MONASH University

Dairy Milk for Skeletal Muscle Health and Sarcopenia in Active Older Adults

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March, 2021

A thesis submitted for the degree of Doctor of Philosophy at

Monash University in 2021

Department of Nutrition, Dietetics and Food

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Thesis Summary

In the ageing population, inadequate protein intake and sedentary behaviour are known to accelerate muscle loss, strength and physical function, a condition known as sarcopenia. Previous studies have focused on modifiable risk factors such as protein supplement interventions (e.g., whey or casein), with or without exercise, to assess the effects on outcomes related to sarcopenia. In comparison, less is known about the role of whole foods, such as dairy milk, in preventing or treating sarcopenia. Dairy milk is a modifiable factor that is easily accessible and is considered a high-quality protein source. Furthermore, most studies have used frail or institutionalised older adults within their study cohorts, with limited consideration of active older adults, who despite having a higher baseline of skeletal muscle and strength than their sedentary counterparts are currently underrepresented in sarcopenia research. The overall aim of this thesis was to investigate the effects of a high-protein dairy milk beverage on outcomes related to sarcopenia in a cohort of active older adults.

A literature review systematically synthesised the current literature regarding dairy milk beverages and dairy-based protein supplementation with or without exercise on healthy older adults to determine how it may impact sarcopenia outcomes. The review highlighted the methodological gaps in the current literature to provide direction to a clinical study that is more relevant to active older adults. It also revealed mixed results regarding protein supplementation and dairy beverages, with or without exercise interventions on improving skeletal muscle mass, skeletal muscle strength and performance outcomes. However, it seemed evident that older adults required higher protein intakes, especially when there is limited distribution across meal periods (i.e., breakfast and lunch).

Two cross-sectional studies were conducted to explore the current anthropometrical, strength, physical activity levels and dietary habits of older adults considered active. The main findings were that age, training status, habitual protein intake and biological sex can explain individual variation in outcomes

related to body composition, strength, and power. Furthermore, daily protein intake, even distribution of protein intake across the three main meals, number of protein meals containing ≥0.4 grams per kilogram of bodymass per meal can assess associations with SMM outcomes, muscle strength and power in a cohort of active older adults.

Then finally, in a randomised control trial that spanned across 12-weeks, the independent and combined effects of a high protein dairy milk beverage provided at breakfast and lunch (or after resistance exercise), with or without progressive resistance training on outcomes of fat-free mass, skeletal muscle strength and power, and physical performance were assessed in a cohort of healthy active older adults. The findings did not support the hypothesis that additional high- protein dairy milk beverage (2x15 g) alone would influence fat-free mass gains, skeletal muscle strength, power or performance compared to control. The high- protein dairy milk beverage with progressive resistance training augmented strength, i.e., 78% leg press, 56% chest press, and 53% *latissimus dorsi* pull down) gains compared to progressive resistance training alone, but there were no significant fat-free mass, power, or physical performance changes. Another critical finding identified increased interleukin -10 cytokine levels in the group that received exercise and the high protein milk drink, indicating a potential anti-inflammatory effect.

The studies within this thesis collectively emphasised the complex nature of ageing and the many cofounding variables (e.g., age, training status, biological sex, and protein intake) that should be considered in the active ageing population. The research presented in this thesis provides novel insight for important methodological considerations for future studies on protein interventions in active older adults, such as; including participants from similar exercise training modalities, standardising baseline diets for all study groups or including participants with reported low habitual intakes (e.g., <1.2 grams per kilogram of bodymass per day), determining lifetime physical activity history, and including other biomarkers that may assist in further understanding of potential mechanisms of sarcopenia.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in a peer reviewed journal, and two submitted for publication. The core themes of the thesis are sports medicine, applied exercise physiology, sports nutrition and ageing. The ideas, development and writing of all the papers in the thesis were the principal responsibility of myself, the student, working within the Department of Nutrition, Dietetics and Food under the primary supervision of Dr. Ricardo Costa and Prof. Judi Porter. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers, and acknowledges input into team-based research.

In the case of Chapters 4 -6 my contribution to the work is presented in Table A :

Table A Thesis including published works declaration					
Thesis chapter	**Publication title**	**Status**	**Nature and % of student contribution**	**Co-author names, nature and % of co-author's contribution**	**Co-author(s), Monash student Y/N**
4	Sarcopenic characteristics of active older adults: A cross-sectional exploration	Published: *Sports Med Open*	Study design, data collection, data analysis, manuscript preparation (60%).	Ricardo Costa: study design, data analysis, manuscript review (25%) Judi Porter: Manuscript review, input into manuscript (10%) Alex Parr: Data collection (5%)	Y (Alex Parr)
5	Protein amount, quality and distribution in active older adults and its effects on outcomes of muscle mass, strength and power: The Active Ageing Study	Published: *International Journal of Sports Science*	Study design, data collection, data analysis, manuscript preparation (60%).	Ricardo Costa: study design, data analysis, manuscript review (25%) Judi Porter: Manuscript review, input into manuscript (10%) Alex Parr: data collection (5%)	Y (Alex Parr)
6	The effects of a high protein dairy milk beverage with or without progressive resistance training on fat-free mass, skeletal muscle strength and power, and functional performance in healthy active older adults: A 12-week randomized controlled trial.	Published: *Frontiers in nutrition*	Study design, data collection, data analysis, manuscript preparation (60%).	Ricardo Costa: study design, data analysis, manuscript review (25%) Judi Porter: Manuscript review, input into manuscript (10%) Alex Parr: data collection (5%)	Y (Alex Parr)
Industry report	Effect of dairy-based supplementation and dairy beverage combined with exercise intervention on skeletal muscle mass, strength and performance outcomes in older adults.	Internal industry publication: *Lion Dairy & Drinks 2018*	Study design, data collection, data analysis, manuscript preparation (60%).	Ricardo Costa: study design, data analysis, manuscript review (20%) Judi Porter: study design, manuscript review (10%) Chris Rach: Data collection and data analysis (5%) Vera Camões-Costa: Data collection and data analysis (5%)	N

Table A Thesis including published works declaration					
Thesis chapter	**Publication title**	**Status**	**Nature and % of student contribution**	**Co-author names, nature and % of co-author's contribution**	**Co-author(s), Monash student Y/N**
Industry report	Systematic review and meta-analysis of the evidence for the relationship between protein (supplement and dairy) and outcomes of skeletal muscle mass, muscle strength, muscle power, and physical performance in active older adults.	Internal industry publication*: Lion Dairy & Drinks 2020*	Study design, data collection, data analysis, manuscript preparation (60%).	Ricardo Costa: study design, data analysis, manuscript review (20%) Judi Porter: study design, manuscript review (10%) Chris Rach: Data collection and data analysis (5%) Vera Camões-Costa: Data collection and data analysis (5%)	N
Appendix	Comments and future directions arising from "The Impact of Dairy Protein Intake on Muscle Mass, Muscle Strength, and Physical Performance in Middle-Aged to Older Adults with or without Existing Sarcopenia"	Published: *Advances in nutrition*	Manuscript preparation, manuscript review (70%).	Ricardo Costa: manuscript preparation, manuscript review (15%) Judi Porter: manuscript preparation, manuscript review (15%)	N

Student signature: Date: 16/03/2021

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: Date: 16/03/2021

Publications during candidature

The following co-authored publications were completed as team-based research during the Ph.D. candidature and are not included within this thesis. My contribution to these publications include data and sample collection and input into each manuscript.

1. Costa, R. J., Camoes-Costa, V., Snipe, R. M., Dixon, D., Russo, I., & **Huschtscha, Z.** (2019). Impact of exercise-induced hypohydration on gastrointestinal integrity, function, symptoms, and systemic endotoxin and inflammatory profile. *Journal of Applied Physiology*, *126*(5), 1281-1291. (Published)
2. Costa, R. J., Camões-Costa, V., Snipe, R. M., Dixon, D., Russo, I., & **Huschtscha, Z.** (2020). The impact of a dairy milk recovery beverage on bacterially stimulated neutrophil function and gastrointestinal tolerance in response to hypohydration inducing exercise stress. *International journal of sport nutrition and exercise metabolism*, *30*(4), 237-248.

Conference Abstracts

1. Huschtscha Z, Russo I, Rhiannon MJ Snipe, Dixon D, Camões-Costa V, Rauch C, Costa R. Exercise-induced hypohydration causes malabsorption of carbohydrate rice pre-exercise meal (ISENC, UK, December 2018)
2. Huschtscha Z, Russo I, Rhiannon MJ Snipe, Dixon D, Camões-Costa V, Rauch C, Costa R. The impact of exercise-induced dehydration on systemic endotoxin and inflammatory cytokine profiles. (ISENC, UK, December 2018)
3. Huschtscha Z, Porter J, Costa R. Does training status and protein intake explain the differences in physical performance between trained endurance and recreationally active older adult males? (Medicine & Science in Ultra-Endurance Sports conferences, South Africa, October 2019)
4. Huschtscha Z, Porter J, Rauch C, Costa R. Is total daily protein and higher activity levels associated with higher lean body mass and muscle function in active older adults? (Sports Dietitians Australia, Australia, October 2019)
5. Huschtscha Z, Porter J, Costa R. Does plasma cytokines at rest have an influence of indices of sarcopenia in an active older cohort? (ISEI, China, November 2019)

The following co-authored abstracts were completed as team-based research during the Ph.D. candidature and are not included within this thesis. My contribution to these abstracts includes assistance with study design and preparation, data and sample collection, data and sample analysis and input into each manuscript.

Scholarships and funding

This research was part of the graduate Research Industry Program (GRIP) within Monash and Lion Dairy & Drinks as an industry partner.

Professional Acknowledgements

Writing this part of my thesis is the most desirable part, as I can express the sincere feelings which originated deep in my heart without any challenge of searching for the right references or using a citation manager software. It is a great pleasure to thank the many people who made this journey possible for me.

First and foremost, I would like to express my gratitude to Dr. Ricardo Costa. Thank you for your support, encouragement, and mentorship throughout this journey. Your dynamism, vision, and commitment for your research to meet the highest standards have truly inspired and motivated me to always strive to learn and be a better researcher. Thank you for providing me with such wonderful and enriching experiences throughout my thesis.

I would also like to express my sincere appreciation to Professor Judi Porter. Judi, you were such a wonderful addition to this team that helped complete this project. Thank you for sharing your wisdom and supporting me throughout this experience. I will forever be grateful for your warm demeanor and words of encouragement when I needed it the most.

To Isabella Russo and Stephanie Gaskell, thank you for your constant encouragement and support throughout this process. It was a privilege to be able to share such amazing experiences with you both, from trekking to the top of Lion's head after an international flight to our catch-ups at Wicked Café.

I would like to thank all staff and students at the Department of Nutrition, Dietetics and Food for all your support over the last four yrs. I am especially thankful to the sports nutrition team: Chris, Alan, and Alex. A special mention also must also go to Leagh, who assisted me with the food orders for my clinical trial.

Finally, this work would not materialise without the finical support of Lion Dairy & Drinks. I would like to also recognise the important roles of Greg Holden and Katrina Strazdins that allowed this project to come to life and for choosing me for the project.

Personal Acknowledgements

I wish to acknowledge the support and great love of my life: Nathan. There are not many couples that can say they both did a PhD at the same time, but we did it. Thank you for being my personal editor, my consigliere, and my sunshine when things felt grey.

To the biggest pillar of strength in my life: My mother. Mum, words cannot express the deep gratitude I have for all your support not only throughout my thesis, but my entire educational career. You are the epitome of strength and resilience - a characteristic I inherited from you, and which certainly came in handy during this thesis. Thank you for your wisdom, encouragement, and nutritional support during my thesis.

To the many friends and family in my life whose support made this possible: Stefanie, Zara, Jess, Bryce and Bendall. Thank you all for being my personal cheerleaders and reveling in my successes, as well as being the emotional pillars and being the shoulders I could always depend on when things got tough for me over the last 4 years.

Lastly, I would like to acknowledge and dedicate this thesis to the people that are no longer with us: my beloved Sister Svetlana, my Baba Slavka, and my Father Victor. My sister Svetlana was always a large advocate for me and pursing my passions and I know is she was here today, she would be so proud of me. Baba never got the opportunity to study and always valued education. She was very curious about the world and even up to the age of 90 years old, she always had a fascination about new things. She loved hearing about my studies and was the biggest advocate for me pursing my PhD. Lastly, my dearest Father, whom I inherited my curious mind from, and love of the sciences. He always encouraged me to keep asking questions and to never stop learning. Anytime things felt tough during these 4 years, I always reminded myself to "*do it for them*"- I drew on that strength to achieve this great achievment in my life. I miss you all so dearly, and truly wish you were here to see what I have accomplised. Although you weren't here, you walked and guided me through this; until we all meet again.

Thesis Format

This thesis commences with the general introduction (Chapter One) which provides background, rationale and justification for the research aims presented in this thesis. This is followed by a systematic review (Chapter Two) which synthesises previous randomised control trials, that examine the effects of interventions including dairy milk and dairy based supplementation with or without exercise protocols on outcomes related to age-related sarcopenia (e.g., skeletal muscle mass, strength and physical performance. This chapter outlines methodological considerations for future research to be applied to active older adults. An additional *letter to the editor* which appears in Appendix A, compliments the findings found in Chapter Two, and further identifies methodological gaps currently in the literature. The general methods are outlined in Chapter Three.

Chapter Four is cross-sectional study, which explores the relationship between age, biological sex, training status, and dietary protein intake on outcomes related to sarcopenia in active older adults.

Moreover, Chapter Five, is a complementary study that further explores potential associations of habitual protein intake, taking into consideration the effect of amount, timing, distribution, and type of protein on outcomes related to skeletal muscle mass, strength, and physical performance in active older adults. Both Chapter Four and Five contextualise the results of intervention studies presented in Chapter Six.

Chapter Six, is a 12-week randomised control study, comparing the effects of a high-protein milk beverage with or without progressive resistance training, on outcomes of fat-free mass, skeletal muscle strength, physical performance and power in active older adults.

Finally, Chapter Seven is the overall discussion that provides a synthesis and further interpretation of the findings of this thesis. Chapter Seven includes a discussion of the contributions to knowledge, strength and limitations, practical implications and directions for future research.

Chapters Three to Six and Appendix A of this thesis are presented in the format as published in peer-reviewed journals, the pages and references have been reformatted for consistency, and margins adjusted and re-formatted as required for submission of this thesis. Abbreviations are defined at first use throughout the thesis, excluded figures and tables, unless indicated. A full list of abbreviations, tables and figures is provided prior to Chapter One. An overlap in content occurs between chapters due to the complementary nature of the studies and links between chapters. Bold type is used to reference other sections within this thesis. The reference list for the in-text citations has been placed at the end of each chapter. This was done due to the nature of the thesis being by publication.

Some discrepancies in the spelling (US or UK), abbreviations and units occur between chapters, due to the specific requirements of the peer-reviewed journals.

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List of abbreviations

1RM	1 repetition maximum
AA	amino acids
ALM	appendicular lean mass
ANOVA	analysis of variance
BCAA	branched chained amino acids
BGL	blood glucose levels
BM	body mass
BMC	bone mineral content
CMJ	counter movement jump
CSA	cross sectional area
CV	coefficient of variation
EAA	essential amino acids
ELISA	enzyme linked immunosorbent assay
EWOSP2	European Working Group of Sarcopenia
FFM	fat-free mass
FM	fat mass
g	gram
HGS	hand grip strength
HR	heart rate
IGF-1	insulin-like growth factor-1
IL-6	interleukin 6
IL-8	interleukin 8
IL-10	interleukin 10
Kg	kilogram
L	litre
METs	metabolic equivalents
µg	microgram
µL	microlitre
min	minute
MJ	megajoule
m	metre
ml	millilitre
mmol	millimole
mOsmol	milliosmole
MPB	muscle protein breakdown
MPS	muscle protein synthesis
MyoMPS	myofibrillar muscle protein synthesis
nmol	nanomole
PRT	progressive resistance training

RDA	recommended daily allowance
RER	respiratory exchange ratio
RPE	rating of perceived exertion
RT	resistance training
RPE	rating of perceived exertion
SMM	skeletal muscle mass
TLM	total lean muscle
TNF-α	tumour necrosis factor alpha
V̇O2max	maximal oxygen uptake
W	watts
WHO	world health organization
WP	whey protein
yrs	years
≥	equal to or greater than
≤	equal to or less than

CHAPTER ONE:

GENERAL INTRODUCTION

1.1 What is sarcopenia

A healthy adult's total body mass comprises 45-50% of skeletal muscle tissue, which plays a fundamental role in whole energy metabolism, maintaining posture and balance, locomotion, and the storage and utilisation of substrates (e.g., amino acids) [1]. Then, from as early as 30 yrs old, there is a decline of skeletal muscle mass (SMM) that occurs at a rate of 0.5-1% per year and a pronounced decrease in skeletal muscle strength (3-5 % loss per year) [2, 3]. This progressive and involuntary decline in SMM and skeletal muscle strength is known as sarcopenia. It is considered one of the most critical factors implicating the progression of loco-motor functional decline, frailty, and disability with ageing [4–6]. Sarcopenia is predicted to affect 10-30% of older adults ≥ 60 yrs and between 11-50% of those aged ≥ 80 yrs, with even higher prevalence rates for those living in institutionalised care [5, 7, 8]. The considerable variation in prevalence data that appears due to the absence of a general consensus of a clinical definition for sarcopenia between different working groups (Table 1). These working groups define sarcopenia with other clinical cut-off points for SMM, skeletal muscle strength, and physical performance outcomes [7, 9, 10]. Such discrepancies make it difficult to compare results across studies in sarcopenic populations. However, despite the variability of clinical cut-off points, sarcopenia poses a growing global concern for health care systems, which may be ill-prepared. For example, in the year 2000, the annual health care cost attributed to sarcopenia and associated complications were estimated at $18 billion in the United States alone [7, 11]. Considering that global demographics indicate that the ageing population (i.e., those that are > 65 yrs) is set to triple by 2050, the application of a conservative estimate of sarcopenia prevalence suggests it will have a substantial impact on the health care system. The effect of the increasing ageing population and medical burden associated with sarcopenia highlight the importance of determining effective interventions for prevention and management at its early stages.

Table 1 Comparison of clinical definitions of sarcopenia amongst different working groups.

Working group	Muscle mass/ muscle quality	Function and strength
EWGSOP2 [7]	ASM: <20 kg for men, <16 kg for women ASM/height^2: <7.0 kg/m^2 for men; <5.5 kg/m^2 for women	Grip strength: <27 kg for men; <16 kg for women 5-time chair stand: >15s Gait speed: ≤0.8 m/s
IWGS [9]	ASM: <7.23 kg/m^2 for men; <5.67 kg/m^2 in women	Gait speed <1.0 m/s and grip strength.
AWGS [10]	ASM/height^2: <7.0 kg/m^2 for men; <5.4 kg/m^2 for women (using iDXA) OR <7.0 kg/m^2 for men, <5.7 kg/m^2 for females (using BIA).	Gait speed: <0.8 m/s Grip strength: <28 kg for males; < 18 kg for females 5-time chair stand

Abbreviations: ASM: appendicular muscle mass; AWGS Asian working group for Sarcopenia; BIA: bioelectrical impedance; *EWGSOP* European Working Group on Sarcopenia in Older People; IWGS international working group on Sarcopenia

Mechanisms causing sarcopenia

Skeletal muscle proteins are in a constant state of turnover, by which they are being simultaneously synthesised and degraded at a rate of 1-2% per day [12]. This turnover allows skeletal muscle tissue to be remodeled, for example, "old" or damaged muscle proteins to be replaced with new muscle proteins, or creating new protein structures to adapt to mechanical loading and stress on muscles from exercise or physical activity [13-15]. SMM is maintained through the dynamic balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB) [13]. Hypertrophy occurs when MPS exceeds MPB (i.e., the net protein balance is positive), whereas skeletal muscle is lost (atrophy) when MPB exceeds MPS [13, 14]. In acute laboratory studies, it is evident that

older adults have a blunted response to the same anabolic stimuli as their younger counterparts. This is known as *anabolic resistance* [16]. Anabolic resistance has been shown to be exacerbated in older adults following reduced physical activity [17], bed rest [18], and during times of illness [19]. For example, a study in healthy older adults (72 ± 1 yrs) observed a 12% decline in lean leg mass and 15% decline in leg strength following 14-days of reduced physical activity (≤ 1500 steps/day) [17]. This decline in skeletal muscle and strength were accompanied with a 30-50% reduction in post absorptive and postprandial MPS rates [17]. For older adults to overcome anabolic resistance and reach the same myofibrillar protein synthetic response in muscle, they need to consume more significant amounts of protein than their younger counterparts [20]. In *vivo,* the stimulation of myofibrillar muscle protein synthesis (MyoMPS) is dependent on a complex, intracellular molecular signaling cascade that activates the mammalian target of rapamycin complex 1 (mTORC1), which is a crucial regulator of MyoMPS [21]. The mTORC1 pathway is activated in response to two primary anabolic cues; through mechanical loading of the skeletal muscle (e.g., resistance training), which causes the release of insulin-like growth factor-1 (IGF-1) that triggers the activation of phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway [22, 23]; and dietary protein (e.g., amino acids), which acts as the building blocks for *de novo* synthesis [22, 23]. In particular, the branched chained amino acid (BCAA), leucine (LEU) can directly activate MyoMPS through the phosphorylation of the mTORC1 complex [22, 23]. When resistance training and protein are combined, it results in a synergistic effect on MyoMPS [24]. If practiced continuously can result in the skeletal muscle remodeling and accretion of muscle protein, such as increasing the cross-sectional muscle area (CSA), muscle thickness, and increases in type II muscle fibers, which is when SMM results in hypertrophy and increased skeletal muscle strength [25, 26].

Ageing is associated with chronic low-grade inflammation, a term known as '*inflammaging*' [27, 28]. Inflammaging is categorised whereby a chronic state of slightly increased plasma levels of pro-

inflammatory mediators, tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, and a reduction of anti-inflammatory cytokines such as IL-10 are commonly observed [27, 28]. Elevated inflammatory cytokines, such as IL-6 and TNF-α, have been associated with lower skeletal muscle mass and reduced strength in healthy community-dwelling older adults (> 70 yrs) [29, 30]. For example, in an observational study, higher levels of IL-6 and C-reactive protein (CRP) were associated with a 2 to 3-fold more significant risk of losing more than 40% of muscle strength (grip strength) in older community dwellers over a 3 year period [30]. The precise mechanisms in human *vivo* in which the elevated inflammatory cytokines affect protein metabolism are unknown. However, numerous animal model studies have shown that IL-6 or TNF-a administration caused an increase in MPB [31], decreased MPS rate, and a reduction of plasma IGF-1 [32]. This suggests that inflammaging may play a role in promoting muscle wasting by suppressing MPS in ageing individuals. However, in multiple observational studies, older adults (>60 yrs) have an inverse dose-relationship between physical activity and systemic inflammatory biomarkers even at modest activity levels [33]. Additionally, exercise intervention studies that include resistance exercise have shown that following 16-24 weeks of training, increased plasma concentrations of IL-10 [34, 35] this suggest that exercise has a role in stimulating anti-inflammatory processes. There are currently limited studies investigating the effect of dietary and exercise interventions on sarcopenia and cytokine outcomes.

While frail, pre-frail, and institutionalised older adults have been the primary focus of much of the sarcopenia research, active older adults who do not have the cofounding variable of sedentary behavior are underrepresented in sarcopenia research. This cohort represents individuals that are in the ≥ 5^{th} decade of life that exercise in at least 150 min/week of light- (e.g., 3-5 metabolic equivalents (METS)) to moderate-intensity (e.g., 6 - 9 METS) physical activity or 75 min/week of vigorous-intensity (e.g., > 9 METS) physical activity [36]. Participation rates in the last three decades of older active adults have progressively increased in endurance and ultra-endurance-

based events [37]. This cohort of active older adults has been suggested to be the exemplars of successful ageing due to their high physical and physiological functioning compared to their sedentary counterparts [38]. Even though physically active older adults have a higher baseline in absolute skeletal and strength than their inactive counterparts, they still show signs of age-related decline in skeletal muscle and stability [39-41]. Although the causal mechanisms of sarcopenia remain elusive, it is likely multifactorial. These include decreases in anabolic hormones (e.g., testosterone, growth hormone), increased inflammation, loss of neuromuscular function, mitochondrial dysfunction, and lipids' infiltration within the skeletal muscle, and changes in muscle protein balance [42, 43]. Due to its complexity, there is currently limited evidence to support the efficacy of pharmacological treatments. Therefore, the current research has focused on MPS response to anabolic stimuli (i.e., exercise and nutrition).

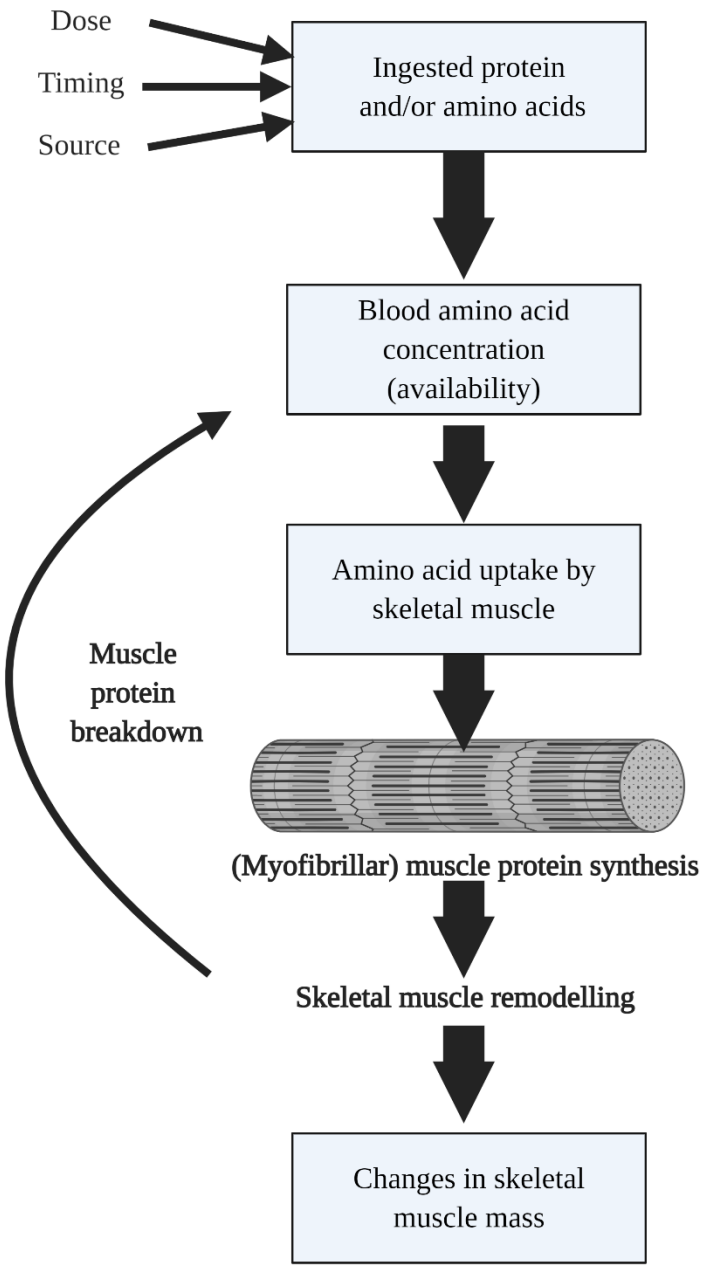

Figure 1. Simplified diagram detailing the role of amino acid availability with changes in protein and/or amino acid ingestion and exercise. Adapted from Witard et al. [68]

Interventions to treat sarcopenia

Protein amount

The consumption of dietary protein provides organic nitrogen and essential amino acids (EAA) used by skeletal muscle for tissue growth, repair, maintenance, and energy homeostasis [44]. As depicted in **Figure 1**, the magnitude of response and amino acid availability is determined by several dietary factors, including dose (amount digested), source of protein and timing [68]. The current recommended daily allowance (RDA) for older adults is 0.8 g per kilogram of body mass per day (g/kg BM/day) for all adults [45]. These protein recommendations are based on short-term nitrogen balance studies in healthy adults that estimate the minimum amount of protein required for 97% of the population to achieve nitrogen balance [46]. These studies are based mainly on adults in their twenties and thirties and thus do not reflect aging's true physiology in older adults. An internationally lead study group (The PROT-AGE group) which includes experts from around the world to represent a range of clinical specialties in ageing (e.g., geriatric medicine, endocrinology, nutrition etc.) have suggested that to meet the metabolic demands associated with ageing, that older adults require higher amounts (e.g., 1.0-1.2g/kg BM/day) than the current recommendations [47]. Additionally, active older adults should consume more (e.g., >1.2g/kg BM/day) to meet the increased metabolic demands of exercise [47]. There have been numerous cross-sectional studies that have found an association with higher protein intakes (e.g., >1.2 g/kg BM/day) to have a positive outcomes of SMM, skeletal muscle strength, and physical function, compared to those with lower protein intakes (e.g., <0.8 g/kg BM/day) [48, 49]. However, limited studies investigate the effects of habitual protein intake and outcomes related to sarcopenia in active older adults.

Protein timing

The 'typical' western diet has an uneven protein distribution throughout the day, with suboptimal protein intakes (e.g., < 10 g) at breakfast and lunchtime [50]. This could be more detrimental in ageing older adults that typically display anabolic resistance. This may, in part, explain the reported

loss of lean body mass observed in older adults that were consuming 'adequate' daily protein (>0.8 g/kg BM/day) intakes [48]. Optimal doses of protein per meal to elicit a near-maximal anabolic response have been observed at ~25-35 g/meal (~10 g EAA) [51, 52] or relative amounts of 0.40 g/kg BM/meal [53]. By spreading out the protein feeding throughout the day at this maximum threshold, it may allow for a continuous stimulation of MPS over the day, leading to skeletal muscle accrual. Considering that the MPS response after protein intake occurs for 4–5 hours after ingestion, consuming protein at each main meal (e.g., breakfast, lunch, and dinner) is a viable strategy [54]. However, most of these findings are mainly drawn from acute laboratory and observational research. There is a lack of data from randomised controlled trials supporting consumption of ≥1.2 g/kg BM/day in a balanced protein distribution on SMM outcomes and physical function in active older adults.

Protein quality

Additionally, the protein quality is another factor that may influence the magnitude of the MPS response and ultimately influences skeletal muscle mass, strength, and performance. Recent studies have shown that protein supplementation that includes high biologically available dairy protein types (e.g., whey and casein) are superior at stimulating MPS in the ageing population compared to plant-based proteins (e.g., soy) [55]. This is due to essential amino acids (EAAs), particularly LEU [56, 57]. LEU acts as a substrate for MPS and directly acts as a key anabolic signal for the activation of the mTORC1 and other protein phosphorylation involved in skeletal muscle anabolic responses [23, 56, 57]. However, despite the abundant amount of research in isolated-protein supplementation, less is known about the role of whole-food protein-containing foods/ drinks in the prevention and management of sarcopenia. Dairy milk, which contains both whey (20%) and casein (80%), also contains other nutrients (e.g., calcium, Vitamin A, E, B-complex, zinc) that may be beneficial to overall health and change the food matrix, resulting in further improvements in digestibility and absorption of food [58]. Two randomised controlled trials

measuring the effect of low-fat dairy post-exercise found significant improvements in skeletal muscle mass and strength gains in healthy young weight-trained males and untrained young females [59, 60]. However, these studies were performed in younger cohorts, whilst studies in older adults are limited. Dairy milk, a rich protein source, high in LEU and widely available, may offer a practical approach to providing an LEU rich nitrogen source to the ageing population to prevent and manage sarcopenia [58]. Considering the physiological changes that occur with aging it would be important to determine the optimal nutrition profile for a dairy beverage and the ideal exercise stimulus needed for this population group to support skeletal muscle mass and function.

Exercise and protein

In particular, resistance training (RT), physical activity is indisputably beneficial for older adults regarding enhancing skeletal muscle mass and strength. A recent systematic review reported that progressive resistance training performed 2-3 times per week at a high intensity (%) resulted in improved physical function and strength in older adults [61]. In acute laboratory studies, dietary protein consumption immediately following resistance training has shown a synergistic effect, where it increases MPS greater than protein or RT alone [13, 62]. Considering that RT has been shown to elevate MPS for 24-48 hours after a single bout of exercise [25], theoretically consuming protein at regular intervals throughout the day following RT would lead to the accumulation of muscle proteins, significant hypertrophy, and skeletal muscle mass response. However, in numerous high-quality RCTs, the impact of protein supplementation and RT on the synergistic effects have been conflicting [63-67]. Many factors are likely to contribute to these varied findings, including heterogeneity in the cohorts (e.g., age, physical activity, and nutritional status). Nonetheless, further research is needed to understand the most effective timing, dose, type, and frequency of protein ingestion to augment resistance training in older adults.

1.2 Current evidence gaps

The aetiology of sarcopenia is complex. The combined results of existing literature on increasing dietary protein to maintain or increase SMM and function are currently inconclusive and sometimes contradictory. Indeed, long-term studies with improved methodologies are needed to fine-tune protein requirements and show the efficacy of increasing protein intake in the active ageing population. There is also inadequate literature on the use of common protein whole foods (e.g., dairy milk) in more longer term studies and considering the distribution and timing of protein and its relation to skeletal muscle mass, skeletal muscle strength, and performance in older active adults. In addition to the need to gather information about the association between dairy products and losses of skeletal muscle mass, skeletal muscle strength, and physical function, nutrition researchers need to further understand the potential factors contributing to these losses. Despite the literature suggesting that certain inflammatory markers may be present in the ageing population, leading to increased risk of sarcopenia, little is known about the long-term effects of nutrition and exercise interventions on this population group's biochemistry. Additionally, there are clear methodological gaps in the current literature for the active ageing population; despite current knowledge of the clinical guidelines for sarcopenia, it is still unknown whether these markers are relevant for the active ageing population.

1.3 Thesis aims and objectives

1) To systematically identify and synthesise results of relevant RCTs assessing the effects of dairy milk beverages and dairy based protein interventions (± physical exercise) on outcomes of SMM, skeletal muscle strength, physical performance, and power in older adults ≥40 yrs.

2) To identify the methodological gaps in current sarcopenia research and provide direction for future research that can be used to apply to active older adults, to help prevent or manage sarcopenia in the active ageing population.

3) To explore potential links between age, physical activity level, dietary protein intake, and biological sex with SMM, strength, power, and physical capacity/ performance in active older adults.

4) To explore protein intake patterns including: daily protein intake, evenness of protein intake across the three main meals, number of protein meals containing ≥0.4 g/kg BM/meal, and the quality of habitual protein intake and assess associations with outcomes of SMM, muscle strength and power in a cohort of active older adults.

5) To determine the independent and combined effects of a high protein dairy milk beverage provided at breakfast and lunch (or after resistance exercise), with or without PRT on outcomes of FFM, skeletal muscle strength and power, and physical performance in a cohort of healthy active older adults.

6) To explore hormonal and inflammatory markers that are relevant in an active ageing cohort that may assist in providing insight into the pathophysiological and potential mechanisms of the experimental interventions.

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CHAPTER 2:

Effect of dairy-based supplementation and dairy beverage combined with exercise intervention on skeletal muscle mass, strength and performance outcomes in older adults.

Internal industry publication: *Lion Dairy & Drinks 2018*

2.1 Background

Ageing is associated with a progressive and involuntary decline in SMM, muscle strength and/or functionality, described as sarcopenia [1]. Sarcopenia can start as early as the 4th decade of life and contribute to 1-2 % loss of SMM per year after 50 yrs old [1, 2]. This age-related loss of SMM is characterised by muscle fibre changes, decreasing the size and number of type II muscle fibres [3, 4]. This change in muscle fibre type and decline in SMM is accompanied by a concomitant and progressive loss in skeletal muscle strength and power [5, 6]. Skeletal muscle strength and power have been shown to decline faster than SMM, with some studies reporting as high as 3% and 5% declines per year, respectively [7].

Moreover, this decline in SMM, skeletal muscle strength and power makes sarcopenia considered one of the most important factors implicating the progression of loco-motor functional decline and disability with ageing [8, 9]. The cause of sarcopenia is likely due to a disruption of skeletal muscle protein turnover, resulting in an imbalance between MPS and MPB, leading to loss of SMM and ultimately muscular function [10]. Achieving a positive protein balance, where MPS exceeds MPB, is a crucial component of skeletal muscle repair and remodelling and accruing SMM to maintain and increase strength and physical function [11]. However, older adults (≥50 yrs) have been observed to have a blunted capacity to respond to anabolic stimuli compared to younger individuals, known as '*anabolic resistance*' [12]. Resistance training (RT) and ingestion of dietary protein foods have been identified as powerful MPS simulators. They can be used to acutely stimulate the biological process favouring a positive protein balance [12]. Over time, the summation of these acute increases in protein synthesis is thought to provide the necessary stimulus to preserve or increase SMM and muscle strength [13].

There are already numerous systematic literature reviews (SLR) and meta-analyses on intervention studies investigating the effects of isolated protein supplementation with RT in the ageing population [14-18]. These studies have yielded conflicting findings regarding the impact of protein supplementation with RT and whether it further augments SMM gains, skeletal muscle strength, performance, and power outcomes in older adults. For example, Morton et al. [15] and Cermack et al. [17] reported protein supplementations ranging from 20-40 g can further augment changes SMM (0.3-0.5 kg) following at least >6 weeks of RT in older adults. Hanach et al. [14] and Ten Haaf et al. [16] did not find any further benefit of protein supplementation beyond RT on SMM outcomes, strength or performance. One potential cofounding variable is the inclusion of frail and institutionalised older adults. For example, two randomised control trials (RCTs) that investigated the effects of exercise and protein supplementation on frail older adults found a significant benefit of protein supplementation with exercise on SMM outcomes and strength [19, 20]. RCTs in healthy older adults have reported mixed findings [14-18]. Nonetheless, active older adults, defined as older adults that regularly engage in physical activity (≥50 yrs, 150 min/week of light- (e.g., 3-5 metabolic equivalents (METS)) to moderate-intensity (e.g., 6-9 METS) physical activity or 75 min/week of vigorous-intensity (e.g., >9 METS) physical activity) [21], still show signs of age-related sarcopenia [22, 23]. Active older adults represent the ideal cohort to study ageing. They offer a high relevance in this ageing research area due to the growing number of participation rates in recreational and elite sporting events [24].

Furthermore, the use of whole foods such as dairy milk, a rich protein source high in leucine and widely available, may offer a practical approach to providing a rich nitrogen source to the ageing population for the prevention and management of sarcopenia [25]. Additionally, dairy-based supplementation of isolated proteins (e.g., whey and casein) is widely used alongside dairy milk

products and as liquid, concentrated, or dried food supplements or ingredients and are widely available on the market [26]. Both dairy milk beverages and dairy-based supplements are rich protein sources, high in leucine, widely available, and may consequently offer a practical approach to providing a leucine-rich protein source to prevent and manage sarcopenia of the ageing population [27].

Review aims

The aim of the current review were to systematically identify and synthesise results of randomised control trials (RCTs) assessing the effects of dairy milk and dairy-based protein beverages (± physical exercise) on outcomes of SMM, skeletal muscle strength, physical performance, and power in older adults ≥40 yrs. This review's secondary aim is to identify the methodological gaps in the current sarcopenia research and provide direction for future research that can be used to apply to active older adults, to help prevent sarcopenia in the active ageing population.

2.2 Methods

A defined search strategy was developed with the assistance of an academic librarian. This strategy was applied to the following electronic databases: Ovid MEDLINE, EMBASE, Cinahl, SportsDISCUS, Web of Science and Scopus. The initial search were carried out in November 2017 and updated as of October 2020. Also, reference lists of included papers were checked for any additional references missed in the search strategy. Methods and reporting were guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [28]. The critical words applied in the database search are shown in **Table 2.0,** and an example of a search strategy in **Appendix B.**

Table 1. Search strategy for the systematic review on the impact of protein supplementation and dairy-based drinks and alternatives on muscle mass, functional or physical performance, strength outcomes and power.

Field one (combine with OR)- Population		**Field two (combine with OR) - Intervention and Comparison**		**Field three (combine with OR)- Outcome**
Keywords: Older or elderly MeSH: Aged	AND	Exp. Amino Acid*/, Dietary protein*, Leucine, Casein, dietary Supplement/*, Whey Vitamins/ mapped to dietary supplement and exp Supplement*- mapped to dietary supplement or as keyword Milk protein* -mapped to caseins and whey proteins	AND	Skeletal muscle mass OR Hypertrophy OR Skeletal Muscle/ Muscle strength/ Exp Hand Strength Grip strength Physical performance Physical performance.mp/ Muscle Power
Same as above *used to retrieve unlimited suffix variations	AND	Milk OR Dairy	AND	Same as above

Original studies using a randomised, controlled trial (RCT) design were considered for the review to evaluate the highest quality evidence. Table 2.1 summarises the criteria used to determine whether studies were eligible for inclusion using the Participant-Intervention-Comparator-Outcomes-Study design (PICOS) format [29]. This was determined by the researchers before commencing the systematic search.

The interventions that were considered for this review can be separated into two domains: 1) dairy milk beverages (or dairy milk alternatives) and exercise intervention, and 2) diary-based supplements (or dietary treatment alternatives) that composed of components of dairy (e.g., whey and casein) and exercise intervention. Interventions excluded from this review were studies that included only a dietary intervention without an exercise intervention. Interventions needed to have a comparative group which had to be either no nutritional intervention or a placebo (e.g., iso-caloric carbohydrate beverage), studies were included if they had two additional comparators (i.e., casein *vs* whey).

Study selection

The database searches were imported into Covidence, and duplicates were manually removed. Study selection and data extraction were performed by two independent reviewers (ZH and VC), and conflicts were resolved by a third reviewer (RC). All studies identified via the database search were assessed against the PICOS, based on the information contained in the title abstract and description. Full articles were obtained from all studies that met the inclusion criteria. Full papers were assessed against the PICOS independently by two reviewers (ZH and VC).

Table 2. Inclusion and exclusion criteria for systematic literature review.

PICOS	Inclusion	Exclusion
Population	Healthy community-dwelling older adults (≥40 yrs) free from acute or chronic disease. Sarcopenic older adults or signs of skeletal muscle loss, not caused by any chronic disease.	Infants and Children. Pregnant or lactating women. Adults with any disease known to cause muscle protein loss that is not sarcopenia (e.g. cancer, cachexia, motor neuron diseases, HIV and autoimmune diseases). Participants identified or categorised as frail. Mobility limited older adults.
Intervention	Dietary treatment or supplement and exercise and/or physical activity. Milk or dairy specific components (including amino acid profile, protein and energy to protein ratio), and exercise and/or physical activity.	Physical activity alone. Diet supplementation in combination with pharmacological intervention or chronic supplementation. Chronic vitamin and mineral supplementation. Studies in which the nutritional intervention is energy restriction to promote weight loss.
Comparator	Placebo, nutritional control, or comparative dietary supplement intervention.	No control or comparative group.
Outcome	**Primary:** Strength outcomes (e.g. Handgrip, leg press, chest press). Power outcomes (e.g. Vertical jump, peak power). Performance outcomes (e.g. $VO_{2max,}$ gait speed, balance, chair raise). Studies were included if they measured skeletal muscle mass plus at least one measure of muscle strength, or power, or physical performance test. **Secondary Outcomes:** Anthropometrics (changes in body composition, lean muscle mass, fat-free mass, and the muscle's cross-sectional area (CSA).	Measurements of anthropometry alone. Including fat-free mass, lean mass, cross-sectional area (CSA).
Study design	Randomised control trials.	

PICOS: Participant- Intervention-Comparator-Outcome-Study design format [29].

Data extraction and synthesis

Data tables were compiled independently for each outcome and standardised to include author names, journal name, year of publication, length of intervention, type of population, gender ratio, mean age, age range, detailed groups with sample size, the protocol of exercise intervention, the protocol of nutritional intervention, skeletal muscle mass, muscle strength, physical performance, and power outcomes. Data were extracted independently by three reviewers (ZH, VC and CR) and cross-checked by the first reviewer (ZH). Missing data from the studies included in a quantitative analysis were sought by emailing corresponding authors of eligible papers. The included studies' results were analysed descriptively, and a meta-analysis was not considered for this systematic review due to the heterogeneity of the included studies.

Assessment of study quality

Risk of bias assessment was performed using the Cochrane Risk of bias tool to determine the methodological quality. This were assessed independently by two reviewers (ZH and CR) [34]. Seven specific domains (sequence generation, allocation concealment, blinding of participants and experiemental researcher, blinding of outcome assessment, incomplete outcome data, selective outcome reporting and ‘other issues’). Any difference of opinion between authors was discussed by the two reviewers, and a consensus were made.

2.3 Results

Search results

The initial database search for dairy milk studies yielded 443 non-duplicated citations. 412 of these were excluded during the title and abstract screening, leaving 98 papers. An additional 4 documents were included before the full-text review, based on the search of a reference list of review papers. Following the full-text screening of 31 papers, 6 met the inclusion criteria (Figure 1). The second search focused on dairy-based supplementation studies found 8232 non-duplicated citations through database searching. 6084 were excluded based on title and abstract. An additional 3 papers were included before the full test review, search of the reference list of review papers. After a full-text review was completed, of 133 papers, 26 met the inclusion criteria (Figure 2).

Results of study quality

The risk of bias assessment for dairy milk beverage and dairy-based supplement studies is shown in Figures 3 and 4, respectively. The results across individual studies indicate that selection bias was not evident in *n*= 12 of the dairy-based supplement studies and *n*= 2 of the dairy milk beverage studies (Figures 3A and 4A). One study in the dairy-based supplement group was high risk as they did not provide any methods of how participants were allocated in the groups [61]. The remaining studies were unclear as they reported that participants were randomized into groups but did not explain the methods further. Potential detection bias was observed in *n*= 11 of the supplement studies and *n*= 3 of the dairy studies due to the lack of sufficient detail of how the intervention and placebo beverages were concealed and blinded from the participants and researchers.

Additionally, another primary source of bias across both reviews was performance bias, with *n*= 12 of the supplement studies and *n*= 4 of the dairy studies not providing any detail of how the primary outcomes' results were blinded from the participants and researchers. Other sources of bias were

compliance bias; *n*= 10 of the supplement studies and *n*= 2 of the dairy studies showed no reporting of compliance, reducing confidence of the results. Overall, the most significant risk of bias across studies was insufficient detail for blinding or randomisation procedures. Therefore the risk of performance bias and detection bias was *'unclear'* (Figures 3B and 4B).

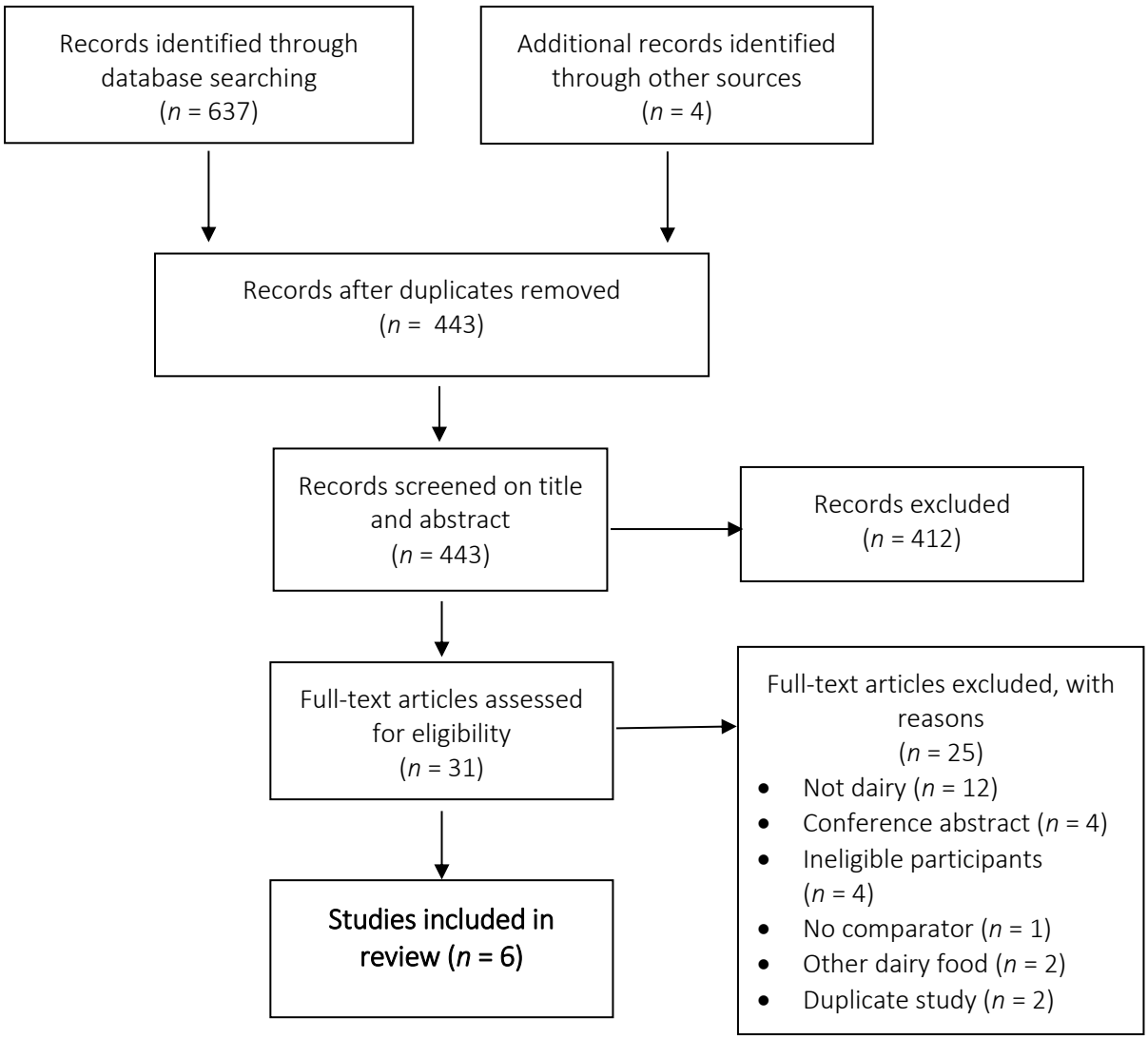


Figure 1. PRISMA flow diagram, showing the inclusion and exclusion of papers for dairy milk beverage studies.

