), besides 24-h metabolic flexibility and appetite whilst controlling for several confounding variables as highlighted above. This contrasts with many previous studies that investigated the combined effects of RE and increased dietary protein on EE in the elderly that typically only measured RMR (Amamou et al. 2017, Campbell et al. 1994, Maltais et al. 2016, Weinheimer et al. 2012). Therefore, as far as the author is aware, data presented in this thesis is the first in older adults to determine the individual and combined effects of RE and whey protein supplementation on multiple components of 24-h energy metabolism.

7.4.7 Comprehensive assessment of markers associated with sarcopenia, metabolic health, and cognitive function

The assessment of multiple biomarkers associated with sarcopenia (i.e., IGF-1, myostatin, cortisol, IL-6, TNF-α and CRP), metabolic health (i.e., fasting glucose, insulin, leptin and 24-h interstitial glucose) and cognitive function (i.e., BDNF and IGF-1) alongside parameters of sarcopenia, cognitive function, and metabolism are key strengths of this thesis. Whilst previous studies have analysed markers of systemic inflammation (Rondanelli et al. 2016, Shahar et al. 2013), myostatin (Bagheri et al. 2020) and IGF-1 (Bauer et al. 2015, Rondanelli et al. 2016) alongside parameters of sarcopenia following RE and increased dietary protein alone, or combined, no study, to the authors knowledge, has also comprehensively examined the array of biomarkers presented in this thesis. Similarly, although studies have investigated the combined effects of these interventions on insulin sensitivity (Bell et al. 2017, Holwerda et al. 2018, Iglay et al. 2007, Leenders et al. 2013, Maltais et al. 2016, Verdijk et al. 2009a, Weinheimer et al. 2012), no study has investigated the individual and combined effects of RE and whey protein supplementation on 24-h glucose control and glucose variability under highly controlled conditions using respiration chambers. Lastly, whilst two previous studies have

examined both cognitive function and BDNF following combined RE and dietary protein (Bell et al. 2019, Formica et al. 2020), the present thesis is the first to examine multiple markers associated with cognitive function (i.e., IGF-1, BDNF, insulin sensitivity, BP, cortisol) alongside a comprehensive assessment of cognitive function following both RE and whey protein supplementation alone, and combined. Collectively, these data are of novelty and add considerable insight to the literature that previously purported mechanisms are not associated with changes in parameters of sarcopenia and cognitive function following RE and whey protein supplementation in healthy older adults.

7.5 Limitations of the thesis

The present thesis has several limitations that warrant further discussion. These will be briefly outlined in the following subsections.

7.5.1 Sample size

In intensive studies of this nature, the trade-off between sample size, resource and completion is an inherent problem. Sample size was calculated *a priori* based on between-group changes in RMR (primary outcome) from a previous respiration chamber study that reported an increase of 109 kcal/d following 16 weeks of RE in older adults (Treuth et al. 1995). The resulting sample size calculation determined 52 participants were required to detect statistically significant between-group differences from a mixed-model ANCOVA with one covariate. However, due to time and budgetary constraints, only 33 participants completed the study for the primary outcome, and 36 participants completed all other measures. Whilst the sample size of the main study of this thesis (**Chapter 4**) in which the primary outcome was investigated was congruent with previous respiration chamber studies (Apolzan et al. 2014, Melanson et al. 2007, Westerterp-Plantenga et al. 2009a), the sample size was unable to detect between-group differences. Based on the reported between-group effect size for RMR, post-hoc power analysis revealed 48 participants would have been required to detect a significant group-by-time interaction. Furthermore, whilst between-group differences were

observed for muscle strength measures, key cognitive outcomes were also considerably underpowered to detect between-group differences (e.g., post-hoc power analysis revealed 64 participants would have been required to detect between-group differences in global cognitive function). Consequently, only within-group differences could be compared, and as such, this data and subsequent conclusions should be interpreted cautiously.

To overcome the lack of statistical power throughout this thesis, RE and whey protein supplementation groups were pooled and compared to pooled non-exercise and control groups, respectively. Whilst this analysis was not planned in the original statistical plan, analysis allowed for determination of the effects of RE and whey protein supplementation on key outcome measures with greater statistical power. However, this did not allow for analysis of whether RE combined with whey protein supplementation augments gains in parameters of sarcopenia, EE, body composition and cognitive function compared to RE alone, which was a key aim of the present thesis. The lack of statistical power may also be a key reason why a repeated theme of the thesis was that dietary protein does not augment gains in outcomes. In partial support, **Chapter 4** demonstrated greater effects of whey protein supplementation versus control for body composition, and **Chapter's 5 and 6** established greater increases in muscle strength and a trend for a greater improvement in global cognitive function, respectively. Thus, it may be that a larger sample size similar to that used in previous studies that observed synergistic effects of RE and increased dietary compared to RE alone (Bell et al. 2017, Rondanelli et al. 2016, 2020, Tieland et al. 2012b) might have elicited synergistic effects on key outcomes in individual studies within this thesis. Though, this hypothesis is purely speculative and requires further examination.

7.5.2 Inclusion of participants who habitually consumed sufficient amounts of dietary protein

As with many previous studies (Björkman et al. 2020, Cramer et al. 2016, Dulac et al. 2020, Holm et al. 2008, Holwerda et al. 2018, Kim et al. 2012, Kirk et al. 2019, 2020, Kukuljan et al.

2009, Leenders et al. 2013, Verreijen et al. 2017, Zhu et al. 2015), a limitation of this thesis was the inclusion of participants who habitually consumed sufficient amounts of dietary protein (>1.0 g/kg/d) at baseline (Bauer et al. 2013, Deutz et al. 2014). It has been hypothesised based on the findings of these studies that a high habitual protein intake may mask the additional effects of increased dietary protein in older adults (Holwerda et al. 2018, Kirk et al. 2019). Consequently, inclusion of only older adults who were protein malnourished at baseline in this thesis might have allowed for augmented effects of whey protein supplementation to be observed and may have made the findings more representative of the target population.

In opposition of the aforementioned, two studies within the literature have observed positive effects of protein supplementation either alone (Norton et al. 2016), or combined with RE (Bell et al. 2017), in healthy older adults habitually consuming ample amounts of dietary protein at baseline (1.1-1.2 g/kg/d). In the present thesis, improvements in body weight maintenance, a reduction in FM, and increases in muscle strength and physical function were observed following whey protein supplementation. Interestingly, unlike many previous studies that failed to observe effects of increased dietary protein in those habitually consuming sufficient amounts (Arnarson et al. 2013, Candow et al. 2006, Chalé et al. 2013, de Carvalho Bastone et al. 2020, Dulac et al. 2020, Englund et al. 2018, Fielding et al. 2017, Gryson et al. 2014, Hofmann et al. 2016, Holm et al. 2008, Holwerda et al. 2018, Kim et al. 2012, Kirk et al. 2019, 2020, Kukuljan et al. 2009, Leenders et al. 2013, Maltais, Ladouceur, and Dionne 2016, Oesen et al. 2015, Ottestad et al. 2017, Shahar et al. 2013, Thomson et al. 2016, Verdijk et al. 2009a, Verreijen et al. 2017), the present thesis, as well as those studies that reported beneficial effects (Bell et al. 2017, Norton et al. 2016), increased dietary protein intake to 1.6 g/kg/d and deviated dietary protein by ≥0.4 g/kg/d. Thus, whilst the inclusion of participants who habitually consumed sufficient amounts of dietary protein may be a limitation of this thesis, the findings highlight dietary protein may still be an effective strategy to attenuate adverse health outcomes with age in non-protein malnourished older adults if the dose provided is sufficient.