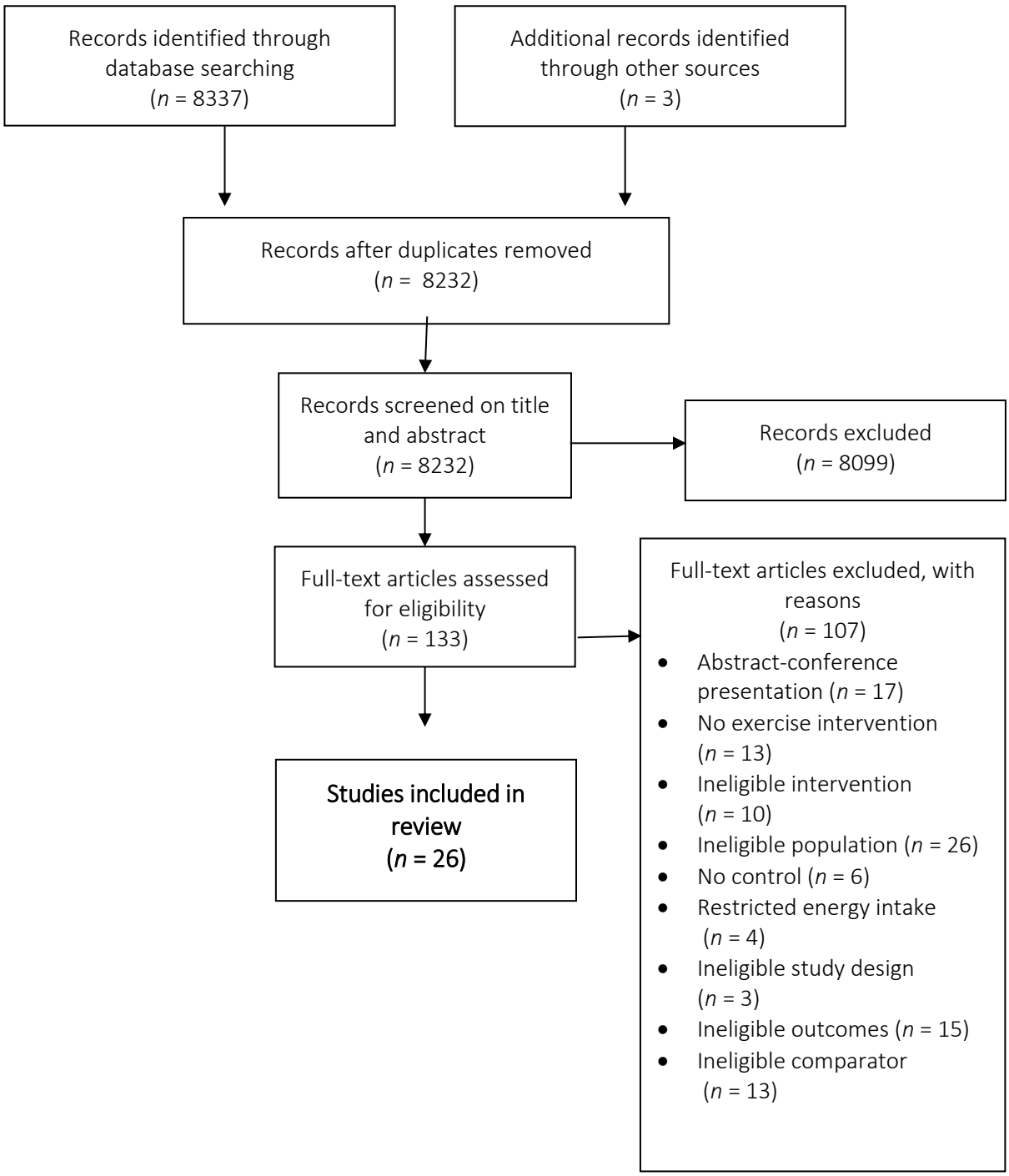
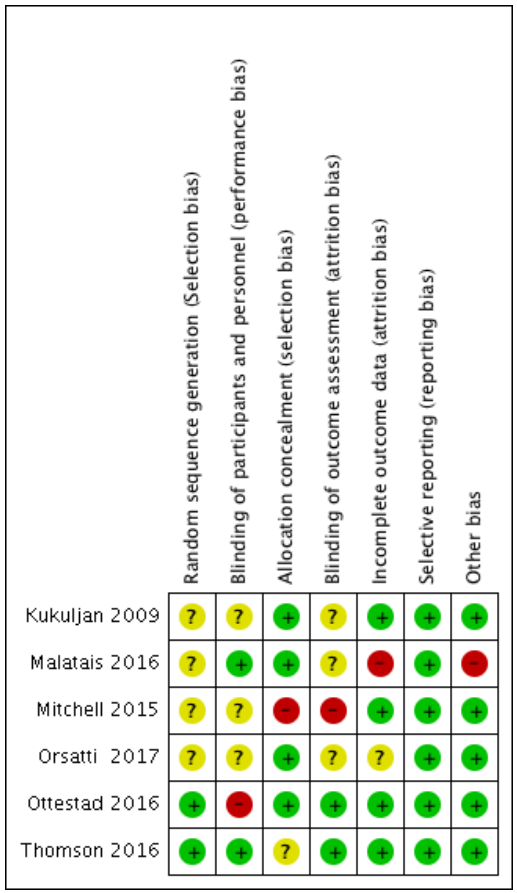


Figure 2. PRISMA flow diagram, showing the inclusion and exclusion of papers for the dairy-based supplement studies.

A.



B.

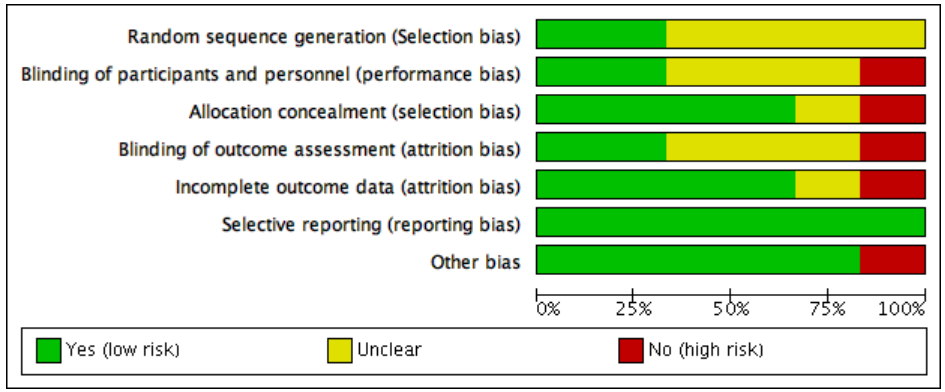
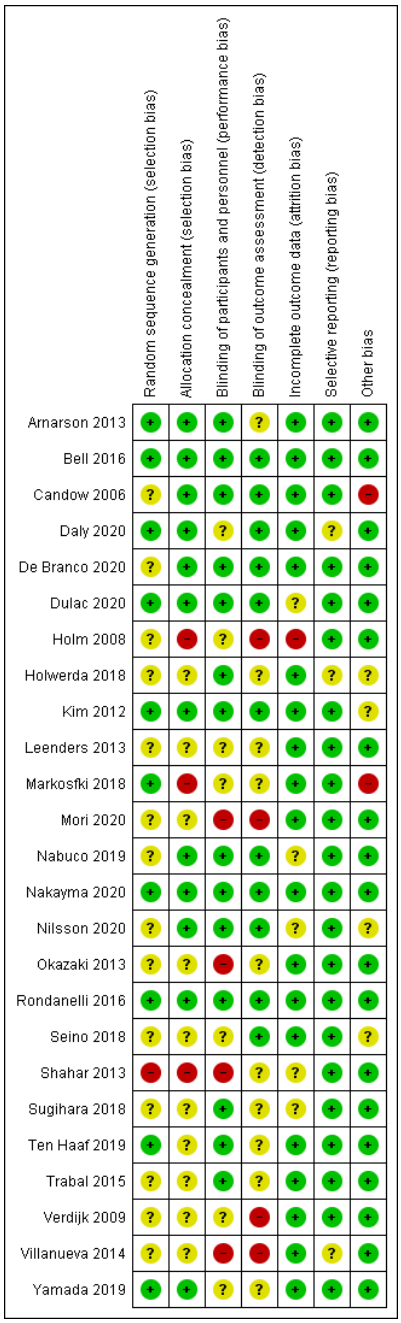


Figure 3. Risk of bias **(A)** summary review of authors' judgements about each risk of bias item for each included study **(B)** review authors' judgements about each risk of bias item presented as percentages across all included dairy studies. (+) Indicates a low risk of bias, (-) indicated a high risk of bias, (?) indicates an unclear risk of bias. Measured using the Cochrane risk of bias tool [30].

A.



B.

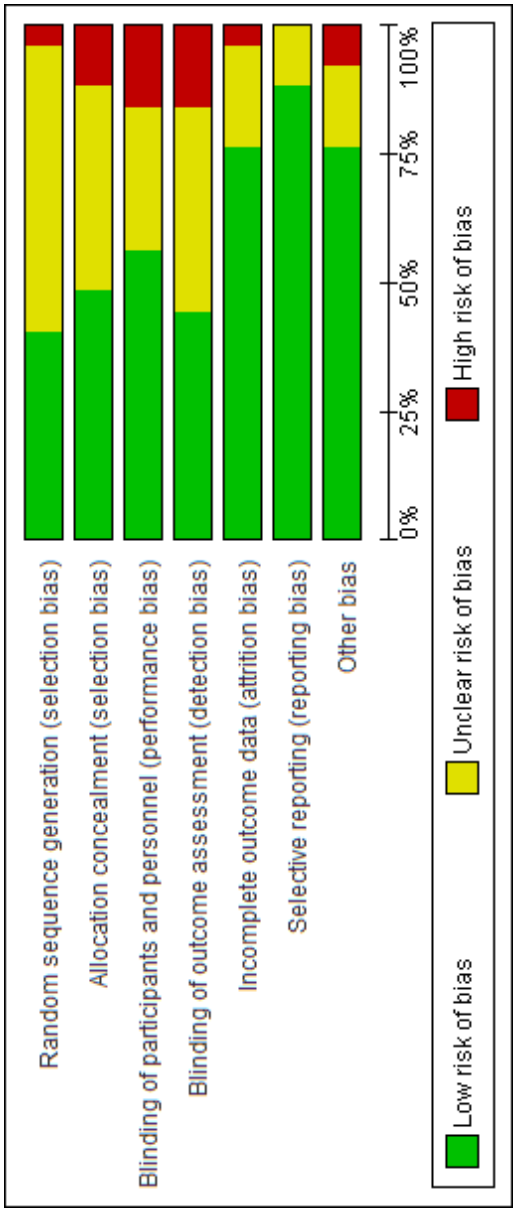


Figure 4. Risk of bias **(A)** summary review of authors' judgements about each risk of bias item for each included study **(B)** review authors' judgements about each risk of bias item presented as percentages across all included supplement studies. (+) Indicates a low risk of bias, (-) indicated a high risk of bias, (?) indicates an unclear risk of bias. Measured using the Cochrane risk of bias tool [30].

Cohort characteristics

The dairy milk beverage studies had participant numbers ranging from 16 to 180 participants in total. The participants' age ranging from 45 to 89 yrs, and the length of the trials was 12 weeks to 6 months. The majority of the study participants were considered healthy community dwellers [31, 33, 35 36]. One study recruited older men (> 60 yrs) who had low muscle mass as determined by an appendicular muscle mass index (MMI) of < 10.75 kg/m^2 [32]. Another study by Orsatti et al. [34] recruited post-menopausal women (> 45 yrs) who had spontaneous amenorrhea for at least 12-months.

Within the dairy-based supplementation cluster, four studies recruited sarcopenic individuals within the community [45, 53, 55, 61]. Yamada et al. [61] was the only study to use a specific clinical diagnosis for sarcopenia (Asian working group of sarcopenia) as a method of inclusion [93]. The remaining studies did not use any diagnostic criteria of sarcopenia. Instead, the loss of skeletal muscle mass or muscle strength was used to determine participation eligibility. For example, Kim et al. [45] defined sarcopenia with the following criteria: low appendicular muscle mass (<6.4 kg/m^2) or a low body mass index (BMI) (below 22 kg/m^2). These results were associated with one of the following two criteria: knee extension strength <1.01 Nm/kg, or walking speed <1.22 m/s. Shahar et al. [55] used criteria based on the assessment for bioelectrical impedance analysis (BIA) by Janssen et al. [94], which included a skeletal muscle mass cut off point of less than 10.75 kg/m^2 for men and 6.75 kg/m^2 for women. Two studies included active older adults [56, 60], two studies included low active older adults defined by <7500 steps per day [47, 40]. The remaining studies recruited healthy community-dwelling participants. The dairy milk beverage studies had participant numbers ranging from 22 to 244, the participants' ages ranged from 41 to 89 yrs and the length of the trials was 8 to 24 weeks.

Intervention characteristics

Nutrition interventions: dairy milk beverage

In the dairy milk beverage studies, Mitchell et al. [33] was the only study to use an unfortified milk drink. The remaining studies enriched the milk drinks with protein (*n*= 3), vitamin D (*n*= 1) or calcium (*n*= 1). Daily protein intake was 13.2 g to 27.0 g, with the dose per serving ranging from 6.6 to 27.0 g. The timing of the dairy beverage varied; post-training (*n*= 4), the remaining included the milk beverage at either breakfast and lunch (*n*= 1) or breakfast and dinner (*n*= 2). In the studies by Kukuljan et al. [31] Thomson et al. [36], the study populations were split into four groups; exercise plus dairy milk beverage, only exercise, only dairy milk, or no intervention (control). Maltais et al. [32] had three groups; exercise plus dairy milk beverage, exercise plus soy beverage, and exercise plus a control (carbohydrate) group. The remaining studies only had two groups.

Nutrition interventions: dairy-based supplement

A substantial variety of supplementation types (e.g. whey protein, casein) were used within the trials for this systematic review. Within the supplement studies the nutritional interventions included; whey protein (*n*= 18), milk protein (*n*= 5), casein (*n*= 2), EAA (*n*= 2) and soy protein (*n*= 1), milk protein concentrate (*n*= 2), collagen protein (*n*= 1), or a multi-ingredient protein supplementation (*n*= 3). A range of 6 g to 62 g of protein was supplemented per day to the protein group, with a range of 5-40 g of protein supplemented at post-training (*n*= 10), breakfast or lunch (*n*= 11), or otherwise not specified (*n*=3*)*. Four of the studies [45, 47, 55, 61] included a 4-armed trial with groups that independently assessed the dairy-based protein supplementation effects.

Exercise interventions

5/6 of the dairy studies included a resistance training program, which varied from 2-3 days a week. The resistance training programs included repetition schemes of 6 to 12, for 3 sets per exercise at

an intensity of 50 to 85% of their 1-repeition maximum. Ottestat et al. [35] was the only study to not provide an exercise intervention; instead, they asked participants to monitor exercises when completed >30 min of aerobic exercise (such as walking) and other moderate or heavy daily activities such as weight training or heavy housework.

The majority (*n*= 15) of the dairy-based supplement studies used resistance training as their exercise intervention. Of these resistance training interventions, four [37, 38. 46. 59] used a progressive resistance training (PRT) program, which involved weight increments of 5-10% per session. Other exercise interventions such as resistance band training (*n*= 4), interval walking (*n*= 1) and bodyweight exercises (*n*= 3), and aerobic exercise (*n*= 1) were used. The frequency of these exercise interventions varied from 2 to 5 days/week.

Effect of dairy milk beverages with exercise on SMM

Changes in SMM were reported by 6 RCTs in the dairy milk beverage studies, which included changes in total lean muscle mass (*n*= 3), fat-free mass (*n*= 1) and changes in the cross-sectional area of the muscle (*n*= 1) (Table 3). In the longest duration intervention within this review, an 18-month intervention by Kukuljan et al. [31] found no beneficial effect by providing a 200 ml fortified milk beverage at breakfast and evening compared to groups that only received either resistance training or the fortified milk beverage. In the aforementioned study, exercise alone seemed to improve total lean muscle (TLM) outcomes, as shown in the MILK+RT (1.6%) and RT (1.2%) groups. There was no additional benefit observed of the milk drink beyond the effect of exercise (Table 3). Two studies evaluated the effects of a chocolate milk-based drink: Malatais et al. [32] compared the effects of a chocolate milk drink (13 g protein, 7 g EAA) compared to a soy milk drink (12 g protein, 7 g EAA) and control (rice milk) in males with a low muscle mass index (<10.75 kg/m^2). All groups received 16-weeks of RT, 3 days a week and had ≥1.0 g/kg BM/day of protein intake at

baseline. All groups significantly increased the different muscle mass measures (e.g. lean body mass, muscle mass index, and total lean muscle) with no significant differences between groups. Contrastingly, Mitchell et al. [33] provided healthy community dwellers with 500 ml of chocolate milk (14 g protein) once a day. They reported a significant increase in the CSA of type I and type II muscle fibres in both the protein and control group, indicating a training effect. They did not provide any baseline protein intake data. Thomson et al. [36] included healthy community-dwelling individuals and compared the effects of enriched low-fat milk (27 g protein), enriched soy milk (27 g protein) and 3 sessions/week of resistance training, reported no statistically significant difference in TLM between groups and when compared to the control group (resistance training only) (2%, 3%, and 1.7%, respectively). Lastly, Orsatti et al. [34] and Ottestad et al. [35] reported no significant changes in SMM outcomes.

Effect of dairy milk-based supplementation with exercise on SMM

Within the supplement studies, 20 reported a significant increase in SMM in the supplement and exercise groups compared to baseline (Table 4). Of those, *n*= 5 reported a significantly higher increase compared to control [40, 48, 49, 53, 56]. The studies that reported significantly higher SMM outcomes compared to the control varied in protein supplementation (18.2-35 g), timing (post-RT, breakfast/ lunch), and study participants (Community dwellers, resistance-trained older adults, sarcopenic). Daly et al. [40] provided 9.1g x 2 of a fortified milk beverage to sedentary adults. In this trial, a statistically significant difference in TLM was observed in the group that received the nutritional intervention (2.4%) compared to the control (1.6 %). Additionally, Mori [48], who provided 22.3 g of whey protein (WP) post-exercise in community dwellers, reported a significantly higher increase in SMI in the WP+RT group (3.3%) compared to RT only (1.6%). Nabuco [49] reported similar findings in females, providing them with 35 g of WP post-exercise. Rondanelli et al. [53] provided sarcopenic individuals 22 g/day of whey with 5 sessions/week of resistance

band training and observed a significant increase in TLM compared to the control that received a carbohydrate drink and exercises only (3.5% *vs* 0%, respectively). Finally, a study in resistance-trained older women also provided participants 35 g of protein post exercise and reported a significantly higher TLM in the WP+RT group than the group that only received RT (4.8 *vs* 2.3%) [56].

Table 3. Main outcomes for skeletal muscle mass and dairy milk beverage intervention trials

Study	Length	Participants	Exercise stress	Dairy and placebo details: Nutrition intervention	Frequency x timing	Study groups	Habitual protein intake at baseline (g/kg BM/day)	Method used	Results
Kukuljan et al. [31]	72 weeks	*n*= 180, Healthy community-dwelling males 50-79 yrs	RT, 3 sets, 10-12 reps at 50% 1RM for 7 exercises, 3 x days a week	1) 200 ml Fortified Milk drink: 6.6 g PRO, 11 g CHO, 2.2 g Fat, 418 kj	Morning and evening	1) MILK (*n*= 43) 2) RT only (*n*= 43) 3) MILK + RT (*n*=43) 4) CON (*n*= 42)	1) 1.2±0.28 2) 1.3±0.32 3) 1.2±0.32 4) 1.3±0.31	DEXA	**TLM** ↑MILK + RT 1.6%, ^{NS}MILK 0.35% ↑ RT 1.2 % ^{NS}CON 0.1%
Maltais et al.[32]	16 weeks	*n*= 26 low muscle mass index, males 65± 5 yrs	RT, 3 sets, 6-8 reps 80% 1RM for 8 exercises, 3 x days a week	1) MILK: Chocolate milk 375 ml: 13.5 g PRO, 37 g CHO, 7 g EAA (3.5 g leucine), 1058 kj. 2) SD: soy drink. 379 ml: 12 g PRO, 39 g CHO, 7 g EAA 3) CON: rice milk	Post training only	1) MILK +RT (*n*= 8) 2) SD +RT (*n*= 8) 3) CON +RT (*n*= 10)	1) 1.0±0.20 2) 1.2±0.20 3) 1.3±0.41	DEXA	**TLM** ↑MILK +RT 3.1% ↑SD + RT 3.4% ↑CON 2.5%
Mitchell et al. [33]	12 weeks	*n*= 16 (47 males, 18 females) healthy community dwellers 74 ± 5 yrs	RT, 3 sets at 75-85% 1RM, 3 x days a week	1) MILK: chocolate milk 500 ml (14 g PRO, 54 g CHO, 5 g fat, 1340 kj) 2) CON: CHO drink (13.2 g CHO)	1 x day (Post RT or with breakfast)	1) MILK + RT (*n*= 8) 2) CON: + RT (*n*= 8)	N/A	CT	**CSA, Type 1** ↑MILK + RT 16% ↑CON: 24% **CSA, Type II** ↑ MILK + RT 22% ↑ CON 18%
Orsatti et al. [34]	16 weeks	*n*= 32 post-menopausal females 50-68 yrs	RT, 3 sets, 8-12 reps, 70% 1RM, 8 exercises, 3 x days a week	1) MILK: 200 ml of skimmed milk +25 g soy protein 2) CHO drink (25g CHO +RT)	Post RT, or same time each day	1) MILK + RT (*n*= 16) 2) CHO drink +RT (*n*= 16)	1) 0.8±0.3 2) 1.0±0.3	BIA	**TLM** ^{NS}MILK + RT 6.1% ^{NS}CON 8.3%
Ottestad et al. [35]	16 weeks	*n*= 36 (12 males, 24 females) healthy community dwellers 70-83 yrs	Regular exercise monitored	1) MILK: Protein enriched milk. 400 ml 696 kj, 20 g PRO, 19.6 g CHO 0.4 g fat)	Breakfast and dinner	1) MILK + RT (*n*= 17) 2) CON: RT only (*n*= 19)	1) 1.0±0.3 2) 1.0±0.3	DEXA	**FFM** ^{NS}MILK +RT 0.9% ^{NS}CON 0.9%
Thomson et al. [36]	12 weeks	*n*= 179 (81 males, 98 females) healthy community dwellers 61.5 ±7 yrs	RT, 3 sets, 8-12 reps, 5 exercises, 2 x days a week	1) MILK: Enriched low fat milk: (27g PRO, 30 g CHO) 2) SM: Enriched soy milk: (27 g PRO, 30 g CHO) 3) OJ + 2 sweet biscuits (500 ml)	1 x day (post training)	3) MILK + RT (*n*= 54) 4) SM: (27 g PRO) + RT (*n*= 64) 5) RT only (*n*= 61)	N/A	DEXA	**TLM** ↑MILK 2% ↑SM 3% ↑CON 1.7%

Abbreviations: BIA: Bioelectrical impedance analysis, CHO: Carbohydrate, CON: control, CT: Computed tomography, CSA: Cross-sectional area, FFM: fat-free mass, RT: Resistance training, DEXA: Dual X-ray absorptiometry, Reps: repetitions, TLM: Total lean mass, PRO: Protein WP: whey protein. ↑= significance increase ↓= significant decrease, and NS = no significant difference from baseline. $^{stat-X}$ no statistical analysis provided or statistical test unclear.

Table 4. Main outcomes for skeletal muscle mass and dairy-based supplement intervention trials

Study	Length	Participants	Exercise stress	Dairy and placebo details		Study groups		Habitual protein intake at baseline (g/kg BM/day)		Method used	Main outcomes %change from baseline
				Nutrition intervention	Frequency x timing						
Arnarson et al. [37]	12 weeks	*n*= 161, (67 males, 94 females) Healthy older adults 65-91 yrs	RT, 3 sets, 6-8 reps, 75-80% 1RM. Load increased 5-10% each week	1) WP: 30 g PRO, 20 g CHO, 709 kj 2) CON: 40 g CHO	1x Post RT only	1) WP +RT (*n*= 75) 2) CON+RT (*n*= 66)		1) 1.0 ± 0.26 2) 0.92±0.30		DEXA	**TLM** ↑WP 1.5% ↑CON 1.8%
Bell et al.[38]	20 weeks	*n*= 49, Healthy community-dwelling males, 73± 1 yrs	RT, 3 sets 10-12 reps, at 65% 1RM increasing to 3x6-8 sets/reps, 80% 1RM HIIT 1x week 10x60s intervals	1) WP: 31 g PRO, 1 g CHO, 2.5 g creatine, 500 IU vitamin D, 400 mg calcium and 1500mg n-3 PUFA, 484.5 kj 2) CON: 22 g CHO	2x 1 hour after breakfast, 1 hour before bed	1) WP + RT (*n*= 25) 2) CON+ RT (*n*= 24)		1) 1.1±0.3 2) 1.2±0.4		DEXA	**TLM** ↑WP 1.3% [NS] CON 0%
Candowe t al. [39]	12 weeks	*n*= 29, Community dwelling males 59-76yrs	RT, 3 sets, 10 reps at 70% 1RM	1) WP: 26 g PRO, 14.7 g CHO 2) CON: 15 g CHO	1x Before or after training	1) WP+ RT (before) (*n*= 9) 2) WP+RT (after) (*n*= 10) 3) CON+RT (*n*= 10)		1) 1.5± 0.2 2) 1.1±0.10 3) 1.3±0.17		Body pod	**TLM** ↑WP (before) 1.2%, ↑WP (after) 1.7% ↑CON 1.0 %
Daly et al. [40]	16 weeks	*n*= 244 inactive females, <7500steps per day 45-65 yrs	Home based body weight workout, 7 exercises (2x10-15 reps)+ balance	1) FMD: 9.1 g PRO, 16.5 g CHO, 418 kj 2) CON: Rice powder 25 g CHO	2x a day (breakfast and lunch/or post training)	1) FMD +RT (*n*= 108) 2) CON+RT (*n*= 136)		1) 1.2 ±0.41 2) 1.2±0.47		DEXA	**TLM** ↑FMD+RT 2.4% ↑CON+RT 1.6% FMD+RT>CON
De Branco et al. [41]	8 weeks	*n*= 34, post-menopausal females 61±7yrs	RT, 1-3 sets, 8-12 reps (70% 1RM), 3x a week	1) WP: 30 g PRO 2) CON: 30 g CHO	Post exercise (3x week) and afternoon	1) WP+RT (*n*= 17) 2) CON+RT (*n*= 17)		1) 0.9±0.2 2) 1.0±0.3		DEXA	**TLM** ↑WP+RT: 2.1% ↑ CON: 1.5%
Dulac et al. [42]	12 weeks	*n*= 60, Sedentary (<120 minutes per week) older males 69 ±7yrs	RT self-selected moderate weight. Increased 2.5-5kg once hit >12 reps	1) WP: 10 g PRO 2) Casein: 10 g PRO 3) CON: 19 g CHO	3x a day breakfast/lunch and dinner	1) WP+RT (*n*= 21) 2) Casein +RT (*n*= 20) 3) CON+RT (*n*= 19)		1) 1.4±0.31 2) 1.34±0.45 3) 1.5±0.36		DEXA	**TLM** ↑WP 2.0% ↑Casein 2.3% ↑ CON, 1.9%
Holm et al. [43]	24 weeks	*n*= 29, Healthy post-menopausal females 55±1yrs	RT, 3 sets, 15 reps	1) WP: 10 g PRO, 31g CHO, 1 g fat, 720 kj 2) CON: CHO 6 g	1x (Post training days only)	1) WP+ RT (*n*= 16) 2) CON+ RT (*n*= 16)		N/A		DEXA	**TLM** ↑WP 1.9% [NS]CON 0.7%
Holwerda et al. [44]	12 weeks	*n*= 41, healthy older males 70±1yrs	RT, First 4 weeks 70% 1RM (8 reps) then 80% 1RM (10	1) WP 21 g PRO, 9.4 CHO, 3 g LEU, 628 kj 2) CON: CHO 24 g	Breakfast and lunch	1) WP +RT (*n*= 20) 2) CHO +RT (*n*= 21)		1) 1.14±0.05 2) 1.2±0.06		DEXA	**TLM** ↑WP+RT 2.6% ↑CHO+RT 1.85%

			reps), 3x days a week								
Kim et al. [45]	12 weeks	*n*= 155 Sarcopenic women, >75yrs	RB + balance and gait training CON: Health education	1)	EAA: 3 g	2x (Not specified)	1) 2) 3) 4)	RB+ EAA (*n*= 38) RB (*n*= 39) EAA only (*n*= 39) CON (*n*= 39)	N/A		BIA	**TLM** NS EAA +RT 1.8% NSEAA 1.1%NS NSRT 1.49% NSCON:1.1%
Leenders et al.[46]	24 weeks	*n*= 53 (Male: 29, Female: 24) Healthy older adults, 68-74yrs	RT, 3 sets, 10 reps, 60% of 1RM for the first 4 weeks, increased to 75-80% of 1RM for the remainder of the trial	1) 2)	MP: 15 g PRO, 7.13 g CHO, 0.5 g fat, 119 kj CON: CHO 18 g	1x (After breakfast)	1) 2)	MP + RT (*n*= Males: 14, females 12) CON +RT (*n*= Males: 15, females 12)	N/A		BIA	**TLM** ↑MT+RT 3% females, 2.2% males, ↑CON 2% females, 1.6% males
Markosfki et al.[47]	24 weeks	*n*= 50, Healthy independent older adults (low-active <7500 steps 65-82yrs	AE, 3x week 45minutes 70% HR treadmill	EAA: 15 g		1 hour after training and same time on non-training days	1) 2) 3) 4)	EAA+AE (*n*= 10) EAA (*n*= 13) PLA+AE (*n*= 11) PLA (*n*= 11)	N/A		DEXA	**TLM** NSEAA +AE 0.2% NSEAA 0.95% NSPLA+AE 0.24% NSPLA 0.70%
Mori et al. [48]	24 weeks	*n*= 75 community dwelling females 65-80yrs	BW, RT 50-70%, 1RM, 3 days a week	1)	WP: 22.3 g PRO, 0.1 g CHO, 0.3 g fat, 2.97 LEU, 92 kcals	After RT	1) 2) 3)	WP +RT (*n*= 24) WP (*n*= 24) RT (*n*= 24)	1) 2) 3)	1.3±0.0 1.3±0.0 1.3±0.0	BIA	**SMI** ↑WP +RT 3.3% ↑ RT 1.6% WP+RT>CON and EX
Nabuco et al. [49]	12 weeks	*n*= 30, community dwelling women 68 ±4 yrs	RT 8.-2 reps (60-70% 1RM, 3 x a week | 1) 2)	WP: 35 g PRO CON: 16 g CHO	After RT	1) 2)	WP + RT (*n*= 15) CON +RT (*n*= 15)	N/A		BIA	**LBM** ↑WP+RT 3.9% ↑ CON+RT 2.0% WP+RT>CON
Nakayama et al. [50]	24 weeks	*n*= 122 (30 males, 92 females) 60-84yrs	Low-to moderate BW exercise (<70% of 1RM), 3 days a week	1) 2)	MPC (10 g PRO, 7g CHO, 68 kcals) CON: CHO 1 6g	After RT	1) 2)	MPC +RT (*n*= 61) CON +RT (*n*= 61)	**1)** **2)**	1.3±0.03 1.2±0.03	InBody	**LBM** ↑MPC + RT 1.5% ↓CON+RT -0.25%)
Nilsson et al. [51]	12 weeks	*n*= 32 community dwelling sedentary males 60-84yrs	unsupervised home based resistance training	1) 2)	WP + casein (40 PRO, 272 kcals) Collagen (40 g PRO, 272 kcals)	With breakfast	1) 2)	WP + casein +R (*n*= 16) Collagen +RT (*n*= 16)	1) 2)	0.97±0.07 1.2±0.12	DEXA	**TLM** ↑WP 0.58 % ↑Collagen 2.0%
Okazaki et al. [52]	12 weeks	*n*= 35, Healthy women, 41-78yrs	60 minutes, Interval walking, 5 or more sets, 3 minutes 40% Vo2 peak, 1 set at 70% vo2 peak for 3 minutes	1)	WP: 7 PRO, CHO, 4.4 g Fat	1x (Post exercise only)	1) 2)	WP+ RT (*n*= 17) RT only (*n*= 18)	N/A		CT	**Quadriceps CSA** ↑WP 1.3% ↑RT 1.4%
Rondanelli et al. [53]	12 weeks	*n*= 130 (Male: 53, Female: 77) Sarcopenic 80 ±7yrs	20 minute sessions, resistant band and ankle weight	1)	WP: 22 g pro, 4.7 g CHO, 0.4 g fat, 2.5IU vitamin D, 4 g LEU	1x (with lunch time meal)	1) 2)	WP+ RT (*n*= 69 CON+RT (*n*= 61)	1) 2)	0.90±0.20 1.00±0.20	DEXA	**TLM** ↑WP +RT 3.5%) NSCON -0.70% WP+RT>CON (GxT)

			exercises, repeated 8 times	2)	CON: CHO 32 g						
Seino et al. [54]	12 weeks	*n*= 78 (13 males, 65 females) community dwellers 73± 4.3 yrs	Resistant bands, 2 sets, 20 reps, 5-6 borg scale	1)	MP: 10.5 g PRO, 9.3 g CHO, 3.9 g fat, 337 mg calcium 478 kj, per 200 ml + additional micronutrient drink	1x (At lunch)	1) 2)	MP +RT (*n*= 40) RT (*n*= 40)	1) 2)	1.4±0.3 1.3±0.2	DEXA	**TLM** ↑MP +RT 1.7% ^{NS}CON 1.3%
Shahar et al. [55]	12 weeks	*n*= 65 (47 males, 18 females) Sarcopenic adults, 60-74 yrs	1) RB, 8 exercises, 30 minutes 2) CON: Relaxation (60 minutes)	SP: 20 g PRO (females), 40 g PRO (males)		1x (Unspecified)	1) 2) 3) 4)	SP+ RB (*n*= 15) RB (*n*= 19) SP (*n*= 15) CON (*n*= 16)	1) 2) 3) 4)	0.84±0.4 0.90±0.14 0.81±0.2 0.90±0.13	BIA	**FFM** ↑RT 5.3% ^{NS}SP + RT 35 0.3% ^{NS}SP -0.5%, ^{NS}CON -3.5%^{ns}
Junior et al. [56]	26 weeks	*n*= 31, RT women, 67±4 yrs	RT, 3 sets 8-12 reps, 8 exercises, 3 days a week	1) 2)	WP: 35 g PRO, 5 CHO, 131kcal CON: CHO 33 g, 134 kcals	Post RT	1) 2)	WP+RT (*n*= 15) CON +RT (*n*= 16)	1) 2)	0.85±0.1 0.81±0.1	DEXA	**TLM** ↑WP+RT 4.8% ↑CON 2.3% WP>CON
ten Haaf et al. [57]	12 weeks	*n*= 114 (95 males, 19 females) active adults with low protein intake (<1.0g/kg BM/day) 67-73yrs	Training for a 4 day walking even (30,40,50km)	1) 2)	MPC: 31 g PRO, 36 g CHO, 1.1 fat CON: CHO 36 g, 1.1 g PRO, 5.2 g fat	With breakfast and post exercise/ or lunch	1) 2)	MPC (*n*= 58) CON (*n*= 56)	1) 2)	0.86±0.23 0.92±0.24	DEXA	**TLM** ↑MPC 0.54% ↑ CON 0.31%
Trabal et al. [58]	12 weeks	*n*= 27 (8 males, 6 females) healthy adults 84 ± 2yrs	Body weight strength exercises, 2 sets, 15 reps at 65% intensity	1) 2)	LEU: 5 g CON: CHO 10 g	2x day (lunch and dinner)	1) 2)	LEU+RT (*n*= 7) CON + RT (*n*= 4)	1) 2)	1.2±0.2 1.2±0.1	MUAMA	^{NS}LEU 1.9%, ^{NS}CON -2.2%
Verdijk et al. [59]	12 weeks	*n*= 26 community dwelling males, 72 ±2yrs	RT, first 4 weeks: 3 sets, 10-15 reps at 60% 1RM, 75% 8-10 reps Last 4 weeks 75-80% 1RM 4 sets at 8 reps	1) 2)	CH (10 g PRO) Water	2x day (before and after training session)	1) 2)	CH+RT (*n*= 13) CON + RT (*n*= 13)	1) 2)	1.1±0.1 1.1±0.1	DEXA and CT	**TLM** ↑CH 1.2% ↑CON 1.0% **Total CSA** ↑CH 1.2%, ↑CON 9%
Villanueva et al. [60]	12 weeks	*n*= 22 Healthy, recreationally active males 68±6yrs	RT, 2-6 sets, 5-6 exercises at 70% 1RM	WP (35 g PRO + creatine monohydrate, 0.3 g/kg for first 5 days then 0.7 g/kg for completion of the study)		1x day (post RT or AM on non-training days)	1) 2) 3)	WP+RT (*n*= 7) RT (*n*= 7) CON (*n*= 8)	N/A		BIA	**TLM** ↑WP+RT 3.2% ↑RT 1.4% ^{NS}CON 1.9%
Yamada et al. [61]	12 weeks	*n*= 122	RT, bodyweight exercises, 20	WP: 10 g PRO, 100 kcals		After breakfast	1) 2)	WP+RT (*n*= 28) RT (*n*= 28)	N/A		BIA	**AMM** ^{NS}WP+RT 0%

	(49 males, 73 females) with sarcopenia or dypenia 84±5 yrs	minutes, 3 sets of 20 reps, 2 times a week	3) 4)	WP (*n*= 28) CON (*n*= 28)	[NS]RT 0.4% [NS]WP -0.69% [NS]CON -4.08%

Abbreviations: AE: aerobic exercise, AMM: appendicular muscle mass, BIA: Bioelectrical impedance analysis, BW: body weight, CH: Casein hydrolysate CHO: Carbohydrate, CON: control, CT: Computed tomography, CSA: Cross-sectional area, FFM: fat-free mass, FMD: fortified milk drink, MUAMA: mid-upper arm muscle area, RB: resistance bands, RT: Resistance training, DEXA: Dual X-ray absorptiometry, Reps: repetitions, TLM: Total lean mass, PRO: Protein, MPC: milk protein concentrate, WP: whey protein

↑= significance increase ↓= significant decrease, and NS = no significant difference from baseline. [stat-X] no statistical analysis provided or statistical test unclear.

Effect on dairy milk and dairy supplementation on outcomes of strength

Handgrip strength

Handgrip strength (HGS) was measured in only one of the dairy milk studies [35]. There were no significant differences in HGS reported in the MILK+RT or control group (Table 6). In the dairy-based supplement studies, *n* =10 studies measured HGS as a measure of muscle strength. Of those studies, only Mori et al. [48] reported a significant difference in HGS in sarcopenic participants that received whey (22 g) and resistance training and no change in the placebo group (19.2% *vs* 0%).

Leg strength

5/6 of the dairy milk studies measured leg strength, which included leg press (n= 4) and knee extension (*n*= 2). 4 of those studies reported a significant increase in leg strength compared to baseline in the groups that received the dairy milk beverage and resistance training [31, 32, 33, 34, 36]. However, there were no significant effects on any of the strength outcomes with dairy milk beyond RT alone (Table 5).

In the supplement studies the method of measurement for leg strength varied, knee extension/ knee flexion (*n*= 14), leg press (*n*= 8), and isometric voluntary contraction (*n*= 1). In leg strength outcomes, 19 of the studies showed a significant increase from baseline (Table 6). Of those, only three studies showed a significant interaction effect. Mori et al. [48] showed a significant increase in maximal knee extension in participants that received 22.5 g/day of WP with 3x/week RT compared to the group that received RT only (17% *vs* -4.5%). Villanueva et al. [60] showed a significant increase in leg strength in both groups that recived RT (72 % v*s* 50%) compared to the control. Finally, Yamada et al. [61] had a 17% improvement in maximal knee extension in the group of sarcopenic women who received resistance training with 10 g of protein compared to the control (-0.13%).

Upper body strength

All of the dairy milk beverage studies measured upper body strength using maximal chest press. Only 4/6 (70%) of the studies reported a significant increase in maximal chest press from baseline in the groups that received dairy milk and resistance training [31, 34, 35, 36]. One study that measured the effects of fortified milk (13.5 g protein) with or without resistance training found that a group of healthy community dwellers who received exercise intervention had a significant main effect compared to the dairy group alone [31]. Within this study, the groups allocated to resistance training and milk intervention increased chest press by 45%, whereas resistance training only saw a 30% increase. The group that received the milk intervention increased their chest press the same as the control group (15%).

Chest press was measured in *n*= 4 of the dairy-based supplement studies [39, 41, 56, 60]. These studies reported significant increases in maximal chest press from baseline, ranging from 7 to 120%. However, there were no reported differences in the protein groups *vs* placebo groups.

Table 5. Main outcomes for skeletal muscle strength outcomes and dairy intervention trials

Study	Duration	Participants	Exercise stress	Dairy and placebo details: Nutrition intervention	Frequency x timing	Study groups	Baseline protein intake (g/Kg BMday)	Method used	Main outcomes
Kukuljan et al. [31]	72 weeks	*n*= 180, Healthy community dwelling males 50-79 yrs	RT, 3 sets, 10-12 reps at 50% 1RM for 7 exercises, 3 x days a week	1) 200 ml Fortified Milk drink: 6.6 g PRO, 11 g CHO, 2.2 g Fat, 418 kj	Morning and evening	1) MILK (*n*= 43) 2) RT only (*n*= 43) 3) MILK + RT (*n*= 43) 4) CON (*n*= 42)	1) 1.2±0.28 2) 1.3±0.32 3) 1.2±0.32 4) 1.3±0.31	Machine 1RM	**CP** ↑ MILK +RT 45% ↑ Exercise 30% ^{NS} MILK 15% ^{NS} CON 15% MILK + RT >MILK **LP** ↑ MILK +RT 30% ↑Exercise 25% ^{NS} MILK 5% ↓CON15%
Maltais et al. [32]	16 weeks	*n*= 26 overweight and sarcopenic males 65± 5 yrs	RT, 3 sets, 6-8 reps 80% 1RM for 8 exercises, 3 x days a week	1) MILK: Chocolate milk 375 ml: 13.5 g PRO, 37 g CHO, 7 g EAA (3.5 g leucine), 1058 kj. 2) SD: soy drink. 379 ml: 12 g PRO, 39 g CHO, 7 g EAA 3) CON: rice milk	Post training only	1) MILK +RT (*n*= 8) 2) SD +RT (*n*= 8) 3) CON +RT (*n*= 10)	1) 1.0±0.20 2) 1.2±0.20 3) 1.3±0.41	Machine 1RM	**CP** ^{NS} MILK +RT 14% ^{NS} SD 8.4% ^{NS} CON 4.0%
Mitchell et al. [33]	12 weeks	*n*= 16 (47 males, 18 females) healthy community dwellers 74 ± 5 yrs	RT, 3 sets at 75-85% 1RM, 3 x days a week	1) MILK: chocolate milk 500 ml (14 g PRO, 54 g CHO, 5 g fat, 1340 kj) 2) CON: CHO drink (13.2 g CHO)	1 x day (Post RT or with breakfast)	1) MILK + RT (*n*= 8) 2) CON: + RT (*n*= 8)	N/A	Machine 1RM	**LP** ↑ MILK + RT 52% ^{NS} CON: 47% **KE** ↑ MILK + RT 51% ^{NS} CON: 35%% **CP** ^{NS} MILK + RT 9.8% ^{NS} CON 4.9%
Orsatti et al. [34]	16 weeks	*n*= 32 post-menopausal females 50-68 yrs	RT, 3 sets, 8-12 reps, 70% 1RM, 8 exercises, 3 x days a week	1) MILK: 200 ml of skimmed milk +25 g soy protein 2) CHO drink (25 g CHO) + RT	Post RT, or same time each day	1) MILK + RT (*n*= 16) 2) CHO drink + RT (*n*= 16)	1) 0.8±0.3 2) 1.0±0.3	Machine 1RM	**CP** ↑ MILK + RT 36% ^{NS} CON 23% **KE** ↑ MILK + RT 36% ^{NS} CON 23%
Ottestad et al. [35]	16 weeks	*n*= 36 (12 males, 24 females)	Regular exercise monitored	1) MILK: Protein-enriched milk. 400ml 696 kj, 20 g	Breakfast and dinner	1) MILK + RT (*n*= 17) 2) CON: RT (*n*= 19)	1) 1.0±0.3 2) 1.0±0.3	Machine 1RM Dynamometer	**CP** ↑ MILK +RT 4.0% ↑CON 4.5%

		healthy community dwellers 70-83 yrs			PRO, 19.6 g CHO 0.4 g fat)						**LP** [NS] MILK + RT 3.9% [NS] CON 4.2% **HGS** [NS] MILK + RT 0.4% [NS] CON 2.0%
Thomson et al. [36]	12 weeks	*n*= 179 (81 males, 98 females) healthy community dwellers 61.5 ±7 yrs	RT, 3 sets, 8-12 reps, 5 exercises, 2 x days a week	1) MILK: Enriched low fat milk: (27 g PRO, 30 g CHO) 2) SM: Enriched soy milk: (27 g PRO, 30 g CHO) 3) OJ + 2 sweet biscuits (500 ml)	1 x day (post training)	1) MILK + RT (*n*= 54) 2) SM (27 g PRO) + RT (*n*= 64) 3) RT only (*n*= 61)	N/A	Machine 1RM Dynamometer	**CP** ↑MILK +RT: 4.0% ↑CON: ↑ 4.5% **LP** ↑MILK+RT 118% ↑ CON 117% **HGS** ↑MILK +RT: 4.7% ↑CON: ↑ 5.9%

Abbreviations: CHO: Carbohydrate, CON: control, CP: Chest press, CT: Computed tomography, HGS: handgrip strength, KE: knee extension, LPD: *lat* pull down, LP: leg press, Reps: repetitions, RT: Resistance training, PRO: Protein, WP: whey protein ↑= significance increase ↓= significant decrease, and NS = no significant difference from baseline. [stat-X] no statistical analysis provided or statistical test unclear.

Table 6. Main outcomes for skeletal muscle strength and supplement intervention trials

Study	Duration	Participants	Exercise stress	Supplement and placebo details: Nutrition intervention	Frequency x timing	Study groups	Baseline protein intake (g/Kg BMday)	Method used	Main outcomes %change from baseline
Arnarson et al. [37]	12 weeks	*n*= 161, (67 males, 94 females) Healthy older adults 65-91yrs	RT, 3 sets, 6-8 reps, 75-80% 1RM. Load increased 5-10% each week	1) WP: 30g PRO, 20g CHO, 709kj 2) CON: 40g CHO	1x Post RT only	1) WP +RT (*n*= 75) 2) CON+RT (*n*= 66)	1) 1.0 ± 0.26 2) 0.92±0.30	dynamometer (N)	**KE** ^{NS}WP 12.% ^{NS}CON 12%
Bell et al.[38]	20 weeks	*n*= 49, Healthy community dwelling males, 73±1 yrs	RT, 3 sets 10-12 reps, at 65% 1RM increasing to 3x6-8 sets/reps, 80% 1RM HIIT 1x week 10x60s intervals	1) WP: 31g PRO, 1g CHO, 2.5g creatine, 500 IU vitamin D, 400 mg calcium and 1500mg n-3 PUFA, 484. kj 2) CON: 22g CHO	2x 1 hour after breakfast, 1 hour before bed	1) WP + RT (*n*= 25) 2) CON+ RT (*n*= 24)	1) 1.1±0.3 2) 1.2±0.4	Machine 1RM	**Sum of all 1RMs** ↑WP+RT 20% WP+RT> CON
Candow et al. [39]	12 weeks	*n*= 29, Community dwelling males 59-76yrs	RT, 3 sets, 10 reps at 70% 1RM	1) WP: 26g PRO, 14.7g CHO 2) CON: 14.7 CHO	1x Before or PT	1) WP+ RT (before) (*n*= 9) 2) WP+RT (after) (*n*= 10) 3) CON+RT (*n*= 10)	1) 1.5± 0.2 2) 1.1±0.10 3) 1.3±0.17	Machine 1RM	**LP** ↑WP+ RT 31%, (before) ↑WP+RT (after) 25%, ↑CON 22% **CP** ↑WP+ RT (before) 38% ↑WP+RT (after) 28% ↑CON 23%
Daly et al. [40]	16 weeks	*n*=244 inactive females, <7500 steps per day 45-65 yrs	Home based body weight workout, 7 exercises (2x10-15 reps)+ balance	1) FMD: 9.1g PRO, 16.5g CHO, 418kj 2) CON: Rice powder 25g CHO	2x a day (breakfast and lunch/or post training)	1) FMD +RT (*n*= 108) 2) CON+RT (*n*= 136)	1) 1.2 ±0.41 2) 1.2±0.47	Machine 1RM Dynamometer	**LP** ↑FMD+RT 24% ↑CON 21% **HGS** ↑FMD+RT 4% ↑CON 3%
de Branco et al. [41]	8 weeks	*n*=34, post-menopausal females 61±7yrs	RT, 1-3 sets, 8-12 reps (70% 1RM), 3x a week	1) WP: 30g PRO 2) CON: 30g CHO	PT and afternoon (3pm)	1) WP+RT (*n*= 17) 2) CON+RT (*n*= 17)	1) 0.9±0.2 2) 1.0±0.3	1RM Dynamometer	**KE** ↑WP+ RT 12% ↑CON 8% **CP** ↑WP+RT 15% ↑CON 18% **HGS**

											↑WP+RT 5% ↑CON 7%
Dulac et al. [42]	12 weeks	*n*=60, Sedentary (<120 minutes per week) older males 69 ±7yrs	RT self-selected moderate weight. Increased 2.5-5kg once hit >12 reps	1) 2) 3)	WP: 10g PRO Casein: 10g PRO CON: 19g CHO	3x a day breakfast/lunch and dinner	1) 2) 3)	WP+RT (*n*=21) Casein +RT (*n*=20) CON+RT (*n*=19)	1) 2) 3)	1.4±0.31 1.34±0.45 1.5±0.36	Machine 1RM	**KE** ↑WP+RT 39% ↑ Casein + RT 34% ↑CON 36%
Holm et al. [43]	24 weeks	*n*=29, Healthy post-menopausal females 55±1yrs	RT, 3 sets, 15 reps	1) 2)	WP: 10g PRO, 31g CHO, 1g fat, 720kj CON: CHO 6g	PT	1) 2)	WP+ RT (*n*=16) CON+ RT (*n*=16)		N/A	Machine 1RM	**KE** ↑WP+ RT 9% ^{NS} CON+RT 1%
Holwerda et al. [44]	12 weeks	*n*=41, healthy older males 70 ±1	RT, First 4 weeks 70% 1RM (8 reps) then 80% 1RM (10 reps), 3x days a week	1) 2)	WP (21g PRO, 9.4CHO, 3g LEU, 628kj) CON: CHO 24g	Breakfast and lunch	1) 2)	WP +RT (*n*=20) CHO +RT (*n*=21)	1) 2)	1.14±0.05 1.2±0.06	Machine 1RM	**LP** ↑WP+RT 15% ↑CON 15% **KE** ↑WP+RT 18% ↑CON 20%
Kim et al. [45]	12 weeks	*n*=155 Sarcopenic women, >75yrs	RB + balance and gait training	EAA: 3g		2x (Not specified)	1) 2) 3) 4)	RB+ EAA (*n*=38) RB (*n*=39) EAA (*n*=39) CON (*n*=39)		N/A	Machine 1RM	**KE** ↑EAA +RT 9.3% ^{NS}EAA 0% ^{NS}RT ↑ 4.5% ^{NS}CON 5%
Leenders et al.[46]	24 weeks	*n*= 53 (29 males, 24 females) Healthy older adults, 68-74yrs	RT, 3 sets, 10 reps, 60% of 1RM for the first 4 weeks, increased to 75-80% of 1RM for the remainder of the trial	1) 2)	MP: 15g PRO, 7.13g CHO, 0.5g fat, 119kj CON: CHO 18g	1x (After breakfast)	1) 2)	MP + RT (*n*=Males: 14, females 12) CON +RT (*n*=Males: 15, females 12)		N/A	Machine 1RM	**KE** ↑MP+RT Males (44%) Females (45%) ↑CON+RT Males (41%) Females (45%) **LP** ↑MP+RT Males (44%) Females (45%) ↑CON+RT Males (41%) Females (45%)
Markosfki et al. [47]	24 weeks	*n*=50, Healthy independent older adults (low-active <7500 steps	AE, 3x week 45minutes 70% HR treadmill	EAA: 15g		PT	1) 2) 3) 4)	EAA+AE (*n*=10) EAA (*n*=13) PLA+AE (*n*=11) PLA (*n*=11)		N/A	Dynamoeter (right leg)	**LP** ↑EAA+AE 16% ^{NS}EAA 1.8% ^{NS} PLA+AE 10.6% ^{NS} PLA 11%

		65-82yrs								
Mori et al. [48]	24 weeks	*n*=75 community-dwelling females 65-80yrs	BW, RT 50-70%, 1RM, 3 days a week	WP: 22.3g PRO, 0.1g CHO, 0.3g fat, 2.97 LEU, 92kcals	PT	1) WP +RT (*n*=24) 2) WP (*n*=24) 3) RT (*n*=24)	1) 1.3±0.0 2) 1.3±0.0 3) 1.3±0.0	Machine 1RM Dynamometer	**KE** ↑WP +RT 17.8% ↑WP 4.1% ↑RT -4.5% WP+RT > WP and CON **HGS** ↑WP +RT 10.8% ↑WP 4.2% ↑RT -4.1% WP+RT > WP
Nabuco et al. [49]	12 weeks	*n*=30, community dwelling women 68 ±4 yrs	RT 8.-2 reps (60-70% 1RM, 3 x a week	1) WP: 35g PRO 2) CON: 16g CHO	PT	1) WP + RT (*n*=15) 2) CON +RT (*n*=15)	N/A	Isokinetic dynamometer (60°/s and 180°/s)	**60°/s** ↑WP + RT 12.9% CON +RT 8.4% **180°/s** WP + RT 11.1% CON +RT 8.4%
Nakayama et al. [50]	12 weeks	*n*=122 (30 males, 92 females) 60-84yrs	Low-to moderate BW exercise (<70% of 1RM), 3 days a week	1) MPC (10g PRO, 7g CHO, 68kcals) 2) CON: CHO 16g	PT	1) MPC +RT (*n*=61) 2) CON +RT (*n*=61)	1) 1.3±0.03 2) 1.2±0.03	KE and KF: Isokinetic dynamometer HGS: dynamometer	**KE** ↑MPC +RT 10.3% ↑Con +RT 12.6% **KF** ↑MPC +RT 10.8% ↑Con +RT 12.5% **HGS** ^{NS}MPC +RT 0% ^{NS}Con +RT -2.1%
Nilsson et al. [51]	12 weeks	*n*=32 community dwelling sedentary males 60-84yrs	unsupervised home based resistance training	1) WP + casein (40 PRO, 272kcals) 2) CON: Collagen (40g PRO, 272 kcals)	With breakfast	1) WP + casein +RT (*n*=16) 2) CON +RT (*n*=16)	1) 0.97±0.10 2) 1.2±0.12	Machine 1 RM Isometric KE (Nm) dynamometer	**LP** ↑WP + RT 14% ^{NS}CON +RT 1.4% **KE** ↑WP +RT 6.8% ^{NS}CON +RT 5% **HGS** ^{NS}WP +RT 3.0% ^{NS}CON +RT 2.3%
Okazaki et al. [52]	12 weeks	n=35 Healthy women, 41-78yrs	60 minutes, Interval walking, 5 or more sets, 3 minutes 40% Vo2 peak, 1 set at 70% vo2 peak for 3 minutes	WP: 7.6g PRO, 32.6 CHO, 4.4g Fat	PT	1) WP+ RT (*n*=18) 2) RT only (*n*=17)	N/A	Hand dynamometer,	**HGS** ↑WP + 19.2% ^{NS}CON 0% WP>CON

Rondanelli et al. [53]	12 weeks	*n*=130 (53 males, 77 females) Sarcopenic, 80 ±7yrs	20-minute sessions, resistant band and ankle weight exercises, repeated 8 times	1) WP: 22g pro, 4.7g CHO, 0.4g fat, 2.5IU vitamin D, 4g LEU 2) CON: CHO 32g	1x (with lunch time meal)	1) WP+ RT (*n*=69) 2) CON+RT (*n*=61)	1) 0.90±0.20 2) 1.00±0.20	Hand dynamometer,	**HGS** ^{NS}WP+ RT 19.2% ^{NS}CON+RT 18.8%
Seino et al. [54]	12 weeks	*n*=78 (13males 65 females) Healthy older adults, 73± 4.3 yrs	Resistant bands, 2 sets, 20 reps, 5-6 borg scale	MP: 10.5g PRO, 9.3g CHO, 3.9g fat, 337mg calcium 478kj, per 200ml + additional micronutrient drink	1x (At lunch)	1) MP +RT (*n*=40) 2) RT (*n*=40)	1) 1.4±0.3 2) 1.3±0.2	Hand dynamometer, (Dominant) Single leg knee extension, dynamometer	**HGS** ^{NS}MP+RT 0.4% ^{NS}RT 2.2% **KE** ^{NS}MP+RT 0.9% ^{NS}RT 4%
Shahar et al. [55]	12 weeks	*n*=65 (47 males, 18 females) Sarcopenic adults, 60-74 yrs	RB, 8 exercises, 30 minutes	SP: 20g PRO (females), 40g PRO (males)	1x (Unspecified)	1) SP+ RB (*n*=15) 2) RB (*n*=19) 3) SP (*n*=15) 4) CON (*n*=16)	1) 0.84±0.4 2) 0.90±0.14 3) 0.81±0. 4) 0.90±0.13	Dynamometer, HG	**HGS** ^{NS}SP + RB -4.0% ^{NS}RB -6.7% ^{NS}SP only -14.0% ^{NS}CON -8.5%
Junior et al. [56]	26 weeks	*n*=31, resistance trained older women, 67±4 yrs	RT, 3 sets 8-12 reps, 8 exercises, 3 days a week	1) WP: 35g PRO, 5 CHO, 131kcal 2) CON: CHO 33g, 134kcals	PT	1) WP+RT (*n*=15) 2) CON +RT (*n*=16)	1) 0.85±0.1 2) 0.81±0.1	Machine 1 RM	**KE** ↑WP+RT 8.5% ↑CON+RT 4.3% **CP** ↑WP+RT 6.3% ↑CON+RT 2.7%
ten Haaf et al. [57]	12 weeks	*n*=114 (95 males, 19 females) active older adults with low protein intake (<1.0g/kgBM/day) 67-73yrs	Training for a 4 day walking even (30,40,50km)	1) MPC: 31g PRO, 36g CHO, 1.1 fat 2) CON: CHO 36g, 1.1g PRO, 5.2g fat	With breakfast and PT or lunch)	1) MPC (*n*=58) 2) CON (*n*=56)	1) 0.86±0.23 2) 0.92±0.24	Isometric voluntary contraction Hand dynamometer,	**MVC** ^{NS}MPC 7.2% ^{NS}CON 8.7% **HGS** ^{NS}MPC 0% ^{NS}CON 1%
Trabal et al. [58]	12 weeks	n=27 (8 males, 16 females) Retirement living healthy older adults, 84 ±2yrs	Body weight strength exercises, 2 sets, 15 reps at 65% intensity	1) LEU: 5g 2) CON: CHO 10g	2x day (lunch and dinner)	1) LEU+RT (*n*=12) 2) CON + RT (*n*=12)	1) 1.2±0.2 2) 1.2±0.1	dynamometer, 1RM	**KF** ^{NS}LEU+RT 42% ^{NS}CON 22%
Verdijk et al. [59]	12 weeks	*n*=26 community dwelling males, 72 ±2yrs	RT, first 4 weeks: 3 sets, 10-15 reps at 60% 1RM, 75% 8-10 reps	1) CH (10g PRO) 2) Water	2x day (before and PT)	1) CH+RT (*n*=13) 2) CON + RT (*n*=13)	1) 1.1±0.1 2) 1.1±0.1	Machine 1RM	**LP** ↑CH 24% ↑CON 24% **KE** ↑CH 27 ↑CON 38%

			Last 4 weeks 75-80% 1RM 4 sets at 8 reps							
Villanueva et al. [60]	12 weeks	*n*=22 Healthy, recreationally active males 68 ±6yrs	RT, 2-6 sets, 5-6 exercises at 70% 1RM	WP (35g PRO + creatine monohydrate, 0.3g/kg for first 5 days then 0.7g/kg for completion of the study)	1x day (post RT or AM on non- training days)	1) 2) 3)	WP+RT (*n*=7) RT (*n*=7) CON: no intervention (*n*=8)	N/A	Machine 1RM	**LP** ↑WP + RT 72.4% ↑RT 50.1% $^{STAT-X}$CON N/A **CP** ↑WP + RT 129% ↑RT 113% CON: N/A WP+RT and RT >CON
Yamada et al. [61]	12 weeks	*n*=122 (49 males, 73 females) with sarcopenia or dypenia	RT, body weight exercises, 20 minutes, 3 sets of 20 reps, 2 times a week	WP: 10g PRO, 100kcals	After breakfast	1) 2) 3) 4)	WP+RT (*n*=28) RT (*n*=28) WP (*n*=28) CON (*n*=28)	N/A	Machine 1RM	**KE** ↑WP+RT 17.3% ^{NS}WP 7.3% ^{NS}RT 1.7% ^{NS}CON -0.13% WP+RT >CON **HGS** ^{NS}WP+RT 4.5% ^{NS}WP 2.1% ^{NS}RT 3.1% ^{NS}CON 1.3%

Abbreviations: CHO: Carbohydrate, CH: casein hydrolysis, CON: control, CP: Chest press, CT: Computed tomography, EAA, essential amino acids, FMD: fortified milk drink, HGS: handgrip strength, KE: knee extension, KF: knee flexion, LEU: leucine, LPD: *lat* pull down, LP: leg press, MPC: milk protein concentrate, Reps: repetitions, RT: Resistance training, PRO: Protein, PT: Post-Training WP: whey protein, ↑= significance increase ↓= significant decrease, and NS = no significant difference from baseline.

$^{stat-X}$ no statistical analysis provided or statistical test unclear.

Performance measure Outcomes

Performance measures in the dairy milk beverage and dairy-based beverage studies varied (Table 7 and 8). Chair stand (CS) was the most common performance measure across the studies. For example, in the dairy-based supplement group, De Branco et al. [41], Dulac et al. [42], Shahar et al. [55] measured the number of repetitions of standing out of a chair in '20 or 30 seconds. Leenders et al. [46], Nilsson et al. [51], Seino et al. [54] and Yamada et al. [61] reported the time it took participants to perform 5 chair stands. No significant change was observed in the *n*= 2 dairy trials that measured CS [32, 35].

Timed up and go (TUG) was only measured in one of the dairy-based beverage studies. Maltais et al. [32] reported a significant decrease in TUG in the soy group (17.8%), compared to dairy milk (1.4%) and control groups (1.4%) was measured in *n*= 8 dairy-based supplement studies. Only *n*= 5 of those studies showed a significant difference in the dairy-based supplement group compared to baseline [38, 41, 50, 54, 57]. Shahar et al. [55] was the only dairy-based supplement study to show a difference between groups in TUG. This study provided 20-40 g of soy protein to sarcopenic adults and found that the soy protein plus exercise and soy protein group alone resulted in a significant increase (62.8% and 72.6%, respectively).

Gait speed was measured in only one of the dairy milk studies. Kukuljan et al. [31] reported a significant decrease in time to complete a 4-meter gait speed course in the MILK+RT and EX groups (Table 7). Gait speed was measured in *n*= 6 dairy-based supplement studies; however, only *n*= 2 saw a significant change in the nutrition intervention group (Table 8).Kim [45] found a statistical improvement in the groups that received 6 g of EAA daily plus resistance training (13.5%) and the resistance training only group (16.3%). There was no significant change in gait speed in the group that received EAA without exercise intervention (5.4%). Villanueva et al. [60] reported significant

improvements in gait speed in the resistance training only group (9.7%) and the resistance training group that also received 35 g of whey post-training (11.0%). In a dairy milk beverage study, Thomson et al. [40] saw a significant change in all the groups (dairy milk: 118%, soy: 61.3%, and control: 117%), with no differences between groups (Table 7).

Table 7. Main outcomes for physical performance measures and dairy intervention studies

Study	Duration	Participants	Exercise stress	Dairy and placebo details: Nutrition intervention	Dairy and placebo details: Frequency x timing	Study groups	Methods used	Main outcomes
Kukuljan et al. [31]	72 weeks	*n*= 180, Healthy community-dwelling males 50-79 yrs	RT, 3 sets, 10-12 reps at 50% 1RM for 7 exercises, 3 x days a week	200ml Fortified Milk drink: 6.6g PRO, 11g CHO, 2.2g Fat, 418kj	Morning and evening	1) MILK (*n*=43) 2) RT only (*n*=43) 3) MILK + RT (*n*= 43) 4) CON (*n*=42)	GS (4m/second)	**GS** ↓MILK +RT -18.0% ↓EX -20% NSMILK -3.0% ↓CON: -7.2
Maltais et al. [32]	16 weeks	*n*= 26 overweight and sarcopenic males 65± 5 yrs	RT, 3 sets, 6-8 reps 80% 1RM for 8 exercises, 3 x days a week	1) MILK: Chocolate milk 375ml: 13.5g PRO, 37g CHO, 7g EAA (3.5g leucine), 1058kj. 2) SD: soy drink. 379ml: 12g PRO, 39g CHO, 7g EAA	PT	1) MILK +RT (*n*=8) 2) SD +RT (*n*=8) 3) CON +RT (*n*=10)	CS (5 reps) TUG	**CS** NS MILK +RT -0.8 % NS SD+ RT -9.3% NS CON: -3.3% **TUG** NS MILK +RT-1.5 % ↓ SD+RT 17.8% NS CON 1.4% **6MW** ↑MILK+ RT: 7.1% ↑ SS+RT ↑25% ↑ CON:↑ 6.6%
Ottestad et al. [35]	16 weeks	*n*=36 (12 males, 24 females) healthy community dwellers 70-83 yrs	Regular exercise monitored	MILK: Protein-enriched milk. 400ml 696kj, 20g PRO, 19.6g CHO 0.4g fat)	breakfast and dinner	1) MILK + RT (*n*=17) 2) CON:RT only (*n*=19)	CS (5 repetitions)	**CS** NS MILK +RT 2.2% NS CON 3.3%
Thomson et al. [36]	12 weeks	*n*=179 (81 males, 98 females) healthy community dwellers 61.5 ±7 yrs	RT, 3 sets, 8-12 reps, 5 exercises, 2 x days a week	1) MILK: Enriched low fat milk: (27g PRO, 30g CHO) 2) SM: Enriched soy milk: (27g PRO, 30g CHO) 3) OJ + 2 sweet biscuits (500ml)	PT	1) MILK + RT (*n*=54) 2) SM: (27g PRO) + RT (*n*=64) 3) (3) RT only (*n*=61)	6MW	**6MW** ↑MILK 118% ↑SM 61.3% ↑CON 117%

Abbreviations: 6MW: 6- meter walk, CS: chair stand, CHO: Carbohydrate, CON: control, area, GS: gait speed, RT: Resistance training, Reps: repetitions, PRO: Protein, PT: Post-training, TUG: time up and go, WP: whey protein. Between baseline and end of the trial: ↑= significance increase ↓= significant decrease, and NS = no significant difference from baseline.

Table 8. Main outcomes for physical performance and dairy-based supplement intervention trials

Study	Duration	Participants	Exercise stress	Supplement and placebo details: Nutrition intervention	Supplement and placebo details: Frequency x timing	Study groups	Method of measurement	Main outcomes %change from baseline
Arnarson et al. [37]	12 weeks	*n*=161, (67 males, 94 females) Healthy older adults 65-91yrs	RT, 3 sets, 6-8 reps, 75-80% 1RM. Load increased 5-10% each week	1) WP: 30 g PRO, 20 g CHO, 709 kj 2) CON: 40 g CHO	1x Post RT only	1) WP +RT (*n*=75) 2) CON+RT (*n*=66)	TUG, 6MW	**TUG** ^{NS}WP+RT -7.4% ^{NS}CON -6.2% **6MW** ^{NS}WP+RT 7.6 ^{NS}CON 9%
Bell et al. [38]	20 weeks	*n*=49, Healthy community-dwelling males, 73± 1 yrs	RT, 3 sets 10-12 reps, at 65% 1RM increasing to 3x6-8 sets/reps, 80% 1RM HIIT 1x week 10x60s intervals	1) WP: 31 g PRO, 1 g CHO, 2.5 g creatine, 500 IU vitamin D, 400 mg calcium and 1500mg n-3 PUFA, 484.5 kj 2) CON: 22g CHO	2x 1 hour after breakfast, 1 hour before bed	1) WP + RT 2) CON+ RT (*n*=24)	TUG, CS (n/30s), GS (6 m)	**TUG** ↑ WP +RT 9.8% ↑ CON: ↑10.5% **CS** ↑ WP 8.3% ^{NS}CON: 0% **6MW** ↑ WP↑ 6.9% ↑ CON: ↑7.7%
Daly et al. [40]	16 weeks	*n*=244 inactive females, <7500 steps per day 45-65 yrs	Home-based bodyweight workout, 7 exercises (2x10-15 reps)+ balance	1) FMD: 9.1g PRO, 16.5g CHO, 418 kj 2) CON: Rice powder 25g CHO	2x a day (breakfast and lunch/or post training)	1) FMD +RT (*n*=108) 2) CON+RT (*n*=136)	GS (4m/second) TUG (seconds)	**GS** ↓FMD+ RT -2.8 ^{NS}CON 0% **TUG** ↓FMD+RT -3.1% ↓CON -2.2%
De Branco et al. [41]	8 weeks	*n*=34, post-menopausal females 61±7yrs	RT, 1-3 sets, 8-12 reps (70% 1RM), 3x a week	1) WP: 30g PRO 2) CON: 30g CHO	Post-exercise (3x week) and afternoon (3pm)	1) WP+RT (*n*=17) 2) CON+RT (*n*=17)	GS (4m/second) CS (n of steps) TUG (seconds)	**GS** ↓WP+ RT -2.8 ^{NS}CON 0% **CS** ↓WP+RT 3.1% ↓CON 2.2% **TUG** ↑WP+RT 5% ↑CON 7%
Dulac et al. [42]	12 weeks	*n*=60, Sedentary (<120 minutes per week) older males 69 (7) yrs	RT self-selected moderate weight. Increased 2.5-5kg once hit >12 reps	1) WP: 10g PRO 2) Casein: 10g PRO 3) CON: 19g CHO	3x a day breakfast/lunch and dinner	1) WP+RT (*n*=21) 2) Casein +RT (*n*=20) 3) CON+RT (*n*=19)	GS (4m.seconds) CS (n of reps)	**GS** ↓WP+ RT -6.2% ↓Casein +RT -3.8% ↓CON -4.2% **CS** ↑ WP+RT 8.8% ↑ Casein +RT 13.3% ↑ CON 12.8%

									TUG ↓WP+RT -5.5% ↓Casein +RT -7.8% ↓CON -5.1%
Holwerda et al. [44]	12 weeks	*n*=41, healthy older males 70 ±1 yrs	RT, First 4 weeks 70% 1RM (8 reps) then 80% 1RM (10 reps), 3x days a week	1) WP (21g PRO, 9.4CHO, 3g LEU, 628kj) 2) CON: CHO 24g	Breakfast and lunch	1) WP +RT (*n*=20) 2) CHO +RT (*n*=21)	GS (4m/second) STS (time)	**GS** ^{NS}WP+RT 0.84% ^{NS}CON 0.81% **STS** ↓WP+RT -6.9% ↓CON -6.6%
Kim et al. [45]	12 weeks	*n*=155 Sarcopenic women, >75yrs	RB + balance and gait training	EAA: 3g	2x (Not specified)	1) RB+ EAA (*n*=38) 2) RB (*n*=39) 3) EAA (*n*=39) 4) CON (*n*=39)	GS (m/s)	**GS** ↑ EAA +RT: 13.5% ^{NS}EAA: ↑ 5.4%^{n} ↑ RT 16.3% ↑ CON 3.4%
Leenders et al. [46]	24 weeks	n= 53 Male: 29, Female: 24 Healthy older adults, 68-74yrs	RT, 3 sets, 10 reps, 60% of 1RM for the first 4 weeks, increased to 75-80% of 1RM for the remainder of the trial	1) MP: 15g PRO, 7.13g CHO, 0.5g fat, 119kj 2) CON: CHO 18g	1x (After breakfast)	1) MP + RT (*n*=Males: 14, females 12) 2) CON +RT (*n*=Males: 15, females 12)	CS (5 repetitions)	**CS** ↓MP (females) -15.0% (males) 16.4% ↓CON (females) -9.2% (Males) 18.3%
Mori et al. [48]	24 weeks	*n*=75 community-dwelling females 65-80yrs	BW, RT 50-70%, 1RM, 3 days a week	WP: 22.3g PRO, 0.1g CHO, 0.3g fat, 2.97 LEU, 92kcals	After RT	1) WP +RT (*n*=24) 2) WP (*n*=24) 3) RT (*n*=24)	GS (4m/sec)	**GS** ↑WP +RT $^{stat\text{-}x}$ ↑WP $^{stat\text{-}x}$ ↑RT $^{stat\text{-}x}$
Nakayama et al. [50]	12 weeks	*n*=122 (30 males, 92 females) 60-84yrs	Low-to moderate BW exercise (<70% of 1RM), 3 days a week	1) MPC (10g PRO, 7g CHO, 68kcals) 2) CON: CHO 16g	After RT	1) MPC +RT (*n*=61) 2) Con +RT (*n*=61)	TUG (seconds) STS (seconds)	**TUG** ↓MPC +RT -4.8% ↓Con +RT -4.1% **STS** ↓MPC +RT -12.1% ↑Con +RT -8.9%
Nilsson et al. [51]	12 weeks	*n*=32 community dwelling sedentary males 60-84yrs	unsupervised home based resistance training	1) WP + casein (40 PRO, 272kcals) 2) CON: Collagen (40g PRO, 272 kcals)	With breakfast	1) WP + casein +RT (*n*=16) 2) CON +RT (*n*=16)	TUG (seconds) GS (4m/second) CS (x5, seconds)	**TUG** ^{NS}WP + RT -3.9% ^{NS}CON +RT -0.96% **GS** ^{NS}WP +RT 6.1% ^{NS}CON +RT 3.2% **CS** ↓WP +RT -8..3% ^{NS}CON +RT -9.4%
Seino et al. [54]	12 weeks	*n*=78 (13 males, 65 females) Healthy older adults,	Resistant bands, 2 sets, 20 reps, 5-6 borg scale	MP: 10.5g PRO, 9.3g CHO, 3.9g fat, 337mg calcium 478kj, per 200ml + additional micronutrient drink	1x (At lunch)	1) MP +RT (*n*=40) 2) RT (*n*=40)	TUG, CS (30seconds) CS (5-reps) GS	**TUG** ↓MP -10.0% ↓CON -11.5% **CS**

		73± 4.3 yrs							↑MP 27.4% ↑CON: 24.4% **CS (5 reps)** ↓MP: -1 27.4% ↓CON: -25.0% **GS** ^{NS}MP: -6.6% ^{NS}CON: 7.3%
Shahar et al. [55]	12 weeks	*n*=65 (47 males, 18 females) Sarcopenic adults, 60-74 yrs	RB, 8 exercises, 30 minutes	SP: 20g PRO (females), 40g PRO (males)	1x (Unspecified)	1) SP+ RB (*n*=15) 2) RB (*n*=19) 3) SP (*n*=15) 4) CON (*n*=16)	CS (20 seconds)	**CS** ↑SP + RT: 62.8% ^{NS}Exercise only 24.0% ↑SP only 72.6% ^{NS}CON 10.3% SP+RT and SP> CON
ten Haaf et al.[57]	12 weeks	*n*=114 (95 males, 19 females) active older adults with low protein intake (<1.0g/kgBM/day) 67-73yrs	Training for a 4 day walking even (30,40,50km)	1) MPC: 31g PRO, 36g CHO, 1.1 fat 2) CON: CHO 36g, 1.1g PRO, 5.2g fat	With breakfast and post-exercise/ or lunch	1) MPC (*n*=58) 2) CON (*n*=56)	GS (4m/seconds) TUG (seconds) CS (seconds)	**GS** ^{NS}MPC 0% ^{NS}CON 0% **TUG** ↓MPC -5.7% ↓CON -5.7% **CS** ^{NS}MPC-7.8% ^{NS}CON -6.3%%
Trabal et al. [58]	12 weeks	*n*=27 (8 males, 16 females) Retirement living healthy older adults, 84±2yrs	Body weight strength exercises, 2 sets, 15 reps at 65% intensity	1) LEU: 5g 2) CON: CHO 10g	2x day (lunch and dinner)	1) LEU+RT (*n*=12) 2) CON + RT (*n*=12)	TUG, CS (unspecified)	**TUG** $^{ns-x}$ LEU 19.3% $^{ns-x}$ CON 11.5% **CS** $^{ns-x}$ LEU: 25.0% $^{ns-x}$ CON 13.0%
Villanueva et al. [60]	12 weeks	*n*=22 Healthy, recreationally active males 68 ±6yrs	RT, 2-6 sets, 5-6 exercises at 70% 1RM	WP (35g PRO + creatine monohydrate, 0.3g/kg for first 5 days then 0.7g/kg for completion of the study)	1x day (post RT or AM on non- training days)	1) WP+RT (*n*=7) 2) RT (*n*=7) 3) CON: no intervention (*n*=8)	GS (400m)	**GS** ↑WP + RT 11% ↑RT 9.7% CON: N/A
Yamada et al. [61]	12 weeks	*n*=122 (49 males, 73 females) with sarcopenia or dypenia	RT, body weight exercises, 20 minutes, 3 sets of 20 reps, 2 times a week	WP: 10g PRO, 100kcals	After breakfast	1) WP+RT (*n*=28) 2) RT (*n*=28) 3) WP (*n*=28) 4) CON (*n*=28)	CS (5-reps) 5MW	**CS** ^{NS}WP+RT -14.1% ^{NS}WP -7.2% ^{NS}RT -5.3% ^{NS}CON 5.3%

Abbreviations: 6MW: 6- meter walk, CS: chair stand, CHO: Carbohydrate, CON: control, area, GS: gait speed, RT: Resistance training, Reps: repetitions, PRO: Protein, PT: Post-training, TUG: time up and go, WP: whey protein. Between baseline and end of the trial: ↑= significance increase ↓= significant decrease, and NS = no significant difference from baseline.

Skeletal muscle power

Table 9 shows the outcomes of muscle power measured by *n*= 3 studies in the dairy-based supplement studies. Bell et al. [38] measured peak power (W) and observed a 106% increase in the WP+RT group. Daly et al. [40] measured static jump (cm) and CMJ (W/kg and cm) and found a significant increase in both the fortified dairy milk and control groups. Daly et al. [40] reported that there was only a significant difference between the groups in the CMJ, where the fortified dairy group showed significantly more significant improvement (6.7%) compared to the control group (3.7%). Lastly, Villanueva et al. [60] used the Margaria power test (W) and only showed significantly more improvement in the WP+RT group, where the increase in power (38%) was higher than the control and the exercise only groups.

Table 9 Main outcomes for skeletal muscle power based on supplement groups

Study	Duration	Participants	Exercise stress	Dairy and placebo details			Method of measurement	Main outcomes
				Nutrition intervention	Frequency x timing	Study groups		
Bell et al. [38]	20 weeks	*n*=49, Healthy community-dwelling males, 73± 1 yrs	RT, 3 sets 10-12 reps, at 65% 1RM increasing to 3x6-8 sets/reps, 80% 1RM HIIT 1x week 10x60s intervals	WP: 31g PRO, 1g CHO, 2.5g creatine, 500 IU vitamin D, 400mg calcium and 1500mg n-3 PUFA, 484.5kj CON: 22g CHO	2x 1 hour after breakfast, 1 hour before bed	1) WP + RT (*n*=25) 2) CON+ RT (*n*=24)	Peak power (W)	**PP** ↑WP+RT 106% ↑CON 112%
Daly et al. [40]	16 weeks	*n*=244 inactive females, <7500 steps per day 45-65 yrs	Home-based bodyweight workout, 7 exercises (2x10-15 reps)+ balance	1) FMD: 9.1g PRO, 16.5g CHO, 418kj 2) CON: Rice powder 25g CHO	2x a day (breakfast and lunch/or post training)	1) FMD +RT (*n*=108) 2) CON+RT (*n*=136)	Force plate	**Static squat jump** ↑FMD + RT 7.0% ↑CON+ RT 5.1% **CMJ (cm)** ↑FMD + RT 6.7% ↑CON+ RT 3.7% FDM+RT>CON+RT **CMJ (W/kg)** ↑FMD + RT 6.8% ↑CON+ RT 4.6%
Villanueva et al. [60]		*n*=22 recreationally active males 68 ±6yrs	RT, 2-6 sets, 5-6 exercises at 70% 1RM	WP (35g PRO + creatine monohydrate, 0.3g/kg for first 5 days then 0.7g/kg for completion of the study)	1x day (post RT or AM on non-training days	1) WP+RT (*n*=7) 2) RT (*n*=7) 3) CON: no intervention (*n*=8)	Margaria power test (W)	↑WP + RT 38.3% ^{NS}RT stat-x ^{NS}CON stat-x WP+RT>CON and RT

Abbreviations: CHO: Carbohydrate, CON: control, CMJ: countermovement jump, FMD: fortified milk drink, RT: Resistance training, Reps: repetitions, PRO: Protein, PP:peak, W: Watts, WP: whey protein ↑= significance increase ↓= significant decrease, and NS = no significant difference from baseline. $^{stat\text{-}X}$ no statistical analysis provided or statistical test unclear.

2.4 Discussion

The current SLR aimed to systematically identify and synthesise results of RCTs assessing the effects of dairy milk beverages and dairy-based protein interventions (± physical exercise) on SMM outcomes, skeletal muscle strength, performance, and power in older adults ≥40 yrs. The secondary aim was to use the current SLR results to guide future research for intervention trials that can be applied to active older adults. Following the SLR, *n*= 6 studies that used dairy milk and *n*= 26 studies that used dairy-based protein supplementation were identified that met the inclusion criteria for this review. Results from the current SLR found that within both the dairy and supplementation studies, that there was limited evidence for the use of dietary protein supplementation alone, as there were insufficient studies investigating the effects of dairy milk and/or dairy-based supplementation alone on outcomes of SMM, skeletal muscle strength, and power and physical performance in older adults. Secondly, there was limited evidence of the use of dairy milk and/or dairy-based supplementation with exercise showing any additional benefits beyond exercise on SMM outcomes, skeletal muscle strength, physical performance and power in older adults. This is likely due to the considerable heterogeneity within the studies. Despite trying for maximum uniformity in the characteristics of the included studies, the participants varied in sex, health status and were provided with different types of exercise (e.g., free-living physical activity or resistance training programs) and nutritional interventions (e.g., ad libitum or controlled, with or without supplementation and/or additional dairy). Moreover, the nutritional interventions also varied in protein and EAA type with or without other macronutrient inclusion, dose, frequency, and the trials' length.

Skeletal muscle mass outcomes

SMM is regulated by the balance of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) [12]. RT and dietary protein are two known anabolic stimuli that can acutely increase MPS

[17]. To increase SMM, MPS needs to exceed MPB, which over time, the summation of periods in positive protein balance can induce skeletal muscle hypertrophy [17]. The main findings of this current review found that *n*= 3 (50%) of the dairy and that *n*= 21 (70%) of the supplement showed a significant increase in SMM with exercise training and nutrition intervention (Tables 4 and 5). A group*time interaction effect of protein supplementation was only found in *n*= 5 of the supplement studies, indicating that the protein supplementation and RT had a more significant impact at increasing SMM than RT alone. These studies varied in participant type, timing, type and dose of protein supplementation with no specific similarities between them that could explain their positive result as distinct from the other studies that did not significantly affect muscle mass with protein supplementation. Of these studies, Daly et al. [40] provided the lowest dose of protein, 9g of a multi-nutrient fortified milk drink to older females at breakfast and lunch. They reported a significantly higher increase in SMM in the milk drink group compared to the control. Similarly, Rondellli et al. [53] provided participants with 22 g of whey protein at lunchtime. In the ageing population, a recent study in older individuals in the Netherlands found that protein intake in community-dwelling individuals was particularly low at breakfast: between 8-10 g protein and lunchtime between 15-23 g [62]. In acute laboratory studies that measure the effects of various protein doses on outcomes of myofibrillar muscle protein synthesis (myoMPS) suggest that 20-30 g of protein (or 0.4 g/kg/meal) has shown to maximize MPS in acute studies [63]. Therefore, although the protein intakes in the studies of Daly et al. [40] and Rondelli et al. [53] were lower than the amount required to maximally stimulate myoMPS, they were consumed with other foods, which may have also contributed to the protein intake at that meal. Reaching maximal MPS allows for skeletal muscle protein remodelling and SMM gains, which over time would potentially lead to changes in skeletal muscle quality and increased strength and muscular performance [64]. Therefore, to achieve 25-30 g of protein at each meal, the best benefits for mMPS and muscle accreditation are providing an extra bolus of protein in the form of a high protein milk beverage at

breakfast, and lunch post-training may be a beneficial strategy.

Despite iDXA being the gold standard for measuring SMM changes, one major limitation in using iDXA in the ageing population is that it cannot measure the muscle's fat infiltration, which is one of the main characteristics of sarcopenia [65]. Therefore, SMM may be overestimated, especially in the case of individuals that may have sarcopenia. A recent meta-analysis indicated that low muscle mass was not associated with functional decline, but low muscle strength was associated with a greater functional decline [66]. It is unknown why these inconsistencies occur. More precise skeletal muscle mass measures are the cross-sectional area (CSA) measured using a CT or MRI. Quadriceps CSA was measured in *n*= 2 supplement studies and *n*= 1 of the dairy studies. CSA may be more of a predictor of functional capacity and strength in the ageing population as it can provide details of muscle density, show fat infiltration, and can be used to detect differences in skeletal muscle fibres [67]. Although it may be highly accurate at measuring skeletal muscle mass changes, this technique is costly and relatively inaccessible in a clinical setting. Therefore, this highlights the necessity to combine outcomes used to define sarcopenia with changes in skeletal muscle mass, strength and performance.

Skeletal muscle strength outcomes

Handgrip strength

Changes in strength with increasing age have been observed to decline faster (~3%) per year compared to the reported loss of SMM [3,4]. Changes in muscle strength can act as an indicator for functional capacity with increasing age [68]. Handgrip strength (HGS) has been used as a diagnostic tool to predict disability, morbidity and mortality before hospitalisation in older populations and those presenting protein-energy malnutrition [68]. One longitudinal study found that HGS among community-dwelling men and women was a strong predictor of hospitalisations after a 10-year follow-up [69]. Another review found a strong correlation with low HGS relative to

hospitalisation and length of stay [68]. In this current review, *n*= 1 (16%) study in the dairy and *n*= 2 (20%) of the supplement studies found a significant increase in HGS from baseline during their experimental trials. Of those, only one study showed a significant effect of protein supplementation with resistance training. Mori et al. [48] provided older (65 to 84 yrs) females with 22 g of whey protein after RT and observed a significantly higher increase in HGS in the group that received the protein (10%) compared to the protein group alone (4%). Considering HGS wasn't a trained outcome, the supplementation and exercise groups' differences can be associated with the nutritional supplementation intervention. Although HGS may be useful in the clinical setting, as it is a valid and reliable measure to predict mortality in the ageing population; in shorter-term clinical trials, it may not be a sensitive and/or useful measure to determine changes in overall skeletal muscle strength in the active older population. Isometric HGS is a poor surrogate for changes in 1-RM bench press [70] and 1-RM leg press [71] in older adults (> 50 yrs). For example, a study by Tieland et al. [71] found no measurable changes in handgrip strength or improvements in leg muscle strength in pre-frail and frail older adults (> 78 yrs) following 24- weeks of a whole-body resistance training program. Therefore, HGS is likely to be more associated with the consequences of sarcopenia, which may be more useful for the initial screening of participants but not effective at measuring the effects of an intervention trial using RT.

Upper and lower body strength

As measured by 1RM testing, maximum muscle strength has been established as a safe and reliable method for measuring strength in older healthy men and women [72]. Increasing maximal strength through RT can result in a robust improvement in skeletal muscle force production and efficiency, resulting in improved overall functionality [72]. In this current review, significant increases from baseline in maximal strength outcomes were observed in 83% (5/6) and 75% (20/26) of the dairy and supplement studies, respectively. Again, only a small number of studies in the supplement

group, 19% (5/26), showed a greater improvement in strength outcomes than control (e.g., exercise or supplement only). Of these studies that showed a significant interaction effect with protein, the amount of protein supplement provided varied (10-35 g), the timing and the type of protein varied also. Previous reviews have found that protein can augment strength gains by 30% more than RT alone [15], whereas others have reported no significant improvement [17]. As well as total habitual protein intake and dose as previously discussed, the moderate effects of changes in maximal strength found in this review may be due to the relative lack of progressive overload in the exercise interventions.

Performance outcomes

This review's performance outcomes were too heterogeneous to draw any confident conclusion between protein consumption and performance change. Outcomes measured within the dairy (*n*= 4) and supplement (*n*= 11) studies reported a variety of performance measures which included: Short Physical Performance Battery (SPPB), usual gait speed, timed-up-and-go (TUG), chair stand and stair climb. These outcomes make up the clinical diagnosis measurements proposed by the European Working Group on Sarcopenia in older adults [73]. Still, they do not necessarily constitute the most appropriate and '*gold standard*' performance measures for active individuals, including the active elderly. Additionally, these measurements are used in the clinical setting as a predictive tool for possible disability and can help monitor function in older people. However, these clinical measurements were mainly used in healthy community populations without many significant outcomes being reported in this review. Measurements such as gait speed, although it is related to leg strength, the relationship is non-linear, explaining how small changes in physiological capacity (e.g., increase in skeletal muscle mass and subsequent strength) may have substantial effects on performance in frail adults. In contrast, significant changes physical performance in healthy adults, may have little to no change to their the physical capacity [74]. For example, Shahar

et al. [55] reported in a cohort of sarcopenic adults a significant increase (63%) in chair stand performance in those that received the protein supplement, which was higher than those in the control group (10% increase). Therefore, it is likely that active older adults would achieve a '*ceiling effect'* in performance outcomes similar to those reported in this study. The studies that included 'active' older adults [57] reported no significant difference in performance measures from baseline. Overall, these performance measures may be used as valid measures in the clinical setting to detect age-related outcomes related to sarcopenia and may be more useful to use as an initial screening of participants, but may not be sensitive enough to see meaningful change in an intervention trial in an older population that is physically active.

Skeletal muscle power outcomes

Skeletal muscle power is the product of the force and velocity of muscle contractions and has been shown to decline faster than muscle strength or SMM in ageing adults [5,6]. Skeletal muscle power has been suggested to be more of a critical indicator of the status of physical functioning in older adults than muscle strength [74] or SMM [75]. This current SLR found few studies using muscle power measures, and of those that did, there was considerable heterogeneity in the methods used. Only Daly [40] reported a significant interaction effect using muscle power outcomes (CMJ) in a fortified milk group compared to the control. Considering that muscle power declines faster in healthy older people than strength, measuring power output may be a better predictor of certain functional activities [76]. These measurements should be considered in future studies measuring skeletal muscle performance in healthy older adult populations, primarily if cohorts are described as ' *healthy*' or '*active*'.

Future studies

In this section, to address our second review question, we highlight some research gaps in the prevailing sarcopenia literature and provide direction for future research to use a dairy milk beverage in a cohort of active older adults. Based on the statistical test, mean, standard deviation, and effect size (i.e., small 0.20, medium 0.50, and large 0.80) and applying a standard alpha (0.05) and beta value (0.80), a sample size of *n*= 36 using a randomized controlled design as per Hanach et al. [14], is estimated to provide adequate statistical power (0.80-0.99) to detect variable differences (G*Power 3.1, Kiel, Germany). A four-armed randomised clinical trial that includes the following groups:

1. Dairy beverage,
2. Resistance training group,
3. Dairy beverage and resistance training group,
4. Control (no intervention).

Such a group design will allow the effects of the dairy milk beverage alone compared to the effects with or without resistance training to be tested on skeletal muscle status markers, function, and performance. Creating controls for baseline, adequate energy, and protein requirements is important to ensure that the results' differences are due to the protein intervention meeting their protein requirements. Additionally, often overlooked, the intervention found to be beneficial needs to be achievable and acceptable to older people in the long-term and incorporate into their dietary pattern that meets all their dietary requirements while enhancing their lives.

Dieatry protein

Protein amount

Protein alone in either dairy beverage or dairy-based supplement studies did not significantly benefit outcomes of SMM, skeletal muscle strength, performance, or power in older adults in the

majority of studies within this review. Previous studies have advocated for higher amounts of protein ≥20 g per meal, or ≥0.4 g/kg BM may be required to stimulate MPS in older adults [77, 78]. Reaching maximal MPS allows for skeletal muscle protein remodeling and SMM gains, which over time would potentially lead to changes in skeletal muscle quality and increased strength and muscular performance [79]. Additionally, this amount of protein's anabolic effect is likely due to the higher amount of essential amino acids (EAA) content in the protein sources (e.g. whey protein and milk). However, within this review, the studies used a range of protein doses (6-40 g), which were above the previously found to be the ideal amount to maximally stimulate MPS [77, 78]. However, there were no consistent differences between the studies' results compared with those using lower protein doses. This indicates that acute effects do not appear to translate to a chronic response. One potential confounding variable is the habitual protein intake at baseline. It is difficult to determine whether the improved changes in the studies included were due to reaching sufficient total daily protein (e.g., ≥1.2 g/kg BM per day) or the intervention itself. One potential way to control this is to provide a baseline control diet that provided sufficient protein. Therefore, future studies should consider providing controlled diets providing sufficient total daily protein to determine whether additional protein in the form of dairy milk has any further benefit.

Protein quality

Protein quality refers to the digestibility and bioavailability of the proteins amino acids [80]. This balance of protein quality seems especially important in the ageing population, where the digestibility and availability of the protein may be a limiting factor when considering anabolic resistance. Protein quality can be determined using a scoring system: digestibility -corrected amino acid score (PDCAAs) or digestible indispensable amino acid score (DIAAS) [81]. Based on these scoring systems, dairy milk and milk proteins (i.e., whey, casein, and milk protein concentrate) are considered the highest quality protein found on the PDCAAs and DIAAs scores. This is most likely

due to the high ratio of BCAAs present in these proteins, particularly LEU. LEU is the primary activator of involved MPS and works as the intracellular '*trigger*' that activates the mTORC1 and other intracellular protein expression pathways targeting MPS and attenuating MPB [82, 83]. Within the current SLR, only one study looked at LEU supplementation alone. Supplementing participants with 10 g/day (2x 5 g doses) of LEU found no significant change in body composition, strength or performance outcomes [53]. Another study supplemented whey protein (22 g protein) with 4 g of LEU and reported statistically significant increases in skeletal muscle mass, strength and performance outcomes [83], supporting the hypothesis that LEU promotes MPS as long as a positive nitrogen balance exists (i.e., sufficient accompanying protein dose). Furthermore, preliminary data from Katsanos et al. [84] found that when participants consumed small amounts of EAA (6.7 g) with higher portions of LEU (2.8 g), a significantly higher increase in MPS was observed, compared to participants that consumed the same amount of EAA with a lower proportion of LEU (1.7 g). This supports the notion that LEU alone should not be considered. Instead, if a lower total protein is available, higher doses of LEU is the critical factor for simulating the maximal response of MPS for increased muscle mass and strength.

Protein timing

Regarding the timing of either the protein sources, most of the studies in this review focused on the timing of the amino acid ingestion after exercise. However, timing may only be effective if adequate protein is given throughout the day. The use of either protein supplementation or dairy milk after exercise training provides EAAs leading to hyperaminoacidemia, which assists in facilitating adaptations of skeletal muscle following exercise training [82, 85]. Additionally, the typical Western diets in older adults have insufficient protein at breakfast and lunch to elicit a significant MPS response during this time [83]. For example, Daly et al. [40] and Dulac et al. [42] provided 9-10 g of protein at breakfast and lunchtime in older adults that sufficiently consumed

adequate protein at baseline ≥1.2 g/kg BM/day. They reported a significant increase in SMM, strength, power, and performance but no significant differences between them. It is unclear whether the participants reached the maximal amount of protein for those meals, as the protein per meal was not reported. However, providing additional dairy protein at times that are usually inadequate in protein with exercise can have an accumulative effect on MPS, eventually leading to a positive protein balance and increased SMM and strength [85, 86]. To achieve 25-30 g of protein at each meal, the best benefits for MPS and muscle accreditation are providing an extra bolus of protein in the form of a high protein milk beverage at breakfast, and lunch post-training may be a beneficial strategy. Suggesting older adults can quickly achieve the appropriate recommended protein amount for skeletal muscle maintenance and/or gain through consumption of dairy milk beverages with appropriate protein composition.

Resistance training program

The ideal resistance training (RT) program's composition to increase skeletal muscle strength, mass, and performance in the ageing population based on this review is unclear. Although older adults can benefit from the strength training, performance and skeletal muscle mass increases from 2-3 sessions/week of resistance training, this review could not identify any exact training regimen due to heterogeneity. Therefore, a large meta-analysis of various types of RT regimes reported that 12-24 weeks of training could increase muscle strength by at least 25% in men and women >50 yrs, with more significant improvements being associated with higher-intensity resistance training (i.e., ≥80% of 1RM) [87]. Although this higher intensity (>80%) has been observed in the current SLR by numerous studies [32, 33, 38, 44], lower intensities of ≤60-75% have also been shown to increase muscle strength. They may help reduce the risk of injury in the older population [31, 34, 37, 39, 41, 46, 47, 48, 49, 58, 60]. Progressive resistance training (PRT), which involved the first couple of weeks starting at a lower intensity (50-60%) and the intensity

increasing until the participants reached 80%, was the most adopted experimental exercise regime. PRT is a type of exercise strength training that intensity increases as the individuals get stronger and has been accepted as a form of RT that is beneficial for treating sarcopenia [88]. Considering that muscular weakness plays a critical role in the contribution of fragility and functional decline that occurs with ageing, it is important to consider the ideal training regimen and adjunct nutritional intervention that may improve strength for future studies.

Strengths and limitations of the systematic review

A significant strength in the current SLR was the strict selection criteria for the included studies, which only included randomised controlled trials that used a placebo or control comparator. However, a limitation is that not all of the included studies employed a double-blinded method or a placebo beverage. This explains why most of the studies within this review received a high score for risk of bias. A second strength is that the whole screening process and data extraction was performed independently and in duplicate. To add strength, any discrepancies were resolved by a third author. This rigorous procedure reduced any potential for researcher error during the data extraction and increased the SLR's strength. A major limitation in interpreting the extracted data were the considerable heterogeneity between studies. This large study variability resulted in a meta-analysis not being possible, and thus a narrative approach was used. Additionally, most studies did not publish the mean change and its accompanying standard deviation, nor the correlation confidence, which described the association between baseline and final measurements across participants. Although a thorough attempt was carried out to contact authors to request missing data or additional details, it was limited by a low response rate, meaning a meta-analysis could not be conducted. An extensive number of outcomes measurements were extracted from included studies to examine body composition, muscle strength and performance. However,

uniformity in outcome measurements is important for delivering high-quality evidence-based on meta-analyses, and it brings a more focused to practical advice for the health care setting. A potential cause of this considerable heterogeneity of results is the lack of general consensus on a definition of sarcopenia between different working groups. As there is no working definition to those with sarcopenia from those unaffected, it is difficult to apply these results across different population groups.

One major limitation within all the studies was that participants' habitual physical activity levels were not taken into consideration. Yet, these could significantly influence the magnitude of the effect of the anabolic response. Therefore, future studies should consider controlling for habitual physical activity and measure cardiorespiratory fitness to ensure participants are not increasing their physical activity during the intervention and any changes in SMM, strength, physical performance, and power the exercise intervention alone [89].

Future studies should also consider exploring relationships with known biochemical markers associated with sarcopenia, such as inflammation and anabolic hormones. Chronic low-grade inflammation is thought to be a causal mechanism for muscle loss in the ageing population. This was supported by a prospective cohort study of ~1000 older adults (mean age 74.6 yrs) and found an association between higher serum levels of interleukin-6 (IL-6) and C-reactive protein (CRP) being associated with increased risk of loss of muscle mass over 3 yrs [90]. The pathophysiology of inflammation leading to increased muscle wasting in the ageing population is poorly understood but possibly causes catabolic effects on the skeletal muscle [91]. Only one study looked at the effects of protein and exercise on inflammation markers [53]. However, this study found no changes in CRP in any groups. Considering CRP is an acute-phase protein and not directly a systemic inflammatory marker, future studies should consider other and more appropriate inflammatory

markers collectively (e.g., pro-inflammatory: IL-1β, TNF-α, and IFN-γ; anti-inflammatory: IL-4, IL-10, and IL-1ra, and TNF-sr; response cytokines: IL-6 and IL-8; chronic inflammatory, stimulating pathologies: LPS, gram-negative endotoxin and anti-endotoxin, sCD14, and LBP), and the effects that nutrition and/or exercise interventions may have on these markers [92].

2.5 Conclusion

The current SLR showed mixed results regarding protein supplementation and dairy beverages, with or without exercise interventions on improving skeletal muscle mass, skeletal muscle strength and performance outcomes in individuals ≥40 yrs. The ageing population may require higher amounts of protein throughout the day, especially when there is little distribution (i.e., breakfast and lunch). However, beyond protein quantity, quality, timing and frequency of the protein beverage should also be considered for future studies and subsequent product development.

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CHAPTER THREE:
GENERAL METHODS

Additional details of the general methods of all experimental studies that are not contained within Chapters Four to Six of this thesis are provided in this chapter. The general methods applied to the design of the study have been based on previous literature in the field of exercise physiology, clinical nutrition, and exercise immunology, whilst also considering the validity and any limitations associated with the method.

3.1 Ethical approval

Approval was obtained from the Monash University Human Research Ethics Committee (Project number: 12812) prior to the commencement of the clinical trial (Appendix B). The purpose and nature of what was conducted during the clinical trial was fully explained to participants, in written form, through the participant information sheet (Appendix C) and verbally, during the telephone screening. Participants were made aware that they were free to withdraw from the clinical trial at any time. All participants provided written informed consent and completed a medical questionnaire prior to the commencement of the clinical trial (Appendix D). This clinical trial was registered with the Australian and New Zealand Clinical Trials Registry (ANZCTR): 12618001088235

3.2 Study population

Active (≥30 minutes, 3 times a week or ≥90 minutes total a week) older (> 50 yrs) males and females were recruited for this study. It is recognised that active older adults are considered to engage in 150 min/week of light (e.g., 3-5 metabolic equivalents (METS)) to moderate-intensity (e.g., 6-9 METS) physical activity, or 75 min/week of vigorous-intensity (e.g., >9 METS) physical activity) [1]. A minimum cut-off point of ≥90 minutes total a week was used to consider those engaging in planned physical activity, who were more likely to engage in a higher leisure physical activity (i.e., walking and household chores), leading to a higher overall physical activity.

Participants were recruited within the local community in Clayton and the surrounding areas in Victoria, Australia. Using posters in surrounding suburbs and targeting local sporting clubs (e.g.

runners groups) via social media, were the employed mediums for recruitment. Potential participants were first screened via telephone and were deemed eligible to initiate and complete the clinical trial if they did not have the following: *1)* dairy protein allergy or known lactose intolerances; *2)* currently using dietary protein supplements; *3)* any injuries preventing safe exercise; *4)* had surgery in the past 12 months; *5)* had an acute coronary (e.g. myocardial infarction) or vascular event in the last year, or uncontrolled coronary heart disease; *6)* had a stroke in the past 2 yrs; *7)* have orthopaedic limitations that limit the participant in the exercise program; *8)* been diagnosed with, or taking medication for thyroid condition; *9)* had weight loss of more than 5% of body weight over the last 6 months; *10)* take medications that could interfere with skeletal muscle mass structure and/or function (e.g., corticosteroids, testosterone replacement or anabolic drugs); *11)* currently undergoing immunosuppressive therapy or hormone replacement therapy; *12)* have any chronic diseases, such as Diabetes Mellitus or gastrointestinal diseases/disorders; *13)* drinks more than 2 standard drinks of alcohol/day, or 14 standard drinks of alcohol/week; *14)* were a smoker; *15)* have a BMI greater than 30 kg/m^2 *16)* had participated in a structured resistance training program in the past 12 months. Once participants were deemed eligible, data were collected during the period September 2018 to January 2020. Participants were required to attend the Be Active Sleep EAT (BASE) facility at Notting Hill for baseline measurements. The participant recruitment and retention for each trial are described in Chapters Four to Six. The following measurements were collected at the baseline, 6 weeks and 12 weeks. This was done at the commencement of the trial; all in the same order for each participant.