7.5.3 Investigation of only healthy older men

Women were excluded from studies within this thesis due to the relatively small sample size and prior work establishing sex differences in primary outcomes, which would have likely resulted in large variability in primary study endpoints (Geisler et al. 2016a, Geisler and Müller 2017). Men were studied due to reported greater annual declines in FFM per annum (3% vs. 1.7%) and shorter life expectancy compared to women (Reid et al. 2008). However, due to the sex differences reported above, the findings presented in this thesis may not be applicable to female older adults. As women account for a large proportion of the older population [55% over 65 years (Office for National Statistics 2018)] and have a high prevalence of sarcopenia (Kim et al. 2016a), the exclusion of women is recognised as a limitation of this thesis.

In addition to the above, the inclusion of only healthy men with a normal-overweight BMI must also be acknowledged as a limitation of this thesis. Individuals with obesity were excluded due to the preventative nature of this thesis, of which one aim was to determine the effectiveness of RE and whey protein supplementation as a strategy to mitigate sarcopenic obesity. Men with obesity were also excluded based on prior work establishing obesity exacerbates sarcopenia (Trouwborst et al. 2018). Furthermore, older adults with obesity display an altered metabolic profile to that of individuals without obesity (Perna et al. 2017), which may have led to large variability in metabolic parameters. For these reasons, the EWGSOP2 advise investigating sarcopenic obesity as a distinct condition (Cruz-Jentoft et al. 2019). However, due to the metabolic differences between older adults with and without obesity, the findings of this thesis do not necessarily translate to older adults with obesity, who make up ~20% of adults over 65 years (Peralta et al. 2018) and are at greater risk of metabolic dysfunction (Wannamethee and Atkins 2015).

7.6 Recommendations for future research

Following the results of this thesis, several identified areas warrant further research. These will be outlined in the subsequent subsections.

7.6.1 Investigation into the mechanism(s) of reduced activity energy expenditure following resistance exercise in older adults

In study 1 (**Chapter 4**), combined RE and whey protein supplementation resulted in a reduction in spontaneous activity whilst participants resided in the respiration chamber. This reduction was hypothesised, based on previous work, to be a compensatory reduction controlled by the neuromodulating factor orexin A to oppose the reduction in FM (Kotz et al. 2017, Perez-Leighton, Billington, and Kotz 2014, Teske, Billington, and Kotz 2010). However, orexin A was not measured in this study; thus, this theory is hypothetical. As the reduction in AEE observed in this thesis following RE is not unique (Hunter et al. 2018, Melanson 2017), further research into the potential mechanism(s) is warranted. Although, it may be that studies that have measured AEE following RE in the elderly are of too short duration for elderly individuals to adjust; therefore, research of longer duration (e.g., 6 months) is warranted to determine the longitudinal effect.

7.6.2 Longitudinal effect of high protein diets on protein balance in older adults

As highlighted in **Chapter 4**, a potential drawback of long-term increased dietary protein intake in the elderly is a reduction in protein balance, particularly in the overnight fasting period. Whilst these findings have also been observed by another recently published study (Højfeldt et al. 2020), this and the present study were of relatively short duration (3 and 12 weeks, respectively). Further research determining the effects of high intakes of dietary protein (~1.6 g/kg/d) on overnight protein breakdown over a longer period is therefore warranted.

7.6.3 Longitudinal effects of resistance exercise and whey protein supplementation on appetite-related hormones in older adults

Whilst the present thesis observed a within-group increase in 24-h subjective hunger when RE groups were pooled and no changes in subjective ratings of appetite when whey protein groups were pooled, this thesis did not measure the diurnal response of key appetite-related hormones such as ghrelin, PYY or GLP-1. Further analysis of these biomarkers is therefore

warranted and will add considerable mechanistic value of the effects of RE and increased dietary protein on appetite in older adults.

7.6.4 Longitudinal effects of resistance exercise combined with whey protein supplementation on energy expenditure in older adults

As emphasised in **Chapter 4**, participants in whey protein supplementation groups were not fed a high protein diet whilst they resided in the respiration chamber during post-intervention testing. This methodology was chosen as the aim of this study was to examine the effects of RE and whey protein supplementation on skeletal muscle/FFM and subsequent effects on energy metabolism, which may have been cofounded if participants were fed a high protein diet whilst residing in the respiration chamber post-intervention. However, due to this, the study was unable to directly determine the long-term effects of a high protein diet in the elderly on 24-h EE, substrate oxidation and appetite. This should therefore be examined in future work.

Moreover, whilst no additive effects of RE and whey protein supplementation occurred for any components of EE, as previously highlighted, the sample size was small and underpowered to observe such effects. Further research with a larger sample size is required to examine whether combined RE and whey protein supplementation augments EE in older adults. Although, as recruitment to respiration chamber studies is challenging due to the expense and time required to obtain such data, a multi-centre trial may be required. Lastly, as women were excluded from this thesis and make up the largest proportion of older adults, individual studies within the present thesis should also be replicated in elderly women.

7.6.5 Relationship between salivary cortisol concentration and sarcopenia

As reported in **Chapter 5**, significant inverse correlations were observed between diurnal salivary cortisol AUC and numerous parameters of sarcopenia at baseline. However, though these results highlight salivary cortisol may be a potential biomarker of sarcopenia, no

changes were observed following RE, despite increases in parameters of sarcopenia being observed, questioning its clinical use. Consequently, further research with a larger sample size is needed to determine whether salivary cortisol correlates with changes in parameters of sarcopenia in a wide range of older adults (i.e., heathy, pre-sarcopenic, sarcopenic). Collection of this data is needed to supplement the findings observed in this thesis to determine whether salivary cortisol may be a potential biomarker of sarcopenia.

7.6.6 Effects of resistance exercise combined with increased dietary protein intake on parameters of sarcopenia and mechanisms of action in older adults

Due to lack of statistical power, this thesis was unable to truly determine whether RE and whey protein supplementation synergistically attenuates sarcopenia. As whey protein supplementation alone elicited greater effects on physical function and muscle strength compared to an isocaloric carbohydrate control when whey protein supplement groups were pooled, which is in contrast to many studies that increased dietary protein by <0.4 g/kg/d (Björkman et al. 2020, Cramer et al. 2016, de Carvalho Bastone et al. 2020, Kim et al. 2012, Kirk et al. 2020, Kukuljan et al. 2009, Verreijen et al. 2017, Zhu et al. 2015), further research using the same experimental methodology (i.e., twice weekly RE combined with a dietary protein intake of 1.6 g/kg/d) with a larger sample size is justified to determine whether increased dietary protein augments RE-induced improvements in parameters of sarcopenia.