3.3 Preliminary measures

Participants' anthropometry, body composition, resting metabolic rate, cardiorespiratory fitness and strength measures were measured in temperature ambient conditions (range 21-23°C) at baseline, 6-weeks, and 12-weeks as described in Chapters Four to Six .

Self-reported diet and physical activity

Participants were required to record all the foods and fluids, using a 3-day food diary (Appendix E) on each main meal (breakfast, lunch and dinner) and any additional snacks (e.g., morning tea, afternoon tea, and supper) ingested on 2 weekdays (Monday-Friday) and one day on the weekend (Saturday/Sunday), that most reflected their usual intake. Participants were required to specify the food and beverage quantities (e.g., g, ml, litres, portion size and number) and qualities (e.g., cooking method, brands of foods-beverages, and types of foods-beverages). The food diary was separated by main meals (e.g., breakfast, lunch and dinner) and snacks (e.g., morning tea, afternoon tea and supper). Nutrient intakes were assessed using a 3-day food diary (2 weekdays and 1 weekend day) and analysed using the Foodworks nutrient analysis software program (Xyris Software, Brisbane, Queensland, Australia), by a qualified sports dietitian. Further methods of dietary analysis are presented in Chapters Four to Six.

Prior to commencing any physical activity, participants filled out a physical activity readiness questionnaire (PAR-Q), in which they self-reported their level of activity as well as their exercise volume (e.g., <once per month, once per month, 2-3 times per week, 4-5 times per week, or > 5 times per week), intensity (e.g., vigorous, moderate and light) and modality of training (Appendix F).

Sample collection and analysis

For consistency, the experimental trial was conducted in the same order as outlined below and following the data collection sheet (Appendix G). Participants were asked to arrive at the lab between 7am-9am, in a fasted state (osmolality: 296 ± 5.6 mOsmol/kg; Osmomat 030, Gonotec, Berlin, Germany; and total body water: 53.3 ± 6.4%; Seca 515 MBCA, Seca Group, Hamburg,

Germany) and after refraining from any vigorous physical exercise 24 hours prior to their initial testing. Height and weight were measured as described in Chapters Four to Six.

Biomarkers and cytokines

Venous blood samples were collected to determine haematocrit in triplicate, and haemoglobin, blood glucose and leukocyte count (i.e., neutrophils, lymphocytes, and monocytes), in duplicate with a 1.2%, 1.8% and 4.6% coefficient of variation (CV); respectively for Chapters Four and 1.1%, 1.5% and 3.0% for Chapter Six. Blood glucose concentration, haemoglobin, total and differential leukocyte counts (i.e., neutrophils, lymphocytes, and monocytes), were determined by HemoCue system (Glucose 201+, Hb201, and WBC DIFF, respectively; HemoCue AB, Ängelholm, Sweden). Haematocrit was determined by aspirating ~500 µg of lithium heparin blood into three capillary tubes, which were sealed using a capillary tube sealant at one end. The capillary tubes were then centrifuged for 2 minutes at 10,000 rpm and then values were determined using a micro-haematocrit reader. Haemoglobin and haematocrit values were used to estimate changes in plasma volume relative to baseline, and used to correct plasma variables.

The remaining heparin whole blood samples were centrifuged at 4,000 rpm for 10 min, within 15 min of sample collection. Aliquots of heparin plasma were places in 1.5 ml microstorage tubes and frozen at -80°C until analysis, except 2 x 50 µl plasma was used to determine plasma osmolality in duplicate (CV 1.1%). Plasma concentrations of IL-2, IL-6, IL-8, IL-10, and TNF-α, were determined by multiplex ELISA (HCYTOMAG-60K, EMD Millipore, Darmstadt, Germany) in duplicate with a CV of 7.1%, 19.2%, 15.0%, 14.4%, 19.1% for Chapter Four and Chapter Six.

Circulating concentrations of cortisol (DiaMetra, Perugia, Italy), insulin (Crux Biolab, Scoresby, Australia), insulin- like growth factor-1 (IGF-1) (Crux Biolab, Scoresby, Australia), testosterone (17b-

OH-4-androstene-3-one; DiaMetra, Perugia, Italy), and estradiol (17β-Estradiol; DiaMetra, Perugia, Italy) were measured by enzyme-linked immunosorbent assay (ELISA) in duplicates with a CV of 6.0%, 8.0%, 13.4% and 6.7% for Chapter Four and Chapter Six. Considering the large individual variation in cytokine responses, the peak pre (baseline) to post (6-weeks and 12-weeks) for pro-inflammatory (TNF-α), response (IL-2, IL-6 and IL-8), and anti-inflammatory cytokines (IL-10) were combined to establish an exercise-associated systemic inflammatory response profile [Chapter Six].

Resting metabolic rate (RMR)

Prior to participants measuring their metabolic rate, participants were fitted with a heart rate monitor (Polar Electro, Kempele, Finland) before being asked to rest quietly lying in a comfortable position for 10 minutes in temperate ambient conditions (22.2 ± 1.4°C). Participants were provided with pillows and blankets for added comfort. Using an indirect calorimeter (Vmax Encore Metabolic Cart; Carefusion, San Diego, CA) with a ventilated hood system, oxygen consumption ($V O_2$) and carbon dioxide ($V CO_2$) production, respiratory exchange ratio (RER), and resting energy expenditure/min (REE) were measured for 30 min at 1 min intervals, with the participants in a supine position and completely rested. The first 5 minutes of data were deleted as an acclimation artefact, based on best practice protocols [2]. From the remaining 25 minutes, a segment of five min consecutive 1 min measures in VCO_2, CO_2 and REE were considered as a steady state and were used to calculate RMR.

Body composition

Total (kg) and relative (%), regional FM and FFM, and bone mineral content (BMC) were assessed by a trained radiographer, using a dual-energy X-ray absorptiometry (iDXA; Prodigy, GE Lunar, Madison, WI; with analysis software 14.10). Appendicular lean mass (ALM) was determined by adding the total arm and leg mass, and then it was adjusted for height (ALM/ht^2). After the

completion of these testing procedures, participants were then provided a standardised (1.4 MJ, 15.3 g protein, 51.7 g carbohydrates, 6.8 g fat) breakfast meal, with the choice of their normal beverages. Participants were then required to rest for 30±15 minutes before commencing their performance testing.

Assessment of physical performance
Incremental bike test

Submaximal aerobic fitness was determined using an incremental bike test, with a cycle ergometer (*Corival,* Lode, Groningen, Netherlands) and a metabolic cart (Vmax Encore Metabolic Cart; Carefusion, San Diego, CA). The initial workload began at 1 watt (W) per kilogram of FFM (W/kg FFM) and increased by 0.5 W/kg FFM every 3 min until participants obtained a RER of 1.000, could not maintain the speed at ≥ 60 RPM or they reached a rating of perceived exertion (RPE) of 15 - 17 [3, 4]. Heart rate (HR) (Polar Electro, Kempele, Finland), RPE, $\dot{V}O_2$, and respiratory exchange ratio (RER) were measured every 3 min in real-time. Cardiorespiratory fitness was expressed as the $\dot{V}O_2$ ml/kg/min at which the RER reached 1.000.

Counter movement jump (CMJ)

Using a force plate (400 series, Force Plate; Fitness Technology, Adelaide, Australia) at a sample rate of 1,000 Hz to measure relative muscle power (Watts/kg), jump height (in centimetres) and velocity (m/sec) during a countermovement jump test (CMJ). Participants were instructed to dip to a self-selected depth and "jump for maximal height". Hands were kept on the hips to minimise any influence of arm swing [5]. Participants were asked to perform 2-3 practice jumps off the force plate to ensure correct technique. Before each set of CMJs, participants started in a full erect standing position in the middle of the force plate. Then participants were asked to perform three attempts of a CMJ with 1-minute rest in-between jumps. The ground reaction platform was

calibrated before each participant assessment. The Force plate was interfaced with computer software (Ballistic Measurement System; Fitness Technology, Adelaide, Australia), where the mean of three jumps was selected for further analysis. The highest mean power (Watts/kg) during the concentric phase and the corresponding jump height (cm) derived from flight time, were calculated based on a previously validated and reliable approach [6, 7].

Gait speed test

A walking course of 4 metres in length was marked on the floor. Participants were instructed to walk from one end of the course to the other at their usual walking pace. The timer began as the participant started walking and the timer was stopped with the first footfall after the 4-metre line. The test was repeated twice and the average of the two was scored. Gait speeds of longer than 5 seconds to walk 4 metres (<0.8m/s) suggested an increased risk of frailty [8].

Assessment of physical strength

Grip strength was measured using a hand Dynamometer (Jamar® Plus+ Digital hand dynamometer; Sammons Preston, Bolingbrook, IL, USA) with participants standing, their elbow by their side and flexed to the right angles, and a neutral wrist position. Participants were asked to keep their arm flexed at 90 degrees and squeeze as hard as they could for a count of 3-seconds. Grip strength was measured three times alternating each hand. The highest value for their dominant and non-dominant hand, were recorded. Participants were not able to view their score until all attempts were complete.

Maximal strength was evaluated using a 1-repetition maximum, test. We used a leg press, *latissimus doris (lat)* pull down and chest press machines (Hammer strength; LifeFitness, Sydney, Australia. The assessments were performed in this sequence. They were standardised and

continuously monitored to guarantee consistency in the maximum strength assessment, during the familiarisation sessions. Each exercise was preceded by a warm-up set (5 – 10 repetitions), with approximately 50% of the estimated load to use on the first attempt for each test. This warm-up was used to familiarise the participants on each weight machine. The testing procedure commenced 3-5 minutes after the warm-up set. After the initial warm-up set, the weight was increased by 5-20% and completion of another 5-6 repetitions at a heavier weight. Participants were then given a 3-5 minute rest. Weight was again increased by 5-20% and the participant was asked to perform 3-5 repetitions, followed by a 3-5 minute rest. The weight was increased again by 5-20% and the participant was asked to perform 1 repetition, followed by a 3-5 minute rest. This was continued until the highest load could be raised in one single repetition, using correct technique. If a participant failed a repetition, the weight was reduced (2.5% - 5% for upper body and 5-10% for lower body exercises) and one other attempt was given. Five minutes recovery was given in-between machines. Participants who reached the maximum weight on the leg press (200 kg), were asked to perform maximum repetitions until failure. This number total was added to 200 kg to provide a 1RM value.

3.4 Experimental design

This 2x2 factorial design study was a 12-week randomised control trial (Chapter Six). The two factors were progressive resistance training (EX) and a high protein milk beverage (DM); each were tested on two levels so that the participants were randomly allocated to one of the four groups: 1) DM, 2) DM+EX 3) EX or 4) control (CON). Randomisation was carried out by a researcher who was blinded to allocation using a block randomization table scheme, with stratification by age and sex. The block sizes were 12x12x12x12 for a total *n*= 48. Additional blocks were added to for dropouts.

Exercise group

Participants allocated into the exercise group were required to attend supervised exercise sessions on three non- consecutive days/weeks, for 12 weeks at the Monash University, Notting Hill BASE facility. The exercise sessions were conducted either in a morning session (between 7.00am to 10.00am) or an afternoon session (between 1.00pm to 3.00pm), to account for participants' work-life schedule. Each resistance training session lasted for 30-45 minutes and consisted of 5 - 10 min self-selected warm-up on the treadmill (Forma Run 500, Technogym, Seattle, Washington, US) or stationary bike (*Corival,* Lode, Groningen, Netherlands). The participants then performed 2 warm-up sets at 25-50% of their working weight for the first set and 60-70% for their second set. The participants were to perform each exercise with an eccentric and concentric load to be performed with two counts each way. They had a rest of 1-2 minutes in between sets and 2-3 minutes in-between exercises. The main resistance exercises (leg press, *lat* pulldown and chest press) were performed to, or near task failure in the range of repetitions provided. If participants could perform the end range of repetitions for the given sets, then the weight was increased by 5% and the participant aimed for the lower end of the range and work up again. Additional exercises including bicep curls, triceps extensions, shoulder raises, calf raises, cable deadlifts, leg curls, back rows, and abdominal exercises were performed on a cable machine (Infinity series Functional trainer, Keiser, Fresno, CA), and rotated throughout the program to ensure the development of muscle balance (Appendix H). The number of repetitions, sets the load were determined from their 1-RM values as outlined in Table 1. This progressive resistance training program (PRT) was designed based on previously published protocols for resistance training exercises to prevent and manage sarcopenia in older adults [9].

During the course of the trial, all exercise sessions were supervised, to ensure correct lifting, to monitor the appropriate amount of exercise and rest intervals and to test for compliance. An

exercise sheet (Appendix H) with the determined weights, reps and sets was filled out by the researcher and maintained throughout the 12 weeks. Participants' 1-RM were assessed at baseline and reassessed at week 6 to adjust the weight lifted accordingly and to account for strength gains throughout the protocol.

Table 1. Progressive resistance training for the active ageing study for A. Main exercises and B. Accessory exercises throughout the 12- week intervention (Chapter Six)

A. Main exercises (e.g., leg press, *lat* pull down, chest press)			
Weeks	**Sets**	**Repetitions**	**Percentage of 1RM**
1-2	3	10-15	50-60%
3-6	3	8-10	69-75%
7-12	3	6-8	75-80%
B. Accessory exercises (e.g., bicep curls, triceps extensions, shoulder raises, calve raises, cable deadlifts, leg curls, back rows, and abdominal exercises)			
Weeks	**Sets**	**Repetitions**	**RPE**
1-2	3	10-15	50-60%
3-6	3	8-10	69-75%
7-12	3	6-8	75-80%

Abbreviations: RPE: rates of perceived exertion.

High protein milk drink group

2 x 250 ml (measured in a 250 ml measuring cup) serves of reduced fat fresh bovine milk specifically formulated by Lion Dairy & Drinks (Melbourne, Australia) were provided to participants. This is equivalent to two glasses of milk per day, which is in line with the current Australian dietary recommendations of 2-3 servings of dairy per day [10]. Participants were encouraged to consume one cup (250 ml) of milk in the morning (with breakfast) and another at lunchtime or after training. Each 250 ml cup of milk contained: Energy 535 kj, 15 g protein, 8.3 g carbohydrates, 8.3 g lactose,

435 mg calcium). Thus, participants were consuming an extra 30 g of protein per day, containing a total of 3.12 g of leucine. The amino acid profile of the intervention product is shown in Appendix I.

Participants assigned to the dairy group were asked to consume 500 ml/ day (Lion Dairy and Drinks) Fresh milk was delivered each week and participants were provided with enough product to last through the week. A measuring cup was provided. Individuals in the dairy intervention group were also provided with food provisions that provided them with 100% of EER and 100% of estimated protein requirements (based on 1.2-1.5 g/kg/ day). Energy requirements were calculated using their resting metabolic rate (RER), which was determined by measuring oxygen consumption at rest using a metabolic cart, then an activity factor (Harris-Benedict) was applied based on their reported exercise. Participants were asked to fill out food diaries to record their food and dairy milk intake (Appendix J). Participants were also asked to return their empty bottles of milk as an indication of compliance.

Activity tracking

All participants were asked to resume their usual lifestyle activity levels, and were required to wear an activity monitor (ActiGraph wGT3X-BT, Actigraph, Pensacola, FL) on their non-dominant wrist. Participants were instructed to wear the activity monitor during the course of the intervention trial from waking to bedtime, and to take the monitor off only when engaged in aquatic activities. A new activity monitor was provided every 2 ± 1 weeks, due to the limited (25 days) battery life. Data were uploaded to analytical software (ActiLife 6 v 6.1.3; Actigraph, Pensacola, Florida, United States). Energy expenditure was calculated using the Freedson algorithm [11]. Cut points (e.g., time in sedentary, light, moderate and vigorous) were determined using the Freedson algorithm (Adult VM3) [11]. The cut point values in ActiLife are based on 60-sec epoch lengths that reflect counts

per minute (CMP). So, the cut points for the different levels of intensity include: Sedentary: 0 - 99 CPM, Light: 100 - 1951 CPM, moderate: 1952 - 5724 CPM, Vigorous: 5725 - 9498 CPM and Very Vigorous: 9499 - ∞ CPM [11]. All calculated data from the Actigraph were downloaded into an Excel spread sheet for each participant. Wear score was assessed and a valid day was defined as ≥80%, the days that were not considered valid were removed and accounted for by providing each participant with a total compliance of wear during the trial. The remaining data were assessed in blocks from 0-6 weeks, 6-12 weeks and total 0-12 weeks. The average time (min/day) for each cut point, as well as total step count was recorded.

Sample size calculation and statistical analysis

To determine the sample size required for the RCT, a power calculation was conducted for fat-free mass and strength, based on the standard deviation of 1.5 kg [12] and 0.37 kg; and using a standard alpha (0.05) and beta value (0.8). A total sample size of *n*= 36 (*n*= 8, per group) was estimated to have adequate statistical precision to detect an increase in FFM and strength values to provide statistical power (G*Power 3.1, Kiel, Germany) (Chapter Six). During the 12-week intervention trial (Chapter Six) some blood samples at certain time points were not able to be collected due to difficulty obtaining the sample. Therefore, only the participants with full data sets within each outcome variable were used in the data analysis and are reflected in each table with corresponding participant numbers in the results section (Chapter Six). More details for each specific statistical method are detailed in Chapters Four to Six.

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CHAPTER FOUR:

Sarcopenic characteristics of active older adults: A cross-sectional exploration.

The following chapter appears on the next page as published in *Sports Medicine Open*

Huschtscha, Z., Parr, A., Porter, J., Costa, R.J. Sarcopenic Characteristics of Active Older Adults: a Cross-Sectional Exploration. *Sports Med – Open.* 2021; 7 (32): 1-5

4.1 Abstract

Background: Ageing is associated with a decline in skeletal muscle mass and function (strength and power), known as sarcopenia. Inadequate dietary protein and inactivity have been shown to accelerate sarcopenia outcomes, occurring at different rates in males and females. Regardless, active older adults who often exceed the exercise guidelines still show signs of sarcopenia. This study aimed to explore associations between age, physical activity, protein intake, and biological sex with skeletal muscle mass, strength, power, and physical capacity/performance in active older adults. Fifty-four active older adults were recruited from this trial, and grouped according to age (middle aged: 50-59 yrs, and older age: ≥60 yrs), exercise volume (low: ≥90-149 min/week, moderate: ≥150-299min/week, and high: ≥300min/week), protein intake (low: <0.8g/kg body mass (BM), moderate: ≥0.8-1.19g/kgBM, and high: ≥1.2g/kgBM), and biological sex (males and females). Skeletal muscle and fat mass (dual x-ray absorptiometry), strength (1-repetition maximum using leg press, chest press, lateral pull down, and hand grip), power (counter movement jump), and general fitness (cardiorespiratory capacity and gait speed) were assessed. Data were grouped based on variables, and a general linear model (ANCOVA) or an independent t-tests was used to determine between group differences. *Results:* 53 of the total participants data were analysed. The middle-aged group had a 18%, 11%, and 10% higher leg press, chest press, and lateral pull down, respectively, compared to the older aged group (p< .05). There were no significant differences between different levels of training volume and any of the outcomes. Higher protein intakes were associated with significantly less body fat mass (p= .005) and a trend towards a higher leg press (p= .053) and higher relative power (W/kg) (p= .056) compared to the moderate and low protein intake groups. Significant differences based on biological sex were observed for all outcomes expect for gait speed (p= .611) and cardiorespiratory fitness (p= .147).*Conclusions:* Contributions of age, physical activity, daily protein intake, and biological sex can explain the individual variation (individual variation refers to the differences among individuals that are either permanent or

change slowly e.g., body fat) in outcomes related to changes in body composition, strength, power, and/or cardiorespiratory fitness in a cohort of active older adults.

Keywords: Strength, power, cardiorespiratory, protein, exercise, body composition.

Key points:

- There appears to be a significant difference in whole body strength outcomes in middle-aged older adults (50-59 yrs) compared to older adults (≥ 60 yrs) that are considered active.
- There were significant differences in outcomes of body composition, strength and performance observed based on biological sex.
- Active older adults have a low risk of adverse outcomes caused by age related sarcopenia.

4.2 Background

The global population is ageing and older adults (i.e., middle-aged: ≥50-59 ys; older: ≥60 yrs) are remaining active much later in life than in previous generations. This is evident in the growing participation rates of both recreational and competitive older adults in endurance events, such as marathons [1] and ultra-endurance marathons [2, 3]. Many studies report that regular physical activity is imperative to mitigate age-related declines in skeletal muscle mass (SMM), strength, power, and general physical performance - known as sarcopenia [4, 5]. For example, a systematic review and meta-analysis of 14 studies concluded that older adults engaging in regular physical activity reduced the odds of acquiring sarcopenia later in life (odds ratio [OR] =0.45; 95% confidence interval [CI] 0.37 – 0.55) [6]. However, active older adults still show signs of declining physical function with increasing age despite often exceeding the physical activity guidelines and exercising significantly more than sedentary older adults (>150 min/week of moderate intensity or 75 min/week of vigorous intensity- or combination) [7, 8]. For example, highly competitive Masters level athletes (≥35 yrs) show declines of declines cardiorespiratory fitness (e.g., $\dot{V}O_2$) and peak power compared to younger trained athletes (i.e., 18 - 27 yrs) in a variety of sporting disciplines [4, 9]. This decline in physical performance is most notable from the age of 50 yrs (middle-aged) until the age of 60 - 70 yrs (older) after which it declines exponentially thereafter [10]. Despite this, the results in recreationally active and Masters athletes regarding SMM and function have been conflicting. Such differences are likely due to small sample sizes, few female athletes, limited outcome assessments (e.g., biochemistry markers), and not taking into consideration lifestyle factors (e.g., not assessing dietary intakes). The rates of decline in SMM and strength are often highly individual and been attributed to intrinsic (e.g., hormonal changes) and extrinsic (e.g., lifestyle choices and social influences) factors, in which physical activity habits, biological sex, and dietary habits play a key impacting role [11]. Despite this, active older adults, while currently underrepresented in sarcopenia research, may provide the ideal cohort to study as they do not

display the extrinsic factor of sedentary behaviour often seen in sarcopenia research [12]. Therefore, understanding how age, habitual exercise, protein intakes, and biological sex may influence outcomes of body composition, strength, power, and physical performance (e.g., cardiorespiratory fitness and gait speed) in an active cohort may provide guidance for future intervention studies.

Although the causal mechanisms of sarcopenia remain elusive, it is likely multifactorial, and include decreases in anabolic hormones (e.g., testosterone), increased low grade chronic inflammation, loss of neuro-muscular function, mitochondrial dysfunction, infiltration of lipids within the skeletal muscle, and changes in muscle protein balance [12, 13]. Therefore, it is likely that the causes outlined, create a disruption of skeletal muscle protein turnover, resulting in an imbalance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB), leading to loss of skeletal muscle mass and ultimately muscular function [14]. For example, a 12-year longitudinal study observed a 12.5% decline in the cross-sectional area (CSA) of the thigh muscle and a 21-35% loss of leg strength of healthy older adults (initial age (mean ± SD): 65.2 ± 4.2 yrs) [15]. However, studies in recreationally active and Masters athletes in regards to SMM have been conflicting. One cross-sectional study found that strength- or power-trained Masters athletes (i.e, 68 ± 0.8 yrs) had a greater muscle retention with age whereas endurance trained older adults (i.e, 70 ± 0.7 yrs) had similar SMM as age-matched controls, and reduced SMM compared to younger sedentary (28 ± 0.1 yrs) or endurance trained individuals [16]. Another cross-sectional study found that older (65 ± 8.5 yrs) adults who reported that they engaged in vigorous physical (50^{th} percentile) activity had a 72% lower risk for having at least one criteria for sarcopenia [17]. In contrast, a study which observed forty high-level recreational Masters (i.e., 40 - 81 yrs) athletes who trained 4-5 times a week found, via magnetic resonance imaging (MRI), that mid-thigh muscle area and quadriceps area did not decline with progressing age [18]. The inconsistencies between these results may be

due to these studies not measuring any other lifestyle factors that may contribute to declines in SMM. For example, A cross-sectional study by ten Haaf et al. [19] found in a cohort of physically active (mean ± SD: 85 ± 53 metabolic equivalents (MET)/week) older (70 ± 4 yrs) adults that over 50% of participants did not meet the protein requirements of 1.2 g/kg BM/day as suggested for active older adults [19]. Furthermore, Nilsson et al. [20] found that older community-dwelling women (67 ± 1.8 yrs) who habitually consumed ≥1.1 g/kg BM/day seemed to have an additional benefit on physical function outcomes when compared to those meeting the recommended daily allowance (e.g., 0.8 g/kg BM/day). Therefore, considering habitual daily protein intake for active older adults is higher (≥1.2 g/kg BM/day) than what is currently suggested for older adults, active older adults may be at an increased risk of muscle decline and strength due to the increased requirements for amino acid (AA) utilisation coupled with the progressive degeneration of SMM associated with ageing [21-23]. Therefore, determining whether there is an association between adequate protein intakes suggested for older adults (e.g., 1.2 g/kg BM/day) has on outcomes of sarcopenia should be explored.

The importance of maintaining strength throughout advancing age is highlighted by the recent shift in the European Working Group of Sarcopenia (EWGOSP2) clinical diagnosis that placed low hand grip strength at the forefront of their criteria [5]. Strength is defined as the ability to generate force, usually through the application of concentric muscle action [24]. Lower limb strength is associated with walking performance, the ability to get in and out of chair, step-climbing speed, and rate of falls [25]. Additionally, the correlation between hand grip strength (HGS) and isometric knee extensor strength allows grip strength to be used as a measure of whole body strength in sedentary older adults [26]. However, a study that looked at the ratio of HGS to quadriceps strength and found it to be significantly lower in the older group compared to the younger group indicating a greater age-related decline in quadriceps strength than HGS [27]. It is unknown whether these

findings would be seen in a cohort of active older adults. Given these research inconsistencies and confounding factors that have not been adequately controlled for in previous research, it would be of interest to include a measure of upper and lower body strength outcomes for active older adults.

Along with declines in skeletal muscle size and strength, declines in skeletal muscle power and cardiorespiratory fitness have also been shown to occur with ageing [28, 29]. In a cross-sectional study of one-hundred community-dwelling older adults (65-89 yrs), skeletal muscle power was reported to decline in men at a rate of 3.5% per year [30]. Additionally, a study of Masters power athletes (i.e., sprinting, throwing, and jumping), aged 35-95 yrs, found skeletal muscle power dropped 1.2% per year, beginning from the age of 70 yrs [31]. The events that involved mostly upper limbs (e.g., shot put and javelin throw), showed the higher rate of decline (1.4% per year), compared to lower limb events (e.g., long jump decreased 1.1%). Additionally, there has been an observed decrease in $\dot{V}O_{2max}$ of 2.2 ml/kg/min (5.5%) per decade in Masters endurance-trained athletes [32]. However, this decline was significantly less than age-matched sedentary controls who had an observed decline of 3.3 ml/kg/min (12% per decade). It appears that regular physical activity during older age may play a role in being protective against outcomes related to sarcopenia in comparison to inactive older adults. Measures of muscle power and cardiorespiratory fitness may provide more reliable indicators of declines in muscle function in older populations that are considered '*active*' [12]. Another major limitation of many previous studies is the relatively few outcomes measured detecting intrinsic (e.g., inflammatory and anabolic resistance) [33, 34] and/or extrinsic (i.e., lifestyle factors) [35] factors, which makes it unclear as to whether these potential factors may contribute more to the skeletal muscle functional decline than ageing alone in active older adults.

To our knowledge the potential single and combined associations between the influences of dietary intake (e.g., protein intake) and exercise status in relation to the outcomes of SMM, strength and power have not been investigated in a homogenous sample of '*active older adults*' (≥50 yrs). While ≥50 yrs is not considered 'older' in the general spectrum of sarcopenia research, it is the age at which declines in SMM and strength begins to be noticeable. Therefore, the present study aimed to explore the association between age, physical activity level, dietary protein intake, and biological sex with SMM, strength, power, and physical capacity/ performance in active older adults (≥50 yrs).

4.3 Methods

Study population

Fifty-four active middle-aged (mean ± SD: age 52.0 ± 2.8 yrs, BM 74.7 ± 16.1 kg, height 1.71 ± 0.10 m) and older adults (mean ± SD: age 64.6 ± 4.3 yrs, BM 74.5 ± 14.1 kg, height 1.71 ± 0.10 m) volunteered to participate in the study. Participants were eligible if they were ≥50 yrs, performed exercise training for recreational fitness and/or sports competitions ≥3/week, for ≥90 min/week, had no functional limitations, were free from chronic disease/disorders, were not taking medications that could interfere with SMM structure and/or function (e.g., corticosteroids, testosterone replacement or anabolic drugs), were not undergoing immunosuppressive therapy or hormone replacement therapy, and were not adhering to structured resistance training program/s. All participants gave written informed consent. The study protocol obtained approval from the local ethics committee (Project number 12812), and the study was performed in accordance with the standards of ethics outlined in the Declaration of Helsinki. Data were collected during the period of September 2018 to January 2020, See Figure 1 for participant flow.

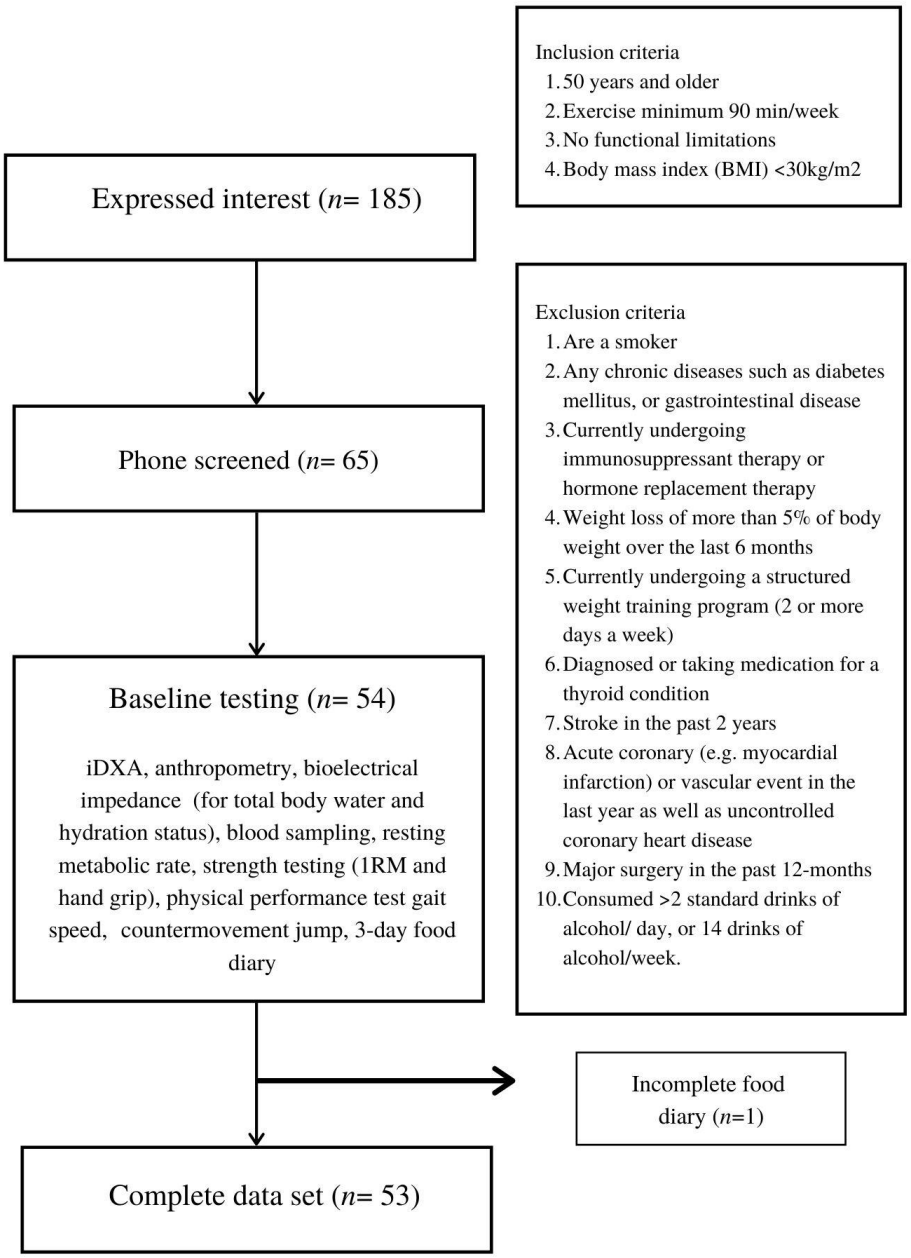

Figure 1. Flow diagram for the identifications, screening, eligibility and participant completion.

Exercise volume

Prior to commencing any physical activity participants filled out a physical activity readiness questionnaire (PAR-Q). They self-reported their level of physical activity including exercise intensity and volume per week, and the modality of exercise. Participants self-reported their level of activity including exercise frequency (e.g., <once per month, once per month, 2-3 times per week, 4-5 times per week, or > 5 times per week), intensity (e.g., vigorous, moderate and light), the time of exercise spent in each intensity and modality of training. All participants were categorised into low (≤149 min/week), moderate (≥150-299 min/week) or high (≥300 min/week) exercise volume [7].

Dietary assessment

Participants were educated and asked to complete a three day food-fluid diary prior to their initial visit. They were required to record all the foods and fluids on each main meal (breakfast, lunch and dinner) and any additional snacks (e.g., morning tea, afternoon tea, supper) ingested on 2 weekdays (Monday-Friday) and one day on the weekend (Saturday/Sunday) that most reflected their usual intake. Participants were required to specify the food and beverage quantities (e.g., g, ml, litres, portions) and qualities (e.g., cooking method, brands of foods-beverages, types of foods-beverages). Data were analysed for outliers using boxplot. Identified outliers were removed prior to comparative data analysis procedures. Food-fluid diaries were analysed using FoodWorks v10.0 nutritional analysis software (Xyris Software, Brisbane, Australia, 2019) based on Australian food composition tables from Australian Food Composition Database (AFCD) 2019. Based on preliminary data participants were then categorised into low (<0.8 g/kg BM/day), moderate (0.8-1.19 g/kg BM/day), and high (≥1.2 g/kg BM/day) protein intake to assess the effects that daily dietary protein intake alone has on outcomes of SMM, strength, power, and physical performance markers [21].

Experimental procedure and measurement of outcomes

For measurements of outcome variables, participants were required to attend the laboratory for the period between 07.00am to 09.00am in a fasted and euhydrated state (296 ± 5.6 mOsmol/kg; 53.3±6.4% TBW; Seca 515 MBCA, Seca Group, Hamburg, Germany), and after avoiding strenuous exercise for a 24 h period. All measurements were performed in the same order for all participants. Height was assessed using a fixed stadiometer (Holtain, Crosswell, Crymych, UK). BM was measured (Seca 515 MBCA) to the nearest 0.1 kg, using standardised anthropometrical procedures. Total (kg) and relative (%) FM and FFM, and bone mineral content were assessed by a trained radiographer using a dual-energy X-ray absorptiometry (Prodigy, GE Lunar, Madison, WI; with analysis software 14.10). Appendicular lean mass (ALM) was determined by adding the total arm and trunk lean mass and then it was adjusted for height (ALM/height^2). Resting metabolic rate (RMR) was determined by measurement of resting oxygen consumption using an indirect calorimeter (Vmax Encore Metabolic Cart; Carefusion, San Diego, CA) in temperate ambient conditions (22.2 ± 1.4°C), and in accordance with best practice guidelines [36]. Prior to commencing the strength, power, and performance measures, participants were provided with a standardised breakfast (1.4 MJ, 15.3 g protein, 51.7 g carbohydrates, 6.8 g total fat). Physical assessment measures commenced ~30 min thereafter.

Blood collection and analysis

Blood glucose concentration, haemoglobin, total and differential leukocyte counts (i.e., neutrophils, lymphocytes, and monocytes), were determined by HemoCue system (Glucose 201+, Hb201, and WBC DIFF, respectively; HemoCue AB, Ängelholm, Sweden) in duplicate from heparin whole blood samples. Coefficient of variation (CV) for blood glucose concentration, hemogobin, and leukocyte counts were 4.1%, 1.8% and 13.6%, respectively. The remaining heparin whole blood

samples were centrifuged at 4,000 rpm for 10 min within 15 min of sample collection. Aliquots of heparin plasma were placed in 1.5 ml microstorage tubes and frozen at-80°C until analysis, except 2 x µl plasma was used to determine P_{Osmol} in duplicate (CV 1.0%), using a freeze point osmometry (Osmomat 030; Gonotec, Berlin, Germany).

Circulating concentrations of cortisol (DiaMetra, Perugia, Italy), insulin-like growth factor-1 (IGF-1) (Crux Biolab, Scoresby, Australia), insulin (Crux Biolab, Scoresby, Australia), testosterone (17b-OH-4-androstene-3-one; DiaMetra, Perugia, Italy), estradiol (17β-Estradiol; DiaMetra, Perugia, Italy) were measured by enzyme-linked immunosorbent assay (ELISA). Plasma concentrations of interleukin (IL)-2, IL-6, IL-1β, tumor necrosis factor (TNF)-α, IL-8, and IL-10 were determined by high sensitivity multiplex ELISA (HCYTOMAG-28SK; EMD Millipore, Darmstadt, Germany). All assays were performed as per manufacturer's specifications, with standards and controls on each plate. The CV for analysed circulating biomarkers was ≤7.2%, and for systemic inflammatory cytokines was ≤13.5%.

Strength Outcomes

Strength was assessed by performing a 1 repetition maximal strength (1-RM) in accordance with previously described procedures [37]. During a familiarisation trial, proper lifting technique was demonstrated, then participants were familiarised with each resistance machine (Hammer strength; LifeFitness, Sydney, Australia) by performing 8-10 repetitions of a light load (~50% of predicted 1-RM). After the successful completion of a further five to six repetitions at a heavier weight selected by the instructor, the workload was increased incrementally until only one repetition with correct technique could be completed. Participants were given 3-5 min rest in-between attempts [38]. The value indicative of 1-RM was the highest load that could be raised in one single repetition using correct technique for leg press, *latissimus dorsi* (lat) pull down, and

bench press. The 1-RMs were normalised by BM (1-RM/BM). Hand grip strength (HGS) was measured using a digital hand dynamometer (Jamar® Plus+ Digital hand dynamometer; Sammons Preston, Bolingbrook, IL, USA). HGS was measured in a standing position with the participants elbow by their side and flexed to a 90° angle and a neutral wrist position. Participants were asked to apply the maximum grip strength three times with both left and right hands, HGS was defined as the highest value for their dominant hand [39].

Submaximal incremental bike test

Submaximal aerobic fitness was determined using an incremental cycle test using a cycle ergometer (*Corival,* Lode, Groningen, Netherlands) and a metabolic cart (Vmax Encore Metabolic Cart; Carefusion, San Diego, CA). The initial workload began at 1 watt (W) per kilogram of Fat-free mass (W/kgFFM) and increased by 0.5 W/kgFFM every 3 min until participants could not maintain the speed at 60 RPM or higher or they reached a rating of perceived exertion (RPE) of 15-17 on the Borg scale [40]. Heart rate (HR) (Polar Electro, Kempele, Finland), $\dot{V}O_2$, respiratory exchange ratio (RER), and RPE were measured every 3 min in real-time. Cardiorespiratory fitness was expressed as Watts/RER. Procedures were adjusted from standard fitness testing procedures [41].

Countermovement jump

A Force plate (400s+ Performance Force plate; Fitness Technology, Adelaide, Australia) was used to measure relative muscle power (W/kg), jump height (cm) and velocity (m/sec) during a countermovement jump test (CMJ). Participants were asked to start in a full erect standing position in the middle of the force plate, then instructed to dip to a self-selected depth and "jump for maximal height". Hands were kept on the hips to minimize any influence of arm swing [42]. Participants were asked to perform three attempts of a CMJ with 1 min rest in-between jumps. The

Force plate was interfaced with computer software (Ballistic Measurement System; Fitness Technology, Adelaide, Australia), where the mean of three jumps was selected for further analysis.

Gait speed measurement

To assess gait speed, a walking course of 4 metres long was marked on the floor. The participant was instructed to walk from one end of the course to the other at their usual walking pace. The timer began as the participant started walking and the timer was stopped with the first footfall after the 4-metre line. The test was repeated twice and the average (of two scores) was determined. Gait speed was reported at meter/second.

Statistical analysis

Data in text and tables are presented as either mean ± SD (descriptive experimental data) or mean and 95% confidence interval (CI) (primary and secondary variable), as indicated. All statistical analyses were performed using IBM SPSS statistics software (Verson 25.0, IBM Corp, Armonk, NY). Prior to analysis, assumptions of normality in the data were tested using the Shapiro-Wilk test and visualisations of normality plots. Variables with multiple groups were examined using a general linear model (ANCOVA) or non-parametric ranked repeated measures where appropriate. A Tukey's post-hoc test was applied to determine between group differences. In addition, adjustments for biological sex were performed using gender as a catergorial variable and stratifying the data. Variables with singular points were examined using an independent t-tests, or non-parametric Mann-Whitney U test. Significance was accepted at $p \leq 0.05$. Additionally, Cohen's d was applied to determine the magnitude of effect size for significance differences, with $d \geq 0.20$ for small, $d \geq 0.50$ for medium, and $d \geq 0.80$ for large effect size.

4.4 Results

Table 1 presents the participant characteristics. Of the fifty-four participants included in the data collection, 53 were included in the analysis, due to a missing food diary (Figure 1). Of the 53 participants 86% were Caucasian, 10% and 14% Asian. The participants from this study came from a variety of sporting backgrounds that were composed of endurance runners and race walkers (61%), cyclists (9%), aerobic gym goers (16%), or a combination of multiple activities (14%). Based on the EWGOS2 clinical diagnosis 6% (*n*= 3) had low HGS and were considered to have probable sarcopenia [5].

Body composition stratified by age, exercise volume, dietary protein intake, and biological sex

The middle-aged group had a higher average bone mineral density compared to the older group, with a moderate association on age 7.6%, *d*= 0.562 (Table 2A). Table 2B shows between group differences of outcomes based on the level of reported exercise volume, there was a trend towards significance with the low training group having 10.5%, and 9.5% higher FM compared to the moderate and high exercise volume groups, respectively. A significant difference was observed between total daily protein intake with BM, FM and ALM/ht^2 (Table 2C). The high protein group (>1.2 g/kg BM/day) weighed-16.1kg (*p*= .025, *d*= 0.386) less than the low protein group. Table 2D presents the differences in body composition based on biological sex. Male participants were 16 kg heavier than female participants (*d*= 1.30), had 28% higher FFM (*d*= 2.34), and showed higher arm (*d*= 3.01) and leg lean muscle mass (*d*= 2.44). Male participant appendicular muscule mass (ALM/h^2) was also significantly higher than female participants (*d*= 2.85). Female participants had significantly higher FM compared to male participants (*d*= 0.921).

Table 1. Participant characteristics

Age, yrs	58.8 (57.0 to 61.0)
Height, m	1.71 (1.70 to 1.75)
Body mass (BM), kg	74.3 (70.2 to 78.3)
BMI, kg/m^2	24.8 (22.0 to 26.0)
RMR, MJ/day	5.8 (5.5 to 6.1)
EWOSP2 category for sarcopenia	
Hand grip, (dominant), kg	
ALM/ht^2	37.0 (33.8 to 40.1)
Gait speed, m/sec	7.7 (7.3 to 8.1)
n= , considered sarcopenic	0.8 (0.8 to 0.9)
Exercise volume	0
min/week	226 (191 to 260)
Dietary protein intake	
g/kg BM/day	1.4 (1.2 to 1.5)
iDXA measurements	
FFM, kg	53.6 (50.6 to 56.7)
FM, %	28.4 (26.0 to 31.0)
Arm lean mass, kg	5.6 (5.1 to 6.1)
Leg lean mass, kg	17.5 (16.4 to 18.6)
Strength, power, and physical performance	
Leg press, kg/BM	1.9 (1.8 to 2.1)
Lateral pull down, kg/BM	1.0 (0.9 to 1.1)
Chest press, kg/BM	0.8 (0.7 to 0.9)
Vertical jump	
Jump height, cm	17.3 (3.0 to 28.0)
Relative power, W/kg	29.5 (28.0 to 31.1)
Velocity, m/sec	1.9 (1.8 to 2.0)
Cardiorespiratory fitness	
Watts/RER,	111 (97 to 127)
Biochemistry	
BGL, mMol/L	4.8 (4.6 to 5.1)
Insulin, ulU/ml	7.1 (3.2 to 20.5)
IGF-1, pg/ml	97 (33 to 160)
Testosterone, ng/ml	1.4 (0.0 to 5.0)
Estradiol, pg/ml	26 (0 to 273)
Cortisol, nmol/L	366 (15 to 964)
WBC, pg/ml	4.8 (2.3 to 7.1)
Neutrophils, pg/ml	2.7 (1.1 to 4.5)
Lymphocytes, pg/ml	2.1 (1.0 to 3.9)
Monocytes, pg/ml	0.3 (0.1 to 1.1)
IL-2, pg/ml	4.1 (0.3 to 10.8)
IL-6, pg/ml	5.9 (0.8 to 36.8)
IL-8, pg/ml	5.9 (0.2 to 28.1)
IL-10, pg/ml	18.5 (1.0 to 50.1)
TNF-α, pg/ml	2.3 (0.4 to 5.6)

Mean (95% CI). **Abbreviations:** ALM: appendicular muscle mass, BGL: blood glucose levels, BMI: Body mass index, BM: body mass, EWSOP2: European Working Group of Sarcopenia 2, FFM: fat-free mass, FM: fat mass, IGF: insulin-like growth factor, IL: interleukin, RER: respiratory exchange ratio, RMR: resting metabolic rate, TNF- α: tumour necrosis factor alpha, W: watts.

Table 2. Body composition of participants based on age (A), exercise volume (B), protein intake (C), biological sex (D)

	BM (kg)	FFM (kg)	FM (%)	Arm lean mass (kg)	Leg lean mass (kg)	BMD	ALM/ht^2
A.							
Middle *n*= 26	74.7 (68.5 to 81.0)	55.3 (51.3 to 59.2)	27.0 (23.5 to 30.6)	5.6 (5.0 to 6.3)	18.0 (16.4 to 19.7)	1.3 (1.2 to 1.3)	7.8 (7.3 to 8.4)
Older *n*= 27	74.5 (69.2 to 80.3)	51.9 (47.3 to 56.6)	30.0 (26.2 to 34.0)	5.7 (5.1 to 6.4)	17.7 (16.1 to 19.2)	1.2 (1.1 to 1.2)	7.6 (7.0 to 8.1)
p-value	.936	.573	.250	.822	.459	.040	.451
B.							
Low *n*= 15	78.0 (70.0 to 86.1)	52.0 (45.4 to 58.3)	35.6 (31.2 to 40.0)	5.5 (4.5 to 6.5)	16.9 (14.7 to 19.1)	1.1 (1.1 to 1.3)	7.7 (6.9 to 8.6)
Moderate *n*= 24	75.8 (70.0 to 82.0)	56.4 (52.0 to 60.7)	25.1 (22.3 to 30.0)	5.8 (5.2 to 6.5)	18.6 (16.7 to 20.3)	1.3 (1.2 to 1.3)	7.8 (7.3 to 8.4)
High *n*= 14	69.5 (60.3 to 79.0)	51.0 (44.7 to 56.7)	26.1 (21.8 to 31.0)	5.4 (4.4 to 6.3)	16.7 (14.2 to 19.1)	1.2 (1.1 to 1.3)	7.5 (6.7 to 8.3)
p-value	.385	.405	.077	.344	.685	.916	.198
C.							
Low *n*= 7	86.1 (70.1 to 102.1)	57.1 (46.8 to 67.6)	35.2 (27.8 to 39.0)	6.7 (4.4 to 9.0)	19.7 (15.5 to 24.0)	1.2 (1.2 to 1.3)	8.6 (7.3 to 10.0)
Moderate *n*= 13	80.6 (72.6 to 92.0)	57.1 (50.7 to 63.5)	31.6 (25.9 to 37.3)	5.4 (4.6 to 6.3)	17.6 (14.6 to 20.6)	1.3 (1.1 to 1.4)	7.6 (6.6 to 8.6)
High *n*= 33	70.0[a] (65.8 to 71.0)	51.8 (47.9 to 55.8)	25.3[a] (22.2 to 28.3)	5.4 (4.8 to 6.0)	17.1 (15.7 to 18.4)	1.2 (1.1 to 1.3)	7.6 (7.1 to 8.0)
p-value	.025	.933	.005	.063	.263	.093	.143
D.							
Males *n*= 36	80.2 (75.5 to 85.0)	59.2 (56.3 to 62.0)	25.8 (23.1 to 28.4)	6.5 (6.2 to 6.9)	19.8 (19.7 to 20.8)	1.3 (1.2 to 1.3)	8.4 (8.0 to 8.7)
Females *n*= 17	64.1 (55.0 to 62.0)	42.4 (39.5 to 45.3)	34.1 (29.2 to 38.5)	3.8 (3.5 to 4.1)	13.4 (12.6 to 14.2)	1.1 (1.0 to 1.1)	6.5 (6.1 to 7.0)

Mean (95% CI). **(A)** Age: middle-age (50-59 yrs) and older (≥60 y); **(B)** Exercise volume: low (≥90-149 min), moderate (≥150-299 min), high (≥300 min); **(C)** Protein intake: low (≤0.8 g/kg BM/day), moderate (≥0.8-1.19 g/kg BM/ day), high (≥1.2 g/kg BM/day); **(D)** Males and females. Between group differences: [a] $p < .05$ vs low. **Abbreviations:** ALM: appendicular muscle mass, BM: body mass, FFM: fat-free mass, FM: Fat mass, BMD: Bone mineral density.

Strength outcomes stratified by age, exercise volume, dietary protein intake, and biological sex

Significantly higher leg press (*d*= 0.758), *lat* pull down (*d*= 0.532), and chest press (*d*= 0.600) 1-RMs were observed in middle-aged compared with and older participants (Table 3A). There was no substantial difference between HGS based on age. Based on exercise volume there was a trend towards significance for 1-RM leg press, with post-hoc analysis indicating a significant differences between groups for low vs. high training volumes (27%, *p*= .012, *d*= 0.902) and low vs. moderate (23%, *p*= .018, *d*= 1.06) exercise groups (Table 3B). There was no significant differences observed for outcomes of strength based on daily protein intake (Table 3C). Male participants presented significantly higher relative 1-RM assessment compared with female participants (Table 3D). Leg press 1-RM was 20% greater in male compared with female participants (*d*= 0.708). Male participants showed 30-33% higher chest press (*d*= 1.42) and *lat* pull down (*d*= 0.4), and HGS (*d*= 2.39), compared with female participants.

Table 3. Strength outcomes stratified by age (A), Exercise volume (B), Protein intake (C) and biological sex (D)

	Leg press (kg/BM)	Chest press (kg/BM)	Lat pull down (kg/BM)	Hand grip, dominant, kg
A.				
Middle *n*= 26	2.2 (2.0 to 2.3)	0.8 (0.8 to 1.0)	1.0 (0.9 to 1.3)	38.7 (34.1 to 43.0)
Older *n*= 27	1.8 (1.5 to 2.0)	0.7 (0.6 to 0.8)	0.9 (0.8 to 1.0)	37.3 (33.5 to 41.0)
p-value	**.008**	**.042**	**.047**	.264
B.				
Low *n*= 15	1.6 (1.3 to 1.9)	0.6 (0.5 to 0.8)	0.9 (0.7 to 1.0)	34.4 (27.7 to 41.0)
Moderate *n*= 24	2.1 (1.9 to 2.4)	0.8 (0.7 to 0.9)	1.0 (0.9 to 1.0)	40.5 (36.3 to 44.6)
High *n*= 14	2.2 (1.9 to 2.4)	0.8 (0.7 to 1.0)	1.0 (0.9 to 1.1)	37.0 (28.3 to 39.7)
p-value	.051	.195	.289	.351
C.				
Low *n*= 8	1.8 (1.4 to 2.1)	0.7 (0.6 to 0.9)	0.9 (0.8 to 1.1)	44.3 (35.8 to 52.8)
Moderate *n*= 12	1.7 (1.4 to 2.1)	0.8 (0.6 to 1.0)	0.9 (0.7 to 1.0)	36.5 (29.4 to 43.7)
High *n*= 33	2.1 (1.9 to 2.3)	0.8 (0.7 to 0.9)	1.0 (0.9 to 1.1)	36.4 (32.7 to 40.1)
p-value	.053	.232	.289	.239
D.				
Males *n*= 36	2.1 (1.9 to 2.2)	0.9 (0.8 to 0.9)	1.1 (1.0 to 1.1)	42.6 (40.0 to 45.3)
Females *n*= 17	1.7 (1.4 to 2.0)	0.6 (0.5 to 0.7)	0.7 (0.7 to 0.8)	25.8 (23.0 to 29.0)
p-value	**. 011**	**< .001**	**< .001**	**< .001**

Mean (95% CI). **(A)** Age: middle-age (50-59 yrs) and older (≥60 yrs); **(B)** Exercise volume: low (≥90-149 min); moderate (≥150-299 min); high (≥300 min), **(C)** Protein intake; low (≤0.8 g/kg BM/day), moderate (≥0.8-1.19 g/kg BM/ day), high (≥1.2 g/kg BM/day); **(D)** Males and females. **Abbreviations:** BM; body mass.

Power and physical performance outcomes stratified by age, exercise volume, dietary protein intake, and biological sex

Comparing age to physical performance and power outcomes (Table 4A), the middle-aged group presented 16% higher relative power (W/kg) (*d*= 0.900) and 10% higher velocity (*d*= 0.755) compared to the older aged group. Additionally, the middle-aged group, showed 30% higher average watts/RER, reflecting a higher cardiorespiratory fitness, compared to the older aged group (*d*= 0.822). Moreover, the middle-aged group jumped on average 6 cm higher compared to the older group (*d*= 1.39). Exercise volume did not influence any substantial difference on any of the physical performance outcomes (Table 4B). Comparing groups based on relative dietary protein intake, there was no significant difference between groups (Table 4C). The comparison of power and physical performance outcomes based on biological sex (Table 4D), indicated that male participants jumped 6 cm higher (*d*= 0.802), had 11% higher relative power (*d*= 0.721), and 13% higher velocity (*d*= 1.11) compared to female participants. There was no statistical significant difference between biological sex for gait speed and cardiorespiratory fitness.

Systemic hormonal and inflammatory cytokine profiles

There were no main significant associations between systematic hormonal or inflammatory cytokine markers on outcomes related to age, training status or protein intake (supplementary material 1-3). There was a significant difference in testosterone, blood glucose, insulin, resting neutrophils and monocytes for biological sex (supplementary material 4).

Table 4. Performance and power outcomes stratified by age (A), Training volume (B), Protein intake (C) and biological sex (D)

	Jump height (cm)	Relative power (W/kg)	Velocity (m/s)	Gait speed (m/s)	Cardiorespiratory fitness, Watts/RER
A.					
Middle *n*= 26	20.3 (18.5 to 22.1)	32.0 (30.0 to 33.6)	2.0 (0.0 to 2.0)	0.8 (0.7 to 0.8)	136 (119 to 533)
Older *n*= 27	14.5 (12.5 to 16.5)	26.4 (24.0 to 29.0)	1.8 (1.6 to 1.9)	0.8 (0.7 to 0.8)	85 (65 to 106)
p-value	< .001	.002	.006	.249	.004
B.					
Low *n*= 15	14.9 (11.5 to 18.4)	26.0 (22.7 to 29.1)	1.7 (1.6 to 1.9)	0.9 (0.8 to 0.9)	89 (64 to 118)
Moderate *n*= 24	18.8 (16.7 to 21.0)	31.3 (28.8 to 33.7)	2.0 (1.9 to 2.1)	0.8 (0.7 to 0.9)	129 (107 to 151)
High *n*= 14	17.3 (14.4 to 20.3)	29.2 (27.6 to 31.0)	1.9 (1.7 to 2.0)	0.8 (0.7 to 0.9)	114 (82 to 146)
p-value	.660	.275	.279	.271	.092
C.					
Low *n*= 8	16.3 (11.2 to 21.3)	27.3 (23.0 to 31.6)	1.9 (1.6 to 2.1)	0.8 (0.7 to 0.9)	107 (65 to 149)
Moderate *n*= 12	17.8 (13.7 to 22.0)	26.0 (19.5 to 32.6)	1.7 (1.4 to 2.1)	0.8 (0.7 to 0.8)	117 (98 to 138)
High *n*= 33	17.6 (16.0 to 19.1)	31.2 (29.6 to 33.0)	2.0 (1.9 to 2.1)	0.8 (0.8 to 0.9)	117 (100 to 129)
p-value	.580	.056	.138	.641	.408
D.					
Males *n*= 36	19.0 (17.1 to 21.0)	30.4 (28.4 to 32.5)	2.0 (1.9 to 2.1)	0.8 (0.7 to 0.8)	20.0 (17.5 to 22.5)
Females *n*= 17	14.1 (11.5 to 16.7)	26.9 (24.0 to 30.0)	1.7 (1.6 to 1.8)	0.8 (0.8 to 0.9)	19.1 (14.6 to 23.5)
p-value	.003	.048	.001	.611	0.147

Mean (95% CI). **(A)** Age: middle-age (50-59 y) and older (≥60 y); **(B)** Exercise volume: low (≥90-149 min); moderate (≥150-299 min); High (≥300 min); **(C)** Protein intake, low (≤0.8g/kgBM/day), moderate (≥0.8-1.19g/kgBM/ day), high (≥1.2g/kgBM/day); **(D)** Males and females. Abbreviations: RER: respiratory exchange ratio, W: watts.

4.5 Discussion

This study aimed to assess the link between self-reported and objective measures of age, physical activity, habitual dietary protein intake, and biological sex as they relate to measures of body composition, strength, power, and physical performance outcomes in a population of active older adults. The main findings were: 1) middle-aged and older adults had no significant differences in body composition, but did display differences in strength, power, and performance; 2) higher exercise volumes (≥150 min/week) in active older adults had a trend towards significance in leg strength and lower body fat, however there were no other significant differences between any other outcomes; 3) higher dietary protein intakes (≥1.2 g/kg BM/day) was linked with lower FM and body weight compared to lower protein intakes (<0.8 g/kg BM/day); and 4) significant differences in body composition, strength, and power outcomes exist between male and female active older participants. There are many cross-sectional studies that examine some of these outcome measures in either community dwellers or frail and institutionalised older adults [43]. However, the current study is the first to comprehensively explore these prospective relationships in a cohort of active older adults. Overall, this study demonstrated that the contributions of age, physical activity, daily dietary protein intake, and biological sex can help explain individual variation in outcomes related to changes in body composition, strength, power, and physical performance.

Based on the EWGSOP2 clinical diagnosis criteria, 6% of the participants had low HGS and were considered to have “probable” sarcopenia. However, when analysed further these participants

were not confirmed to have sarcopenia based on muscle quantity [5]. This prevalence for sarcopenia is substantially lower than the 1-29% reported rates for community dwelling adults over the age of 50 yrs [44]. A similar study by Fien et al. [45] found that in a cohort of 156 Master's athletes, 3.8% were considered to be below the sarcopenic HGS cut-off points. The difference in results is likely due to the larger sample size and the higher level of training of the Masters' athletes compared to the more recreationally active older adults used in the current study. Considering that the prevalence of sarcopenia in aged-care facilities have been reported as high as 41% [44], our data suggests that active middle-aged and older adults levels of SMM, skeletal muscular strength, and physical function place them at very low risk of adverse health effects caused by sarcopenia on ageing, and that even higher levels of training may mitigate this [46].

Ageing is often associated with a decline in FFM, strength and physical performance that is accelerated with sedentary behaviour [47]. The effects on ageing alone on these outcomes in Masters or recreationally active adults, is less defined. In the current study, there was no observed differences in body composition based on age. However, for strength, skeletal muscle power (e.g., CMJ), and physical performance outcomes, the middle-aged group had ≥ 10% higher strength (i.e., 1-RM leg press, chest press, and lateral pull down), jumped 28% higher, and had 30% higher cardiorespiratory fitness compared to the older-aged group. The decline in muscle power with ageing has been indicated to decline more rapidly than SMM and muscular strength, and therefore muscle power may be more of a substantial indicator of physical function with ageing, especially

in the active population [30]. The CMJ is a weight-bearing activity where the participant needs to accelerate their body mass. It is therefore similar to the challenges an individual faces during the execution of performing activities of daily living, such as getting out of a chair or climbing up stairs [48]. A cross-sectional study by Pearson et al. [29] found that peak power (W) declined with increasing age at a similar rate between elite Masters weight lifters and controls (1.2% and 1.3% per year); while muscular strength declined at a similar but lower rate (0.6% and 0.5% per year). The cause of reduced force-and power-producing capabilities relative to muscle size with ageing appears to be attributed to a reduction in Type II fast-twitch muscle fibre size. A original study by Lexell et al. [49] observed from cross-sections of the *Vastus Lateralis* in 30 male cadavers that older adults (71 ± 1 yrs) had on average 18% less muscle fibre size, and 25% decline in total muscle fibres compared to younger adults (30 ± 6 yrs). The difference in total muscle size was purposed to be accounted by a marked reduction in the number of myofibrils in the older muscle (478,000 vs 364,000). In support of this, a more recent study by Nilwik et al. [50] found that type II muscle fibres were substantially smaller (29%) in older (71 ± 1 yrs) versus a young (23 ± 1 yrs) population. The decrease in individual muscle fibres, in particularly Type II, could potentially explain the observed lower muscle strength and lower body power in the older-aged group compared with the middle-aged group in the current study. Additionally, in relation to age, there was a significant difference in cardiorespiratory fitness with the average fitness levels being 30% less in the older group than the middle-aged group. There have been numerous cross-sectional and longitudinal studies reporting a decrease in $\dot{V}O_2$ max with age, irrespective of training status and type of activity

(e.g., endurance and power athletes) [32, 51]. These studies have reported a rate of decline in sedentary individuals to be approximately 10% per decade [28, 52], whilst even highly active individuals have a decline of ~5% [53 - 55]. A study by Stathokostas et al. [28] in a 10-year longitudinal study in healthy older adults (73.5 ± 6.4 yrs; 28 women, 72.1 ± 5.3 yrs) observed a 14% and 7% decline in VO_{2max} in men and women, respectively. Additional analysis showed that age-related changes in VO_{2max} were not significantly related to physical activity [28]. Irrespective of the mechanisms, this indicates that decreasing muscle power and strength and cardiorespiratory fitness in older age may be cause for concern and it is clear that activity alone may not mitigate these changes occurring with age. Additionally, the maintenance of muscle power and cardiorespiratory fitness in older adults may be an important target for intervention, given its implications for ambulation and physical function in older adults.

The current recommendations based on the World Health Organisation (WHO) suggest that all adults should engage in at least 150 minutes of moderate activity throughout the week to support bone, joint and muscle health, which in turn may reduce functional limitations prevent falls and promote independent living [7]. There were no significant associations between exercise volume and any of the outcomes. This is likely due to the population that were included in this study were mostly above a high threshold for exercise volume (e.g., >150 min/week). However, there was a notable trend towards significance, with the results indicating that individuals who achieved the recommended exercise volume (≥150 - 299 min per week) or higher (≥300 min per week), when

compared to the lower training group (≥90 - 149 min/week), had a trend towards a significant greater leg strength and lower body fat in those that achieved the recommended exercise volume or more. Considering that the low training group had the highest proportion of females (50%, supplementary material 2), and females typically present with ~10% higher body fat, are generally produce less power and strength compared to men, this could explain the trend between the groups, more than training alone [56, 57]. It is likely that there was not a more significant difference in results as the participants within this cross-sectional study were considered 'active', this is highlighted by the fact that there were no significant differences in cardiorespiratory fitness (CRF) observed between groups. Furthermore, a cross-sectional study by Marcos-Pardo et al. [17] found that a measured sedentary time over 300 min/week found a significant association for increased risk factors for lower functional performance and failing at least one variable associated with sarcopenia. inadequate physical activity levels have also been associated with an increase risk in chronic diseases (e.g., type II Diabetes Mellitus), obesity and unfavourable cardiovascular markers [58, 59]. A limitation for this study is the lack of comparison made with an age-matched less active comparison group. Therefore, despite there being a trend in body fat percentage and leg strength between the exercise groups, it is not known if these are any better than the general population and if the larger proportion of females were the cause of the significant difference. Higher protein intakes (e.g., ≥1.2 g/kg BM/day) have been advocated for physically active older adults to overcome the increased requirements of AA utilisation due to exercising, alongside increased requirements caused by ageing [21]. This study found that higher intakes of protein (≥1.2 g/kg BM per day) were

associated with lower body weight, lower fat mass compared to the moderate and low protein intake groups. The effect size comparing the low and moderate intake was small for the same variables ($d < 0.5$). Our findings partially support the assumption that physically active older adults may require higher amounts of protein (≥1.2 g/kg BM per day) than the current recommendations (0.8 g/kg BM per day) and may be a key factor in preventing the decline in muscle strength in older adults [60, 61]. There have been numerous observational studies that have correlated protein intake in relation to sarcopenia to explore diet-muscle health relationships [61]. However, the majority of these studies are conducted in free-living community dwellers, with limited studies in physically active older adults. One of the few studies in active older adults compared protein intakes and physical function in active (150 min/week of moderate-to-vigorous activity) older (67.5 ± 1.8 yrs) women and found a significant difference in self-reported physical function - as measured using hand grip strength and short physical performance battery (SPPB) - in women with higher protein intakes (>1.1 g/kg BM per day) compared to low protein intakes (0.8 g/kg BM per day) [21]. Additionally, a recent systematic review and meta-analysis of observational studies assessing protein intake with various physical performance outcomes found that reasonably high (1.0 g/kg BM per day) and very high protein intakes (>1.2 g/kg BM per day) were associated with more favourable lower-limb physical performance ($p < .050$) and lower limb strength ($p = .050$) when compared to low protein (<0.8 g/kg BM per day) in community dwelling older adults [61]. Furthermore, after supplementation of protein for 12-weeks in physically active older adults who habitually consumed low protein (<1.0 g/kg BM/day) d a significant increase in physical

performance, lean body mass and a decrease in FM was observed after race walking training [62]. Physical activity increases the sensitivity of skeletal muscle to the anabolic properties of protein consumption, and the active older population, which exercises at higher amounts than the general population, requires more protein [59, 63]. However, total protein intake *per se* may not be sufficient enough to support active ageing. Numerous studies have indicated that distribution, timing and quality of protein are important to consider [64]. Therefore, future studies should focus on these factors alongside total protein intake in an active older population.

The results of this study based on gender found that males had significantly more FFM, ALM and regional muscle mass (arms and legs). The findings of this study strengthen the already abundant results of previous studies that have reported significant sex differences in lean muscle mass, as estimated by DEXA, MRI and CT [57, 65, 66]. On average FFM was 28% higher in males than in women with a large effect size. This gender difference remained after adjusting for height (ALM/ht^2) where men had 25% more SMM compared to women. The muscle distribution between genders showed that women had 41% less arm SMM and 31% less leg SMM. These findings support previous works by Gallagher and Heymsfield [66] , which reported that females have a larger proportion of their appendicular muscle mass in their lower extremities in comparsion to males (as estimated by DEXA). Additionally, Janssen et al. [65] reported similar gender differences in upper (40%) and lower body (33%) muscle mass based on MRI images. This difference in muscle distribution when adjusting for differences in total body mass is the likely cause for the observed

differences in strength outcomes. For example the observed gender differences in lower body strength (~30%) which are smaller than those observed for upper body strength (~50%). The differences in skeletal muscle mass, strength and power between the genders is most likely due to the greater capacity for muscular hypertrophy as a results of higher levels of circulating testostrone seen in males [67]. This is highlighted in the differences in the biochemical parameters observed between biological sex (supplementary material 4). Where males have 8 times greater levels of testostrone than females. Additionally, considering the clear physiological differences between genders, future intervention studies should consider differentiating between gender.

The declines in SMM and strength observed with increasing age may be due to the changes in systemic anabolic hormones (e.g., testosterone and IGF-1), and chronic low-grade inflammation (e.g., *'inflammaging'*). [67-70]. Inflammaging is characterised by a chronic low grade systemic inflammatory cytokine response such as tumour necrosis factor-α (TNF-α) and interleukin (IL)-6, and a decrease in anti-inflammatory cytokines such as IL-10 [71, 72]. Additionally, there is an inverse dose-relationship that has been observed in multiple observational studies between the level of physical activity and such systematic inflammatory biomarkers [73]. For example, Colbert et al. [74] observed in a large scale study (*n*= 2964) an inverse relationship between higher levels of self-reported physical activity (≥180 min/week) and inflammatory biomarkers including C-reactive protein (CRP) and IL-6 after adjusting for body fat. However, in the current study there were no observed differences in any of the systemic anabolic hormones or inflammatory cytokines

associated with age, training status or protein intake (supplementary materals 1-3). Nonetheless, intervention studies have reported signficiant effects of exercise and reducing inflammatory biomarkers in the ageing population [73, 74]. For example, Kohut et al. [75] reported a significant decrease in IL-18, CRP, and IL-6 in older adults (>64 y) after performing aerobic exercise 3 days a week, for 45 minutes at 65-80% $VO_{2peak.}$ Therefore, considering that within the current study cohort they were considered active within particiopants habitual lifestyles, this healthy active older adult population may have been too similar to detect the modest group differecnes in systemic hormonal and/or inflammatory profiles seen in previous studies with greater participant variability.

The strengths of this current study is the use of a comprehensive outcomes measured including; FFM, strength, physical performance outcomes taking into consideration more relevant markers for active ageing cohort (e.g., CMJ), and hormonal and inflammatory parameters. This is the first study to assess the contributions of age, physical activity, daily protein intake, and biological sex can explain the individual variation in outcomes related to changes in body composition, strength, power, and/or cardiorespiratory fitness in a cohort of active older adults. The current study included the use of a 3-day food diary to infer daily dietary intake patterns and self-reported physical activity to estimate total physical activity levels. While these are acknowledged to be potential limitations due to the nature of using self-reported measures, based on the reported intake of the same cohort in a intervention study [76], it was observed that the energy and protein intakes of the 3-day food diaries did not change in the control groups over the course of a 12-weeks

trial. For example, average relative protein intakes were reported to be 1.6 g/kg BM/day at baseline and 6-weeks and 1.4 g/kg BM/day at 12-weeks without any significant differences. Therefore, considering that the 3-day food diary used was consistently shown to be similar over 12-weeks it is likely to reflect their true food intake in the current study. Furthermore, the average self-reported physical activity levels of the current study were 228 min/week. In the same intervention study [76], when physical activity was objectively measured using a ActiGraph accelerometer for 12-weeks, the mean time in moderate (3.00-5.99 metabolic equivalents (METS)) physical activity was 200-317 min/day and mean time in vigorous (≥6 METs) physical activity was 16-21 minutes/ day. Considering that the accelerometer measured all physical activity, including incidental activity, it is likely that the self-reported physical activity at baseline within this current study was indeed a reliable method of measurement and did not involve over reporting of physical activity which is commonly observed in studies [77]. This is likely due to the inclusion of active individuals that engage in regular structured physical activity, therefore can more reliably recall habitual physical activity. Lastly, it is acknowledged that this current study is a relatively small sample size. However, we believe that this study may provide initial preliminary data to help contextualise future intervention trials and identifies methodological gaps in sarcopenic research in active older adults.

4.6 Conclusion

This study showed that the contributions of age, physical activity, daily protein intake, and gender can help explain individual variation in outcomes related to changes in body composition, strength,

power, and performance in a cohort of active older adults. Further comparisons indicated that this cohort is at a low risk of adverse outcomes caused by sarcopenia. Strength and power outcomes were influenced by age, training status, protein intake, and biological sex.

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Supplementary Material 1. Supplementary material of participants based on age.

	Middle *n*= 26	Older *n*= 27	*p*-value
Females, %	37	22	
Age, yrs	53.3 (52.2 to 54.5)	64.6 (63.0 to 66.4)	.016
Height, m	1.72 (1.67 to 1.76)	1.72 (1.67 to 1.76)	.926
Weight, kg	74.7 (68.5 to 81.0)	74.5 (69.2 to 80.3)	.936
BMI, kg/m^2	24.7 (23.4 to 26.0)	25.0 (24.0 to 26.2)	.788
Self-reported Exercise			
Ex volume, min/week	228 (183 to 274)	227 (172 to 283)	.979
Resting Metabolic Rate			
MJ/day	6.0 (5.6 to 6.4)	5.6 (5.1 to 6.0)	.177
Daily protein intake			
g/kg BM/day	1.4 (1.2 to 1.5)	1.3 (1.1 to 1.5)	.837
Biochemistry			
BGL, mmol/L	4.9 (4.5 to 5.4)	5.0 (4.8 to 5.3)	.195
Insulin, ulU/ml	7.5 (4.7 to 9.5)	6.7 (5.8 to 8.0)	.450
IGF-1, pg/ml	72 (26 to 118)	224 (61 to 509)	.366
Testosterone, ng/ml	1.0 (0.5 to 1.6)	1.9 (1.2 to 2.6)	.457
Estradiol, pg/ml	30.5 (5.3 to 55.6)	26.6 (3.6 to 49.5)	.683
Cortisol, nmol/L	361 (284 to 438)	393 (297 to 488)	.615
WBC $x10^9$	4.8 (4.3 to 5.3)	5.6 (5.0 to 6.2)	.037
Neutrophils $x10^9$	2.5 (2.2 to 3.0)	3.0 (2.6 to 3.2)	.168
Lymphocytes $x10^9$	2.0 (1.7 to 2.2)	2.1 (2.0 to 2.6)	.160
Monocytes $x10^9$	0.3 (0.2 to 0.4)	0.3 (0.3 to 0.4)	.294
IL-2, pg/ml	4.6 (2.9 to 5.8)	3.7 (2.8 to 4.6)	.531
IL-6, pg/ml	5.0 (0.9 to 8.3)	6.7 (2.5 to 11.4)	.557
IL-8, pg/ml	5.7 (1.9 to 7.5)	6.6 (3.3 to 10.0)	.742
IL-10, pg/ml	17.6 (11.0 to 25.6)	19.4 (13.7 to 25.8)	.545
TNF-α, pg/ml	2.2 (1.3 to 2.7)	2.3 (2.0 to 2.8)	.545

Mean (95% CI). Age: middle-age (50-59 y) and older (≥60 y). **Abbreviations:** BGL: blood glucose levels, BM, body mass, BMI: body mass index, IGF: insulin-like growth factor, IL: interleukin, m, metres, MJ: Megajoules, WBC: white blood cells.

Supplementary Material 2. Supplementary material of participants based on training volume.

	Low *n*= 15	Moderate *n*= 24	High *n*= 14	*p*-value
Females, %	50	21	35	
Age, yrs	60.1 (56.0 to 63.0)	58.4 (55.6 to 62.3)	59.0 (57.0 to 61.0)	0.720
Height, m	1.72 (1.66 to 1.80)	1.74 (1.70 to 1.80)	1.7 (1.61 to 1.80)	.065
Weight, kg	78.0 (70.0 to 86.1)	75.8 (70.0 to 82.0)	69.5 (60.3 to 79.0)	.285
BMI, kg/m^2	25.3 (23.3 to 27.8)	24.5 (23.3 to 26.0)	24.1 (23.7 to 25.5)	.631
Daily protein intake				
g/kg BM/day	1.2 (1.1 to 1.4)	1.3 (1.1 to 1.5)	1.5 (1.2 to 1.8)	.243
Resting metabolic rate				
MJ/ day	5.7 (5.1 to 6.3)	6.0 (5.5 to 6.5)	5.5 (5.0 to 6.0)	.160
Biochemistry				
BGL, mmol/L	5.2 (4.8 to 5.6)	4.9 (4.5 to 5.1)	4.8 (4.5 to 5.2)	.251
Insulin, ulU/ml	6.6 (4.0 to 9.2)	7.2 (5.2 to 9.2)	7.1 (6.1 to 8.1)	.456
IGF-1, pg/ml	125 (46 to 204)	155 (40 to 271)	160 (64 to 385)	.985
Testosterone, ng/ml	1.0 (0.3 to 1.7)	1.9 (1.3 to 2.5)	1.2 (0.5 to 1.9)	.184
Estradiol, pg/ml	22.1 (14.0 to 58.2)	32.5 (6.0 to 59.0)	29.0 (4.0 to 53.0)	.813
Cortisol, nmol/L	353 (266 to 440)	400 (294 to 507)	364 (257 to 470)	.884
Neutrophils x10^9	2.8 (2.1 to 3.4)	2.7 (2.3 to 3.1)	2.5 (1.9 to 3.0)	.508
Lymphocytes x10^9	2.1 (1.7 to 2.3)	1.9 (1.6 to 2.2)	2.2 (1.7 to 2.6)	.864
Monocytes x10^9	0.3 (0.2 to 0.3)	0.3 (0.3 to 0.4)	0.3 (0.3 to 0.4)	.519
IL-2, pg/ml	3.8 (1.9 to 5.7)	4.4 (3.0 to 5.0)	4.3 (3.0 to 5.7)	.864
IL-6, pg/ml	5.9 (0.2 to 12.0)	5.3 (1.2 to 9.4)	6.7 (2.6 to 11.0)	.895
IL-8, pg/ml	5.9 (2.0 to 9.8)	4.2 (2.0 to 6.1)	9.0 (2.8 to 15.1)	.164
IL-10, pg/ml	20.6 (12.6 to 28.6)	19.4 (13.2 to 25.7)	14.3 (7.4 to 21.3)	.462
TNF-α, pg/ml	2.6 (1.7 to 3.5)	2.2 (1.6 to 2.7)	2.1 (1.5 to 2.7)	.464

Mean (95% CI). Exercise volume: low (≥90-149 min), moderate (≥150-299 min), high (≥300 min). Between group differences: [a] $p < .05$ vs low. **Abbreviations:** BGL: blood glucose levels, BM, body mass, BMI: body mass index, IGF: insulin-like growth factor, IL: interleukin, m, metres, MJ: Megajoules, WBC: white blood cells.

Supplementary Material 3. Supplementary material of participants based on daily protein intake

	Low *n*= 8	Moderate *n*= 12	High *n*= 33	*p-valuep*-value
Females, %	0	20	50	
Age, yrs	61.8 (54.1 to 69.5)	56.1 (53.2 to 59.0)	56.6 (52.1 to 62.0)	.170
Height, m	1.70 (1.60 to 1.80)	1.73 (1.67 to 1.80)	1.71 (1.67 to 1.80)	.699
Weight, kg	86.1 (70.1 to 102.1)	80.6 (72.6 to 92.0)	70.0 (65.8 to 71.0)[a]	.025
BMI, kg/m^2	27.5 (24.5 to 29.0)	26.0 (23.4 to 28.4)	23.5 (22.6 to 24.5)[a]	.001
Self-reported exercise				
Ex volume, min/week	200 (119 to 280)	162 (108 to 217)	258 (210 to 305)	.091
Resting metabolic rate				
MJ/day	5.8 (4.7 to 7.0)	6.0 (5.1 to 7.0)	5.7 (5.4 to 6.1)	.450
Biochemistry				
BGL, mmol/L	5.3 (4.7 to 5.9)	4.8 (4.5 to 5.2)	5.0 (4.8 to 5.2)	.402
Insulin, ulU/ml	7.6 (5.3 to 9.1)	7.7 (4.8 to 10.5)	6.7 (5.8 to 7.9)	.722
IGF-1, pg/ml	138 (50 to 228)	136 (44 to 229)	154 (75 to 410)	.976
Testosterone, ng/ml	1.5 (0.3 to 2.8)	1.1 (0.4 to 1.7)	1.7 (1.1 to 2.2)	.330
Estradiol, pg/ml	14.0 (1.8 to 26.1)	21.8 (15.7 to 59.5)	35.5 (12.7 to 58.4)	.560
Cortisol, nmol/L	313 (143 to 482)	380 (277 to 483)	374 (298 to 449)	.752
Neutrophils x10^9	2.9 (2.5 to 3.4)	2.8 (2.3 to 2.9)	2.6 (2.3 to 2.9)	.553
Lymphocytes x10^9	2.3 (1.6 to 2.9)	2.3 (1.9 to 2.7)	2.0 (1.8 to 2.3)	.238
Monocytes x10^9	0.3 (0.2 to 0.5)	0.4 (0.2 to 0.5)	0.3 (0.3 to 0.4)	.547
IL-2, pg/ml	3.2 (0.7 to 5.7)	3.9 (2.6 to 5.2)	4.4 (3.4 to 5.4)	.580
IL-6, pg/ml	1.5 (0.1 to 3.1)	3.8 (0.8 to 6.8)	7.5 (3.7 to 11.3)	.258
IL-8, pg/ml	1.9 (0.9 to 2.9)	5.2 (0.7 to 9.5)	7.1 (4.2 to 10.1)	.150
IL-10, pg/ml	17.0 (2.8 to 31.1)	18.3 (11.8 to 24.8)	18.8 (13.3 to 24.4)	.872
TNF-α, pg/ml	1.8 (1.7 to 2.6)	2.6 (1.8 to 3.3)	2.2 (1.7 to 2.7)	.475

Mean (95% CI). Protein intake: low (<0.8 g/kg BM/day), Moderate (≥0.8-1.19 g/kg BM/day), high (≥1.2g/kg BM/day). Between group differences: [a] $p < .05$ vs low. **Abbreviations:** BGL: blood glucose levels, BM, body mass, BMI: body mass index, IGF: insulin-like growth factor, IL: interleukin, m, metres, MJ: Megajoules, WBC: white blood cells.

Supplementary Material 4. Supplementary material of participants based on biological sex

	Males *n*= 36	Females *n*= 17	*p-valuep*-value
Age, yrs	59.1 (57.0 to 61.4)	58.3 (55.0 to 62.0)	.702
Height, m	1.77 (1.74 to 1.80)	1.62 (1.60 to 1.65)	< .001
Weight, kg	80.2 (75.5 to 85.0)	64.1 (55.0 to 62.0)	< .001
BMI, kg/m^2	25.1 (24.1 to 27.0)	24.3 (22.0 to 26.0)	.101
Self-reported exercise			
Ex volume, min/week	229 (193 to 265)	213 (134 to 291)	.374
Resting metabolic rate			
MJ/day	6.2 (5.8 to 6.5)	5.0 (4.7 to 5.3)	< .001
Daily protein intake			
g/kg BM/day	1.3 (1.2 to 1.5)	1.3 (1.1 to 1.5)	.935
Biochemistry			
BGL, mmol/L	5.1 (4.8 to5.3)	4.7 (4.4 to 5.1)	.050
Insulin, ulU/ml	8.0 (6.6 to 9.2)	5.6 (4.8 to 6.3)	.050
IGF-1, pg/ml	174 (27 to 377)	108 (31 to 185)	.545
Testosterone, ng/ml	2.1 (1.6 to 2.4)	0.2 (0.0 to 0.6)	< .001
Estradiol, pg/ml	31.3 (9.0 to 53.5)	23.2 (1.6 to 48.2)	.657
Cortisol, nmol/L	368 (294 to 443)	366 (473 to 360)	.658
Neutrophils $x10^9$	2.8 (2.5 to 3.1)	2.5 (1.9 to 3.0)	.050
Lymphocytes $x10^9$	2.1 (1.8 to 2.4)	2.1 (1.7 to 2.5)	.545
Monocytes $x10^9$	0.4 (0.3 to 0.4)	0.3 (0.2 to 0.3)	< .001
IL-2, pg/ml	4.1 (3.2 to 5.0)	4.4 (2.7 to 6.2)	.657
IL-6, pg/ml	5.8 (2.5 to 9.2)	5.8 (1.2 to 10.4)	.658
IL-8, pg/ml	5.6 (3.0 to 8.3)	6.1 (2.6 to 9.5)	.092
IL-10, pg/ml	18.0 (13.1 to 23.0)	22.0 (14.4 to 29.1)	.140
TNF-α, pg/ml	2.3 (1.8 to 2.7)	2.4 (1.6 to 3.2)	.828

Mean (95% CI). **Abbreviations:** BGL: blood glucose levels, BM, body mass, BMI: body mass index, IGF: insulin-like growth factor, IL: interleukin, m, metres, MJ: Megajoules, WBC: white blood cells.

CHAPTER FIVE:

Protein amount, quality, and distribution in active older adults and its effects on outcomes of skeletal muscle mass, strength and power

The following chapter appears on the next page as published in the *International Journal of Sports Science.*

Huschtscha, Z., Parr, A., Porter, J., Costa, R.J. Protein amount, quality, and distribution in active older adults and its effects on outcomes of skeletal muscle mass, strength and power: the active ageing study. *IJSS.* 2021;11 (1): 6-17

5.1 Abstract

This study aimed to investigate the associations of habitual protein intake in a cohort of active older adults including: daily relative protein intake, distribution of protein intake across main meals, and number of meals providing ≥0.4 g/kg body mass (BM) on outcomes of fat-free mass (FFM), leg power, leg strength, and handgrip strength (HGS). This was a cross-sectional study (2018-2020) where data were obtained and analysed from *n*= 53 active older adults (≥50 yrs; ≥90 min/week of self-reported physical activity). Daily absolute (g) and relative protein (g/kgBM/day) intake, absolute and relative protein intake per meal, the number of meals that provided 0.4 g/kgBM, and the protein intake distribution were calculated for each participant through a 3-day food diary assessment and analysis. Appendicular muscle mass index (ALM/ht^2; dual x-ray absorptiometry), leg strength (1-repetition maximum using leg press), leg power (force plate countermovement jump) and HGS (dynamometer) were assessed. An independent t-test was used to test statistical significance between groups based on protein intake. Pearson's correlation determined differences between protein intakes with lean muscle mass and strength outcomes. *Results:* Daily protein intake was (mean ± SD) 1.4 ± 0.4 g/kg BM/day, with the coefficient of variation of main meals calculated at 0.46 (0.41-0.51), and the average number of meals that provided ≥0.4g/kgBM was 1.1 ± 0.8 meals. There was a moderate but significant positive correlation between number of meals per day providing ≥0.4g/kgBM, and number and leg press (r = .301, p< .05), significant for males (r= .591, p= .029), but not females (r=.262 , p= .196). There was also a small significant association between the number of total protein and dairy serves per day and leg strength (r= .290, p= .035; r= .372,

p= .006, respectively). No significant differences were observed for outcomes of HGS or FFM and any of the habitual dietary protein measures. *Conclusion:* In a cohort of active older adults who achieve greater protein intakes than the current recommendations, a minimum of 1 meal containing ≥0.4 g/kg BM of protein and higher intakes of dairy based foods may be required to achieve favourable outcomes in leg strength.

Key words: Sarcopenia; protein intake; muscle strength; muscle power

Key points:

- Active older adults in this current study reported a high average intake of daily protein 1.4 ± 0.4 g/kg BM/day.
- There was a small but significant positive correlation between the number of meals providing ≥0.4 g/kg BM per meal and leg press strength for males, but not females.
- A minimum of 1 meal providing ≥0.4 g/kg BM of protein may provide positive outcomes of leg press.

5.2 Background

Sarcopenia is categorised as an age-related progressive decline in skeletal muscle mass (SMM), strength, and function [1]. SMM is directly influenced by the balance between the rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB), collectively known as muscle protein turnover [2]. Achieving a positive protein balance, where MPS exceeds MPB, is a crucial component of skeletal muscle repair and remodeling in response to anabolic stimuli, as well as in accruing SMM to maintain strength and physical function [3]. Older adults (≥65 yrs) have been observed to have a blunted capacity to respond to anabolic stimuli compared to younger individuals, known as '*anabolic resistance*' [4]. Early signs of sarcopenia such as declines in SMM and strength have been observed to commence as early as the 5th decade of life [5]. Anabolic resistance in the ageing population can be overcome by lifestyle factors such as physical activity and increasing dietary protein. These two factors have been the focus of numerous intervention studies in prevention and management of age related sarcopenia [6, 7]. However, the majority of the recent research that has been conducted on frail and institutionalised older adults, and it is uncertain whether these changes commonly associated with ageing reflect the true physiology of ageing muscle or are more a picture of disuse atrophy or inadequate protein intake and other medical considerations (i.e., disease associated) [8]. Interestingly, active older adults that exercise either competitively or recreationally, still show signs of a progressive loss of SMM, skeletal muscle strength and physical performances in speed and power events [9]. Multiple aspects of protein intake have been shown to have promising relevance in the ageing population. These include, total daily protein amount, intake of protein per meal, distribution of protein along the day and protein quality [4, 6]. The majority of research has been conducted in acute laboratory settings and to date there is a lack of evidence in clinical studies to support this contention. Therefore, active older adults who do not have the confounding variable of sedentary behaviour typically seen in

sarcopenia research may provide an indication of the importance of these aspects of protein intake and their association with outcomes of SMM and function.

A longitudinal study in older adults have shown that protein intakes (1.0-1.2 g/kg body mass (BM)/day) higher than the current guidelines (0.8 g/kg BM/day) have positive associations with SMM, physical function and skeletal muscle strength [10]. However, there have also been conflicting results with other studies that have found no association between higher protein intakes and these outcomes [11, 12]. The PROT-AGE study group have suggested that higher amounts of daily protein intakes (e.,g ≥1.2 g/kg BM/day) would be required for older adults that are considered physically active in order to maintain a positive protein balance [13]. In contrast, a cross-sectional study by ten Haaf et al. [14] found in a cohort of physically active (mean ± SD: 85 ± 53 metabolic equivalents (MET)/week) older (70 ± 4 yrs)) adults that over 50% of participants did not meet the protein requirements of 1.2 g/kg BM/day. This may indicate that active older adults may be at an increased risk of muscle decline and skeletal muscle strength due to the increased requirements for amino acid (AA) utilisation coupled with the progressive degeneration of SMM associated with ageing [4, 15]. In a meta-analysis by Morton et al. [16], minimal effects of protein intakes up to 1.6 g/kg BM/day on outcomes of fat-free mass (FFM) in healthy community dwelling older adults were reported. However, there has yet to be any studies that compared the habitual total protein intake amount on outcomes of SMM, skeletal muscle strength, and physical performance in a cohort of active older adults.

In recent studies, there has been a move towards protein intake per meal, as there is a clear saturable dose-dependent response of AA availability and MPS, which indicate that once MPS has been maximally stimulated there is no further augmentation with higher amounts of protein [17]. This limit has been observed to be 0.4 g/kg BM/meal [17] or 25-35 g (~10 g essential AA) of protein

for older adults [18-20]. This recommendation poses problems for many older adults whose dietary protein is distributed inequitably across meals, with the majority of protein intake at the evening meal [20]. Furthermore, typical Western [12] diets in older adults have insufficient protein at breakfast and lunch to elicit a significant MPS response during this time period [21]. To express the evenness of protein distribution throughout the day, many studies have used a coefficient of variation (CV), where a lower value indicates a more consistent protein distribution [22]. For example, a study by ten Haaf et al. [14] categorised participants according to protein distribution as follows:even (CV < 0.43); intermediate (CV = 0.43-0.62), and uneven (CV > 0.62). The main findings of this study found that an even (CV < 0.43) spread of protein throughout the day was associated with higher gait speed, compared to the intermediate group only, but there were no associations between other performance markers (i.e., chair stand and HGS). Regular intake of protein throughout the day increases the opportunities to maximally stimulate MPS [22, 23]. This accumulation of MPS stimulation throughout the day is likely to lead to long term positive protein balance and subsequently leading to a net positive protein balance which may facilitate adaptations in SMM, skeletal muscle strength, and performance outcomes in active older adults [22, 23]. Therefore, the literature suggests that not only total protein amount, but '*per meal*' amount and frequency of protein intake, may be associated with skeletal muscle mass and strength in ageing adults. Studies conflict in regards to the degree of influence from protein distribution and the number of meals providing adequate protein, especially when this is due to the infrequent use of relative protein intake and absolute protein intake, which cause a major hindrance to comparison [15, 18, 22]. This one-size-fits-all approach for absolute protein intake does not account for differences in body size, and particularly the differences often observed in lean muscle mass and size between biological sexes in active older adults [24]. Therefore, assessing the relative protein intakes across meals is proposed to be more feasible to assess the differencesin habitual protein intake comparing biological sex due to the differences in body mass.

Lastly, the quality of the protein sources that active older adults habitually consume should be taken into consideration. Protein quality is determined by the essential AA profile, digestibility, and AA bioavailability, which dictate the anabolic potential of the dietary protein source [25, 26]. Animal based protein (e.g., dairy, lean meats) are considered better quality proteins due to their higher bioavailability and increased ability stimulate MPS compared to plant based proteins [27]. For example, Lord et al. [28] observed in healthy older women (55-75 yrs) that protein intake from animal sources was on the only independent predictor of SMM, explaining 19% of its variance. Similarly, a higher dairy consumption (≥2.2 servings per day) was associated with a significantly greater lean muscle mass, HGS strength and physical performance in a cross-sectional study of healthy older women (70-85 yrs) [29]. Furthermore, a 3-year longitudinal study in healthy community dwellers found that there was a significant association between total protein and animal protein and changes in lean muscle (mean (SE) 8.76 (3.00) kg and 8.82 (3.01) kg) and appendicular lean muscle (ALM (5.31 (1.64) kg and 5.26 (1.65) kg, respectively)) ($p < .01$) [11]. However, there are limited studies in active older adults and determining if their dietary protein quality may be associated with any outcomes related to sarcopenia.

With the previous discussion point in mind, the purposes of the present study to explore protein intake patterns including: total daily protein intake, evenness of protein intake across the three main meals (e.g., distribution of protein), number of protein meals containing ≥0.4 g/kg BM/meal, and protein quality and 2) to assess associations with outcomes of fat-free mass (FFM), skeletal muscle strength and power in a cohort of active older adults. We hypothesised that these aspects of protein intake would be positively associated with greater FFM, skeletal muscle strength, and skeletal muscle power.

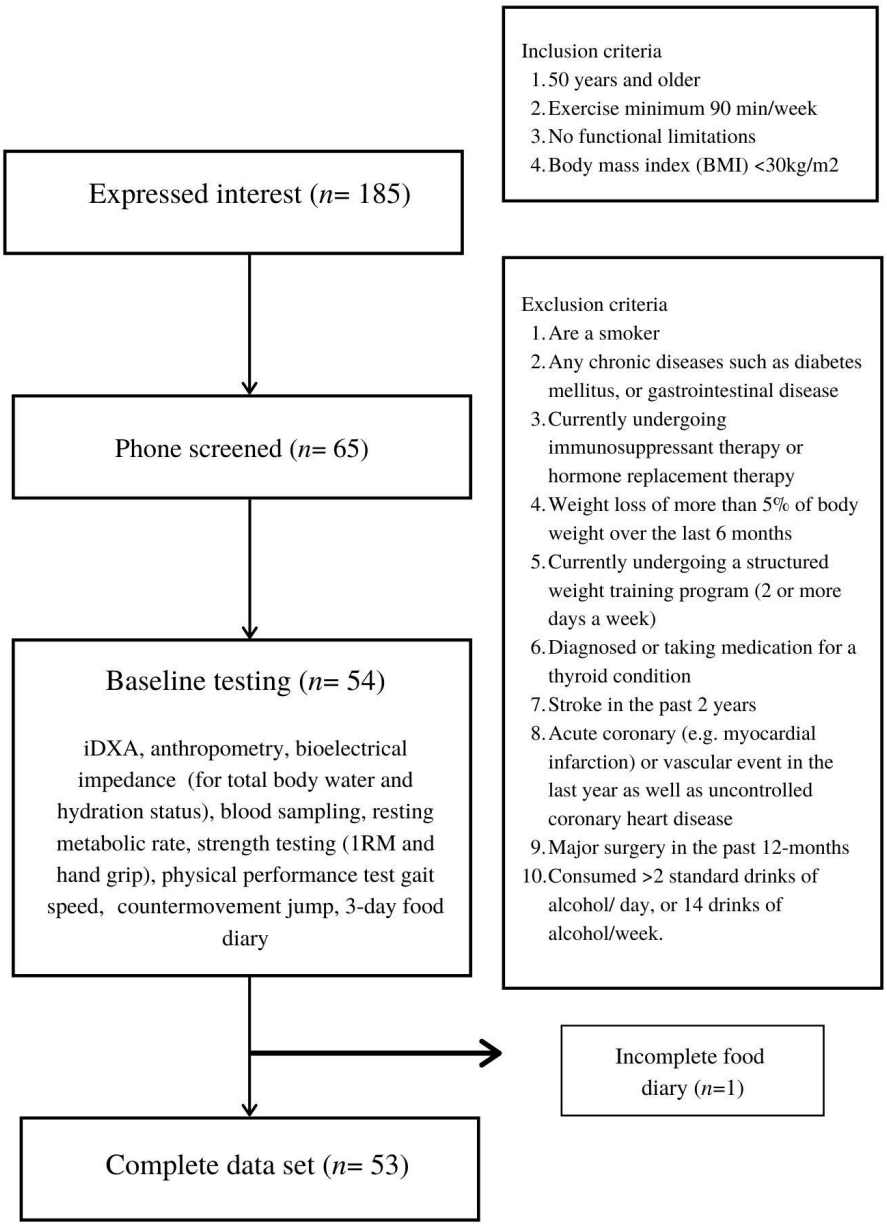

Figure 1. Flow diagram for the identifications, screening, eligibility and participant completion.

5.3 Methods

Active middle-aged and older males (*n*= 36; mean ± SD: age 59.1 ± 6.7 yrs, BM 80.2 ± 13.8 kg, height 1.77 ± 0.07 m), and females (*n*= 18; mean ± SD: age 58.3 ± 7.1 yrs, BM 64.0 ± 11.2 kg, height 1.61 ± 0.06 m) volunteered to participate in the study. Participants were eligible if they were ≥50 yrs, trained for recreational fitness and/or sports competitions at least ≥3 occasions/week, for more than 90 min/week, had no functional limitations, were free from chronic disease, were not taking medications that could interfere with SMM (e.g., corticosteroids, testosterone replacement, or anabolic drugs), were not currently undergoing immunosuppressive therapy or hormone replacement therapy, and did not currently undergo any structured weight training program. All participants were initially screened for eligibility based on the study criteria (**Figure 1**). After participants were deemed eligible all participants gave written informed consent. The study protocol obtained approval from the local ethics committee (Project number 12812). Data were collected during the period of September 2018 to January 2020. See Figure 1 for participant flow.

Physical activity

Participants filled out a physical activity readiness questionnaire (PAR-Q), which they self-reported their level of activity including exercise frequency (e.g., <once per month, once per month, 2-3 times per week, 4-5 times per week, or > 5 times per week), intensity (e.g., vigorous, moderate and light) and modality of training.

Dietary assessment

Participants were educated and asked to complete a 3-day food diary prior to their initial visit. They were required to record all the foods and fluids on each main meal (breakfast, lunch and dinner) and any additional snacks (e.g., morning tea, afternoon tea, supper) ingested on 2 weekdays

(Monday-Friday) and one day on the weekend (Saturday/Sunday) that most reflected their usual intake. Participants were required to specify the food and beverage quantities (e.g., g, ml, litres, portions) and qualities (e.g., cooking method, brands of foods-beverages, types of foods-beverages). These diaries were analysed by an Accredited Practising Dietitian using FoodWorks v10.0 nutritional analysis software (Xyris Software, Brisbane, Australia, 2019) based on Australian food composition tables from Australian Food Composition Database (AFCD) 2019. The average values for the 3 days for total energy, macronutrients and calcium intake were obtained and then total dietary protein intake distributed across breakfast, lunch, dinner, and snacks and per kg/BM were determined. Data were analysed for outliers using boxplot (SPSS statistics software, Version 25.0, IBM Corp, Armonk, NY). Identified outliers were removed prior to comparative data analysis procedures. Protein intake per meal and per day were expressed as absolute (g/day, g/meal) and relative to body mass (g/kg BM/day and g/kg BM/meal). Daily protein intake were compared to 0.8 g/kg BM/day and 1.2 g/kg BM/day. The protein sources that contributed to the participants' intake were also quantified based on the Australian Guide to Healthy Eating [30]. Protein intake were separated by animal based and plant based sources. Animal based proteins included 65 g beef, 80 g chicken, 100 g (edible portion) of eggs, 65 g processed meats (e.g., salami, ham), 100 g seafood. Plant based protein sources included; 30 g nuts and/or seeds, 170 g tofu, beans or legumes [30, 31]. Dairy foods included 250 ml cow's milk, 40 g hard yellow cheese, 120 g soft white cheeses (e.g., ricotta) and 200 g yoghurt. Dairy alternatives included beverages that included the equivalent calcium content to 250 ml of bovine dairy milk (300 mg calcium) [30, 31].

Anthropometry and body composition

For measurements of outcome variables, participants were required to attend the laboratory for the period between 7.00 am to 9.00 am in a fasted and euhydrated state (plasma osmolality: 296 ± 5.6 mOsmol/kg; 53.3 ± 6.4% TBW; Seca 515 MBCA, Seca Group, Hamburg, Germany), and after avoiding strenuous exercise for a 24 h period. Height was assessed using a fixed stadiometer (stadiometer; Holtain, Crosswell, Crymych, UK) and BM was determined (Seca 515 MBCA, Seca Group, Hamburg, Germany) to the nearest 0.1 kg. FFM was obtained by a trained radiographer using a dual-energy X-ray absorptiometry (iDXA, Prodigy, GE Lunar, Madison, WI; with analysis software 14.10). Appendicular lean mass (ALM) was determined by adding the total arm and trunk lean mass and then it was adjusted for height (ALM/height^2). Relative fat mass (%), and absolute fat-free mass (kg) were also obtained from the iDXA. To comply with ethical procedures, prior to commencing the strength, power, and performance measures, participants were provided with a standardised breakfast (1.4 MJ, 15.3 g protein, 51.7 g carbohydrates, 6.8 g fat). Physical assessment measures commenced approximately 30 min thereafter.

Leg strength, skeletal muscle power and handgrip strength

Leg strength was assessed by performing a 1 repetition maximal strength (1-RM) using a 90° leg press machine (Hammer strength; LifeFitness, Sydney, Australia) using previously described procedures [34]. Testing began after a familiarisation trial where proper lifting technique was demonstrated, then participants were familiarised with each resistance machine by performing 8-10 repetitions of a light load (~50% of predicted 1-RM). After successful completion of a further five to six repetitions at a heavier weight selected by the instructor, the workload was increased incrementally until only one repetition with correct technique could be completed. Participants

were given 3-5 min rest in-between attempts [33]. The value indicative of 1-RM was the highest load that could be raised in one single repetitions using correct technique for leg press.

A Force plate (400s+ Performance Force plate; Fitness Technology, Adelaide, Australia) was used to measure relative muscle power (W/kg) during a countermovement jump test (CMJ). Participants were asked to start in a fully erect standing position in the middle of the force plate, then instructed to dip to a self-selected depth and "*jump for maximal height*". Hands were kept on the hips to minimize any influence of arm swing [34]. Participants were asked to perform three attempts of a CMJ with 1 min rest between jumps. The Force plate was interfaced with computer software Ballistic Measurement System; Fitness Technology, Adelaide, Australia), where the mean of three jumps were further analysed.

HGS was measured using a digital hand dynamometer (Jamar® Plus+ Digital hand dynamometer; Sammons Preston, Bolingbrook, IL, USA). HGS was measured in a standing position with the participants elbow by their side and flexed to a 90° angle and a neutral wrist position. Participants were asked to apply the maximum grip strength three times with both left and right hands, HGS was defined as the highest value for their dominant hand [35].