Furthermore, it is important to note that most studies in the literature investigated the effects of protein supplementation to achieve a higher protein diet. However, consumption of protein supplements daily does not replicate the habitual eating behaviour of this population. It is also questionable whether older individuals would consume these supplements longitudinally. Additional research employing the recommended daily protein dosing regimen as previous described using food items that older adults habitually consume is needed to understand if consuming this level of dietary protein is feasible to attenuate sarcopenia. These areas of further research should be examined in both men and women.

Also, as no inflammatory or hormonal biomarkers correlated with changes in parameters of sarcopenia, further research into the mechanisms that govern RE and protein-induced changes in these outcomes in older adults is warranted. In particular, as Morton and colleagues (2018b) have established an association between intramuscular androgen receptor content and muscle hypertrophy following RE in young adults, examination in older adults may provide valuable mechanistic data.

7.6.7 Effects of resistance exercise and whey protein supplementation on cognitive function and mechanisms of action

The findings of **Chapter 6** emphasise the need for further research into the effects of RE and dietary protein on cognitive function in older adults. At present, no dose-response study has been conducted to determine the optimal dose of dietary protein to aid specific cognitive domains. In the present thesis, the rationale for the dose employed was based on the association between cognitive decline and sarcopenia (Chang et al. 2016, Cipolli, Yassuda, and Aprahamian 2019, Peng et al. 2020). However, a longitudinal study in older adults utilising different dosing regimens would enhance our understanding of the dose-response effects. Furthermore, similar to that of previous experimental chapters highlighted, the sample size of the abovementioned study was too small to detect between-group differences. Therefore, a trial of similar methodological design with a larger sample size would enhance current knowledge of the individual and combined effects of RE and whey protein supplement on cognitive function in older adults. A final recommendation for future work investigating the effects of RE and increased dietary protein intake on cognitive function in older adults is to examine the mechanisms of action. As few trials have investigated the combined effects of these interventions on cognitive function in older adults (Bell et al. 2019, Formica et al. 2020, Rondanelli et al. 2020, van de Rest et al. 2014), and neither of these comprehensively assessed potential mechanisms of action, further research with a larger sample size than the present thesis is required to determine mechanistic pathways. Such data would be of value to

the literature to aid healthcare professionals to scientifically recommend RE and dietary protein to attenuate cognitive decline.

7.7 Conclusion

The evidence presented in this thesis supports the use of RE as the primary lifestyle intervention to mitigate age-related declines in EE and parameters of sarcopenia. For example, whilst RE resulted in small increases in aspects of EE such as RMR (36 ± 14 kcal/d), when extrapolated over a year, this would result in an increased EE of 13,140 kcal, which may be significant over time to prevent age-related declines in TEE and adiposity. Additionally, RE resulted in significant improvements in parameters of sarcopenia, including muscle strength (~30%), mass (2.3%), and physical function (3.4%). Considering these parameters decline by ~1-3%, ~0.5-1%, and ~0.5% per annum around mid-life, respectively, and are associated with numerous adverse effects on heath, these improvements, if maintained, are significant and may offset several years of sarcopenia-associated deterioration of health. Consequently, RE throughout the lifespan, and particularly in those individuals in their later years, should be promoted by government and healthcare professionals globally.

In addition to RE, increased dietary protein intake via whey protein supplementation may also be recommended as a safe method for older adults to alleviate declines in muscle strength, physical function and sarcopenic obesity, and aid aspects of cognitive functioning. Notably, increased dietary protein intake may be of particular importance to those unable to perform RE. For example, whey protein supplementation increased 4 m gait speed by ~0.08 m/s and decreased FM by ~0.5 kg. Since low physical function and increased adiposity are two key factors that drive age-related chronic disease, these findings are important to those groups. However, it should be noted that whey protein supplementation alone offers far inferior protective effects against sarcopenic muscle loss than performing RE.

Finally, the data in this thesis does not support the need for combined RE and whey protein supplementation to mitigate age-related sarcopenia, sarcopenic obesity, metabolic disease nor cognitive decline. Although, it should be noted that whey protein supplementation did not adversely affect any markers compared to RE alone. Due to the notably small effect between the two interventions and the lack of statistical power to detect post-hoc differences, further RCTs with a far larger sample size in participants with inadequate habitual protein intakes (~0.8 g/kg/d) is required to determine whether increased dietary protein augments the effects of RE on multiple health outcomes in older adults. Study of the mechanisms of how these interventions may attenuate age-related declines in these conditions is also warranted.

7.8 Dissemination of PhD findings to research participants

In line with ethical considerations and to improve public engagement in research, participants who participated in this study have received a 3-page individual result sheet from their participation in the research study, which includes baseline measures and their individual change after 12 weeks. Additionally, participants have also received a summary of the main research findings of the thesis and will be emailed when manuscripts that arise from this thesis are published.

7.9 Personal PhD reflection

Ever since completing my master's degree in 2013, I wanted to continue in academia and pursue a PhD. I had enjoyed my research modules at undergraduate and postgraduate levels, so continuing further was always the right option for me. In the time between 2013 and starting this PhD in 2017, I had several unsuccessful attempts at securing a scholarship to conduct a PhD, which I found difficult, but this was not going to stop me pursuing my dream. Therefore, I spent this time gaining further research experience as a research assistant/associate at various academic institutions and was excited to start my journey once I had been made an offer to conduct a PhD in an area I was extremely interested in.

With the four years of research experience behind me prior to starting the PhD, I was slightly naïve in the early stages of the PhD programme and thought I would complete the PhD with no problems. However, how wrong I was, and I soon realised why such a small percentage of the population had completed a doctorate degree. Nevertheless, I knew that an important aspect of successfully running a clinical trial is planning. I had planned the research the best way I could, and I believe this helped the trial be the success it was. Furthermore, I knew communication skills and trust with research participants was a highly needed skill to run a research study, and I also believe the skills I gathered in previous roles contributed to the very high adherence to both the exercise and nutritional interventions.

Looking back, there were, however, several areas I believe I could have made the programme easier, which I will take into my future career as a researcher. Firstly, I did not begin to explore the data, especially the respiration chamber data, until late into the PhD programme. The main reason for this was due to the double-blinded nature of the study design. However, this led to a significant amount of continuous data analysis to occur in one period of time. If I was to analyse this data as the research went on, trends and errors picked up along the way would have been identified earlier. Whilst this would not have impact the thesis findings, it would likely have eased the data processing time and allowed for further reading earlier around trends and issues I had seen.

In addition, a significant lesson I learnt during the PhD process was the time required for research activities which are not observed in research papers or within this PhD thesis. For example, in this trial, over 250 men aged >60 years were formally screened; however, I was in contact with >750 men over email or telephone discussing this trial. Additionally, I was in regular communication with trial participants throughout the 1.5 years running this PhD trial. Together, this accounted for a significant number of hours every week, and by the time of the end of the PhD programme, I had experienced burnout and trial fatigue. Therefore, moving

forward and running a large trial in the future, I have learnt sufficient time and staffing is required to run such a study, and this will be factored into future research grants and planning.