Statistical analysis

Data in text and tables are presented as either mean ± SD (descriptive experimental data) or mean and 95% confidence interval (CI) (primary and secondary variables), where indicated. All statistical analyses were performed using IBM SPSS statistics software (Version 25.0, IBM Corp, Armonk, NY). Prior to analysis, assumptions of normality in the data were made using Shapiro-Wilk test and visualisations of normality plots. Variables with singular points were examined using independent t-tests or non-parametric Mann-Whitney U tests'. Significance was accepted at $p \leq .05$. To test

associations between protein intake and CV of protein, and number of meals containing ≥0.4 g/kg BM with outcomes of strength, muscle mass and power, Pearson's correlation analyses were used. Significance was accepted at p≤ .05. Additionally, when significant differences were identified a Cohen's *d* analysis were applied to determine the magnitude of effect size for significance differences, with *d* ≥ .20 for small, *d*≥ .50 for medium, and *d*≥ .80 for large effect size.

Table 1. Characteristics of the total active ageing sample of older adults and subgroups of males and female participants.

	All *n*= 53	Males *n*= 36	Females *n*= 17	*p*-value
Age, yrs	59 (57 to 61)	59 (57 to 61)	58 (55 to 62)	.702
Height, m	1.71 (1.70 to 1.75)	1.77 (1.74 to 1.80)	1.62 (1.60 to 1.75)	< .001
BM, kg	74 (70 to 78)	80 (75 to 85)	64 (55 to 62)	< .001
BMI, kg/m^2	25 (22 to 26)	25 (24 to 26)	24 (22 to 26)	.250
Exercise volume, min/week	226 (191 to 260)	229 (193 to 265)	213 (134 to 291)	.374
iDXA measurements				
FFM, kg	54 (50 to 57)	59 (56 to 62)	42 (39 to 45)	< .001
FM, %	28 (26 to 31)	26 (23 to 28)	34 (29 to 38)	< .001
ALM/ht^2	7.7 (7.3 to 8.1)	8.4 (8.0 to 8.7)	6.5 (6.1 to 7.0)	< .001
Leg strength, leg power, and hang grip strength				
Leg press, kg/kg BM	1.9 (1.8 to 2.1)	2.1 (1.9 to 2.2)	1.7 (1.4 to 2.0)	.011
Relative power, W/kg BM	29 (28 to 31)	30 (28 to 32)	27 (24 to 30)	.048
HGS, kg	37 (33 to 40)	42 (40 to 45)	25 (23 to 29)	< .001

Mean (95% CI). Abbreviations: ALM: appendicular muscle mass, BM: body mass, BMI: body mass index, FFM: fat-free mass, FM: fat mass, and W: watts.

5.4 Results

Participant characteristics

Table 1 presents the participant characteristics. Of the 54 participants included in the data collection, 53 were included in the analysis, due to an incomplete food diary (Figure 1). Participants were 86% Caucasian and 14% Asian Participants were only aerobically trained and from a variety of sporting backgrounds including endurance runners and race walkers (61%), cyclists (9%), aerobic gym goers (16%), or a combination of multiple activities (14%). Based on the European Working Group of sarcopenia 2 (EWGOS2) clinical diagnosis 6% (*n*= 3) had low HGS and were considered to have probable sarcopenia [1].

Macronutrient intakes and total protein intake

Average daily energy and macronutrients are presented in Table 2. All participants reported 3 main meals, 2 participants (1 male and 1 female) did not consume snacks. Total energy, carbohydrates, fat and relative protein intake (g/kg BM/day) in males were significantly higher than in females (Table 2). Over the 3-day measurement period 81% of males and 100% of females, had ≥0.8 g/kg BM/day of protein intakes (Figure 2). Relative protein intake was on average 1.3 (1.2 to 1.5) g/kg BM/day for males and 1.4 ± 0.1 g/kg BM/day for females. However, only 60% of females and 72% of males met the threshold for protein based on 1.2 g/kg BM/day (Figure 2).

Protein distribution

Based on absolute protein intake per meal, males had significantly higher intakes of protein at breakfast, but not at any other meal (p= .002, d= .950) (Table 2). There were no significant differences between groups based on relative protein intake. The mean number of daily meals providing at least 0.4 g/kg BM ranged from 0 to 2.8, while 4.1% of men and no women consumed two or more meals per day providing at least 0.4 g/kg BM of protein on each of the 3 days days.

Meal-specific relative protein intakes are presented in **Figure 2**. The CV for the whole cohort was 0.46 (0.41 to 0.51). The CV was higher in the females compared to the males, although not statistically significant (*p*= .422*)*. Protein intake at breakfast, lunch, dinner, and snacks contributed 0.26, 0.38, 0.56 and 0.058 g/kg BM/meal for males and 0.19, 0.57, 0.86, 0.073 g/kg BM/meal for females (**Table 2**). On a meal-basis the proposed threshold for maximal MPS (0.40 g/kg BM) was met for the majority males (85%), and females (89%) at dinner only (**Table 2, Figure 2**). **Figure 3** indicates within group differences of protein at different meals and snacks. There were significant differences in protein intake for both groups with lunch ≥ breakfast, and dinner ≥ lunch and breakfast. Snacks were not consumed as a single meal; therefore is it not possible to determine whether dietary protein MPS thresholds were met for individual snacks.

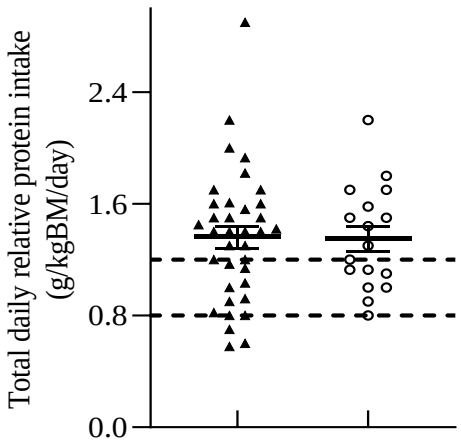

Figure 2. Total daily relative protein intake for ▲ males ○ females. Scatter plots display individual means. Dashed lines indicates recommended daily allowances for dietary protein allowance of 0.8 g/kg BM/day and 1.2 g/kg BM/day.

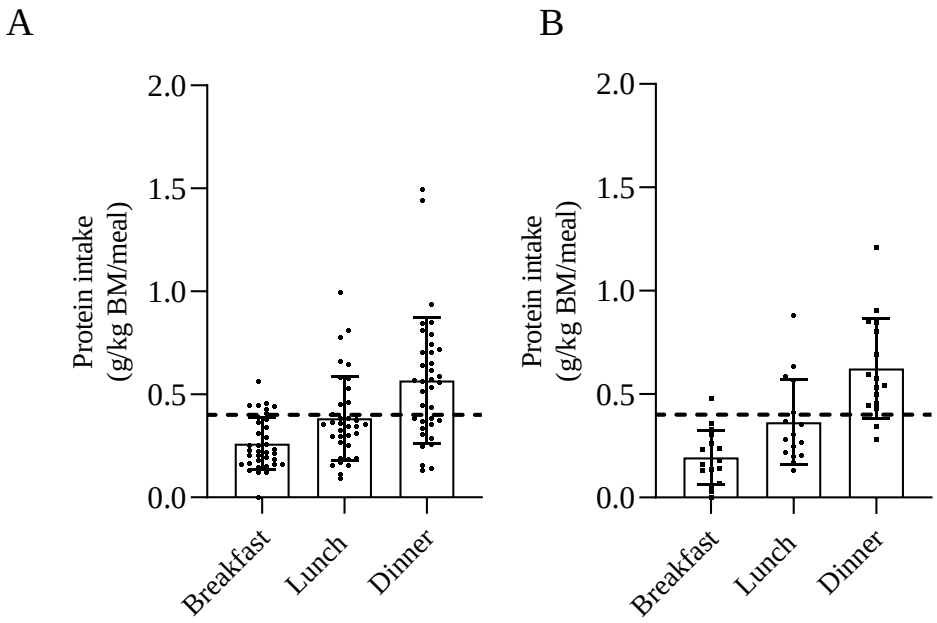

Figure 3. Meal protein distribution intakes in **(A)** Males and **(B)** Females. Dashed line represents threshold protein intake of 0.4 g/kg BM/ meal, suggested for maximal stimulation of MPS in older adults.

Table 2. Daily mean dietary intake data obtained from 3-day food diaries for active older adults and subgroups of male and female participants.

	All	Males	Females	*p* –value
	n= 53	*n*= 36	*n*= 17	
Energy and Macronutrient intake				
Energy, MJ	2.1 (1.9 to 2.3)	2.2 (2.0 to 2.3)	1.8 (1.6 to 2.0)	**.019**
Carbohydrates, g	204 (184 to 225)	221 (195 to 247)	168 (143 to 196)	**.015**
Fat, g	81 (75 to 87)	86 (79 to 93)	70 (59 to 80)	**.011**
Protein, g	98 (91 to 106)	106 (96 to 116)	83 (74 to 92)	**.006**
Protein, g/kg BM	1.4 (1.2 to 1.5)	1.3 (1.2 to 1.5)	1.4 (1.2 to 1.6)	.789
Absolute protein intake per meal				
Protein at breakfast, g	173 (15 to 20)	20 (17 to 24)	18 (15 to 20)	**.002**
Protein at lunch, g	28 (24 to 32)	30 (25 to 35)	14 (18 to 30)	.157
Protein at dinner, g	43 (37 to 48)	44 (36 to 51)	40 (33 to 48)	.557
Protein snacks, g	4.7 (3.7 to 5.8)	4.7 (3.4 to 6.5)	5.0 (3.3 to 6.5)	852
CV of protein	0.46 (0.41 to 0.51)	0.43 (0.37 to 0.48)	0.52 (0.41 to 0.65)	.078
Relative protein intake per meal				
Protein at breakfast, g/kg BM	0.2 (0.2 to 0.3)	0.2 (0.2 to 0.3)	0.2 (0.1 to 0.3)	.200
Protein at lunch, g/kg BM	0.4 (0.3 to 0.4)	0.4 (0.3 to 0.4)	0.4 (0.3 to 0.5)	.888
Protein at dinner, g/kg BM	0.6 (0.5 to 0.7)	0.6 (0.5 to 0.7)	0.6 (0.5 to 0.8)	.577
Protein at snacks, g/kg/BM	0.1 (0.0 to 0.1)	0.1 (0.0 to 0.1)	0.0 (0.0 to 0.1)	.265
Number of protein serves, >0.4 g/kg	1.1 (0.9 to 1.3)	1.0 (0.7 to 1.3)	1.2 (0.9 to 1.6)	.422

Mean (95% CI). Abbreviations: BM: body mass, CV: coefficient of variance, MJ: megajoule.

Protein quality

The types of protein foods that were consumed based on a 3-day food diaries are presented on Table 3. There were no differences between groups in relation to protein serves or the source of protein. Both groups received the majority of their protein serves from animal based sources (1.9 *vs*. 1.8 serves) for males and females, respectively. Both groups had similar dairy serves intakes with the majority of their dairy source deriving from milk (60-65%).

Table 3. The average number of protein serves per day, categorised by source (animal or plant) and dairy based and dairy alternatives obtained from a 3-day food diary for active older adults and subgroups of male and female participants.

Average protein serves based on quality	All *n*= 53	Males *n*= 36	Females *n*= 17	*p*-value
Total Protein serves/ day	2.7 (2.4 to 3.0)	2.8 (2.5 to 3.2)	2.4 (1.8 to 3.0)	.573
Animal based/day	1.9 (1.6 to 2.2)	1.9 (1.6 to 2.2)	1.8 (1.2 to 2.3)	.573
Plant based/day	0.8 (0.6 to 1.0)	0.8 (0.5 to 1.1)	0.6 (0.3 to 1.0)	.405
Total dairy serves/day	2.1 (1.7 to 2.5)	2.3 (1.8 to 2.5)	1.7 (0.9 to 2.5)	.131
Milk/day	1.3 (1.0 to 1.6)	1.5 (1.1 to 1.8)	1.0 (0.5 to 1.5)	.143
Cheese/day	0.4 (0.2 to 0.6)	0.5 (0.2 to 0.7)	0.4 (0.0 to 0.7)	.633
Yoghurt/day	0.3 (0.2 to 0.4)	0.4 (0.2 to 0.5)	0.2 (0.1 to 0.3)	.218
Dairy alternatives/ day	0.1 (0.0 to 0.1)	0.0 (0.0 to 0.1)	0.1 (0.0 to 0.2)	.764

Mean (95% CI).

Associations between aspects of protein intake and skeletal muscle mass and strength

The correlations between aspects of protein intake and physical measures are presented in Table 4. The number of daily meals providing ≥0.4 g/kg BM of protein had a small inverse association with ALM/ht^2 (r= -383, p= .005) across the whole cohort, and in males (r= -.385, p= .020). There was a significant positive association between number of meals providing >0.4 g/kg BM/day and 1-RM leg strength in males (p< .001) and when the data was pooled with females (r= .301, p= .029). There were no significant correlations for women in any of the outcomes. However, when the data were pooled there was a small negative correlation between HGS and number of meals with >0.4 g/kg of protein per meal (r= -.322, p= .019). No other significant correlations were found. There was a significant positive association between leg strength and number of total protein serves (animal and plant based) per day (r=.290, p= .035) and number of dairy serves per day (r= .372, p= .006).

Table 4. Correlations between mean daily intake of protein, mean coefficient of variants of protein across main meals, mean number of meals providing at least 0.4 g/kg BM protein, protein quality and skeletal muscle mass, leg strength, relative leg power, and hand grip strength in active older adults and subgroups of males and females.

	ALM/ht^2			LP Kg/kg/BM			MP W/kg			HGS kg		
	All *n*= 53	Males *n*= 36	Females *n*= 17	All *n*= 53	Males *n*= 36	Females *n*= 17	All *n*= 53	Males *n*= 36	Females *n*= 17	All *n*= 53	Males *n*= 36	Females *n*= 17
Daily protein intake, g/kg BM/day	-0.203	-0.280	-0.049	0.290*	0.591*	0.286	0.204	0.012	0.477	-0.318	-0.257	-0.083
CV of protein intake at main meals	-0.254	-0.073	0.321	0.021	-0.201	0.081	0.208	0.204	0.375	-0.202	-0.318	0.030
Number of meals per day providing ≥0.4 g/kg BM	-0.383*	-0.385*	-0.297	0.301*.	0.591*	0.262	0.046	0.092	0.059	-0.322*	-0.318	-0.269
Number of protein serves per day	-0.203	0.228	-0.180	0.290*	0.120	-0.226	0.137	0.398	0.211	-0.199	0.00	0.193
Number of animal based protein serves per day	-0.004	0.109	-0.182	0.115	0.144	0.073	-0.043	-0.139	-0.079	0.200	0.204	0.201
Number of dairy serves per day	-0.008	-0.351	-0.100	0.372*	0.146	0.326	0.100	-0.148	0.280	0.127	0.091	-0.018

Mean (95% CI). **Abbreviations:** ALM: Appendicular muscle mass, BM: body mass, HGS: handgrip strength, MP: muscle power, LP: leg press, and W: watts. * $p< 0.05$ significant correlation

5.5 Discussion

This cross-sectional study explored protein intake patterns including, daily protein amount, distribution of protein intake across the three main meals, number of meals containing ≥0.4 g/kgBM/meal of protein, protein quality, and associations between these protein intake patterns and outcomes of FFM, skeletal muscle strength and power in a cohort of active older adults.. In support of the hypothesis, the results of this current study observed positive associations between number of meals/day providing ≥0.4 g/kg BM and 1-RM leg press. When stratified based on biological sex this positive association was only significant for males and not females. Furthermore, there was a significant positive association with total protein serves and dairy milk protein serves and 1-RM leg strength. Contrary to the initial hypothesis, the remaining outcomes showed an inverse association between dietary protein patterns and outcomes of ALM/ht^2 and HGS, but no significant association with skeletal muscle power. Overall, these findings suggest that the number of high protein meals and the quality of the protein may further improve outcomes of skeletal muscle leg strength in active older adults that already consume high amounts of protein (≥1.4 g/kgBM/day).

The current study observed no significant positive association between relative total daily protein intake with outcomes of ALM/ht^2, leg strength, leg power, or HGS. Similarly, Gingrich et al. [12] observed in a cohort of healthy community dwellers (75-85 yrs) no associations between total daily protein intake (mean ± SD: 0.97 ± 0.28 g/kg BM/day) and outcomes of skeletal muscle index, leg power, leg muscle strength, or HGS. Another study of older (81 ± 6 yrs) community dwellers found no association between higher total daily protein intakes (≥1.0 g/kg BM/day) and outcomes of HGS, physical function (Short Physical Performance Battery (SPPB)), and quality of life [36]. Moreover, Houston et al. [11] found no cross-sectional association in ALM in older community dwellers (70-

79 yrs) consuming 0.8 or 1.2 g/kg BM/day of protein. In contrast, the Women's Health Initiative (WHI) [10] compared the total daily protein intake of menopausal women (50-79 yrs) with outcomes of strength (i.e., HGS). The findings of the WHI study observed that women with higher total daily protein intakes (1.19 g/kg BM/day) had a small, but significantly higher HGS (24.7 kg vs 21.1 kg, $p < .036$) compared to those that reported lower intakes of protein (<0.9 g/kg BM/day) [10]. Additionally, a systematic review and meta-analysis found that very high (1.5 g/kg BM/day) and high (1.3 g/kg BM/day) protein intakes were associated with a small but significant effect (95%CI: 0.67, 0.56 to 0.82) on outcomes of knee extensor strength, walking speed and SPPB, when compared to low protein intakes (<0.8 g/kg BM/day) [37]. The absence within the current study of a positive effect of a higher total daily protein intake on these parameters might be explained by the fact that average relative protein (1.4 ± 0.43 g/kg BM/day) intake was higher than the proposed alternative protein intake for active older adults (1.2 g/kg BM/day), and was met by the majority of females and males in the current cohort (60% and 72%, respectively); this is much higher than previous reports of protein intakes in active older adults [14]. Additionally, our sample population was healthy and active, and a low portion of the sample size (6%) had low HGS [1]. Comparing these findings to the systematic review that reported lower HGS (24.3 kg), and older population (~74 yrs). These findings may suggest that associating protein intakes with outcomes of SMM, strength, and performance may only be relevant for older (> 70 yrs) populations with lower functional parameters.

There was an uneven distribution of protein observed in this study with smaller amounts of protein reported at breakfast (17.7 ± 9.3 g) compared to lunch (28.1 ± 19.8 g) and dinner (42.7 ± 19.8 g). These observations are consistent with previously reported studies in protein intake in older adults (72-88 yrs) which have observed a skewed intake with the majority of daily protein consumed

towards the evening mealtime [38, 39]. The degree of protein distribution based on the coefficient of variation (CV) for protein intake among the main meals, from this study showed that males had a slightly more balanced distribution (0.43 ± 0.16) compared to females (0.52 ± 0.23), however there was no significant differences between groups (p= .078). Additionally, there was no significant correlation between the balance of protein at main meals and any of the outcomes of muscle mass or function. Similarly, Gingrich et al. [12] did not observe any statistical significant association between protein CV and outcomes of muscle mass, strength and power. The Gingrich et al. [12] study had a similar cohort to the present study (i.e., more homogenous and '*healthier*' study group) and reported a CV that was similar for males (0.51 ± 0.19) and females (0.55 ± 0.17). In contrast, Bollwein et al. [21] categorised participants into non-frail, pre-frail and frail and aimed to determine whether protein distribution was associated with frailty. Their main findings were based on protein skewness, showing that the participants categorised as *'frail'* had a significantly higher CV (0.76 (0.18-1.33)) compared to the pre-frail (0.76 (0.07-1.29)) and non-frail (0.68 (0.15-1.24)) indicating a more skewed protein intake in the frail group. This was despite no significant difference comparing total daily protein intake across groups (~1.1 g/kg BM/day). The rationale for the per-meal recommendations are largely based on acute studies that investigate the maximal saturation of AA per meal on outcomes of MPS [15, 17]. These studies have indicated that the MPS response is dose-dependent and protein intakes above this saturation point are irrelevant as MPS does not increase with larger protein doses (e.g. >45 g/meal) [17]. Therefore, the provision of a protein dose that will elicit MPS to the maximum degree over the day may directly impact skeletal muscle mass and function. However, the results from this study do not support this notion and it may be only relevant for adults that are considered frail and as observed in the study by Bollwein et al. [21]. Considering the participants in this study and Gringrich et al. [12]. were considered healthy and active, the physical activity alone may be sufficient to elicit a MPS and mitigate the

age-related anabolic resistance. Therefore, active older adults that are consuming adequate amounts of total daily protein may offset the negative consequences of skewed protein distribution, as seen in the current literature.

The number of daily meals providing ≥0.4 g/kg BM of protein had a small inverse association with ALM/ht^2 in males and across the whole cohort for HGS. Indicating the more meals providing ≥0.4 g/kg the lower the HGS and ALM/ht^2. Considering that this cohort were considered healthy and free from functional decline, with a low proportion (6%) to have low HGS [39] it is likely that these measurements (HGS and ALM/ht^2) may not represent true physiological change in an active older population. Therefore, HGS and ALM/ht^2 may not be sensitive enough to detect any clinical relevance in relation to skeletal muscle strength and FFM in an active ageing cohort, therefore it is likely that the inverse relationship within this cohort does not hold any practical significance in this cohort. Direct measures of leg strength may be more practically significant. In this study there was a small positive association with number of meals providing the proposed amount to maximally stimulate MPS and 1-RM leg press for the whole cohort and when stratified based on biological sex, only men had a significant association. The average number of meals providing the amount of protein (e.g., 0.4 g/kg BM per meal) to maximally stimulate MPS was 1.1 (95% CI:0.86 to 1.3) with the evening meal reaching this threshold for the majority of participants (69%), whereas lunch was met by 28% and breakfast by 13%. There have been observational studies that have reported a positive association of protein intake per meal and greater outcomes of leg muscle mass [15] and physical function [41]. Of these, an observational study [15] found that in a cohort of older adults (50-85 yrs) consuming 1 or 2 meals with ≥30 g (~0.38 g/kg BM per meal) of protein was associated with higher peak knee strength (383 and 434 Newtons (N) and leg lean mass (15.2 and 16.6 kg) compared to those consuming zero meals (377 N and 15.0 kg) containing ≥30 g of protein [15].

Gaytán González et al. [40] observed that older adults (≥60 yrs) consuming ≥2 meals/day containing ≥30 g of protein had a lower risk of disability compared to those that consumed 0-1 meals. Contrastingly, Gringrich et al. [12] did not find any statistical significant differences with those consuming ≥0.4 g/kg BM of protein and outcomes on FFM, skeletal muscle strength, and skeletal muscle power. Another cross-sectional study of healthy older adults (68.7 ± 6.3 yrs) found no differences in outcomes of appendicular lean mass when comparing those eating at least one meal containing ≥25 g of protein and those who had none [41]. Although there are mixed results, studies that found a positive association between number of meals and relative muscle outcomes had cohorts that were consuming less total daily protein than the recommended amount (i.e. <0.8 g/kg BM). Additionally, it is difficult to compare the results across studies as they differ in study design (e.g., longitudinal vs cross-sectional), outcomes (frailty, grip strength), age and physical health (frail, community-dwelling compared to trained older adults in this study) of the participants. The results of this study suggest that active older adults consuming adequate amounts of daily protein (including 1 meal/day of ≥0.4 g/kg BM) results in favourable outcomes in leg strength for males, but not females. However, considering the low compliance rate (37%) of meals reaching ≥0.4 g/kg BM it is uncertain whether if there was a comparator group with higher compliance if this association would differ. A larger sample size would overcome this limitation in future research.

Although there have been numerous cross-sectional and observational studies reporting mixed findings of the association between dietary protein patterns related to outcomes of SMM and physical performance, this is the first study to assess the relationship of these outcomes in a cohort of active older adults. The threshold of 0.4 g/kg BM/meal derives from acute studies that measure the maximum amount of protein required to reach the threshold for MPS, using high quality single type protein sources (i.e., whey protein isolate) in healthy older men (~71 yrs) [17]. Considering

that this study measured real-life examples of meals that contain different types of protein in mixed meals that may have altered the AA absorption kinetics. For example carbohydrate co-ingestion has been shown to delay protein digestion and absorption [42]. Furthermore, co-ingestion of certain vitamins and micronutrients have been shown attenuate or impair MPS [43, 44]. It is likely that differences in meal composition effects the absorption kinetics of protein, possibly effecting long term FFM, skeletal muscle strength, and skeletal muscle power outcomes. Therefore, higher amounts of protein in mixed meals may be required to elicit the same response in a mixed meal compared to the suggested amount derived from single protein studies.

When considering the protein quality of the habitually consumed meals, there was no significant differences between males and females. Both groups received the majority of their protein sources from animal based proteins (70%). There were no significant differences between the dairy serves with the majority of the dairy sources deriving from milk (60%) followed by cheeses (20%). Furthermore, there was a small but significant association between the total number of protein serves and the number of dairy serves per day and leg strength, but no associations observed with number of animal protein serves. Similar findings have been observed, where there was a significant associations between higher dairy serve consumption (≥2.2 servings per day) and significantly greater FFM, HGS and physical performance in a cohort of healthy older women (70-89 yrs) [29].

Furthermore, intervention studies within our laboratory have shown that a high-protein dairy milk beverage with progressive resistance training (PRT) program, lead to significantly greater adaptations in maximal strength, that were greater than PRT alone, after 12-weeks in a cohort of active older adults [45]. The components of dairy based foods that lead to the maintenance and

improvement of strength and function are still unknown. Dairy based foods contain high levels of the branched-chained amino acids (BCAAs), leucine. Leucine acts as a substrate for MPS and also directly acts as a key anabolic signal for the activation of the mammalian target of rapamycin complex 1 (mTORC1) and other protein phosphorylation involved in skeletal muscle anabolic responses [46]. In addition, dairy milk foods contain other bioactive components (e.g., calcium, whey, casein, vitamin A, E) that may act synergistically with protein to improve outcomes of skeletal muscle mass [47]. Nonetheless, the participants within this study did not achieve the Australian Dietary Guidelines for milk, yoghurt, cheese and or/ alternatives for their age groups (2.5 to 3.5 serves for men, and 4 serves for women), therefore adding additional dairy products to habitual diet may be a promising strategy to improve protein intake in active older adults.

The current study included the use of a 3-day food diary to infer daily dietary intake patterns. While these are acknowledged to be potential limitations due to the nature of using self-reported measures, based on the reported intake of the same cohort in a intervention study from this study group [45] it was observed that the energy and protein intakes of the 3-day food diaries did not change in the control groups over the course of a 12-week trial. For example, average relative protein intakes were reported to be 1.6 g/kg BM/day at baseline and 6-weeks and 1.4 g/kg BM/day at 12-weeks without any significant differences. Therefore, considering that the 3-day food diary was used and was consistently shown to be similar over 12-weeks it is likely to reflect their true food intake in the current study. Lastly, it is acknowledged that this current study included a relatively small sample size. However, we believe that findings may provide initial preliminary data to help contextualise future intervention trials and identifies methodological gaps in sarcopenic research in active older adults.

5.6 Conclusion

This study showed that total protein intake, CV of protein intake, and daily number of meals containing ≥0.4 g/kg BM of protein and protein quality may provide an explanation for variations in outcomes related to FFM, strength and power in a cohort of active older adults. Total protein intake may not be strongly correlated to these outcomes in individuals who already meet and exceed the adequate amount of total protein required for active older adults (>1.2 g/kg BM/day). However, higher protein intakes may offset any negative associations commonly observed from skewed protein intakes. Further comparisons indicate that a minimum of 1 meal containing ≥0.4 g/kg BM of protein and a higher consumption of dairy intake may be required to result in favourable outcomes in leg strength.

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CHAPTER SIX:

The effects of a high protein dairy milk beverage with or without progressive resistance training on fat-free mass, skeletal muscle strength and power and functional performance in healthy active older adults: a 12-week randomized control trial.

The following chapter appears on the next page as accepted for publication in *Frontiers of Nutrition*

Huschtscha Z, Parr A, Porter J, Costa RJ. The Effects of a high-protein dairy milk beverage with or without progressive resistance training on fat-free mass, skeletal muscle strength and power, and functional performance in healthy active older adults: a 12-week randomized controlled trial. Front Nutr. 2021; 8:644865.

6.1 Abstract

The study aimed to investigate the independent and combined effects of consuming a high protein dairy milk beverage, twice daily, with or without a progressive resistance training (PRT) program on outcomes of age related sarcopenia, in healthy active older (≥50 yrs) adults. In this 12-week, 2x2 factorial study, participants were randomly allocated into one of four groups: Dairy milk beverage (DM), exercise and dairy milk beverage (EX+DM), exercise alone (EX), and control (CON). The EX group underwent a 12-week whole-body PRT schedule (x3 sessions/week) and the high protein dairy milk beverage (DM) was consumed twice daily (30g protein/day). At week 0, 6, and 12, body composition (iDXA), strength (1-repetition maximum (1RM): leg press, chest press, lateral (*lat)* pull down, and handgrip), power (counter movement jump), cardiorespiratory fitness (VO_2), and physical performance (gait speed) were measured. Before measures, blood samples were collected to determine immune (i.e., leukocyte trafficking and inflammatory cytokines) and hormonal (i.e., insulin, cortisol, IGF-1, testosterone, and estradiol) profiles. Participants (*n*= 37) completed the study within the controlled experimental conditions. Protein intake increased in EX+DM (mean±SD: 1.2±0.2 to 1.8±0.4g/kg BM/day) and DM (1.3±0.5 to 1.8±0.6g/kg BM/day) groups during the intervention. Absolute fat-free mass increased in *EX*+DM (mean [95% confidence interval]: 0.65 [0.25, 1.0] kg) and EX (0.49 [-0.44, 1.40] kg) groups (*p*<.001)*,* compared to DM (-0.54 [-1.6, 0.05] kg). Relative fat mass *d*ecreased (group*time, *p*=.018*)* in DM (-1.8 [-3.3, -0.35] %) *and EX+DM* (-1.3 [-2.3,-0.31*]%),* which was a greater reduction than the control (CON) (0.10 [-0.80, 1.0] % group (*p*<.01). Relative maximal strength increased in both EX and EX+DM (≥35%; *p*<.05) groups, but not in DM and CON groups. The change in 1RM strength outcomes was higher in

EX+DM compared to all other groups (53-78%; p<.01). There was an increase in resting plasma IL-10 concentration in EX+DM (88%), compared to all the other groups (p=.016). No other differences in systemic inflammatory cytokines were observed. There were no significant changes in all hormone concentrations measured between all groups. In conclusion, a high protein dairy milk beverage providing additional protein did not further enhance the effects of PRT on outcomes of fat-free mass, power, or physical performance. However, there was a significant augmentative effect for high protein dairy milk consumption on changes to maximal strength outcomes during PRT in healthy active older adults.

Keywords: Exercise, leucine, calcium, inflammatory cytokines, insulin, insulin-like growth factor, testosterone, estradiol, cortisol.

Keyfindings:

- There was a significant increase in FFM, and maximal strength (e.g., leg press, chest press and *lat* pull down) in both groups that recived 12-weeks of PRT (EX+DM and EX).
- Relative maximal strength was augumented in the group that recived the high protein dairy milk beverage and exercise, which was greater than all other groups.
- There was an increase in resting plasma IL-10 concentration in EX+DM compared to all the other groups

6.2 Background

There has been considerable research exploring the age-related decline in skeletal muscle mass and function (e.g., strength, power, and performance measures), collectively known as sarcopenia [1, 2]. The multifactorial (e.g., training status, biological sex, age, and nutrition status) and dynamic (e.g., hormonal and immunological) pathophysiological process of sarcopenia is complex, and currently there is limited evidence to support the efficacy of pharmacological treatments [4, 5]. Therefore, there has been an increased interest in modifiable lifestyle factors such as exercise (e.g., resistance training) and nutrition (e.g., dietary protein), for treatment and management of age-related sarcopenia. To date, there have been numerous randomized controlled trials and meta-analyses that have consistently reported that progressive resistance training (PRT) can effectively improve gains in fat-free mass (FFM) (~1.2 kg), maximal strength (≥ 25%), and physical functional performance (e.g., gait speed) in older adults (> 50 yrs) [6-8]. There is no consensus regarding the effect protein supplementation (e.g., whey protein, casein, essential amino acids (EAA), and/or leucine) on augmenting further adaptations of FFM, skeletal muscle strength, and power following PRT [9 - 11]. Many studies are confounded by the inclusion of frail institutionalized or sedentary community-dwelling adults often referred to as 'older adults' who are predominantly aged ≥ 60 yrs, the varied use of supplementation (e.g., type, form, dose, and frequency) and outcome measures, and the large variation of baseline habitual protein intakes [2, 9, 10]. Active older adults that regularly engage in physical activity (≥ 50 yrs, 150 min/week of light- (e.g., 3 - 5 metabolic equivalents (METS) to moderate-intensity (e.g., 6-9 METS) physical activity, or 75 min/week of vigorous-intensity (e.g., > 9 METS) physical activity) [12], either recreationally or competitively, still show signs of age-related sarcopenia [13]. Although active older adults do not have the confounding variables attributed to frailty, sedentary behavior, and/or disease (pathogenic hormonal and/or inflammatory status), they are currently underrepresented in sarcopenia

research. While ≥ 50 yrs is not considered 'older' in the spectrum of sarcopenia research, it is the age at which sarcopenia begins to be noticeable [6-8]. Furthermore, given the potential efficacy of pairing PRT with protein supplementation, nutritional interventions in active older adults are limited and require further exploration to examine their effectiveness in this population.

Higher daily protein intakes (> 1.2 g/kg BM/day) have been suggested for active older adults exceeding the current recommended dietary allowance (RDA) of protein (e.g., 0.8 g/kg BM/day), to overcome the increased requirements of amino acid utilization from the exercise stimulus and the blunted response to muscle protein synthesis (MPS) known as '*anabolic resistance*' [14, 15]. Moreover, evenly-distributed relative and absolute protein intakes per meal have been advocated, as there is an observed maximum capacity for the utilization of EAAs [16]. Optimal doses of protein per meal to elicit a near-maximal response have been reported at ~25-35 g/meal (~10 g EAA) [17, 18] or relative amounts of 0.40 g/kg BM/meal [19]. Numerous cross-sectional studies have observed skewed distributions of protein intake across the day, often not reaching the adequate threshold at breakfast and lunchtime for older adults [20, 21]. The unevenness of protein distribution across the day has been associated with higher levels of frailty [22] in older (≥75 yrs) community-dwelling individuals. However, in a cohort of 'healthier' older adults (75-85 yrs) there were no observed associations between protein distribution and the outcomes of skeletal muscle mass and strength [23]. Noting that these findings are mostly drawn from observational research, there is a lack of data from randomized controlled trials supporting consumption of ≥ 1.2 g/kg BM/day in a dietary intake with balanced protein distribution on outcomes of skeletal muscle mass and physical function in healthy active older adults.

The majority of recent research regarding protein requirements for older adults derives from single-type protein supplementation sources (e.g., whey protein isolate) [10, 11]. However, there

has been increased interest in the use of whole foods (e.g., dairy milk) as a protein source to facilitate gains in skeletal muscle mass and strength with PRT in older adults [24]. Dairy milk (e.g., bovine), which comprises both whey (20%) and casein (80%), is considered a high quality protein source, as it contains all the EAAs and high levels of leucine [25]. There is limited research using dairy milk alone, without additional fortification, in older adults [26-29]. Studies in younger athletic populations have shown promising outcomes using unfortified dairy milk beverages on outcomes of skeletal muscle mass, strength, and physical function [30, 31]. Despite these observed benefits of dairy milk beverages in younger active adults, an investigation of the effects of dairy milk on these same outcomes in active older adults remains a research gap.

Aging is characterized by a decline in anabolic hormones (e.g., testosterone and insulin-like growth factor-1 (IGF-1), and a state of chronic low-grade inflammation, a term known as '*inflammaging*' [32, 33]. Raised levels of systemic inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, and a reduction of anti-inflammatory cytokines such as IL-10 are a common feature [32, 33]. Low-grade inflammation in older adults has been associated with the acceleration of the aging process, leading to an decline in skeletal muscle mass and function [33, 34]. In multiple observational studies, older adults (> 60 yrs) have an inverse dose-relationship between physical activity and systemic inflammatory biomarkers even at modest activity levels [34]. In exercise intervention studies that have provided resistance training, raised plasma concentration of IL-10 has been reported following 16-24 weeks of training in older adults [35, 36], suggesting exercise has the ability to prompt anti-inflammatory processes. Considering dairy milk contains components associated with anti-inflammatory (e.g., casein-derived bioactive peptides) and immunomodulatory effects, together with resistance training, this combination may act synergistically to reduce *inflammaging* [24]. However, the majority of studies exploring this

interaction are mainly based on observational findings with limited evidence in the effects of dietary and exercise interventions on anabolic and cytokine outcomes.

The current study aimed to determine the independent and combined effects of a high protein dairy milk beverage provided at breakfast and lunch (or after resistance exercise), with or without PRT on outcomes of FFM, skeletal muscle strength and power, and physical performance in a cohort of healthy active older adults. We sought to evaluate the study hypothesis that providing a high protein milk on its own would maintain outcomes of FFM, skeletal muscle strength and power, and physical performance compared to those that receive no intervention (e.g., control). In comparison, we hypothesized that a high protein dairy milk beverage in conjunction with PRT would further enhance the effects of PRT leading to augmented gains in FFM, skeletal muscle strength and power, and improved physical performance compared to PRT alone.

6.3 Methods

The study protocol obtained approval from the Monash University Human Research Ethics Committee (Project number 12812), in accordance with the Helsinki Declaration for human research ethics. Informed written consent was obtained from all participants before they were enrolled in the trial. The study was registered with the Australian and New Zealand Clinical Trial Registry as ANZCT12618001088235.

Participants and study design

Older adult males and females, ≥ 50 yrs, with no age upper-limit, performing exercise training for recreational fitness and/or sports competitions (e.g., endurance runners, aerobic gym goes) , ≥ 3 structured exercise sessions/week, totaling ≥ 90 min/week of structured exercise duration, plus additional unstructured physical activity that accounted for meeting the Australian physical activity guidelines [37], were recruited from metropolitan Melbourne and surrounding areas in Victoria, Australia. Interested participants were initially screened over the telephone and excluded based on the following criteria: *1)* dairy protein allergy or known lactose intolerances; *2)* currently using dietary protein supplements; *3)* any injuries preventing safe exercise; *4)* had surgery in the past 12 months; *5)* had an acute coronary (e.g. myocardial infarction) or vascular event in the last year, as well as uncontrolled coronary heart disease; *6)* had a stroke in the past 2 yrs; *7)* have orthopedic limitations that limit the participant in the exercise program; *8)* been diagnosed with, or taking medication for thyroid condition; *9)* had weight loss of more than 5% of body weight over the last 6 months; *10)* take medications that could interfere with skeletal muscle mass structure and/or function (e.g., corticosteroids, testosterone replacement or anabolic drugs); *11)* currently undergoing immunosuppressive therapy or hormone replacement therapy; *12)* have any chronic diseases, such as Diabetes Mellitus or gastrointestinal diseases/disorders; *13)* consumed more

than 2 standard drinks of alcohol/day, or 14 drinks of alcohol/week; *14)* were a smoker; *15)* had a BMI greater than 30 kg/m^2 *16)* had participated in a structured resistance training program in the past 12 months. Once participants were deemed eligible, data were collected during the period September 2018 to January 2020.

A total of 65 participants expressed interested in participating, of these 51 were eligible to participate, and were randomly assigned into one of 4 groups: high protein dairy milk beverage alone (DM), exercise and high protein dairy milk beverage (EX+DM), exercise alone (EX), and control (CON) (Figure 1). Participants in CON were free-living, with self-selected physical activity and food/ fluid intakes that were assessed in the laboratory at baseline, 6 and 12-weeks as per other groups. Randomization was carried out by a researcher blinded to allocation using a block randomization table scheme with stratification by age and sex. Of the 51 randomized participants *n*= 5 ceased the trial due to restrictions imposed by the COVID-19 pandemic. Other reasons for withdrawal from the study are provided in Figure 1. Due to the time line of the data and sample collection, participants did not liaise with each other within or outside experimental procedures. In case of close contact with participants (e.g., cross over time during PRT), participants were advised not to discuss study participation with others.

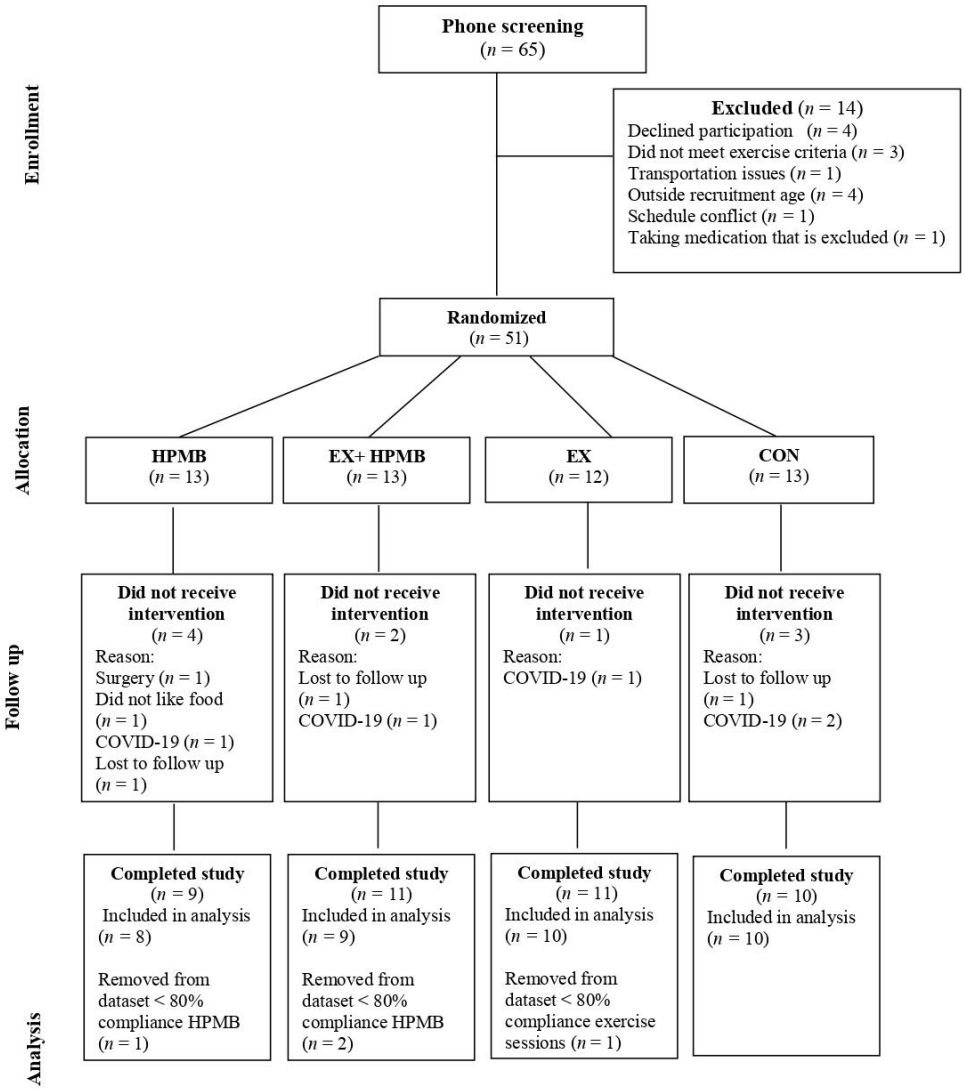

Figure 1. Flow diagram for participant identification, screening, eligibility, and completion.

Preliminary data

Prior to commencing any physical activity, participants filled out a physical activity readiness questionnaire (PAR-Q), which they self-reported their level of activity including exercise volume and type. Participants were asked to complete a three day food-fluid diary prior to their baseline visit, and again at 6 and 12 weeks during the intervention, as previously described [38]. Food-fluid diaries were analyzed using FoodWorks v10.0 nutritional analysis software (Xyris Software, Brisbane, Australia, 2019) based on the Australian Food Composition Database (AFCD) 2019. Total energy, macronutrients and calcium intake were obtained, then dietary protein intake distributed across breakfast, lunch, dinner, and snacks was extracted. Protein intake per meal and per day was expressed as absolute (i.e., g/day, g/meal) and relative to body mass (i.e., g/kg BM/day and g/kg BM/meal).

High protein dairy milk beverage

Participants assigned to DM and EX+DM were asked to consume 500 ml/day (2 x 250 ml) of reduced fat (1.5%) fresh dairy milk (Complete dairy; Lion Dairy & Drinks, Melbourne, Australia). Participants were provided with a measuring cup and asked to consume 250 ml of dairy milk in the morning (with breakfast) and another at lunchtime (or supervised after resistance exercise in EX+DM, consumed within 10-minutes of completing their session). Each 250 ml cup of dairy milk contained: Energy 535 kJ, 15.0 g protein (1.57 g leucine), 8.3 g carbohydrates (8.3 g lactose), 3.8 g fat, and 435 mg calcium. The participants received food provisions to deliver 100% of total daily estimated energy requirements and 100% of total daily estimated protein requirements (~1.2 g/kg BM/day), over the entire duration of the experimental procedure, facilitated by an Accredited Practicing Dietitian. Energy requirements were calculated using participants resting metabolic rate (RMR) scaled by an activity factor based on reported exercise (1.37 ± 0.09). RMR was determined by

indirect calorimeter (Vmax Encore Metabolic Cart; Carefusion, San Diego, CA) in temperate ambient conditions (22.2 ± 1.4°C), and in accordance with best practice guidelines [39]. Intake compliance of daily food provisions and dairy milk, and consumption of other foods/fluids, was recorded using a food-fluid diary. Milk bottles were returned weekly prior to collecting the participants subsequent week's dairy milk and food provisions. An outline of the study protocol is depicted in Figure 2.

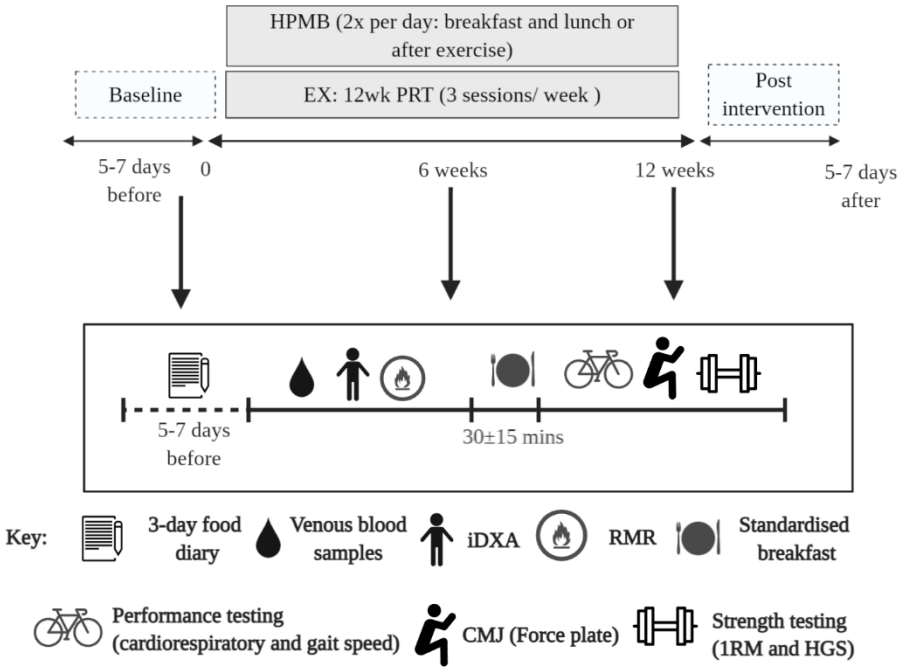

Figure 2. Schematic illustration of the experiemental procedures and study. DM, high protein milk beverage; EX+DM, exercise + high-protein milk beverage; EX, exercise; CON, control.

Exercise protocol

Participants allocated to EX+DM and EX were required to attend supervised PRT sessions, on three non-consecutive days per week, for 12 weeks at the research laboratory. These sessions were

conducted either in a morning session (7.00am to 10.00am) or afternoon session (1.00pm to 3.00pm), to account for participants' work-life schedule. During the course of the trial, all exercise sessions were instructed by a strength and conditioning qualified investigator to ensure correct lifting, to monitor the appropriate amount of exercise and rest intervals, and to check compliance. Each training session (30 ± 15 min) consisted of full body resistance training, which included leg press, *latissimus dorsi* (*lat*) pull down, and chest press (Hammer strength, LifeFitness, Sydney, Australia). Additional exercises including bicep curls, triceps extensions, shoulder raises, calve raises, cable deadlifts, leg curls, back rows, and abdominal exercises were used on a cable machine (Infinity series Functional trainer, Keiser, Fresno, CA), and rotated throughout the program to ensure the development of muscle balance. During the first 2 weeks of the PRT program, participants completed 3 sets of 10-15 repetitions of 50-60% of their 1-repetition maximum (1RM) with 2-min rest intervals. For the following 4 weeks, the training volume was set at three sets of 8 - 12 repetitions at an intensity of 69-75% of 1RM, which increased to 80-95% for 6-8 repetitions the remaining 6 weeks. Each week weight progressively increased by 5-10%. For all exercises, participants were instructed to perform each repetition in a slow, controlled manner, with a rest of 2 min between sets. Testing for participants' 1RM occurred at baseline and week 6, weights were adjusted according to their new 1RM to account for strength gains throughout the protocol. Participants in the exercise groups (i.e., EX+DM and EX) had 2 ± 5 days between their last exercise session and their mid-and post-assessment to minimise a carry-over effect. Exercise compliance was determined by the number of sessions attended. All participants in the exercise groups (i.e., EX+DM and EX) were instructed to continue their normal physical activity outside the PRT program.

Activity tracking

All participants were asked to resume their usual lifestyle activity levels, and were required to wear an activity monitor (ActiGraph wGT3X-BT, Actigraph, Pensacola, FL) on their non-dominant wrist. Participants were instructed to wear the activity monitor during the course of the intervention trial from waking to bedtime, to take the monitor off only when engaged in aquatic activities. A new activity monitor was provided every 2 ± 1 weeks due to the limited (25 days) battery life. Data were uploaded to analytical software (ActiLife 6 v 6.1.3; Actigraph, Pensacola, Florida, United States). A valid day was defined as ≥80% wear time.

Anthropometry and body composition

Participants arrived at the laboratory between 07.00am and 09.00am in a fasted state (plasma osmolality: 296 ± 5.6 mOsmol/kg; Osmomat 030, Gonotec, Berlin, Germany; and total body water: 53.3 ± 6.4%; Seca 515 MBCA, Seca Group, Hamburg, Germany). All participants were required to avoid strenuous exercise for a 24 h period prior to all laboratory assessments. Height was assessed using a fixed stadiometer (Holtain, Crosswell, Crymych, UK). Body mass (BM) was measured (Seca 515 MBCA) to the nearest 0.1 kg, using standardized anthropometrical procedures. Total (kg) and relative (%) FM and FFM, and bone mineral content (BMC) were assessed by a trained radiographer using a dual-energy X-ray absorptiometry (iDXA; Prodigy, GE Lunar, Madison, WI; with analysis software 14.10). Appendicular lean mass (ALM) was determined by adding the total arm and leg mass, and then it was adjusted for height (ALM/ht^2).

Submaximal incremental bike test

To track changes in cardiorespiratory fitness along the experimental timeline, submaximal aerobic fitness was determined using an incremental bike test using a cycle ergometer (*Corival,* Lode,

Groningen, Netherlands) and a metabolic cart (Vmax Encore Metabolic Cart; Carefusion, San Diego, CA). Procedures were adjusted from standard fitness testing protocols [40]. The initial workload began at 1 watt (W) per kilogram of FFM (W/kg FFM) and increased by 0.5 W/kg FFM every 3 min until participants could not maintain the speed at ≥ 60 RPM, they reached a rating of perceived exertion (RPE) of 15-17, and/or obtained a respiratory exchange ratio (RER) of 1.000 [41]. Heart rate (HR) (Polar Electro, Kempele, Finland), RPE, $\dot{V}O_2$, and RER were measured every 3 min in real-time. Cardiorespiratory fitness was expressed as the $\dot{V}O_2$ ml/kg/min at which the RER reached 1.000.

Counter movement jump (CMJ)

A Force plate (400s+ Performance Force plate; Fitness Technology, Adelaide, Australia) was used to measure relative muscle power (Watts/kg), jump height (in centimeters) and velocity (m/sec) during a countermovement jump test (CMJ). Participants were asked to start in a full erect standing position in the middle of the force plate, then instructed to dip to a self-selected depth and to perform a static squat jump. Hands were kept on the hips to minimize any influence of arm swing [42]. Participants were asked to perform three attempts of a CMJ with 1-min rest in-between jumps. The Force plate was interfaced with computer software (Ballistic Measurement System; Fitness Technology, Adelaide, Australia), the best of the three jumps was selected for further analysis.

Gait speed measurement

To assess gait speed, a walking course of 4 meters length was marked on the floor. The participant was instructed to walk from one end of the course to the other at their usual walking pace. The timer began as the participant started walking and the timer stopped with the first footfall after

the 4-metre line. The test was repeated twice and the fastest time of the two scores was recorded [1].

Skeletal muscle strength outcomes

Strength was assessed by performing a 1RM according to previously described protocols [43]. During a familiarization trial, proper lifting technique was demonstrated, then participants were familiarized with each resistance machine (Hammer strength; LifeFitness, Sydney, Australia) by performing 8-10 repetitions of a light load (~50% of predicted 1RM). After the successful completion of a further 5-6 repetitions at a heavier weight selected by the instructor the workload was increased incrementally until only one repetition with correct technique could be completed. Participants were given 3-5 min rest in-between attempts [44]. The value of 1RM was the highest load that could be raised in one single repetitions using correct technique. The leg press, *lat* pull down, and bench press exercises measured. The 1RMs were normalized by body weight (1RM/BM). Hand grip strength (HGS) was measured using a digital hand dynamometer (Jamar® Plus+ Digital hand dynamometer; Sammons Preston, Bolingbrook, IL, USA). HGS was measured in a standing position with the participants elbow by their side and flexed to 90°, and a neutral wrist position. Participants were asked to apply the maximum grip strength by squeezing the dynamometer with as much force as possible using their dominant hand. This was repeated 3 times with a 1 min rest in-between attempts. HGS was defined as the highest value for their dominant hand [45].

Blood collection and analysis

Blood glucose concentration, hemoglobin, total and differential leukocyte counts (i.e., neutrophils, lymphocytes, and monocytes), were determined by HemoCue system (Glucose 201+, Hb201, and WBC DIFF, respectively; HemoCue AB, Ängelholm, Sweden) in duplicate from heparin whole blood

samples. Coefficient of variation (CV) for blood glucose concentration, hemoglobin, and total leukocyte counts were 3.0%, 1.5% and 4.6%, respectively. Hematocrit was determined using the capillary method in triplicate (CV: 1.1%) from heparin whole blood samples and using a microhematocrit reader (ThermoFisher Scientific). Hemoglobin and hematocrit values were used to estimate changes in plasma volume relative to baseline, and used to correct plasma variables. The remaining heparin whole blood samples were centrifuged at 4,000 rpm (1500 g) for 10 min within 15 min of sample collection. Aliquots of heparin plasma were places in 1.5 ml microstorage tubes and frozen at -80°C until analysis, except 2 x µl plasma was used to determine plasma osmolality in duplicate (CV 1.1%).

Circulating concentrations of cortisol (DiaMetra, Perugia, Italy), insulin (Crux Biolab, Scoresby, Australia), insulin- like growth factor-1 (IGF-1) (Crux Biolab, Scoresby, Australia), testosterone (17b-OH-4-androstene-3-one; DiaMetra, Perugia, Italy), and estradiol (17β-Estradiol; DiaMetra, Perugia, Italy) were measured by enzyme-linked immunosorbent assay (ELISA). Plasma concentrations of TNF-α, IL-6, IL-8, IL-2, and IL-10 were determined by high sensitivity multiplex ELISA (HCYTOMAG-28SK; EMD Millipore, Darmstadt, Germany). All assays were performed as per manufacturer's specifications, with standards and controls on each plate. The CV for analyzed circulating biomarkers was ≤ 7.2%, and for systemic inflammatory cytokine profile was ≤ 13.5%. Systemic cytokine profile was established, as previous described [46].

Statistical Analysis

Only participants that attended ≥ 80% of the PRT sessions and consumed ≥ 80% of the DM beverage over the 12-week intervention were included in the data analysis. Based on the statistical test, mean, standard deviation, and effect size (i.e., small 0.20, medium 0.50, and large 0.80) for

outcomes of FFM, skeletal muscle strength and physical performance and applying a standard alpha (0.05) and beta value (0.80), a sample size of *n*= 36 (*n= 8,* per group) using a randomized controlled design as reported in Hanach *et al.* [10], is estimated to provide adequate statistical power (0.80-0.99) to detect variable differences (G*Power 3.1, Kiel, Germany). Data in the text and tables are presented as either mean ± SD or mean and 95% confidence interval (CI), as indicated. For clarity, data in figures are presented as mean ± standard error of the mean (SEM). Only participants that completed the experimental design, adhered to the controlled intervention conditions, and with full data sets within each specific variable were used in the data analysis, as indicated in the tables and figures legends. All data were checked for distribution using Shapiro-Wilks test of normality. Variables with singular data points were examined using a one way ANOVA, or non-parametric Kruskal–Wallis test when appropriate. Variables with multiple data points were examined using a two-way repeated-measures ANOVA with a matrix including group (DM, EX+DM, EX, and CON), time (baseline (week 0), week 6, and week 12). Assumptions of homogeneity and sphericity were checked, and when appropriate, adjustments to the degrees of freedom were made using the Greenhouse-Geisser correction method. Significant main effects were analyzed using a post hoc Tukey's HSD test. Statistics were analyzed using SPSS statistical software (V.25.0, Chicago, Illinois, USA) with significance accepted at $P \leq 0.05$. Furthermore, correlations between changes in primary variables (e.g., FFM, skeletal muscle strength, power and physical performance) and inflammatory and hormone markers were conducted at 6 and 12-weeks. This was carried out with a Pearson's or Spearman's correlation test, based on the data distribution. Significance was accepted at $P \leq 0.05$. Additionally, Cohen's *d* was applied to determine the magnitude of effect size for significance differences, with $d \geq 0.20$ for small, $d \geq 0.50$ for medium, and $d \geq 0.80$ for large effect size.

6.4 Results

Baseline characteristics

The participants from this study came from a variety of sporting backgrounds, including endurance runners/ race walkers (61%), cyclists (9%), aerobic gym goers (16%) or a combination of multiple activities (14%). The dropout rate for the current study was 20% between all 4 groups, with dropout reasoning depicted in **Figure 1**. At the end of the study, groups were composed as follows: DM *n*= 8, EX+DM *n*= 9, EX *n*= 10, and CON *n*= 10. Participant baseline variables, based on group allocation, are summarized in **Table 1**. There were no significant differences in baseline characteristic variables between groups.

Progressive resistance training and food provisions compliance

In EX+DM and EX, the PRT was well tolerated with the average compliance for participants in the exercise program being 89% (95% CI: 85 to 93%), and did not differ between groups (*P*= 0.538). In DM and EX+DM, the average compliance for the high protein dairy milk beverage provisions, according to food diaries and milk bottle returns was 93% (88 to 97%), and did not differ between groups (*P*= 0.969). Average adherence to the standardised meal plan and food provisions, based on food diaries, was 81% (76 to 85%), and did not differ between groups (*P*= 0.821).

Table 1. Baseline characteristics of the participants according to randomized group selection.

	DM *n* = 8	EX+DM *n* = 9	EX *n* = 10	CON *n* = 10	*p*-value
Males, *n*	7	6	8	7	
Females, *n*	1	3	2	3	
Age, yrs	59.7 (52.9 to 67.0)	63.6 (57.4 to 70.0)	58.0 (53.0 to 67.0)	56.1 (51.5 to 60.6)	.147
Height, m	1.7 (1.6 to 1.8)	1.7 (1.6 to 1.7)	1.7 (1.7 to 1.8)	1.6 (1.6 to 1.7)	.105
BM, kg	78.3 (65.6 to 91.0)	71.6 (63.0 to 80.1)	70.0 (64.4 to 89.5)	67.8 (63.2 to 72.4)	.333
BMI, kg/m^2	24.6 (22.2 to 27.0)	24.9 (22.0 to 27.8)	25.3 (22.5 to 28.1)	24.1 (23.0 to 25.3)	.876
Self-reported structured exercise, min/week					
	233 (125 to 340)	189 (144 to 264)	273 (210 to 336)	215 (137 to 293)	.378

Mean (95% CI). **Abbreviations:** BM: body mass, BMI: body mass index, EX+DM: exercise and high protein milk beverage, DM: High protein milk beverage EX: Exercise, CON: control

Dietary intake

A group*time interaction (p= .048) was observed for energy intake, indicating a significant increase in DM (19%) and EX+DM (22%) groups at 12-weeks compared to baseline (Table 2). The consumption of the high protein dairy milk lead to a significant increase in absolute (g/day) (p= .001) and relative protein intake (g/kg BM/day) (p< .001) in DM and EX+DM groups at 6 weeks (38% and 35%, respectively) and 12 weeks (44% and 45%, respectively). Similarly, there was a group*time interaction for calcium intake (p= .007). Further analysis indicated that DM and EX+DM had a significant increase in calcium intake at 6 week (88% and 99%, respectively) and 12 weeks (120% and 112%, respectively) compared to baseline (Table 2). Based on protein intake relative to

BM, a group*time interaction effect was observed for protein (g/kg BM) at breakfast (*P*= 0.005) and dinner (p= .012), and towards significance at lunch (p= .055). Further analysis indicated that compared to baseline, protein at breakfast significantly increased at 6- and 12-weeks in DM (≥ 44%) and EX+DM (≥ 55%) compared to EX and CON. There was a significant decrease of relative protein intake (g/kg) at dinner in EX+DM (50%) and EX (10%) groups, at 6-weeks and 12-weeks compared to baseline.

Table 2. Baseline values and the mean within-group changes at week 6 and 12 for **(A)** Total dietary energy and macronutrient intake and **(B)** relative protein intake based on each meal according randomized allocation

	DM	EX+DM	EX	CON
A. Total energy and macronutrient intake				
Energy intake, MJ/day				
Baseline	8.6 (6.7 to 10.6)	8.1 (6.0 to 9.3)	9.1 (7.0 to 10.0)	9.6 (9.0 to 10.5)
6 weeks	10.0 (7.6 to 14.0)	9.4 (6.0 to 13.7)**	8.6 (5.7 to 11.4)aa	9.6 (4.3 to 13.0)
12 weeks	11.0 (8.0 to 14.0)**	9.5 (7.5 to 12.4)**aa	8.9 (4.3 to 11.8)aa	9.0 (6.1 to 12.0)aa
Total protein intake, g/day				
Baseline	101 (81.7 to 120)	86.4 (67.6 to 105)	95.4 (77.1 to 113)	108 (91.0 to 127)
6 weeks	125 (105 to 148)**	115 (72.0 to 161)**	94.0 (54.3 to 119)ab	112 (52.2 to 159)ab
12 weeks	127 (118 to 152)**	123 (94.0 to 153)**	106 (57.0 to 145)ab	98 (62.0 to 145)ab
Relative protein, g/kg BM/day				
Baseline	1.3 (1.0 to 1.7)	1.2 (1.0 to 1.4)	1.4 (1.0 to 1.7)	1.6 (1.2 to 2.0)
6 weeks	1.7 (1.2 to 0. 2.9)**	1.6 (1.0 to 2.2)**aa	1.2 (0.80 to 1.9)aa	1.6 (0.9 to 2.5)aacc

12 weeks	1.8 (1.4 to 3.2)**	1.8 (1.2 to 1.8)**aa	1.4 (0.70 to 1.8)aa	1.4 (0.9 to 1.4)aacc
Protein, %				
Baseline	27.0 (19.4 to 34.5)	29.1 (20.6 to 37.5)	23.0 (17.0 to 27.0)	32.2 (26.8 to 37.6)
6 weeks	35.5 (26.0 to 57.0)**	34.0 (23.0 to 46.0)**	21.5 (10.3 to 31.0)*ab	32.3 (17.0 to 48.3)ac
12 weeks	37.0 (28.4 to 37.0)**	38.6 (31.0 to 39.0)**	24.4 (14.0 to 38.0)*ab	29.3 (17.5 to 29.3)abc
Carbohydrates, g/day				
Baseline	206 (128 to 308)	192 (80 to 297)	229 (108 to 331)	202 (155 to 249)
6 weeks	304 (187 to 305)**	106 (68 to 144)**	237 (187 to 363)ab	208 (68 to 305)ab
12 weeks	218 (93 to 330)**	100 (48 to 151)**	267 (171 to 384)ab	210 (68 to 348)ab
Fat, g/day				
Baseline	81.6 (66.0 to 97.0)	70.5 (53.5 to 87.7)	78.6 (67.0 to 90.5)	92.0 (81.5 to 102)
6 weeks	64.0 (33.0 to 83.0)	16.3 (-100 to 132)	70.0 (24.0 to 98.0)	66.0 (44.0 to 122)
12 weeks	64.0 (35.0 to 100)	33.6 (-90.0 to 159)	75.0 (29.3 to 74.5)	87.4 (51.2 to 118)
Calcium mg/day				
Baseline	1011 (675 to 1347)	749 (394 to 1104)	1117 (807 to 1426)	1022 (789 to 1256)

6 weeks	1911 (1467 to 2458)**	1629 (941 to 2726)**	1006 (547 to 1613)aabb	1262 (582 to 2074)aabb
12 weeks	2037 (1449 to 2849)**	1695 (1245 to 2704)**	1135(605 to 1592)aabb	1134 (462 to 2151)aabb
B. Relative protein intake based on each meal				
Protein at breakfast, g/kg BM				
Baseline	0.25 (0.10 to 0.40)	0.21 (0.12 to 0.30)	0.30 (0.15 to 0.40)	0.30 (0.21 to 0.36)
6 weeks	0.36 (0.20 to 0.60)**	0.33 (0.30 to 0.40)*	0.22 (0.0 to 0.50)	0.28 (0.10 to 0.50)
12 weeks	0.34 (0.10 to 0.60)**	0.36 (0.20 to 0.50)*	0.25 (0.10 to 0.40)*	0.31 (0.10 to 0.50)
Protein at lunch, g/kg BM				
Baseline	0.40 (0.21 to 0.60)	0.31 (0.18 to 0.43)	0.40 (0.25 to 0.60)	0.37 (0.22 to 0.51)
6 weeks	0.50 (0.30 to 0.70)*	0.50 (0.40 to 0.60)#	0.34 (0.10 to 0.50)	0.45 (20 to 0.70)
12 weeks	0.50 (0.30 to 0.70)*	0.44 (0.30 to 0.50)**	0.31(0.10 to 0.0.70)b	0.40 (0.10 to 0.60)abc
Protein at dinner, g/kg BM				
Baseline	0.50 (0.31 to 0.65)	0.64 (0.50 to 0.80)	0.74 (0.40 to 1.1)	0.64 (0.50 to 0.80)
6 weeks	0.40 (0.20 to 0.50)	0.35 (0.20 to 0.50)*	0.70 (0.30 to 1.0)*b	0.70 (0.20 to 1.2)ab
12 weeks	0.40 (0.30 to 0.41)	0.35 (0.20 to 0.50)*	0.60 (0.40 to 1.0)b	0.60 (0.30 to 1.2)b

Mean (95% CI). **Abbreviations**: BM: Body mass, CON: control, EX: Exercise, EX+DM: exercise and high protein dairy milk beverage, DM: high protein dairy milk beverage, MJ: Megajoule. DM: *n* = 8, EX+DM: *n* = 9, EX: *n* = 10, CON: *n* = 10.Within group changes. Between group changes: $^{aa}p < .01$ or $^{a}p < .05$ vs. DM; $^{bb}p < .01$ or $^{b}p < .05$ vs. EX+DM; $^{cc}p < .01$ or $^{c}p < .05$ vs EX.

Physical activity

At baseline, the reported physical activity did not differ between groups (Table 1). The average compliance based on the wear time for the Actigraph was 93% (90-95%), and did not differ between groups (p= .754). Based on the analysis of the accelerometer over the intervention period, there was no differences between groups for amount of hourly kcals, time in sedentary or time in light physical activity (Table 3).

Body composition

A significant group*time interaction was observed for BM (p= .029), absolute FFM (p= .051) and absolute and relative FM (p= .013 and p= .043, respectively) (Table 4, Figure 3). BM decreased in DM, at week 6 (-2.2 kg) and week 12 (-2.7 kg), which significantly reduced more than all other groups at both time points. Absolute FFM significantly increased in EX+DM at both time points (week 6 and 12) and in EX at week 12. This increase was significantly greater than DM, which showed a significant decline in FFM at week 6 (-0.26%) and week 12 (-0.96%). Absolute FM significantly decreased over time at week 6 in DM and at 12-weeks in DM, EX+DM and EX groups. DM had the greatest loss in absolute FM at 6-weeks (-1.4 ± 1.2 kg) and 12-weeks (-2.1 ± 2.6 kg). At 12-weeks there was a significant decline in relative FM of ≥ 1.0% in the DM, EX+DM and EX groups compared to baseline. No significant main effects or interactions were observed for regional body composition, ALM/ht^2, BMD or resting metabolic rate (Table 4).

Table 3. Average daily physical activity measured by accelerometer over the 12-week experimental procedure.

	DM	EX+DM	EX	CON
Sedentary time, min/ day				
0-6 weeks	923 (844 to 978)	863 (672 to 1035)	782 (351 to 933)	844 (697 to 941)
6-12 weeks	904 (848 to 978)	896 (706 to 1064)	785 (152 to 1017)	877 (785 to 985)
Time in light physical activity, min/ day				
0-6 weeks	281 (193 to 366)	340 (158 to 842)	368 (245 to 883)	280 (230 to 363)
6-12 weeks	282 (214 374)	352 (136 to 938)	359 (215 to 900)	275 (208 to 342)
Time in moderate physical activity, min/ day				
0-6 weeks	171 (130 to 218)	206 (130 to 315)[a]	219 (153 to 280)[a]	216 (174 to 323)[a]
6-12 weeks	170 (131 to 213)	210 (115 to 317)[a]	217 (128 to 310)[a]	214 (158 to 277)[a]
Time in vigorous physical activity, min/day				
0-6 weeks	11.1 (0.0 to 65.2)	19.0 (0.0 to 58.0)	13.3 (0.0 to 43.8)	24.5 (0.50 to 55.4)
6-12 weeks	13.8 (0.0 to 60.0)	18.0 (0.0 to 47.6)	22.1 (0.0 to 45.3)	32.2 (0.00 to 57.3)
Total steps, *n*				
0-6 weeks	24,582 (9,793 to 11,112)	13,360 (9,723 to 18,505)	14,670 (11,734 to 17,000)	14,126 (11,787 to 19,928)
6-12 weeks	12,323 (10,000 to 15,145)	13,811 (8,742 to 20,175)	14,516 (10,700 to 17,510)	14,152 (11,220 to 15,686)

Mean (95% CI). **Abbreviations:** vigorous physical activity. DM: *n*= 8, EX+DM: *n*= 9, EX: *n*= 10, DM: *n*= 8, CON: *n*= 10.

Between group changes: [a]*p*< .05 vs. DM.

Table 4. Baseline values and within-group changes at week 6 and 12 for total body and regional composition, bone mineral density, and resting metabolic rate according to randomized allocation.

	DM	EX+DM	EX	CON
Total body FFM, %				
Baseline	73.0 (67.4 to 79.4)	69.9 (60.1 to 79.1)	75.7 (40.2 to 80.5)	76.0 (69.1 to 82.2)
6 weeks	75.7 (62.0 to 84.2)	70.1 (54.0 to 87.3)	75.7 (61.2 to 89.3)	76.5 (62.7 to 89.5)
12 weeks	75.5 (60.4 to 87.0)	70.5 (53.6 to 86.4)	76.6 (62.4 to 89.4)	76.4 (63.6 to 91.7)
Total body FM, %				
Baseline	26.6 (20.3 to 32.9)	31.5 (21.6 to 41.4)	25.5 (19.9 to 31.2)	24.6 (18.1 to 31.1)
6 weeks	25.2 (16.0 to 38.0)**	31.5 (17.3 to 48.0)	25.1 (11.7 to 39.4)	24.4 (10.3 to 37.7)
12 weeks	25.0 (12.9 to 40.1)**	31.0 (14.0 to 47.0)**a	24.5 (11.3 to 37.9)a	24.7 (9.8 to 37.2)
Arm LM, kg				
Baseline	5.1 (3.0 to 7.0)	5.1 (3.0 to 6.9)	6.3 (4.0 to 8.6)	5.6 (3.3 to 8.4)
6 weeks	6.0 (3.2 to 7.5)	5.1 (3.0 to 7.0)	6.3 (4.0 to 9.0)	5.6 (3.3 to 8.4)
12 weeks	6.0 (3.1 to 8.0)*	5.2 (3.0 to 7.2)	6.5 (3.8 to 9.2)	5.7 (3.3 to 8.4)
Arm FM, kg				
Baseline	2.0 (1.6 to 3.7)	2.3 (1.1 to 4.3)	2.0 (0.8 to 4.0)	1.8 (1.3 to 2.8)
6 weeks	2.0 (1.4 to 3.5)	2.3 (0.8 to 4.1)	2.1 (0.8 to 3.7)	1.8 (0.7 to 2.8)
12 weeks	2.0 (1.1 to 3.4)	2.4 (1.0 to 4.3)	2.1 (1.0 to 4.0)	1.9 (0.9 to 3.0)
Leg LM, kg				
Baseline	18.8 (12.0 to 23.0)	16.1 (11.0 to 20.4)	18.5 (14.1 to 24.3)	17.0 (11.8 to 23.2)

6 weeks	18.6 (12.2 to 23.0)	16.1 (11.3 to 21.0)	18.6 (14.2 to 25.0)	17.1 (12.0 to 24.4)
12 weeks	18.4 (12.1 to 22.3)	16.1 (11.0 to 20.5)	18.5 (14.5 to 24.6)	17.0 (12.0 to 23.1)
Leg FM, kg				
Baseline	5.6 (3.6 to 8.0)	5.6 (3.1 to 10.0)	5.5 (2.4 to 13.0)	6.3 (2.5 to 14.0)
6 weeks	5.5 (4.2 to 7.5)	5.5 (3.0 to 10.1)	5.6 (2.2 to 13.0)	6.3 (2.3 to 14.0)
12 weeks	5.1 (3.4 to 7.3)	5.5 (3.0 to 10.4)	5.3 (2.0 to 13.0)	6.3 (2.4 to 14.0)
Trunk LM, kg				
Baseline	26.0 (17.6 to 29.6)	22.6 (14.7 to 28.1)	26.0 (20.5 to 35.0)	25.1 (16.5 to 35.0)
6 weeks	26.0 (17.6 to 30.0)	23.0 (15.1 to 28.0)	26.0 (21.0 to 34.5)	25.1 (18.4 to 34.2)
12 weeks	26.0 (17.6 to 30.0)	23.0 (15.0 to 28.0)	26.2 (20.1 to 35.0)	24.2 (12.0 to 34.0)
Trunk FM, kg				
Baseline	11.6 (4.7 to 21.5)	13.0 (3.5 to 23.6)	11.0 (2.2 to 20.0)	7.8 (2.7 to 11.0)
6 weeks	10.5 (2.3 to 22.0)	12.2 (2.4 to 23.5)	10.54 (2.0 to 19.6)	7.7 (2.7 to 11.0)
12 weeks	10.2 (3.0 to 21.5)	12.0 (2.2 to 23.0)	10.6 (1.9 to 20.6)	7.8 (2.2 to 11.0)
ALM/HT^2				
Baseline	7.7 (6.5 to 9.0)	7.3 (5.1 to 9.1)	8.3 (5.8 to 11.2)	7.8 (5.6 to 9.4)
6 weeks	7.7 (6.6 to 8.6)	7.4 (5.2 to 9.0)	8.3 (5.8 to 11.2)	7.8 (5.7 to 9.8)
12 weeks	7.6 (6.5 to 8.4)	7.4 (5.2 to 9.0)	8.3 (5.8 to 11.0)	7.8 (5.7 to 9.3)
BMD, g/cm				
Baseline	1.2 (1.0 to 1.4)	1.1 (0.9 to 1.4)	1.3 (1.0 to 1.6)	1.2 (0.9 to 1.4)
6 weeks	1.2 (1.0 to 1.4)	1.1 (0.9 to 1.4)	1.3 (1.0 to 1.6)	1.2 (0.9 to 1.4)
12 weeks	1.2 (1.0 to 1.4)	1.2 (0.9 to 1.5)	1.3 (1.0 to 1.4)	1.1 (0.1 to 1.4)

RMR, MJ/ day				
Baseline	6.1 (4.2 to 7.6)	5.5 (4.5 to 7.3)	6.3 (4.3 to 8.4)	5.7 (4.7 to 7.4)
6 weeks	6.1 (4.2 to 7.6)	5.5 (4.2 to 6.7)	6.3 (4.3 to 8.0)	5.8 (4.7 to 7.4)
12 weeks	6.0 (4.0 to 7.8)	5.4 (4.3 to 6.7)	6.2 (4.3 to 8.0)	5.6 (4.4 to 7.2)

Mean (95% CI). **Abbreviations:** ALM/HT: appendicular muscle mass/ height, BM: Body mass, BMD: bone mineral density, FFM: fat-free mass, FM: fat mass, LM: lean mass, MJ: Megajoule, RMR: resting metabolic rate. DM: *n*= 8, EX+DM: *n*= 9, EX: *n*= 10, CON: *n*= 10.Within group changes: ***p*<.01 or ***p*<.05 vs baseline. [a] *p*< .05 vs. DM.

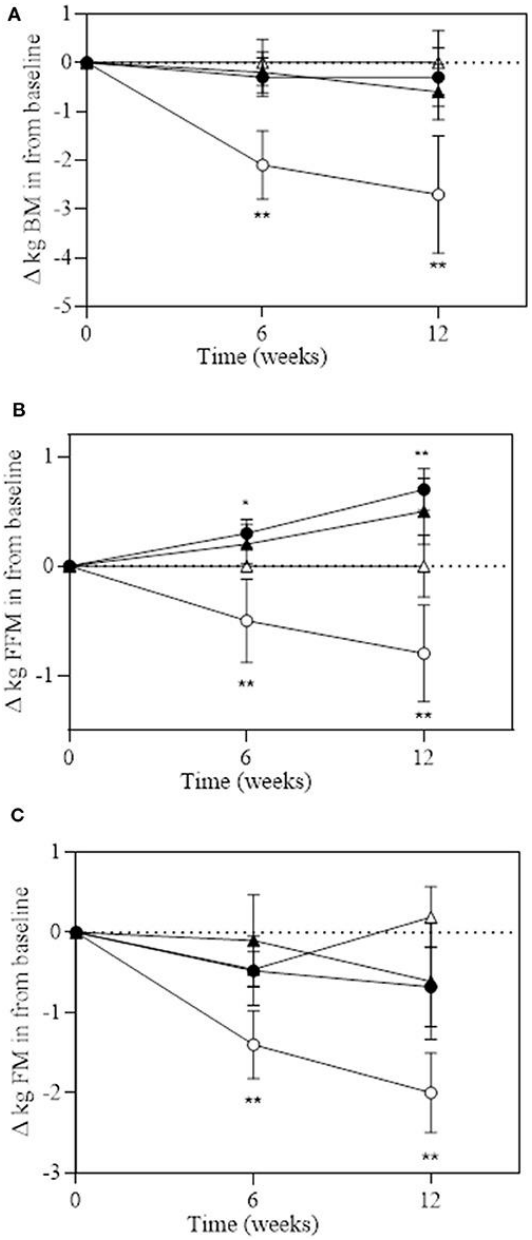

Figure 3. Change over 6 and 12-weeks intervention trial from baseline for (A) total body mass (kg) (B) Fat-free mass (kg) (C) fat mass (kg) according to group DM:ʘ, EX+DM: ●, EX: ▲, CON: Δ). Mean ± SEM: ** p< .01 and baseline.

Skeletal muscle strength

There was a significant group*time interaction for absolute and relative maximal 1RM leg press (p< .001 and p= .006), chest press (p< .001 and p< .001), and *lat* pull down (p= .007 and p< .001) (**Figure 4**). A significant change in absolute maximal 1RM strength was observed in EX+DM and EX group at 6 and 12-weeks from baseline (**Figure 4**). The significant change in relative strength was observed in EX+DM (range: 53-78%) and EX (35-36%) groups from baseline to 12-weeks (**Figure 5**). The change in relative 1RM strength was greater in EX+DM compared to all other groups at 12-weeks. There was no significant main effects or interactions for HGS (p= .561).

Skeletal muscle power and physical performance

For outcomes of muscle power (i.e., CMJ), cardiorespiratory fitness (i.e., submaximal $\dot{V}O_2$), and physical performance (i.e., gait speed), there were no significant main effects or interactions from baseline to week 12 (**Table 5**).

Systemic hormonal and inflammatory cytokine profiles

There were no main effects or interactions observed for any of the hormonal biomarkers measured (**Table 6**). There was a group*time interaction for IL-10 (p= .016) (**Table 7**), associated with the increase observed for the EX+DM group at 6-weeks (88%) and 12-weeks (46%). This increase was significantly higher than all the other groups (p< .01). There were no main effects or interactions observed for any other immune biomarkers measured. There was no significant correlation between the changes in any of the primary outcomes (e.g., FFM, skeletal muscle

strength, power and physical performance) and any of the systemic hormonal and inflammatory cytokine markers.

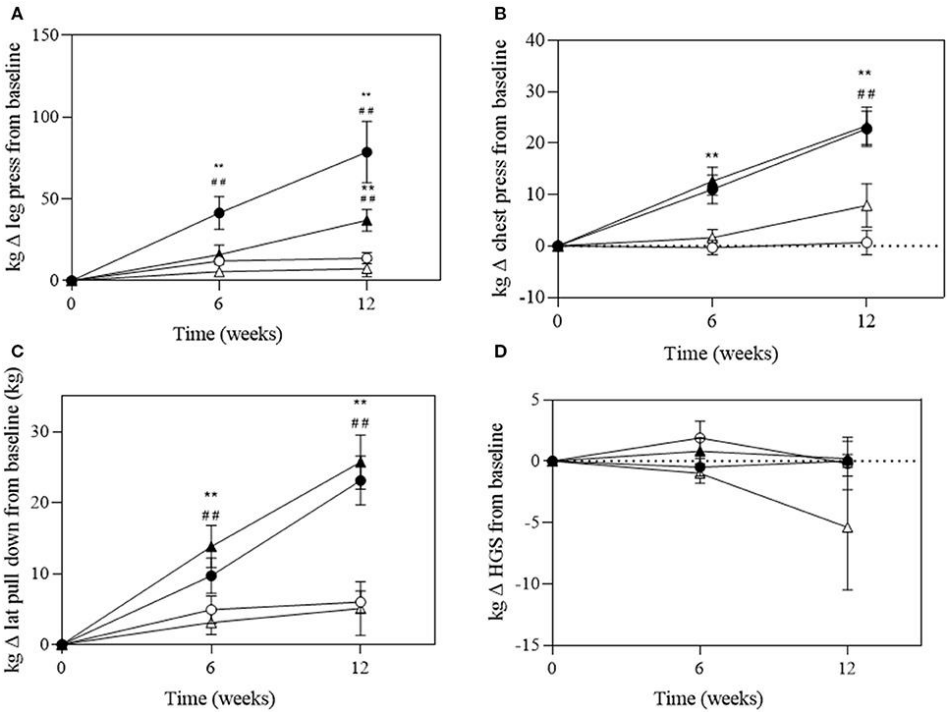

Figure 4. Change of absolute strength (kg) at 6 and 12-weeks intervention trial from baseline for **(A)** lower body (leg press), **(B)** upper body (chest press) and **(C)** back (*lat* pull down) strength **(D)** handgrip strength (HGS) according to group. (DM:○, EX+DM: ●, EX: ▲, CON: Δ). Mean ± SEM: **p< .01 vs. baseline. $^{\#\#}p$< .01 vs 6 week.

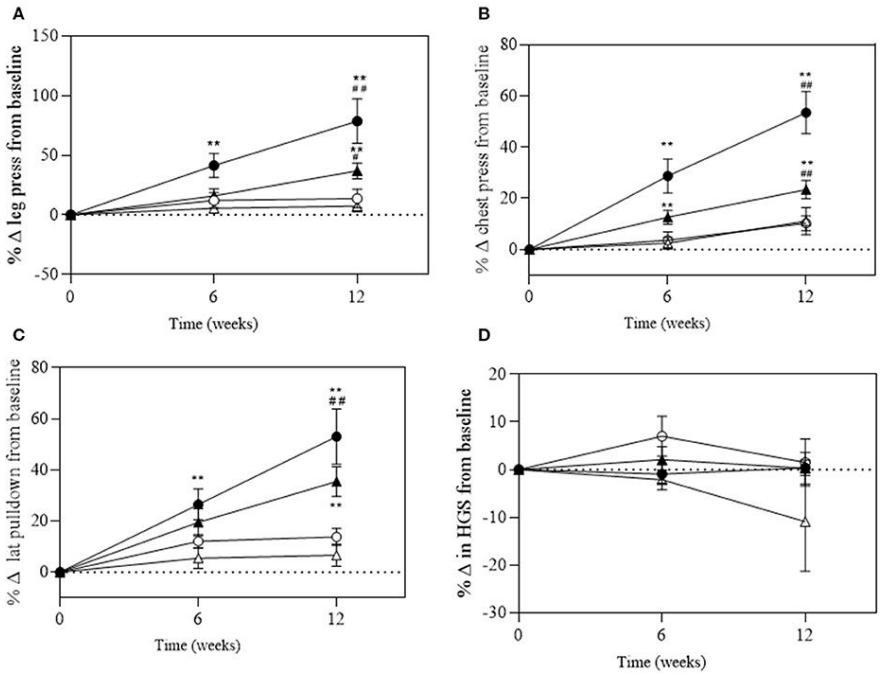

Figure 5. Change of relative (kg/BM) at 6 and 12-weeks intervention trial from baseline for (A) lower body (leg press), (B) upper body (chest press) and (C) back (*lat* pull down) strength (D) handgrip strength (HGS) according to group. (DM:○, EX+DM: ●, EX: ▲, CON:Δ). Mean ± SEM: **p< .01 vs baseline, ## *p*< .01 vs 6 weeks

Table 5. Baseline values and the mean within-group changes at week 6 and 12 in skeletal muscle power, cardiorespiratory fitness, and physical function outcomes in the according to randomized allocation.

	DM	EX+DM	EX	CON
CMJ, cm				
Baseline	17.1 (12.1 to 22.1)	13.5 (15.5 to 22.6)	16.7 (13.5 to 20.2)	17.0 (15.0 to 18.5)
6 weeks	17.3 (7.5 to 22.3)	14.0 (3.3 to 22.1)	18.0 (12.0 to 24.0)	18.2 (10.3 to 26.0)
12 weeks	17.6 (6.7 to 24.0)	15.1 (2.3 to 29.0)	18.5 (13.3 to 24.0)	19.1 (11.0 to 25.2)
CMJ, W/kg BM				
Baseline	28.9 (23.3 to 34.4)	25.4 (21.0 to 29.8)	31.7 (29.0 to 34.4)	29.7 (25.7 to 33.7)
6 weeks	28.5 (16.7 to 34.1)	25.4 (14.5 to 35.0)	31.0 (24.6 to 40.0)	30.2 (20.4 to 38.5)
12 weeks	29.0 (16.3 to 33.0)	28.2 (15.0 to 38.0)	31.4 (27.2 to 36.0)	30.5 (20.6 to 37.0)
Gait speed, m/sec				
Baseline	0.95 (0.80 to 1.10)	0.81 (0.65 to 1.00)	0.90 (0.77 to 0.90)	0.80 (0.74 to 0.83)
6 weeks	0.83 (0.70 to 1.00)	0.84 (0.70 to 1.00)	0.85 (0.60 to 1.00)	0.84 (0.70 to 1.00)
12 weeks	0.81 (0.60 to 0.90)	0.81 (0.60 to 0.90)	0.80 (0.60 to 1.0)	0.80 (0.70 to 0.90)
Submaximal VO_2, ml/kgBM/min				
Baseline	17.0 (11.6 to 22.3)	15.6 (9.7 to 21.5)	21.5 (17.0 to 26.0)	25.0 (17.0 to 33.0)

6 weeks	17.6 (7.0 to 28.3)	17.0 (5.0 to 28.6)	20.0 (13.0 to 27.0)	27.2 (15.6 to 39.0)
12 weeks	18.2 (8.5 to 27.6)	18.0 (5.7 to 30.0)	20.2 (12.0 to 28.6)	24.0 (11.0 to 37.1)

Mean (95% CI). **Abbreviations:** BM: body mass, CMJ: countermovement jump, FFM: fat-free mass, *VO*: maximal oxygen consumption, W: watts. DM: *n*= 8, EX+DM: *n*= 9, EX: *n*= 10, CON: *n*= 10.

Table 6. Baseline values and the mean within-group changes at week 6 and 12 in biochemistry and hormonal markers according to randomized allocation.

	DM	EX+DM	EX	CON
Blood glucose, mMol/L				
Baseline	5.0 (4.3 to 5.4)	5.0 (4.5 to 5.5)	5.1 (5.0 to 5.3)	4.7 (4.1 to 5.4)
6 weeks	4.5 (4.0 to 6.0)	4.1 (3.7 to 6.1)	4.6 (3.2 to 6.2)	4.7 (3.7 to 5.8)
12 weeks	4.5 (3.0 to 6.0)	4.8 (3.2 to 6.5)	5.1 (4.3 to 6.0)	4.7 (3.4 to 6.1)
Insulin, mmol/L				
Baseline	8.9 (4.0 to 14.0)	6.4 (4.4 to 8.3)	5.3 (3.6 to 7.0)	7.4 (5.0 to 10.0)
6 weeks	9.2 (1.0 to 18.0)	6.2 (2.7 to 9.6)	5.2 (2.0 to 8.5)	6.5 (2.0 to 11.2)
12 weeks	5.8 (0.6 to 24.0)	6.6 (3.2 to 10.0)	5.5 (2.6 to 8.4)	7.6 (3.0 to 12.3)
IGF-1, pg/ml				
Baseline	148 (28 to 268)	118 (28 to 264)	56 (17 to 94)	34 (22 to 47)
6 weeks	171 (15 to 328)	220 (0 to 654)	115 (0 to 310)	57 (16 to 100)
12 weeks	150 (0 to 344)	219 (0 to 622)	54 (0 to 26)	117 (52 to 183)
Estradiol, pg/ml				

Baseline	60 (18 to 138)	35 (42 to 113)	42 (7 to 91)	14 (2 to 31)
6 weeks	52 (0 to 151)	8 (0 to 145)	29 (0 to 122)	6 (0 to 34)
12 weeks	38 (0 to 147)	17 (0 to 159)	2 (0 to 128)	9 (0 to 38)
Testosterone, ng/ml				
Baseline	2.0 (0.7 to 3.1)	1.3 (0.3 to 2.3)	2.6 (1.5 to 3.7)	1.4 (0.4 to 2.3)
6 weeks	1.9 (0.2 to 3.4)	1.3 (0.2 to 2.4)	2.7 (0.4 to 5.1)	1.5 (0.3 to 2.4)
12 weeks	2.0 (0.2 to 3.6)	1.2 (0.0 to 2.9)	2.8 (0.9 to 4.8)	1.5 (0.1 to 2.8)
Cortisol, nmol/L				
Baseline	396 (241 to 551)	393 (227 to 559)	301 (229 to 372)	397 (179 to 615)
6 weeks	254 (0 to 472)	344 (46 to 642)	365 (197 to 543)	374 (62.0 to 745)
12 weeks	383 (68 to 698)	404 (135 to 674)	333 (214 to 451)	275 (0 to 676)

Mean (95% CI). **Abbreviations:** IGF-1: Insulin like growth factor-1. DM: *n*= 8, EX+DM: *n*= 9, EX: *n*= 10, CON: *n*= 10.

Table 7. Baseline values and the mean within-group changes at week 6 and 12 in cytokine response according to randomized allocation.

	DM	EX+DM	EX	CON
Leukocyte x10^9				
Baseline	5.8 (5.4 to 6.4)	5.3 (4.0 to 7.0)	5.3 (4.6 to 6.0)	4.7 (4.0 to 5.4)
6 weeks	5.0 (3.2 to 7.0)	5.0 (3.0 to 7.5)	4.6 (3.2 to 6.0)	3.8 (1.5 to 5.0)
12 weeks	4.8 (2.3 to 7.4)	5.0 (3.0 to 7.5)	5.9 (4.0 to 7.8)	4.0 (1.5 to 6.4)
Neutrophils x10^9				
Baseline	3.1 (2.3 to 4.0)	2.5 (1.7 to 3.4)	3.1 (3.0 to 3.5)	2.4 (2.8 to 3.0)
6 weeks	3.1 (1.8 to 4.5)	2.2 (1.0 to 3.5)	3.7 (2.0 to 3.4)	2.1 (1.5 to 3.6)
12 weeks	3.2 (1.4 to 5.2)	2.2 (0.70 to 3.0)	2.8 (1.5 to 4.5)	2.0 (1.5 to 3.3)
Lymphocytes x10^9				
Baseline	2.5 (2.0 to 3.0)	2.3 (1.4 to 3.1)	1.9 (1.5 to 2.4)	1.8 (1.5 to 2.1)
6 weeks	2.1 (1.0 to 3.2)	2.1 (0.7 to 3.3)	1.7 (1.0 to 2.5)	1.3 (0.1 to 2.4)
12 weeks	1.7 (0.3 to 3.2)	2.0 (0.5 to 3.2)	1.7 (0.4 to 3.2)	1.4 (0.5 to 2.4)
Monocyte x10				
Baseline	0.4 (0.3 to 0.4)	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.4)	0.4 (0.2 to 0.6)
6 weeks	0.4 (0.2 to 0.5)	0.3 (0.1 to 0.5)	0.3 (0.1 to 0.5)	0.3 (0.0 to 0.7)
12 weeks	0.4 (0.3 to 0.6)	0.3 (0.1 to 0.5)	0.4 (0.1 to 0.8)	0.3 (0.0 to 0.7)
Neutrophil/ Lymphocyte ratio				
Baseline	1.4 (0.7 to 2.0)	1.1 (0.7 to 1.5)	1.7 (1.4 to 2.0)	1.2 (0.9 to 1.6)

6 weeks	1.4 (0.5 to 2.3)	1.1 (0.5 to 1.8)	1.4 (0.0 to 2.1)	1.3 (0.6 to 2.1)
12 weeks	1.1 (0.5 to 3.0)	1.0 (0.5 to 1.6)	1.9 (0.9 to 2.9)	1.1 (0.6 to 1.7)
IL-2, pg/ml				
Baseline	4.2 (1.9 to 6.5)	3.5 (1.9 to 5.1)	4.2 (3.0 to 5.4)	5.0 (2.5 to 7.6)
6 weeks	3.5 (0.0 to 7.3)	4.0 (1.2 to 6.7)	4.5 (2.2 to 7.0)	10.7 (0.0 to 21.4)
12 weeks	3.4 (0.2 to 6.7)	3.1 (1.0 to 6.7)	3.6 (1.1 to 6.2)	6.0 (0.5 to 12.0)
IL-6, pg/ml				
Baseline	13.0 (0.5 to 25.6)	2.0 (1.1 to 3.0)	8.0 (2.2 to 13.2)	4.0 (0.3 to 7.2)
6 weeks	11.6 (0.0 to 30.6)	2.4 (0.0 to 4.0)	7.1 (2.2 to 15.2)	3.8 (0.2 to 8.4)
12 weeks	11.5 (0.0 to 21.5)	2.0 (0.6 to 3.7)	7.2 (0.0 to 16.0)	3.7 (0.0 to 8.1)
IL-8, pg/ml				
Baseline	10.0 (2.0 to 18.0)	1.9 (1.3 to 2.6)	5.0 (2.6 to 7.0)	4.8 (0.7 to 10.3)
6 weeks	10.5 (0.0 to 22.5)	1.4 (1.2 to 3.7)	4.6 (1.5 to 7.2)	4.8 (0.0 to 11.8)
12 weeks	9.0 (0.0 to 22.1)	1.0 (0.8 to 3.2)	4.0 (0.0 to 9.0)	4.4 (0.0 to 11.3)
IL-10, pg/ml				
Baseline	16.0 (8.2 to 23.5)	14.1 (2.4 to 25.7)	20.4 (10.0 to 31.1)	18.6 (6.0 to 31.5)
6 weeks	16.0 (8.2 to 23.6)[b]	23.4 (3.3 to 43.4)*	20.4 (9.8 to 31.0)[b]	18.7 (5.4 to 42.0)[b]
12 weeks	16.0 (8.0 to 23.5)[b]	19.0 (1.0 to 136)*	20.3 (9.4 to 31.3)[b]	18.7 (5.6 to 31.0)[b]
TNF-α, pg/ml				
Baseline	1.8 (1.5 to 2.3)	1.8 (1.4 to 2.3)	2.3 (1.4 to 3.2)	2.4 (1.1 to 3.6)
6 weeks	2.0 (1.4 to 2.8)	2.4 (1.6 to 3.3)	2.1 (0.6 to 3.7)	2.1 (0.0 to 4.7)
12 weeks	2.0 (1.3 to 3.0)	1.8 (1.2 to 2.0)	3.4 (1.3 to 4.1)	2.0 (0.0 to 4.3)

Systematic inflammatory response profile				
Baseline	48.0 (21.0 to 75.0)	16.5 (3.4 to 29.5)	41.0 (23.5 to 58.1)	37.7 (14.4 to 61.0)
6 weeks	48.2 (6.0 to 90.0)	29.0 (4.4 to 53.5)	42.0 (12.0 to 72.1)	32.1 (0.7 to 81.4)
12 weeks	40.6 (4.2 to 77.0)	24.5 (0.1 to 49.0)	32.3 (0.0 to 71.0)	33.0 (0.2 to 72.2)

Mean (95% CI). **Abbreviations:** IL: interleukin, IGF-1: Insulin-like growth factor-1. DM: *n*= 7, EX+DM: *n*= 8, EX: *n*= 8, CON: *n*= 9. *p< .05 vs baseline. [b]p< .05 vs. EX+DM.

6.5 Discussion

This study aimed to determine the independent and combined effects of a high protein dairy milk beverage provided at breakfast and lunch (or after resistance exercise), with or without PRT on outcomes of FFM, skeletal muscle strength and power, and physical performance in active older adults. In conflict with the hypotheses, a high protein dairy milk beverage did not influence gains in FFM, skeletal muscle strength, power or performance compared to control. In accordance with the hypotheses, a high protein dairy milk beverage provided and consumed twice daily, in conjunction with PRT, resulted in significant increases in strength (i.e., 78% leg press, 56% chest press, and 53% *lat* pull down) compared to PRT alone, but did not further augment changes in FFM, power, or physical performance. Moreover, the consumption of high protein dairy milk beverage during the PRT period resulted in significant increased levels of cytokine IL-10, suggesting an anti-inflammatory effect at this intervention; but did not result in any anabolic hormone enhancements compared to other interventions or control. Overall, these results suggest that the consumption of a high protein dairy milk beverage, in combination with PRT, elicits greater effects on skeletal muscle strength outcomes than consuming the dairy milk beverage or PRT in isolation. This suggests that the DM does not seem to impact FFM, power, or physical performance any more than the CON, in healthy active older adults. Therefore, a high protein dairy milk in combination with PRT may be an effective strategy in the prevention and management of age related sarcopenia in the active aging population.

The progressive decline in strength and FMM begins to be detectable from the age of ≥ 50 yrs [1], the rate of loss that occurs in skeletal muscle mass and strength is between 1-2% and 1.5-5.0%, per year respectively [47-49]. Maintaining skeletal muscle strength is a key factor to maintaining functional capacity and independent living with increasing age [1]. However, even in very physical active older adults (e.g., training ≥ 4-5 session per week), there have been observed declines in leg

strength of 3-5% per year [49]. In the current study, there was a significant increase in maximal 1RM lower and upper body strength observed in both groups that received PRT (EX+DM: ≥ 53% and EX: ≥ 35%). These findings align with previous studies that show maximal 1RM leg strength increases of > 25% after 12-weeks of resistance training in older adults [50, 51]. The improvement in maximal relative muscle strength as measured using 1RM (e.g., leg press, chest press, and *lat* pull down) was significantly higher in EX+DM (53-78%) compared to EX (35-36%), DM (4-7%), and CON (7-11%), indicating a interaction effect. These findings align with a recent meta-analysis that reported protein supplementation (20±18 g protein/day) further augments strength (33%), as measured by 1RM leg press, in community dwelling older adults (≥ 45 yrs) [11]. However, these findings contradict previous exercise intervention studies that have not observed protein supplementation to further increase gains in maximal 1RM leg strength during resistance exercise training in healthy community-dwelling and active older adults, compared to placebo or exercise only groups [50, 51]. One possible explanation for the positive finding from the current study, compared to the aforementioned studies, is the difference in the mean age (58 ± 7 yrs), compared to those in the previous studies (≥ 70 yrs). These age-related discrepancies may be due to the presence of anabolic resistance and the decreased work/power capacity that occurs with increasing age [52]. Secondly, the amount of daily protein consumed in the supplement groups may have been inadequate to illicit a significant strength adaptation between groups. For example, cohorts receiving additional milk servings were consuming 1.3 to 1.4 g/kg BM/day of protein at baseline [50, 51]. Although this is higher than recommendations for older adults (≥ 1.2 g/kg BM/day) to treat sarcopenia, it is below (1.6 g/kg BM/day) the threshold recommended to support significant changes in muscle size and strength during prolonged resistance training in healthy active adults that are novice to weight training [53-56]. Furthermore, in this current study, the addition of the high protein dairy milk beverage increased the protein intake in the EX+DM and DM group to 1.7 g/kg BM/day and 1.9g/kg BM/day, respectively. While this is much higher than the

reported amount needed for those that are novice to weight-training, (e.g., 1.3-1.8 g/kg BM/day) the lack of a further significant effect may be influenced by the CON and EX groups that were consuming high habitual protein intakes throughout the study intervention (e.g., ≥ 1.4 g/kg BM/day) [57]. Although the EX group did habitually consume a higher amount than expected (1.4 g/kg BM/day), the EX+DM group still showed a significantly greater increase in maximal strength than the EX group. This may suggest that increasing protein intake by this magnitude with PRT can lead to additional adaptations in skeletal muscle strength, as previously reported in younger adults [53]. The findings of this study may indicate that higher protein (e.g., ≥ 1.6 g/kg BM/day) intakes than those currently recommended for active older adults (≥ 1.2 g/kg BM/day) may be required to see optimal strength adaptations. This requirement is in accordance with nutrition guidelines for strength and power athletes for adaptations in skeletal muscle strength following resistance training [55-56].