Lastly, in the latter stages of 2019, Coronavirus disease (COVID-19), which is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in Wuhan, China. In Mach 2020, the disease was classified by the World Health Organisation as a global pandemic, which led to a national lockdown in the United Kingdom. As a result, the university laboratories were closed, which resulted in the final piece of data collection analysing appetite-related markers not being analysed. Consequently, a change thesis structure was therefore required, as in the originally thesis planning, a specific appetite chapter was planned. This was unfortunate, as appetite and anorexia of ageing is a particular interest of mine. Nevertheless, I learnt to adapt quickly, which led to the appetite data I had already analysed being amalgamated into the EE and body composition chapter and a new exploratory cognitive function chapter being created.

Overall, the PhD experience was a fantastic journey which I pleasantly enjoyed, and I have developed many transferable skills which I will take with me into my future career. It's a process I would highly recommend to any aspiring scientist looking to gain the required qualifications and skills needed to begin a journey into scientific research. To end, the best piece of advice I would provide to someone aspiring to complete a PhD is to ensure you choose a topic you are extremely passionate about and want to answer the research question(s) proposed, and to begin reading and writing early. Also, it's important to ensure the PhD does not take over your personal life and to make sure you celebrate the successes throughout the journey.

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Appendices

Appendix A: Ethical approval certificate

Certificate of Ethical Approval

Applicant:

Corbin Griffen

Project Title:

Effects of 12 weeks resistance training and whey protein supplementation on multiple indices of sarcopenia in older men

This is to certify that the above named applicant has completed the Coventry University Ethical Approval process and their project has been confirmed and approved as Medium Risk

Date of approval:

31 August 2017

Project Reference Number:

P59723

Appendix B: NHS GafREC approval

Some content has been removed on data protection grounds

Appendix C: Participant information sheet

Participant Information Sheet (Version 3.0)

Study title: **Effects of 12 weeks resistance exercise and whey protein supplementation on multiple indices of sarcopenia in older men**

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information please do not hesitate to contact us.

Principle Investigator: Corbin Griffen (PhD Student, Centre for Applied Biological & Exercise Sciences, Coventry University)

Other scientists working on the study: Professor Derek Renshaw (Professor of Translational Physiology, Coventry University), Dr John Hattersley (Head of Human Metabolism Research Unit, University Hospitals Coventry & Warwickshire) and Professor Michael Duncan (Professor of Sport and Exercise Science, Coventry University).

What is the study about?

This study will investigate whether participating in twice weekly supervised resistance exercise (weighted exercise) and increasing protein intake (through twice daily supplementation), for 12 weeks has beneficial effects on multiple measurements related to sarcopenia. Sarcopenia is defined as the loss of muscle mass, strength and reduction in the ability to perform everyday tasks as a consequence of ageing.

Why is this study important?

After the age of 50, individuals lose between 0.5-1% of their muscle mass and 3% of their muscle strength each year. Over time, these losses can lead to sarcopenia, which affects approximately 10% of individuals over the age of 60 years globally. Sarcopenia has been associated with an increased risk of falls, cardiovascular disease, type 2 diabetes, hospitalisation and a reduction in cognitive function. This highlights the need for research on interventions which can slow the rate of muscle loss due to ageing. Both resistance exercise and increasing daily protein intake are two strategies shown to improve outcomes related to sarcopenia in older adults. However, further research investigating the combined effects of resistance exercise and increasing protein intake on multiple outcomes related to sarcopenia is required.

What is still unclear is:

- **IF** the effects of combined resistance exercise and increasing protein intake improves multiple outcomes related to sarcopenia?
- **HOW** these benefits occur?
- **WHETHER** the effects of combined resistance exercise and increasing protein intake improves metabolic health and cognitive function?

We'd like your help to find this out.

Who can enter the study?

We are looking for **52 men** who are aged between 60 and 80 years.

Unfortunately, you will NOT be able to volunteer for this study if:

- You are a current smoker or ex-smoker who ceased smoking less than 6 months ago
- Your body mass index (BMI) is less than 18.5 kg/m^2 (underweight) or greater than 30 kg/m^2 (obese)
- You currently participate in resistance exercise (using weights) regularly
- You are not weight stable (lost or gained more than 3 kg in the last 6 months)
- You are currently, or have previously taken part in another research project within the last 6 months involving a dietary and/or exercise intervention
- You are currently taking protein or amino acid supplements
- Your GP has told you that you have a disease related to your heart, kidneys, lungs, gut, blood, brain, thyroid, or have been diagnosed with cancer or diabetes
- You are predisposed to gout, or have a recent history of gout within the last 2 years
- You have high blood pressure which is not controlled
- You are prescribed (daily) non-steroidal anti-inflammatory medication, hormonal medication, cholesterol lowering medication, diabetic medication, or beta blockers
- You are lactose intolerant or allergic to wheat or potatoes
- You have a history of falls
- You have muscular disorders or injuries, or are unable to walk unaided

We will check your eligibility for the study by asking you to complete a health and lifestyle questionnaire. However, if you are in doubt about whether or not you are suitable to volunteer, please do not hesitate in contacting the principal investigator of the study.

What do we aim to do?

Our aim is to determine whether participating in supervised resistance exercise twice a week, in addition to consuming a 25 g whey protein supplement immediately after breakfast and lunch daily for 12 weeks has beneficial effects on muscle mass, strength and physical, metabolic and cognitive function in men aged 60-80 years. We will also assess a number of markers found in blood and saliva that are linked to sarcopenia and metabolism.

Our study design will help us to understand whether combining resistance exercise and increasing protein intake is more beneficial to our study outcomes than participating in resistance exercise alone, increasing protein intake alone or not altering physical activity levels or daily protein intake.

Therefore, if you are eligible for this study, you will be allocated to **one** of the following four groups:

1. **Resistance exercise and protein supplementation**
   The resistance exercise and whey protein supplementation group will perform supervised resistance exercise two times per week for between 45-60 minutes in duration for 12 weeks. Participants randomised to this group will also be required to consume a 25 g powdered whey protein supplement to be consumed after breakfast and lunch daily for 12 weeks.

2. **Resistance exercise and placebo supplementation**
   The resistance exercise and placebo supplementation group will perform supervised resistance exercise two times per week for between 45-60 minutes in duration for 12 weeks. Participants randomised to this group will also be required to consume 23.75 g of placebo powder (containing no protein) to be consumed immediately after breakfast and lunch daily for 12 weeks.

3. **Protein supplementation only**
   The protein supplementation only group will be required to consume a 25 g powdered whey protein supplement to be consumed immediately after breakfast and lunch daily for the 12-week intervention.

4. **Control group**
   The control group will be required to consume 23.75 g of placebo powder (containing no protein) to be consumed immediately after breakfast and lunch daily for the 12-week intervention.

The test foods used in this study will appear and taste similar. We will provide recipes of how to consume your test foods, but you will be given freedom to choose to your own particular taste. You will be randomly allocated to one of these groups and, you will therefore be unable to request which group you will be allocated to. We will ask that you **do not** change your normal physical activity and dietary habits throughout the study.

What do I have to do?

You will be asked to attend visits at Coventry University and University Hospitals Coventry & Warwickshire Human Metabolism Research Unit on 4 occasions (in total) during the study. We will send you maps and information of where each visit will take place.

- **Visit 1**: Metabolic assessment (Human Metabolism Research Unit, University Hospitals Coventry & Warwickshire) - **at the beginning of the study**
- **Visit 2**: Strength and functional performance testing (Coventry University) – **at the beginning of the study**
- **Visit 3**: Metabolic assessment (Human Metabolism Research Unit, University Hospitals Coventry & Warwickshire) – **after the 12-week intervention**

- **<u>Visit 4</u>**: Strength and functional performance testing (Coventry University) – **after the 12-week intervention**

You will be required to avoid strenuous exercise 48 hours before each testing visit. Parking at University Hospitals Coventry & Warwickshire will be covered by the Human Metabolism Research Unit and free parking will also be available for visits to Coventry University.

In addition to the above testing visits, if you are randomised to either of the resistance exercise groups, you will be required to attend Coventry University two times per week for 12 weeks for supervised resistance exercise sessions with a qualified gym instructor.

The measurements that will be taken during the study are detailed below.

<u>Strength and Functional Performance Assessment (Coventry University)</u>

The following assessments will be taken at each strength and functional performance testing visit.

- **Body composition –** You will be asked to stand on a plate which will assess your total muscle and fat mass.
- **Handgrip strength –** You will be asked to sit on a chair and grip a handgrip dynamometer as hard as you can for no more than 5 seconds.
- **Muscle strength –** You will be asked to perform a strength test on your legs
- **Short Physical Performance Battery –** You will be asked to perform 3 different tests. These will include:
  - Time to rise from a chair and return 5 times
  - Normal walking speed over 4 metres
  - 3 different balance tests including feet together and feet apart.
- **6-meter usual walking speed –** You will be timed walking 6 meters at your normal walking speed twice.
- **6-minute walk test –** You will walk at your normal walking speed for 6 minutes.
- **Questionnaires –** During the study duration we will ask you to complete questionnaires assessing your physical activity levels and dietary patterns.
- **Physical Activity Monitor –** We will ask you to wear an accelerometer for 7 days (which looks like a watch on your wrist).

<u>Metabolic assessment (Human Metabolism Research Unit, University Hospitals Coventry and Warwickshire NHS Trust)</u>

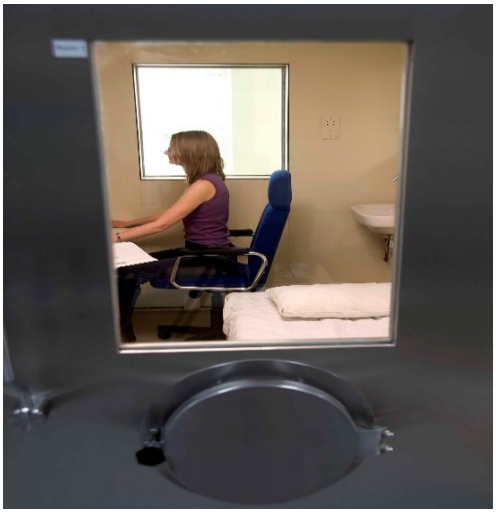
You will be required to stay in a whole-body calorimeter for 24 hours to enable measurement of a number of markers related to metabolic health and sarcopenia. Research staff will be present within the unit throughout the day, and nursing staff will check hourly throughout the night. Before entering the calorimeter, we will assess your muscle content by asking you to sit inside a small pod. For this assessment, tight clothing (e.g. cycling shorts or swimming trunks) and a cap will be required to be worn.

Calorimeters are “bedsit” style rooms; each has a desk, chair, fold-down bed, toilet, sink, telephone and an internet connected computer/television. There is an intercom to speak to

the resident in the second room, as well as the staff in the containing laboratory. The rooms have 3 windows; 1 with an outside view, 1 to the neighbouring room and 1 in the door facing the researchers. There are three 2-way hatches that are air locked which are used for passing food/personal items, samples and taking blood tests. The unit is situated within the heart of the University Hospitals Coventry & Warwickshire, next to a 24-hour ward with the full security and medical safety.

You will be left to live freely within the calorimeter with the exception of set times for meals, light exercise, blood and saliva collection, cognitive function tests and rest. All meals will be provided to you by the research team. Unrestricted access to drinking water will be provided; however, no alcohol, tea, coffee or other caffeinated beverages will be allowed to be consumed as these may affect the study outcomes. Fruit teas are allowed to be consumed and will be provided by the unit.

Before entering the calorimeter, a qualified nurse will insert a cannula into a suitable vein for repeated blood samples to measure markers related to both metabolic health and sarcopenia over the 24 hours. Approximately 115 ml (22 teaspoons) of blood will be taken from the cannula at each 24-hour visit. You will also be asked to wear a continuous glucose monitor to enable monitoring of your sugar levels.



Whole-body calorimeter at the Human Metabolism Research Unit, University Hospitals Coventry & Warwickshire NHS Trust

During the metabolic assessment, you will also be asked to perform a battery of cognitive tests which will assess your reaction time and how you process tasks. The test will last for approximately 30-40 minutes on a tablet device.

Please note, only 40 out of the final 52 participants will complete the 24-hour metabolic assessment for this study. Priority will be given to the first 40 participants. Participants who do not perform the 24-hour metabolic assessment will be required to attend a 3-hour visit at University Hospitals Coventry and Warwickshire NHS trust for a fasted blood test, a muscle mass measurement and a cognitive function test.

What are the benefits of taking part?

We cannot guarantee that taking part in this study will benefit you individually. However, we hope that the results of this study will inform guidelines of an intervention that can slow the rate of muscle loss in men aged 60-80 years. Through taking part in this study, you will receive a thorough examination of your metabolic health and performance of a range of

exercise/functional performance tests in relation to the general population by exercise and metabolism specialists. The metabolic assessment will use a state-of-the-art scientific instrument, which is only available in two locations in the UK. This data will be provided to you in the form of a mini health MOT form at the end of the study. Participants randomised to the exercise intervention will also receive free supervised exercise sessions at Coventry University gym by a qualified exercise professional. All participants who complete the 12-week study will also receive a £20 Waterstones voucher as a thank you for participating.

Are there any side effects or risks of taking part in the study?

The protein and placebo supplements used in this study will be provided by commercial and reputable companies and are fit for human consumption. Both the protein and placebo supplements will be given in a dose that can be achieved from your normal diet (95 kcal per supplement). In this respect, we do not anticipate any side effects. The supplements may contain very small traces of egg, gluten, soy and milk as they are produced in laboratories with other nutritional products. Therefore, if you are highly sensitive to these, we recommend you do not participate in this study.

The exercise sessions will be conducted in accordance with guidelines set by the American College of Sports Medicine (ACSM) for older adults. There is a potential risk of injury and discomfort from participating in resistance exercise. These risks will be minimised thorough familiarisation of each exercise, warm up and cool down procedures before and after each session, monitoring of your perceived exertion and supervision of all exercise sessions by qualified exercise professionals.

The continuous glucose monitor fitted during the metabolic assessment is a minimally invasive device that is attached to the skin; there is a small risk of skin irritation from the adhesive plaster and minor discomfort from a number of finger pricks to calibrate the device. It is normal that you may feel some discomfort whilst a blood sample is taken, and the cannula is inserted, and there is a slight risk of bruising. Only experienced nursing staff and trained research scientists will conduct cannulation and blood drawing to minimises these risks.

Will my details be confidential?

Any personal information (e.g., name, address, contact details) supplied by you during the study, will be handled by trained research staff and will be treated as strictly confidential (e.g. stored in locked filing cabinets, within a room with restricted access). All participants will be assigned a random 3-digit number when enrolled onto the study, and all paperwork, samples and results related to the study will be coded with this number to protect your identity.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part, you are still free to withdraw at any time and without giving a reason. If you chose not to take part in the study, or withdraw from the study at any time, this will not affect your future healthcare. An expression of interest does not commit you to participation.

What will happen to the results of the research study?

The results of this research study will be published in scientific journals and presented at national and international scientific conferences. You will not be identified personally in any

output related to this study and you will be provided with a summary of the research findings after the data has been analysed.

Who has reviewed this study?

This study has been reviewed and approved by the Ethics Committee at Coventry University and by Research & Development at University Hospitals Coventry & Warwickshire NHS Trust. The procedures in place for the study adhere to the Code of Conduct of the British Association of Sport and Exercise Sciences.

What if I have more questions?

If you have any further questions please get in touch with the principal researcher for the study, Corbin Griffen, e-mail: [redacted] or telephone

What happens next?

If you would like to proceed with the study, please complete the health and lifestyle questionnaire attached and return via email to [redacted]

Thank you for showing interest in this study and for taking the time to read this information sheet.

Appendix D: Informed consent form

Informed Consent Form

Volunteer study ID: | | | |

Study title: Effects of 12 weeks resistance training and whey protein supplementation on multiple indices of sarcopenia in older men

	Please initial
1. I confirm that I have read and understood the participant information sheet.	
2. I have been given the opportunity to ask questions about the study and my participation.	
3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason.	
4. I understand that all the information I provide will be treated in confidence. I understand that any data gained may be used in scientific publication but that the data will be anonymous.	
5. I agree to take part in the research project.	

(Name in BLOCK Letters:):
……..

Signature of volunteer …….

	D	D	M	M	M	Y	Y	Y	Y
DATE:									
E.g.	*0*	*1*	*J*	*A*	*N*	*2*	*0*	*1*	*7*

Scientist/researcher taking consent (I confirm that the volunteer above has been given a full verbal and written explanation of the study)
Name of researcher (PRINT): ……..
Signature of researcher: ……..

	D	**D**	**M**	**M**	**M**	**Y**	**Y**	**Y**	**Y**
DATE:									
E.g.	*0*	*1*	*J*	*A*	*N*	*2*	*0*	*1*	*7*

PLEASE PROVIDE A PHOTOCOPY OF THIS SHEET TO THE PARTICIPANT AND RETAIN ORRIGNAL FOR STUDY RECORDS

Appendix E: Recruitment poster

Are you a generally healthy male aged 60-80 years?

If so, you are being invited to participate in a cutting-edge research study investigating the impact of a 12 week exercise and/or nutrition intervention on outcomes related to healthy ageing.

Benefits of participating:

- Opportunity for 12 weeks free exercise sessions with a qualified personal trainer
- You will receive a unique and detailed health assessment and metabolic profile using state-of-the-art scientific equipment
- £20 Waterstones voucher on completion of the study

Volunteers will need to: Attend 4 test visits over 3 months (2 x 2 hour visits at Coventry University & 2 x 24 hour metabolic assessments at University Hospitals Coventry & Warwickshire NHS Trust) • Provide blood, saliva & urine samples • Have body composition, muscular strength & performance + metabolic & cognitive function assessed.

Interested in finding out more? Please contact Corbin Griffen

Some content has been removed on data protection grounds

Appendix F: Health and lifestyle questionnaire

HEALTH AND LIFESTYLE QUESTIONNAIRE

The following questionnaire will help us to determine if you are eligible to participate in the research study. Please complete each question in full.

	DAY		MONTH			YEAR			
Date questionnaire completed									
Example	**1**	**0**	**J**	**U**	**N**	**2**	**0**	**1**	**7**

Your Contact details

Title: **Forename(s):** **Surname:**	**Address:** **Postcode:**
Landline telephone number:	**Mobile telephone number:**
E-mail address:	**Tell us the best way to contact you:**

About you:

Date of Birth:		
Age today:		
Approximately how tall are you?		**BMI:**
What is your approximate weight?		**Office use only:**

Has your weight changed in the last 3 **months? IF YES, by how much?**

Q1. Do you smoke? If you are an ex-smoker, when did you stop? A. (**if previous smoker**, please give further details of approximate no. of cigarettes smoked per day and no. of years smoked)

BRIEF MEDICAL HISTORY
(please be aware that we do NOT have access to your medical notes and any relevant information you can provide is very helpful)

For the following questions, please tell us **<u>if your GP has diagnosed you</u>** with existing or previous history of:

Q2. Heart disease (e.g. Angina), stroke or any other disease of the circulation (such as Reynaud's disease)? IF yes please give further details
Q3. Diabetes (either type 1 or type 2)? IF yes please give further details
Q4. Cancer IF yes please give further details
Q5. A liver or kidney complaint / disease? IF yes please give further details

Q6. A digestive disease (such as Chron's, coeliac irritable bowel, colitis)? IF yes please give further details
Q7. Neurological disorder, severe muscular injuries or a thyroid condition? IF yes please give further details
Q8. Respiratory conditions, including asthma: IF yes please give further details
Q9. Any other illness or conditions we need to be aware of? IF yes please give further details
Q11. Has your doctor diagnosed you as having high cholesterol? IF yes please give further details
Q12. Has your doctor diagnosed you as having high blood pressure? IF yes please give further details

Q13. Do you have any known food intolerances (e.g. lactose intolerance), food allergies, or other allergies? IF yes please give further details
Q14. Are you claustrophobic?

MEDICATIONS

Q15. Have you been prescribed cholesterol lowering drugs (e.g. statins, simvastatin, atorvastatin)?
Q16. Have you been prescribed blood pressure medications (e.g. alpha blockers, beta blockers, ACE inhibitors, Ca^{2+} channel blocker)
Q17. Have you been prescribed anti-diabetic medications (e.g. insulin, exenatide, liraglutide) or oral hypoglycemic agents / oral antihyperglycemic agents (e.g. glipizide, metformin, pioglitazone)?

Q18. Are you currently on any long-term medication; including aspirin / steroids, antihistamines, anti-inflammatory medication, pain relief?

A. (**if yes**, please give further details)

Q19. Do you regularly take non prescribed pain relief, anti-inflammatory?

A. (**if yes**, please give further details of brand, dose, duration of use)

Q20. Are you currently prescribed hormone replacement therapy?

Q21. Do you currently take any other medications?

DIETARY OR SUPPLEMENT USE

Q22. Are you on a therapeutic diet? or '*dieting'* with the intention of substantial weight loss?

A. (**if yes**, please give further details regarding the dietary regime)

Q23. Do you currently consume protein and/or amino acid supplements, or any other nutritional supplements?

A. (**if yes**, please give further details)

Q24. Have you been involved in an **exercise** or **dietary intervention** trial in the last 6 months? If yes, please provide more details.

EXERCISE AND PHYSICAL ACTIVITY

Q25. Do you currently participate in resistance exercise (using weights) regularly or have done within the last 6-12 months?
Q26. Do you regularly participate in any other regular exercise more than two times per week?
Q27. Has your GP told you for any reason why you should not participate in an exercise programme?

OFFICE USE ONLY – OFFICE USE ONLY – OFFICE USE ONLY

Is the volunteer eligible to proceed in the trial?

YES NO

Name of scientist / research nurse (PRINT): …….

Signature of the scientist / research nurse: ……

	D	D	M	M	M	Y	Y	Y	Y
DATE:									

Appendix G: Supplement nutrient and amino acid sheet

Appendix H: Supplement log

CRF 10. Missed sachet sheet

Please complete the table of any missed sachets throughout the 12 weeks.

Date	Time (morning/lunch)	Reason

Appendix I: Example exercise training log (up to session 8)

Resistance exercise log – Sarcopenia study

Participant ID:

Group Allocation:

Session Number	Date	Attended?
1) 1RM (1) – W0		
2) RT Session 2 – W1		
3) RT Session 3 – W2		
4) RT Session 4 – W2		
5) RT Session 5 – W3		
6) RT Session 6 – W3		
7) RT Session 7 – W4		
8) RT Session 8 – W4		
9) 1RM (2) – W5		
10) RT Session 10 – W5		
11) RT Session 11 – W6		
12) RT Session 12 – W6		
13) RT Session 13 – W7		
14) RT Session 14 – W7		
15) RT Session 15 – W8		
16) RT Session 16 – W8		
17) 1RM (3)		
18) RT Session 18 – W9		
19) RT Session 19 – W10		
20) RT Session 20 – W10		
21) RT Session 21 – W11		
22) RT Session 22 – W11		
23) RT Session 23 – W12		
24) RT Session 24 – W12		

Session 1) 1RM
Date:

Warm up	**Machine set up**	**Heaviest weight (1RM)**	**Comments**	**RPE**
Bike – 5 minutes		50 rpm		
Whole-body dynamic exercises – 5 minutes				
Main session				
Leg Press				
Chest Press				
Leg extension				
Shoulder Press				
Hamstring Curl				
Lat row				
Cool Down				
5 minute of bike – self-selected pace				
Whole body static exercises				

Session 2) Resistance training session **(60% 1RM).**
Date:

Warm up	**Machine set up**	**Intensity/Weight**	**Target reps**	**Sets**	**Reps completed**	**Comments**
Bike – 5 minutes		50 rpm				
Whole-body dynamic exercises – 5 minutes						
Main session						
Leg Press			**12-15 (final set failure)**	**3**		
Chest Press			**12-15 (final set failure)**	**3**		
Leg extension			**12-15 (final set failure)**	**3**		
Shoulder Press			**12-15 (final set failure)**	**3**		
Hamstring Curl			**12-15 (final set failure)**	**3**		
Lat row			**12-15 (final set failure)**	**3**		
Cool Down						
5 minute of bike – self-selected pace						
Whole body static exercises						

Session 3: RT Session **(60% 1RM).**
Date:

Warm up	**Machine set up**	**Intensity/Weight**	**Target reps**	**Sets**	**Reps completed**	**Comments**	**RPE**
Bike – 5 minutes		50 rpm					
Whole-body dynamic exercises – 5 minutes							
Main session							
Leg Press			**12-15 (final set failure)**	**3**			
Chest Press			**12-15 (final set failure)**	**3**			
Leg extension			**12-15 (final set failure)**	**3**			
Shoulder Press			**12-15 (final set failure)**	**3**			
Hamstring Curl			**12-15 (final set failure)**	**3**			
Lat row			**12-15 (final set failure)**	**3**			
Cool Down							
5 minute of bike – self-selected pace							
Whole body static exercises							

Session 4: RT Session **(70% 1RM).**
Date:

Warm up	**Machine set up**	**Intensity/Weight**	**Target reps**	**Sets**	**Reps completed**	**Comments**	**RPE**
Bike – 5 minutes		50 rpm					
Whole-body dynamic exercises – 5 minutes							
Main session							
Leg Press			**12-15 (final set failure)**	**3**			
Chest Press			**12-15 (final set failure)**	**3**			
Leg extension			**12-15 (final set failure)**	**3**			
Shoulder Press			**12-15 (final set failure)**	**3**			
Hamstring Curl			**12-15 (final set failure)**	**3**			
Lat row			**12-15 (final set failure)**	**3**			
Cool Down							
5 minute of bike – self-selected pace							
Whole body static exercises							

Session 5: RT Session **(70% 1RM)**.
Date:

Warm up	**Machine set up**	**Intensity/Weight**	**Target reps**	**Sets**	**Reps completed**	**Comments**	**RPE**
Bike – 5 minutes		50 rpm					
Whole-body dynamic exercises – 5 minutes							
Main session							
Leg Press			**12-15 (final set failure)**	**3**			
Chest Press			**12-15 (final set failure)**	**3**			
Leg extension			**12-15 (final set failure)**	**3**			
Shoulder Press			**12-15 (final set failure)**	**3**			
Hamstring Curl			**12-15 (final set failure)**	**3**			
Lat row			**12-15 (final set failure)**	**3**			
Cool Down							
5 minute of bike – self-selected pace							
Whole body static exercises							

Session 6: RT Session **(70% 1RM)**
Date:

Warm up	**Machine set up**	**Intensity/Weight**	**Target reps**	**Sets**	**Reps completed**	**Comments**	**RPE**
Bike – 5 minutes		50 rpm					
Whole-body dynamic exercises – 5 minutes							
Main session							
Leg Press			**8 (final set failure)**	**3**			
Chest Press			**8 (final set failure)**	**3**			
Leg extension			**8 (final set failure)**	**3**			
Shoulder Press			**8 (final set failure)**	**3**			
Hamstring Curl			**8 (final set failure)**	**3**			
Lat row			**8 (final set failure)**	**3**			
Cool Down							
5 minute of bike – self-selected pace							
Whole body static exercises							

Session 7: RT Session **(80% 1RM)**.
Date:

Warm up	**Machine set up**	**Intensity/Weight**	**Target reps**	**Sets**	**Reps completed**	**Comments**	**RPE**
Bike – 5 minutes		50 rpm					
Whole-body dynamic exercises – 5 minutes							
Main session							
Leg Press			**8 (final set failure)**	**3**			
Chest Press			**8 (final set failure)**	**3**			
Leg extension			**8 (final set failure)**	**3**			
Shoulder Press			**8 (final set failure)**	**3**			
Hamstring Curl			**8 (final set failure)**	**3**			
Lat row			**8 (final set failure)**	**3**			
Cool Down							
5 minute of bike – self-selected pace							
Whole body static exercises							

Session 8: RT Session **(80% 1RM).**
Date:

Warm up	**Machine set up**	**Intensity/Weight**	**Target reps**	**Sets**	**Reps completed**	**Comments**	**RPE**
Bike – 5 minutes		50 rpm					
Whole-body dynamic exercises – 5 minutes							
Main session							
Leg Press			**8 (final set failure)**	**3**			
Chest Press			**8 (final set failure)**	**3**			
Leg extension			**8 (final set failure)**	**3**			
Shoulder Press			**8 (final set failure)**	**3**			
Hamstring Curl			**8 (final set failure)**	**3**			
Lat row			**8 (final set failure)**	**3**			
Cool Down							
5 minute of bike – self-selected pace							
Whole body static exercises							

Appendix J: 3-day diet record

3-day dietary record

Please complete a dietary record of everything you eat and drink for 3 days (**2 days during the week and 1 weekend day**). Please be as accurate and as honest as possible. If you need more space, please use back of piece of paper.

Example

VOL_ID:	Date: 29 JULY 2017		Day of week (circle below)					Typical? (circle)		Other important detail:
	Mon	Tue	Wed	Thur	Fri	Sat	Sun	YES	NO	
Meal / eating event	**Time (24hr format)**	**location**	**Food item – identify full description, brand, type (i.e. low fat, sugar free etc.)**			**Preparation method (e.g. boiled, grilled etc.)**	**Estimate amount**	**Leftovers? ..how much**		**Additional info identified during multiple pass**
Breakfast	07:30	Home	Cornflakes Milk Orange Juice Coffee, milk, 1 sugar				50g bowl ½ Pint / 284 ml 200ml glass 50ml milk	NO		
Snack	10:45	Coffee shop	Coffee, milk, 1 sugar Chocolate muffin				50ml milk 1 medium 1 bag- 25g	NO		
Lunch			Ham sandwiches, crisps (salt and vinegar), Kit Kat, Glass of orange cordial				1 bag- 25g 2 slices brown bread, Kit Kat chunky 250 ml orange cordial	NO		

VOL_ID:	Date:		Day 1)		Day of week (circle below)			Typical? (circle)		Other important detail:
	Mon	Tue	Wed	Thur	Fri	Sat	Sun	YES	NO	
Meal / eating event	Time (24hr format)	location	Food item – identify full description, brand, type (i.e. low fat, sugar free etc.)			Preparation method (e.g. boiled, grilled etc.)	Estimate amount	Leftovers? ..how much		Additional info identified during multiple pass

VOL_ID:	Date:		Day 2)		Day of week (circle below)			Typical? (circle)		Other important detail:
	Mon	Tue	Wed	Thur	Fri	Sat	Sun	YES	NO	
Meal / eating event	Time (24hr format)	location	Food item – identify full description, brand, type (i.e. low fat, sugar free etc.)			Preparation method (e.g. boiled, grilled etc.)	Estimate amount	Leftovers? ..how much		Additional info identified during multiple pass

VOL_ID:	Date:		Day 3)		Day of week (circle below)			Typical? (circle)		Other important detail:
	Mon	Tue	Wed	Thur	Fri	Sat	Sun	YES	NO	
Meal / eating event	Time (24hr format)	location	Food item – identify full description, brand, type (i.e. low fat, sugar free etc.)			Preparation method (e.g. boiled, grilled etc.)	Estimate amount	Leftovers? ..how much		Additional info identified during multiple pass

Appendix K: Actigraph log

Actigraph Instruction Sheet

Please wear this monitor on your **dominant** wrist for the next 7 days. The monitor is an accelerometer and it will monitor your activity levels. Please carry on your week as normal and do not adjust your activity levels due to wearing the monitor. The monitor should be worn both during the day and overnight. The monitor should be placed snug to the wrist to pick up movement.

Please note: The accelerometer will not display any information during the study period

When you take the monitor off (e.g. for showering or swimming), please write down the approximate time you took it off and put it back on in the table below. This will help us to determine any periods of non-wear when the data is analysed. Please also write down the final time the monitor was taken off. Finally, please bring this piece of paper back with you when your return the monitor at your next visit.

Day	Time taken off	Time put back on	Normal day activity? (yes/no) – if no why?
1			
2			
3			
4			
5			
6			
7			

Final time taken off:			

Appendix L: Timeline for 24-h metabolic testing

Day 1	
Time	**Activity**
2000	Meal
2130	Blood Pressure and Hunger/ Sleep Scales Blood sample 7 SALIVA sample 5
2200	SWIPE glucose meter Lights out, apply external blind

Day 2	
0650	Wake SWIPE SALIVA sample 1 & 8 Prepare for RMR
0700	Resting on bed for 60min
0805	SALIVA sample 2 Exchange Urine bottle and **weigh** Blood Pressure and Hunger/ Sleep Scales
0815	Cannulation - Blood sample 1 (and 8) Prepare for Exercise
0830	Start Exercise step 75 bpm for 30m
0900	Blood sample 2 Blood Pressure and Hunger/ Sleep Scales
0915	Meal
1000	Start Cognition test
1225	Blood Pressure and Hunger/ Sleep Scales SALIVA sample 3
1230	Blood Sample 3
1240	Meal
1355	Blood Pressure and Hunger/ Sleep Scales SWIPE Blood Sample 4

1445	Start **Exercise** step 75 bpm for 30min
1700	**SALIVA** sample 4
1715	Blood Pressure and Hunger/ Sleep Scales **Blood** sample 5
1720	**Meal**
1835	Blood Pressure and Hunger/ Sleep Scales **Blood** sample 6
1915	Start **Exercise** at 75 bpm for 30min
1955	Urine container exchange and **weigh** Blood Pressure and Hunger/ Sleep Scales **SWIPE**
2000	**SALIVA** sample 6

2005	Exit Room
(Remove cannula) **SWIPE** Remove CGM	

Appendix M: Pilot chamber data

Vol	RMR (kcal/d)	Total EE (kcal)	PAL
1	1837	2559	1.39
2	1562	2373	1.52
3	1295	1900	1.47
4	1343	2088	1.55
5	1604	2317	1.44
		Mean	1.47
		SD	0.06

Appendix N: Appetite visual analogue scales

Satiety Visual Analogue Scale

Participant Study number: | Date: | Time:

Please mark with a pen on each line depending on how you feel at this moment.

Hunger

I am not hungry at all — How hungry do you feel? — I have never been more hungry

Satiety

I am completely empty — How satisfied do you feel? — I cannot eat another bite

Fullness

How full do you feel?

Not at all full — Totally full

Appetite

How much do you think you can eat?

Nothing at all — A lot

Appendix O: Copyright agreement with Cambridge Cognition

Content removed on data protection grounds

Content removed on data protection grounds