The current study provided participants with a 15 g protein (1.57 g leucine) dose at breakfast and lunch (or after resistance exercise). This significantly increased relative protein intakes at those meal times (≥ 25%) in DM and EX+DM compared to baseline. The provision of this protein dose at these time points was based on previous reports suggesting that the distribution of protein is often inadequate at those times in older adults [57]. Additionally, cross-sectional reports have indicated that in healthy active older adults that consume sufficient protein, if one meal reaches this purposed threshold, it may be sufficient to elicit favorable results in FFM, skeletal muscle strength and power, and physical performance [58]. Considering the significant increase in FFM mass and strength was observed in EX+DM compared to other studies that only provided protein supplementation post-training [50, 51], this may suggest that the distribution of protein may be more relevant in older adults already consuming adequate amounts of total daily protein (i.e., ≥ 1.2 g/kg BM/day). Resistance training acutely sensitizes skeletal muscle mass to anabolic effects of

ingested protein [59]. When considering a chronic response, PRT and nutritional supplementation has an additive effect on skeletal muscle strength [11, 58]. Therefore, regular intakes of protein throughout the day increases the number of opportunities to maximally stimulate myofibrillar MPS. This accumulation of myofibrillar MPS stimulation throughout the day is likely to lead to long term positive protein balance, which may facilitate adaptations in skeletal muscle mass and strength in active older adults. The findings of this current study may indicate that older adults who are already active and consuming an adequate amount of protein may need to consider the distribution of protein to gain further benefits from PRT.

There was a significant increase in absolute FFM in EX+DM (1.2%) and EX (0.85%) groups at 12-weeks from baseline; whereas the DM group had a significant decrease in FFM (-1%) over the course of the intervention trial. The absence of any greater increase in FFM from consumption of additional protein in DM aligns and conflicts with previous findings in studies that investigated PRT and protein intake in healthy community dwellers [11, 26, 28, 52] and active older adults [9, 51]. The discrepancies amongst these studies are likely due to the large heterogeneity within the study designs, such as the use of supplementation (e.g., plain dairy milk and protein fortified dairy milk), methods of outcomes measured (e.g., iDXA, BIA, MRI), and participant fitness status (e.g., community dwelling and institutionalized). Furthermore, there is emerging evidence to suggest that whole foods such as dairy may exert a greater stimulatory effect on MPS than isolated protein supplements. For example, a review by Burd et al. [60] compared between studies the MPS response to different protein sources, and showed that skim milk was greater compared to whey protein or casein. Dairy milk also contains non-protein components that act directly as anabolic signaling molecules and can regulate nutrient activity due to the 'food matrix effect' [61]. However, more studies are needed to confirm this, especially in older active adults. Furthermore, while there were no significant interaction effects observed on outcomes of FFM, this present study confirms

that a PRT can increase FFM by 0.8-1.2% in active older adults. Although not statistically significant, this has translational practice significance in the clinical setting, as the reported loss of skeletal muscle mass observed in older adults is 1-2% per year [46-48]. This could indicate a saving of 1 - 2 yrs of skeletal muscle mass with a 12-week PRT intervention, and therefore has great practical and clinical significance for potentially reducing age-related muscle loss in older adults.

Aging is associated with the redistribution of FM; characterized by an increase in the abdominal region (visceral fat) and a decrease in appendicular (mostly subcutaneous fat) [3]. This study observed the greatest loss in absolute FM in DM (-2.0 kg), followed by the EX+DM and EX groups (-0.68 kg and -0.61 kg, respectively). The greatest loss of FM was observed in the trunk region in both the DM and EX+DM group (5-10%). While not statistically significant, these findings align with previous exercise and protein intervention trials [62, 63]. One of the most cited plausible mechanisms proposed to contribute towards the decrease in FM is: dietary protein stimulates the release of satiety hormones, increases the thermic effect of food, and stimulates protein-induced alterations in gluconeogenesis [64]. In the current study, the DM group increased their RMR by 1% at 6-weeks; although this finding did not attain statistical significance, it could explain the significant FM and BM loss observed at that time point. Additionally, the greater FM loss observed in both groups that received the DM may be due, in part, to the greater calcium intake (~983 mg/day) in the EX+DM (941 ± 316 mg) and DM (1011 ± 331mg). Increasing dietary calcium, either through whole foods or supplementation, has been shown to increase fat oxidation [65] and fat excretion in the digestive tract [66]. However, the findings of this current study contrast with findings from Kukjujan et al. [26], who observed a significant increase in FM (1.3 kg) in healthy older community dwellers (50-70 yrs) that received a fortified milk beverage to consume twice daily for 18-months. The FM gain observed in the study by Kukjujan et al. [26] was likely due to the addition of extra energy intake (836 kj; 200 kcals), which may have led to the excess energy intake leading

to gain in FM. This highlights one of the strengths of the current study, where there was a provision of food for 12-weeks, controlled for energy intake and provided 100% of estimated total daily energy requirements, and estimated protein intakes for the participants in both the EX+DM and DM group. Dietary control and food provisions also accounted for the extra energy provided by the high protein dairy milk beverage. It is important to acknowledge that other studies have failed to provide adequate dietary controls. Therefore, the increase in calcium and protein intakes from the DM may potentially explain the increased loss in FM observed in this current study despite the participants' balanced energy intake. Lastly, in relation to physical activity the DM had the lowest moderate physical activity and highest sedentary physical activity compared to the other groups (Table 3). Therefore, the losses of FM and BM observed in DM cannot be due to differences in habitual physical activity leading to increases in energy expenditure. Overall, there were no significant differences for markers (e.g., RMR, calcium, physical activity and protein intake) individually, but combined they could have a substantial effect, which may explain the significant decrease in BM and FM observed in the DM compared to the other groups.

The finding that the high protein dairy milk beverage did not enhance the effects of PRT measures of HGS and performance outcomes (e.g., gait speed, counter movement jump and cardiorespiratory fitness) are consistent with two meta-analyses that have reported mixed findings in regards to benefit of additional dairy milk protein/ or protein supplementation on outcomes of physical performance [10, 67]. In the current study, the lack of a significant change is likely due to the examination of active older adults who have physical performance measures higher than community dwellers or frail older adults, who are the typical participants in sarcopenic research [68]. This highlights the lack of research in active older adults who are still prone to age related sarcopenia [68]. For example, Dulac et al. [69] recruited sedentary (< 120 min/week of physical activity) older (69 ± 7 yrs) males and found significant gains in all groups for HGS (4 to 10%) and

gait speed (-4 to -5%). Similarly, Daly et al. [63] found a significant increase in HGS (10 to 14%) and gait speed (-3%) in inactive (< 7500 steps/day) females. Considering that the self-reported baseline physical activity levels for this current study were 227 ± 31 min/week, which is higher than the level of physical activity that is considered '*active*' for older adults (e.g., 150 min/week of light-to moderate-intensity physical activity or 75 min/week of vigorous-intensity) [12], and higher than the previously mentioned studies, could indicate that active older adults in this current study reached a '*ceiling effect'* in the outcomes of performance*,* similarly observed in high functioning and highly trained older adults [68]. For example, the average HGS at baseline was 37 kg and 42 kg, for females and males, respectively. This is higher than previous findings in community dwelling females (≥ 27 kg) [69] and males (≥ 38 kg) [70]. Moreover, gait speed has been found to have a non-linear relationship between leg strength, as indicated by a wide population variance (e.g., 22%) [68]. Therefore, any changes in skeletal muscle mass and strength in active older adults is unlikely to show an improvement in gait speed or HGS. A review by Beaudart et al. [70] proposed a gait speed test over a course of 400 m would be more clinically relevant and sensitive to detect change in active older adults than a 4-m distance. Previous works have suggested that measures of muscle power (e.g., CMJ) should be considered more clinically relevant in the active older population, due to power declining at a faster rate than strength [71]. Within this current study, there was an average increase by 10% in jump height in the groups that received PRT. Although this finding was not statistically significant, it aligns with Daly et al. [63], where a significant change in the CMJ height with a change of 3-6% was reported. The difference of results is likely due to the much larger sample size per group (*n*= 108) within the study by Daly et al. [63]. Overall, the measurements of HGS and gait speed have been used as valid measurements in the clinical setting to detect age-related declines related to sarcopenia, and may be more useful to use as an initial screening of participants, but may not be sensitive enough to detect meaningful change in an intervention trial in an older population that is physically active.

Considering the role of systemic inflammatory responses in the pathophysiology of age related sarcopenia [32, 33], the current study employed a human cyto/chemokine panel to determine intervention induced change in these immune response markers. Using a high-sensitivity multiplex assay, the results of the current study found a significant increase in plasma IL-10 concentration in the EX+DM group (81%) at 12-weeks, which was greater than in EX (-6%), DM (-5%) and CON (-8%) groups, and without any significant changes to any of the other cytokine markers measured (e.g., TNF-a, IL-2, IL-6, IL-8). Previous studies have indicated that the anti-inflammatory cytokine IL-10 is the most sensitive cytokine marker in response to exercise stress, unlike pro- (TNF-a) and response- (IL-6 and IL-8) cytokines showing no to minimal responses to acute exercise [72 - 79]. For example, studies that evaluate the cytokine response to resistance training in older adults have reported a significant increase in resting plasma IL-10 concentration (23-50%) following 16-24 weeks of resistance training compared to controls (no training) [36-37]. The significant difference increase of plasma IL-10 concentration, suggesting a greater systemic anti-inflammatory effect in EX+DM compared to the EX group is a novel finding, and the mechanism/s for such an outcome are yet unknown. Some plausible mechanisms have largely been explored in *vitro* and in *vivo* models and could be possibly be explained by the addition of the high protein milk beverage. In particular, the addition of branched-chained amino acids (BCAAs) found in dairy milk acts as a substrate for the synthesis of short-chained fatty acids (SCFAs) such as butyrate [80]. Butyrate and other derived SCFAs from BCAAs (e.g., isobutyric acid, 2-methylbutyric acid, and isovaleric acid) increase the expression of IL-10 lymphocyte cells in the gut [81]. Additionally, other constituents of dairy milk have immunomodulatory and anti-inflammatory properties (e.g., immunoglobulins, lactoferrin, α-lactalbumin), which may act upon cytokine up- or down-regulation [24]. Galactooligosaccharides (GOS), which is a prebiotic substrate derived from lactose found in dairy milk [82-84] have been found to promote the increase in the bacterial counts of *Bifidobacteria* and *Lactobaclli*, which have

an anti-inflammatory effects [83]. GOS derived from dairy milk were found to play a direct role in the regulation of CD4+ T-cells, which are involved in the proliferation of IL-10 cytokines, however this study was limited in animal models [84]. Lastly, research has previously found that individuals who consume diets that are higher in GOS following a bout of strenuous physical activity, showed lower levels of intestinal fatty acid binding protein (I-FABP: an indirect marker of intestinal epithelial injury and regulatory point for luminal bacterial endotoxin translocation and subsequent systemic inflammatory responses) compared to those that followed a low GOS diet (e.g., low FODMAPs) [72]. These studies along with our current findings indicate a potential link between protein intake and skeletal muscle health in older adults. However, further research is needed to understand the mechanistic potential for dairy milk.

Anabolic resistance in aging individuals may be due to the changes in systemic anabolic hormones with increasing age (e.g., testosterone and IGF-1), which has a direct correlation with the onset of sarcopenia [85, 86]. In addition, the decrease in estrogen levels associated with menopause, may also play a role in the decline in skeletal muscle mass and skeletal muscle strength in aging females [87]. In the current study, there were no significant changes in the outcomes related to systematic resting hormonal markers in any of the groups. Previous studies have found that resistance training alone can increase circulating levels of IGF-1 [88] and testosterone [89] in older adults. In contrast, other studies have not found such effect [90 , 91]. In the current study, there was an increase in testosterone in the both groups that received PRT (> 7%). However, there was only an increase in IGF-1 in the EX+DM group (80%) at 12-weeks from baseline. West & Phillips [92] found in young males (18-30 yrs) that the associated effects (e.g., increase in skeletal muscle strength) of resistance training on circulating anabolic hormones was modest, and explained 8-12% of the variance for changes in lean skeletal muscle. Considering that older adults have lower resting anabolic circulating hormones than their younger counterparts, the effect of PRT may be even less.

Nonetheless, in older adults even a modest effect on skeletal muscle strength or skeletal muscle may have practical implications, as even small improvements could result in increased functional capacity for those at risk of sarcopenia.

Overall, the strengths of this study lies in its randomized controlled design, high study retention (≥ 80%), and compliance rate (≥ 80%) to the intervention, and the comprehensive outcomes measured including FFM, strength, power, and physical performance accounting for outcomes that may be more relevant in an active aging cohort (e.g., CMJ). This study also controlled for variables such as dietary intake, through the use of providing food previsions and monitoring food intake through diaries. Physical activity was monitored over the course of the clinical trial to ensure changes in outcomes were due to the exercise intervention and not due to participants increasing habitual exercise outside the trial, which other studies have failed to implement. The measurement of blood markers (e.g., cytokines and hormones) provided an extensive insight into the pathophysiology and potential mechanisms of the interventions. Previous nutrition and exercise intervention studies have shown significant interaction effects on sarcopenia outcomes (i.e., FFM, strength and performance) with a study size of 6 - 196 participants' per group, in two to four group studies [9, 10]. In the present study a larger sample size may have accounted for identifying subtle significant difference between the outcomes measured. From a clinical and practical perspective these smaller changes that may have been observed in a larger sample size, would possibly be of no clinical relevance beyond the magnitude of change that has already been observed in this current study that was sufficiently statistically powered. One limitation that should be acknowledged is that physical activity at baseline was self-reported. Therefore, it is unknown if non-exercise activity or exercise-activity, which can be significant components of energy expenditure increased during the intervention period. This could have resulted in an increased energy deficit leading to more significant weight-loss as observed in DM [93]. However, the most important limitation of this study is the large habitual protein intakes in the EX and CON groups

(e.g., 1.4-1.6 g/kg BM per day). These participants were not provided a control diet based on previous studies that have suggested that up to 50% of active older adults do not meet the the protein requirements of 1.2 g/kg BM/day [94]. Therefore, an assumption was made that protein intake would be lower and unevenly distributed than our dietary intervention. However, protein intake between groups was comprehensively managed, assess and analyzed; and a strength in comparison to previously published investigations of a similar nature [9 , 10]. Furthermore, unlike previous studies that used isolated protein supplementation, this study used whole foods (i.e., dairy milk), which are commercially available and accessible. The addition of 500 ml of dairy milk translates to two additional serves of dairy, consistent with the Australian Guidelines to Healthy Eating [95], which may provide a cost-effective solution to providing a high-quality protein source and subsequent amino acids and were well-tolerated (compliance: 93 ± 8%).

6.6 Conclusion

This study showed that a daily consumption of two high protein milk beverages at breakfast and lunch (or after PRT) significantly enhanced the effects of PRT on skeletal muscle strength outcomes in active older adults, that already have high levels of protein intake (≥ 1.2 g/kg BM/day). There was a significant increase in FFM following the PRT, but no augmented effect with the high protein dairy milk beverage. There was a significant decrease in FM, in those consuming the high protein milk beverage. Potentially the influence from the protein, or other constituents of the high protein milk beverage (e.g., calcium) may have contributed to the significant reduction in FM observed. Additionally, EX+DM led to a significant increase in resting anti-inflammatory cytokine (i.e., IL-10), which all may play a significant role in improving skeletal muscle mass and strength outcomes in active older adults (e.g., inflammaging). Finally, these results suggest that consuming a high protein milk beverage at times that are usually inadequate in protein with PRT can facilitate gains in muscle strength, FFM and reductions in FM, and is well-tolerated by active older adults. Overall, the

findings of this study are novel and define opportunities for future interventional studies examining age-related sarcopenia in the healthy active aging population.

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CHAPTER SEVEN:

GENERAL DISCUSSION

7.1 Summary of key findings

The overall aim investigated in this thesis is part of an overarching relationship between dietary protein in the form of a high protein dairy milk beverage, with or without PRT on outcomes related to sarcopenia (e.g., SMM, skeletal muscle strength, power and physical performance) in a cohort of active older adults. To address this, a systematic review of the literature was (Chapter two), conducted in two parts: 1) to systematically identify and synthesise results of RCTs assessing the effects of dairy milk beverages and dairy-based protein interventions (± physical exercise) on outcomes of SMM, skeletal muscle strength and power, and performance in older adults ≥40 yrs; and 2) to identify the ideal formulation of a dairy milk beverage, considering dose, timing and nutritional quality to support the maintenance or gains in SMM, skeletal muscle strength and power in active older adults. Limited studies used dairy milk only. Therefore, the SLR expanded to include dairy-based protein supplementation to determine an ideal protein formulation for a dairy milk beverage. The major methodological issues that were identified include i) large heterogeneity between studies; ii) failure to consider biochemical parameters (e.g., hormonal and cytokine); iii) failure to control for dietary intake; iv) the use of physical performance measures that may not be suitable for a 'healthy' or 'active' population ((e.g., Short Physical Performance Battery (SPPB) and gait speed)); v) failure to assess potential confounding changes in cardiorespiratory fitness (CRF). The methodological gaps in the current studies included i) lack of inclusion of active older adults and ii) lack of milk only studies. These methodological issues and gaps were further emphasised in a *letter to the editor,* as shown in Appendix A in response to the most recent SLR. As described in Chapter Three, the general methods are a result of this inclusion of the limitations.

Two cross-sectional studies were conducted to understand the current anthropometrical, strength, physical activity levels and dietary habits of older adults that are considered active to help contextualise intervention studies results. The main findings of Chapter Four were i) Middle-aged

(50-59 yrs) and older adults (≥60 yrs) had no significant differences in body composition, but did display differences in skeletal muscle strength, power, and performance; ii) There were no significant differences observed on any of the outcomes when comparing low, moderate or high self-reported physical activity; iii) higher dietary protein intakes (≥1.2 g/kg BM/day) was associated with a trend towards lower FM and higher skeletal muscle leg strength in active older adults; iv) significant differences in body composition, skeletal muscle strength, and power outcomes exist between male and female active older participants. Overall, this chapter found that in a cohort of active older adults, contributions of age, physical activity, daily protein intake and biological sex can explain individual variation in outcomes related to body composition, strength, and power.

Chapter Five explored the relationship between protein intake and outcomes of SMM, strength and power. The main findings of this chapter were that active older adults that consumed and at least 1 meal containing ≥0.4 g/kg BM of protein per day and enough habitual protein (e.g., ≥1.2 g/kg BM/day) were associated with favourable outcomes in maximal leg strength in males, but not females. There was a moderate positive association between the protein and dairy serves and leg strength outcomes.

Finally, the findings of the central chapter of this thesis (Chapter Six) did not support the hypothesis that additional protein supplementation alone in the form of a high protein dairy milk beverage would enhance gains in FFM, skeletal muscle strength, power or performance compared to control. In contrast, per the hypothesis, a high protein dairy milk beverage provided and consumed twice daily, in conjunction with PRT, resulted in significant increases in strength (i.e., 78% leg press, 56% chest press, and 53% *lat* pull down) compared to PRT alone, but did not further augment changes in FFM, power, or physical performance. Moreover, an observed increase in resting levels of cytokine IL-10 suggests an anti-inflammatory effect in the group that received the DM+EX.

In this final chapter, findings from previous chapters are summarised in terms of the main outcomes explored throughout this thesis; fat-free mass, skeletal muscle strength, skeletal muscle power and physical performance. Findings from all included chapters are summarised within each topic, and build on their individual discussions which include the main variables explored throughout this thesis (e.g., protein intake, physical activity, age etc.). The summary of the findings build on the current knowledge, explore potential mechansims, and aim to link all intervention chapters together (i.e., Chapters Two to Six). This is followed by discussing the research strengths and limitations, as well as recommendations for future research and practice.

7.1.1 Outcomes of skeletal muscle mass; fat-free mass

Skeletal muscle is a highly dynamic tissue in a constant state of turnover [1]. It is widely accepted that the age-related decline observed in SMM and skeletal muscle strength is due to the disruption of muscle protein balance, leading to a structural imbalance between MPS and muscle MPB, where there is an increase in the breakdown of skeletal muscle proteins [2]. There is abundant evidence that PRT is an essential component in any intervention to increase FFM, skeletal muscle strength and reduce age-related changes in body composition and physical performance [3]. Although PRT is considered fundamentally anabolic, to achieve a positive net gain in SMM outcomes, dietary protein interaction is needed [4]. For this reason, the current literature has a greater focus on nutritional interventions and resistance training to enhance the acute anabolic response (e.g., MPS), which augments long-term SMM adaptations such as muscle hypertrophy and skeletal muscle strength [5-7]. The findings from the randomised control trial within this thesis (Chapter Six) found a significant increase in absolute FFM in EX+DM (1.2%) and EX (0.85%) groups at 12-weeks from baseline. In contrast, the DM group had a significant decrease in relative FFM (-1%). There were no significant interaction effects observed, which did not support the study hypothesis. Following these findings, in Chapter Two, it was found that 50% (3/6) of the dairy studies and 80%

(21/26) of dairy-based supplement studies significantly increased FFM from baseline with an exercise effect, and significant increases in FFM ranged from 1.6 to 22%. However, only 19 % (5/26) of the dairy-based supplement studies and none of the dairy studies showed an interaction effect. The mixed results across studies could likely be due to individual variations within participants, differences in measurement methods, differences in habitual protein intake, and differences in participant characteristics.

Individual variation

The changes in FFM were highly variable between participants in the EX (-0.3 to 3.6 %) and EX+DM (0.0 to 3.8%) groups (Chapter Six). Similar ranges of variability have been reported in previous studies. One retrospective analysis found a variation of muscle size from −11 to 14 % following a 20–24-week PRT in healthy community-dwelling adults (19 to 78 yrs) [8]. Similarly, a meta-analysis of 111 resistance training intervention studies observed a wide range of heterogeneity in SMM measures (0 to 7.2 kg) in healthy adult males [9]. One possible consideration is the baseline differences of FFM. There is a clear difference in baseline FFM, fat mass, skeletal muscle strength, power and physical performance that is largely governed by differences in training status, biological sex, and age, as discussed in Chapter Four. For example, the findings from Chapter Four observed in a cohort of *n*= 54 active older adults that males had 25% more relative FFM compared to women (Chapter Four). This finding is supported by numerous cross-sectional studies that have reported significant sex differences in FFM [10-12]. While females have a lower initial FFM and strength than males, previous intervention studies in older adults have reported conflicting findings as to whether females can gain relative FFM to the same degree as males. For example, Da Boit [13] found in older community-dwelling adults (>65 yrs) that underwent 18-weeks of resistance training that males increased FFM (34%) significantly more than females (9%). In contrast to these findings, Churchward-Venne et al. [14] observed in older adults (>70 yrs) undergoing 24 weeks of resistance

training that there were no significant differences in strength or muscle mass in males compared to females. This was despite the females in this cohort having a significantly lower initial muscle mass and strength than males in this study mentioned above [14]. While differences in biological sex may explain the large variation response in FFM within the main findings of this thesis (Chapter 6), when considering the changes in FFM in those that received PRT, females gained more FFM (1.2 %) over the 12-week intervention trial, compared to males (0.8%). Therefore, within this current thesis, the differences in biological sex in active older adults do not support females' inability to gain relatively more FFM compared to males.

Another potential explanation for the considerable individual variation may be the concept of "*muscle memory*". Muscle memory refers to the observation that prior exercise training results in the increased ability to gain FFM and strength in individuals who have previously undergone resistance training than a novice to the practice [13]. Within this thesis, there was the inclusion of participants who did not participate in structured resistance training within a 12-month period to account for this phenomenon based on previous studies protocols [14-18]. However, one review suggests that this muscle memory might be longer lasting in humans for up to 15 yrs [19]. Regular resistance training in a long-term training period increases the number of myonuclei in the cell, which results in an increase in muscle fibres [19]. When an individual experiences detraining, it leads to muscle atrophy but does not decline in myonuclei [20]. Unfortunately, previous lifetime exercise history was not accounted for in the current participants. Therefore, it may be that a higher level of "muscle memory" within the study participants with different training backgrounds may have contributed to variation in increases in FFM.

Despite the novel aspect of the inclusion of active older adults, the participants from this study came from a variety of sporting backgrounds but all aerobic/ endurance focused including

endurance runners/ race walkers (61%), cyclists (9%), aerobic gym-goers (16%) or a combination of multiple activities (14%). The differences in the training modality, frequency, and intensity within the study participants' habitual physical activity outside the study design could have affected their ability to gain FFM and strength throughout the 12-weeks, due to an interference effect [21]. The interference effect postulates that when an individual undergoes resistance training and concurrent endurance training, it can result in overreaching and overtraining, leading to competing adaptations within a long-term training program [21]. From a molecular perspective, endurance training results in the phosphorylation of activated protein kinase (AMPK) and proliferator-activated receptor gamma coactivator (PGC-1) protein levels to promote mitochondrial biogenesis, which increases oxidative capacity, substrate utilisation and capillary density [22]. Resistance training induces the phosphorylation of the anabolic AktmTOR signalling cascade, resulting in myofibrillar MPS and skeletal muscle hypertrophy [23]. Therefore, the inclusion of endurance athletes undergoing PRT training could have interfered with each other and produced inferior FFM gains. In an early *vivo* study conducted in rats showed that when subjected to endurance-like stimulation it activated the AMPK/PGC1- streams and inhibited the AktmTOR and its downstream targets [24]. In this model it suggests that concurrent training of endurance exercise may inhibit the pathways that are important for muscle growth (e.g., AktmTOR), which may lead to a decreased capacity for skeletal muscle hypertrophy. While this may explain the interference effect's potential mechanisms, this theory remains elusive in humans [25, 26]. Furthermore, Wilson et al. [21] observed significant decrements in hypertrophy and strength in those trained concurrently in endurance running, but not only moderate decreases in those who participated in endurance cycling. This may be due to cycling being more biomechanically like the significant strength and power measures often undertaken (e.g., leg press, vertical jump).

Furthermore, there is a difference in muscle contraction (eccentric vs concentric) within the difference sporting modalities, which may create more muscle damage in running than in cycling. For example, Koller et al. [27] observed a significantly higher amount of skeletal muscle damage following ultra-marathoners (67 km) compared to modest amounts of muscle damage in long-distance cyclists (230 km). While previous studies have indicated that physical inactivity is one of the major causes of age-related sarcopenia [28], training type and modality may influence the capacity to gain FFM. This may explain why there was such an individual variation within the response to FFM within the study groups that received PRT. Future studies should consider recruiting active older adults from similar training modalities to reduce this potential cofounding bias.

Methods of measurement

The DXA is considered a 'gold standard' method to provide an accurate FFM measure, fat mass, and bone mineral density. Therefore, it is often used in the clinical and research setting to assess body composition changes in intervention studies, as evident in the SLR (Chapter Two). In Chapter Two, most studies reported absolute changes in body composition to establish conclusions about their interventions' outcomes. Chapter Six reported both absolute and relative FFM changes that occurred over the 12-week intervention trial and the ALM/ht^2. Interestingly, there was a significant change in absolute FFM; however, when considering changes in relative FFM and ALM/ht^2, there was no significant change. Participants were asked to come in euhydrated, at baseline hydration status as measured by plasma osmolality (296 ± 5.6 mOsmol/kg), indicated that they were slightly dehydrated. There were no significant changes in ALM/ht^2 or regional FFM. This may suggest that the significant differences reported in whole-body FFM may be due to body hydration fluctuations. However, this was accounted for, and there were no significant differences in total body water over the 12-weeks. More precise SMM measures are the cross-sectional area (CSA) measured using a CT or MRI. Quadriceps CSA was measured in *n*= 4 supplement studies and *n*= 2 of the dairy

studies (Chapter Two). Although it may be highly accurate at measuring skeletal muscle mass changes, it is costly and relatively inaccessible in a clinical setting. Therefore, this highlights the necessity to combine outcomes used to define sarcopenia with changes in skeletal muscle mass, strength, and performance. Furthermore, one major limitation in the use of iDXA in the ageing population is that it cannot measure the muscle's fat infiltration, which is one of the main characteristics of sarcopenia [29]. Therefore, skeletal muscle mass may be overestimated, especially in individuals who may have sarcopenia and may explain the considerable variation of FFM and even skeletal muscle strength increases throughout the studies.

Total protein intake

Dietary protein is an important anabolic input that can stimulate MPS and inhibit MPB, resulting in a net positive protein balance [1]. This is an important contribution to the maintenance of FFM, skeletal muscle strength and power, and physical function. The current recommended daily allowance for older adults is 0.8 g/kg BM/day [30]. This recommendation is based on nitrogen balance studies of mostly younger adults (20-30 yrs) to meet the minimum requirements to achieve nitrogen balance within 2 standard deviations. It does not consider the age-related physiological changes that result in an attenuated capacity of protein utilisation for MPS (i.e., *anabolic* resistance) [31]. The PROT-AGE study group has suggested that older adults > 65 yrs should consume at least 1.0 g/kg BM/day [32]. Moreover, active older adults should consume even more, i.e., ≥ 1.2 g/kg BM/day of protein, to comply with the synergistic effects of exercise and protein intake on muscle protein synthesis [32]. There were likely no significant differences in FFM in the groups receiving the PRT due to the already high habitual protein intakes by most participants at baseline. For example, in Chapter Five, it was shown that active older adults reported a high average intake of daily protein 1.4 ± 0.4 g/kg BM/day at baseline, with 60% of females and 72% of males consuming ≥ 1.2 g/kg BM/day of protein-based on 3-day food diaries.

This baseline protein intake is higher than what has been previously reported in intervention trials in active older adults (1.0 - 1.1 g/kg BM/day) [33, 34]. Additionally, ten Haaf [35] assessed the effects of 12-week daily protein supplementation on outcomes related to sarcopenia in physically active older (67-73 yrs) adults that were consuming low protein intakes (e.g., <1.0 g/kg BM/day). In this study, those that consumed an additional 31 g of milk protein a day increase FFM more than the placebo group (0.93 % vs 0.44%). Therefore, considering that the habitual daily protein intake across all the study groups was already adequate (e.g., >1.2 g/kg BM/day), this may explain why there were no significant differences between groups in outcomes of FFM.

Overall, there was a significant increase in FFM of 0.8-1.2% following 12-weeks of PRT, without any significant differences between groups. While there were no significant differences between groups, this has a translational practice in a clinical setting. Considering that the loss of SMM observed in older adults is reported as 1-2% per year from the age of 50 yrs, this could indicate that 12-weeks of PRT, 3 days a week could save 1-2 yrs of SMM. Furthermore, understanding an individual's sensitivity to a certain type of RT may enable individually tailored exercise training programs to optimally improve or maintain healthy muscle function throughout the lifespan.

7.1.2 Outcomes of skeletal muscle strength

During this candidature, there were changes to the European Working Group of Sarcopenia's (EWGOS2) clinical definition, where the measurement of strength became at the forefront of the diagnosis [36]. From the ages of ≥ 50, changes in skeletal muscle strength have been observed to decline at a faster rate (3-5 %) than skeletal muscle mass [37]. Declines in skeletal muscle strength directly correlate to functional performance and can be the defining moment for older adults maintaining their physical independence [38]. The main findings of the intervention trial presented in Chapter Six showed that an increase in protein intake from 1.2 g/kg BM/day to 1.8 g/kg BM/day

(mean ±SD) after 12-weeks of milk protein supplementation with resistance training in physically active older adults was associated with ≥25% increase in maximal strength (e.g., leg press, chest press and lat pull down). There was an observed synergistic effect for the groups that received the high protein milk and PRT on relative maximal strength outcomes (Chapter Six). However, there were no significant differences in absolute strength outcomes. The total strength results are consistent with previous studies that have reported increases in maximal leg strength of >25% following 12-weeks of resistance training in older adults [33, 34]. Despite the use of protein supplementation to augment adaptations in skeletal muscle mass and strength have long been advocated, the results of various studies have been somewhat contradicting [14-18]. For example, in Chapter Two, only 19% (6/26) of the included studies showed a synergistic effect of protein with resistance training and skeletal muscle strength. Of these studies that did show a significant synergistic effect of protein with RT, they varied in protein doses (e.g., 10-35 g), timing (morning and lunchtime, post-training), type of supplement (e.g., whey protein, casein) and participant type (e.g., community dwellers, active and inactive older adults). The differences in results from the current thesis and previously reported studies may be due to protein distribution, the total dietary protein and the type of protein used (e.g., dairy milk).

Protein distribution

Evenly distributed relative and absolute protein intakes per meal have been advocated, as there is an observed maximum capacity for utilising EAAs [37]. It appears that optimal doses of protein per meal to elicit a near-maximal response have been reported at ~25-35 g/meal (~10 g EAA) [38, 39] or relative amounts of 0.40 g/kg BM/meal. Studies in frail and community-dwelling older adults have observed skewed protein intakes, with most of their protein intake at the evening meal [40]. In Chapter Five, it was observed that active older adults also consumed an uneven distribution of protein at breakfast (17.7 ± 9.3 g) compared to lunch (28.1 ± 19.8 g) and dinner (42.7 ± 19.8 g). For

this reason, the high-protein dairy milk beverage was provided at breakfast and lunch (or after resistance exercise). This alteration in protein distribution may explain the significant interaction effect in the current study (Chapter Six) compared to most previously reported intervention studies that only provided participants with protein post-exercise (Chapter Two). However, likely, this mechanism may only occur when total protein intake is above adequate (e.g., >1.2 g/kg BM/day). For example, ten Haaf et al. [35] provided 2x 18 g dose of milk protein supplement at breakfast and lunchtime in physically active older (>67 yrs), adults that had low habitual protein intakes (e.g., <1.0 g/kg BM/day) undergoing 12-weeks of resistance training and did not report any significant interaction effect on outcomes of strength or muscle function compared to placebo. The mechanisms underlying the improved relative skeletal muscle strength in Chapter Six are likely due to the enhanced protein synthesis and reduced protein breakdown from the increased amino acid availability provided by the high-protein milk beverage. While resistance training alone directly initiates MPS via mechanical loading through the activation of IGF-1 signalling, the net balance will remain negative unless protein feeding occurs [5]. The regular intakes of protein throughout the day, as provided in the EX+DM group (Chapter Six), provided more opportunities to maximally stimulate MyoMPS, which likely lead to long term positive protein balance, which may explain why there was a synergistic effect of the high-protein dairy milk on outcomes of strength, compared to the EX only group, that did not have an evenly disrupted protein intake. Myofibrillar hypertrophy occurs due to an increased number of myosin/actin filaments inside each sarcomere [41]. This leads to increased strength and size of the contractile until ((actin, myosin, troponin, and tropomyosin), thus leading to increased strength observed [41]. This is different from sarcoplasmic hypertrophy, leading to increased sarcoplasm and non-contractile proteins (α-actinin, desmin, dystrophin, myomesins, nebulin, titin, and vinculin), which do not lead to an increase in muscle strength and force [42]. Therefore, changes in FFM can occur without any subsequent changes in skeletal muscle strength. This may explain why there were increases in skeletal muscle strength in

the EX+DM group, which were greater than the EX-group, without any significant differences in skeletal muscle hypertrophy. Therefore, it may be that the synergistic effects observed from the high-protein dairy milk beverage may have increased the synthesis of the contractile proteins leading to an increase in maximal relative strength.

Total protein intake

As expected, the groups that received the high-protein milk beverage significantly increased their daily protein intake by 40-50% compared to baseline (Chapter Six). In Chapter Six, it has been acknowledged that the habitual dietary daily protein intakes in all groups were above the suggested requirement for active older adults at baseline (≥ 1.2 g/kg BM/day), which may have cofounded, as there was no significant difference between the EX+DM and EX groups total protein intake after 12-weeks [33]. Furthermore, a meta-analysis indicated that total daily protein intakes of >1.6 g/kg BM/day do not further benefit from resistance or strength training on FFM outcomes in older adults [15]. However, these findings are based on healthy community-dwelling older adults. Therefore, it is difficult to determine whether these guidelines apply to the higher physical and physiological demands in active older adults. For example, a study in younger (18-40 yrs) endurance-trained (≥6 days/wk, ~1–1.5 h/day) males found that the estimated average requirement and the upper 95% CI for protein were 2.1 and 2.6 g/kg/day, respectively based on amino acid oxidation levels, following 24- hours post-exercise [43]. In a similar study in young male bodybuilders, the protein requirements were estimated at 1.7 and 2.2 g/kg BM/day [44]. Although these studies were conducted in younger adults, likely, active older adults (e.g., >50 yrs) that need to overcome the anabolic resistance of ageing and the oxidative losses of whole-body amino acids may even require more protein than what is considered "maximal" for active older adults.

Protein quality

Currently, most intervention studies have focused on isolated milk proteins (e.g., whey protein isolate), investigating their potential roles in augmenting adaptions from exercise training (Chapter Two). However, the use of plain-unfortified high-protein dairy milk is novel and has not previously been researched in a cohort of active ageing adults (Chapter Six). Milk contains the highest score on the PDCAAS and DIAAS rating system, which is a score based on the quality of proteins based on their digestibility, net protein utilisation and biological value [45]. Dairy also contains both whey (20%) and casein (80%), both of which include the highest leucine content of all other protein sources at 11% and 9%, respectively [46, 47]. Leucine is the only amino acid that directly affects skeletal muscle anabolism through the activation of the mTORC1 signalling in response to increased EAAs [47]. While Leucine is an essential factor for MPS, most of the studies included in the SLR (Chapter Two) also had high-quality protein sources at higher doses that contained high levels of leucine and did not show significant results in SMM, strength and physical performance. Therefore, the differences in skeletal muscle strength could also be due to dairy milk's other components (e.g., calcium).

Milk is a complex food that contains several nutrients (e.g., calcium, phosphorus, magnesium, Vitamin A, E and water-soluble B-vitamins) as well as bioactive components (e.g., β-lactoglobulin, immunoglobulins, lactoferrin), and components of the milk fat globule membrane (MFGM) that are beneficial for human health [46]. Although the focus of this thesis was the component of protein that is found in dairy milk, these other components that are not found in isolated protein sources (e.g., whey protein isolate) may alter the absorption and digestibility of the protein, known as the' food matrix effect' [48]. Furthermore, the food matrix effect may influence the post-exercise stimulation of MPS and the skeletal muscle's remodelling. For example, Burd et al. [49] compared studies of the MPS response to different protein sources and showed that skim milk

simtulated MPS 10-30% greater compared to whey protein or casein. Another example is from a study that assessed the impact of whole eggs, compared to iso-nitogenous egg whites, and found a significantly higher response in MPS after the consumption of whole eggs compared to egg whites, following resistance training in health younger males [50]. The authors in this aforementioned study concluded that other componets in the whole egg that are not found in the egg whites (e.g., lipids, cholesterol, vitamins, minerals) may have contributed to the increase in MPS observed [50]. Therefore, the inclusion of a high-protein dairy milk beverage that includes other nutrients may have played a role in the observed syngerstic effect on strength outcomes observed in this current study, that were not seen in previous studies only using isolated proteins (Chapter Two).

In this current thesis, the potential food matrix effect of dairy may be explained by the significant increase in IL-10 in the EX+DM group, as discussed in Chapter Six. The addition of BCAAs, which are found in dairy milk, acts as a substrate for SCFAs such as butyrate. Butyrate increases the expression of IL-10 lymphocyte in the gut [51]. Other potential mechanisms includes the presence of galactooligosaccharides in dairy milk, which increases the bacterial counts of *Bifidobacteria* and *Lactobacilli* through a prebiotic effect [52]. GOS directly impacts the regulation of CD4+ T-cells, which are involved in the proliferation of IL-10 cytokines. However, this study is only in animal models [53].This potential anti-inflammatory effect observed from the dairy milk beverage consumption in this current thesis may explain the significant difference observed in maximal strength between the EX+DM and DM groups. IL-10 is responsible for suppressing the pro-inflammatory cytokine response in skeletal muscle tissue [54] by reducing the activation of inflammatory cytokines (IL-6, TNF-α and IL-1β) [51]. In an *vitro* study, it was observed that after co-culturing muscle cells with macrophages with IL-10, muscle cells were then stimulated for 24-hours to induce the M2 phenotype. This study found that the cells with IL-10 and M2 present increased

myoblast proliferation and MyOD (myoblast determination protein 1) expression [55]. This *vitro* study indicates that IL-10 is required for average muscle growth and regeneration of muscle following injury [55]. Therefore, this may explain the potential mechanisms of the dairy milk beverage and the subsequent increase in IL-10, leading to a greater muscle strength gain in the DM+EX group. This may directly affect the expression of skeletal muscle mass and, therefore, the adaptations in strength training. However, further research is needed to understand dairy milk's mechanistic potential and the food matrix effect.

7.1.3 Outcomes of physical performance and HGS

In sarcopenia research, the decline in physical performance is often associated with functional decline, which often leads to adverse health outcomes of decreased mobility, increased risk of falls, loss of independence and increased risk of morbidity [56]. Throughout this thesis, these physical parameters often used in sarcopenia research may not be suitable/or relevant in measuring clinically significant differences in active older adults [Appendix A, Chapter Two, Three, and Five]. HGS strength and gait speed are considered reliable diagnostic tool for predicting disability, morbidity, and mortality before hospitalisation in older populations [57]. However, changes in HGS and gait speed may not offer the same sensitivity in a cohort of active older adults that often exceed physical activity recommendations [Appendix A]. For example, in Chapter Six, there was no significant change observed for HGS or gait speed following the 12-week intervention in any group. This contrasts with other studies in sedentary older males (69 ± 7 yrs) and females (50 to 70 yrs) that have reported significant improvements in HGS and gait speed following an exercise and nutrition intervention trial [58, 59] and in support of the majority of the studies included in Chapter Two, in healthy community dwellers not finding any significant outcomes on physical performance. The high level of baseline physical activity within the cohort of the included studies was higher than what is considered '*active*' for older adults (e.g., 150 min/week of light-to moderate-intensity

physical activity or 75 min/week of vigorous-intensity) [60], and higher than the previously mentioned studies, could indicate that active older adults in this current study reached a '*ceiling effect*' in the outcomes of performance*,* similarly observed in a high functioning and highly trained older adults [Appendix A]. Furthermore, while these measurements are considered reliable and predictive tools for possible disability and functional decline in clinical settings, they may be more useful in the initial screening of participants to detect sarcopenia status rather than significant changes in an intervention trial in active older adults.

7.1.4 Outcomes of Skeletal muscle power

Previous works have suggested that muscle power measures (e.g., CMJ) should be considered more clinically relevant in the active older population due to power declining faster than strength [61]. Although gait speed is a physical performance measure of muscle power, it has little relevance to performance measures in an active ageing cohort, as previously discussed. This can be demonstrated in Chapter Four, where the older aged group showed significantly lower outcomes of jump height (-28%), relative power (-17%) and velocity (-20%) compared to the middle-aged group, but no significant difference in gait speed was observed. Furthermore, CMJ has been found to correlate well with other methods commonly used to assess the risk of falls (e.g., chair stand) [62]. In this current thesis, there was an increase of the CMJ by 10-12% (cm and W/kg BM, respectively) in the EX+DM group only, although these findings were not significant, they are in-line with previously reported studies [59]. For example, Daly [59] reported a significant increase (7%) in CMJ (cm) in the group that received a fortified milk drink, which was higher than the control group (5%). The results and the current thesis's differences may be due to the larger sample size, *n*= 244 in the study mentioned above. However, Villanueva [63] that included *n*= 22 recreationally active males, also reported a significant synergistic effect where the whey protein + creatine group increased their CMJ (W/kg) greater (38%) than the control group (5%). The difference between

Villanueva [63] and the current study that could explain the difference in results could be due to the inclusion of creatine within the supplement, which is consistently known to increase muscle power, especially in the lower limbs [64], the homogenous study sample compared to this current thesis, or that muscle power was specially trained with the inclusion of plyometric exercises.

Furthermore, the loss of skeletal muscle power cannot be explained by muscle atrophy alone. The cause of muscle power has been attributed to the loss of type II fast-twitch muscle fibre size [61]. This was demonstrated in an original study by Lexell et al. [65] that observed in 30 male cadavers a 18% decline in muscle fibre size, and a 25% decline in total muscle fibre size in the cross section of the *Vastus Lateralis* in older adults (70 ± 1 yrs) compared to younger males (30±6 yrs) [65]. Nonetheless, the CMJ is a simple, valid, and reliable measure of lower-body power. It mimics similar physical challenges that older adults undertake daily living activities, such as getting out of a chair or climbing up stairs [66]. Therefore, although potential cofounders (e.g., sample size, heterogonous sample or supplement ingredients) assessing muscle power in active older adults using methods such as the CMJ should still be considered in future research.

Body fat

With increasing age, there are changes to the distribution of FM, characterised by an increase in the abdominal region (visceral fat) and a decrease in appendicular (mostly subcutaneous fat) [3]. This change in fat distribution around the abdominal area can increase the risk of comorbidities, such as insulin resistance and cardiovascular disease [67]. An unexpected finding within the main study chapter (Chapter Six) was that there was a significant decline at 12-weeks in FM in the DM (1.6%), followed by the EX+DM (0.5%) compared to baseline. While there seemed to be a sizeable inter-individual variation within the DM group (-6.1 to 0.9 kg), the BF loss was overall significantly greater than any other group. The greatest loss of FM was observed in the trunk region in both the

DM and EX+DM group (5 - 10%) (Chapter Six). While not statistically significant, these findings align with previous exercise and protein intervention trials [59, 68]. High protein diets help promote a reduction in overall body fat percentage through the maintenance and increase in lean muscle mass at the expense of fat mass in participants with similar activity levels [69, 70]. This was shown in Chapter Four, where participants with higher daily protein intakes (≥1.2 g/kg BM/day) had 6 and 9% less body fat than those that had moderate (≥0.8-1.19 g/kg BM/ day) or low protein intakes (≤0.8 g/kg BM/day). Both the DM and EX+DM groups had an increase of protein by 10% from baseline. At 12-weeks, both groups had >37% of their total energy derived from protein (Chapter Six), which protein diets that include protein contribution >30% are considered high [71]. This significant increase in dietary protein could have stimulated the release of satiety hormones (e.g., CCK, GLP-1, YYY) or increased the thermic effect of food, which is higher in protein (e.g., 20%–35%) than other macronutrients (e.g., carbohydrates 5-15%), therefore may contribute to more significant energy expenditure during rest [72-74]. However, there was no substantial change in RMR observed in any group (Chapter Six). As appetite and the satiety hormones were not measured, it is uncertain if this is the cause of the significant weight-loss observed. However, another possible mechanism is the significant increase of calcium intake in the DM (+1026 mg/day) and EX+DM (+946 mg) groups from baseline (Chapter Six). Increasing dietary calcium through whole foods or supplementation has been shown to increase fat oxidation [75] and fat excretion in the digestive tract [75]. This has been demonstrated in *vitro,* and *vivo* studies, where a decrease in intracellular Ca^{2+} induced by dietary intake suppresses calcitro-phic hormones (e.g., 1,25-dihydroxyvitamin D); this by virtue increases the expression of fatty acid synthase, which results in the increase of lipid synthesis [77, 78]. A cross-over study, where participants were provided with high dairy diets, showed an increase in fat oxidation (30 g/ day or 270 kcal/day) using a whole-room calorimeter [79]. Similarly, a 12-month trial showed a high calcium diet (1000-1400 mg/day) significantly increased fat oxidation rates [80]. Although these mechanisms are plausible reasons

as to why there was a significant loss in FM in the DM, it does not explain why there was no significant decrease in FM in the DM+EX group that were also consuming the same amount of dairy milk.

Another potential reason why there was a significant FM loss and loss of FFM observed in the DM group could be simply that the participants' food provisions were insufficient. This could be due to the study design's potential limitations in Chapter Six, such as self-reported physical activity at baseline, which was used to apply a physical activity level score for total energy expenditure to determine the baseline diet's appropriate energy needs. This could have been overestimated by the participant, leading to an energy deficit. However, when considering the physical activity levels that were objectively measured using the ActiGraph accelerometer for 12-weeks, the mean time in moderate (3-5.99 metabolic equivalents (METS)) physical activity was 200-317 min/day and mean time in vigorous (≥6 METs) physical activity was 16-21 minutes/ day. Since the ActiGraph measures all physical activity during the 12-weeks, including incidental activity, it is likely that the self-reported physical activity at baseline is a accurate measure of this cohorts physical acitiy and is not over-reported as what is commonly observed in studies [81]. Furthermore, the inclusion of active older adults that engage in regular structured training programs are more likely to reliably recall habitual physical activity.

Lastly, participants in the DM group only contacted the researcher once a week to pick up their food provisions. There was not regular monitoring of the participants' weight to ensure that the diet/food provision was sufficient to meet their energy needs. To increase compliance with the dietary protocol, best practice guidelines suggest regular (once a week) follow-ups either face-to-face or via telephone [82]. While a strength of this study design in Chapter Six is the use of the control diet that has not been implemented before in other sarcopenia studies (Chapter Two) to

confirm whether the FM loss was from the dairy milk alone and not the control diet, future studies should consider using the control diet in all the groups (e.g., EX and CON) to assess the differences.

7.2 Contributions to knowledge

The findings from this thesis have implications for researchers studying health and performance outcomes in active older adults, for health professionals working within this athletic population (e.g., sports dietitians), and the active older adults themselves.

The implications for researchers studying active older adults within a clinical setting is that active older adults do not have the cofounding variable of sedentary behaviour that is commonly observed in sarcopenic research. Therefore, studying older adults who regularly engage in physical activity, either competitively or recreationally, allows researchers to understand the real physiological impact their intervention may have without the cofounding variable. Secondly, the knowledge of the baseline physical, dietary, and biological profile of active older adults determined in Chapters Four and Five provides researchers with a preliminary understanding of active older adults baseline profiles to implement appropriate future studies.

For health professionals working with active older adults, this thesis provides novel insight to consider individual factors (e.g., age, training status, protein intake) when implementing nutritional or exercise interventions within active older populations. Furthermore, it also highlights the use of whole foods over supplementation to increase protein intake that may be as effective as isolated proteins.

In the main study, while there were no significant interaction effects observed on outcomes of FFM (Chapter Six) this study does confirm that a PRT program can increase FFM by 0.8 – 1.2% in active

older adults **(Chapter Six).** Although not statsically significant, this has translational practice significantr in a clinical setting, as the reported loss of skeletal muscle mass observed in older adults is 1 - 2% per year [37]. This could indicate a saving of 1 - 2 yrs of skeletal muscle mass with a 12-week PRT intervention, and therefore has great practical and clinical significance for potentially reducing age-related muscle loss in older adults.

Finally, this study also provides novel insight for active older adults themselves. The population of those that are >50 yrs make up a considerable proportion of the total global and Australian population [1] as this cohort of older adults are also predicated on living much longer than the previous generations [60]. This generation of older adults has been shown to have higher expectations for wellness and independence in late life [83]. Therefore, this thesis confirms other studies [14-17] that engaging in regular physical activity (resistance training) and consuming adequate protein can maintain and potentially facilitate gains in skeletal muscle mass, strength and performance and thus preventing age-related sarcopenia that may lead to functional decline later in life.

7.3 Overall strengths and limitations

The studies included within this thesis are the first to compressively assess outcomes related to age-related sarcopenia with a high-protein milk beverage in active older adults. The main study (Chapter Six) presented in this thesis included a comprehensive study design that measured outcomes including FFM, skeletal muscle strength and power, and physical performance, biological parameters and accounting for more relevant outcomes in an active ageing cohort.

An overall limitation of the research presented in this thesis includes the high habitual protein intakes observed in the EX and CON groups. This may have cofounded the results as there was no

significant difference in relative protein intakes across groups. While strength in the study design is the controlling of food intake via providing participants food provisions, a limitation is that participants may have had a diet that was different in quality than their usual habitual intake and insufficient energy, which may have led to weight loss seen in the DM group. Physical activity at baseline was also self-reported and may have been caused for error, especially in Chapter Four and Five, where baseline physical activity was correlated with sarcopenia outcomes. This also limited the ability to present the ActiGraph data at baseline value and did not reflect whether those in the EX groups reduced their usual physical activity due to the PRT's attendance 3/days a week. Future studies should consider objectively measuring physical activity 2 weeks before the clinical trial's commencement to establish a more accurate baseline, as previously reported [35].

7.4 Suggestions for future research

The studies described in this thesis extend our current understanding of the baseline characteristics and help further understand potential successful interventions in the maintenance and positive increase in skeletal muscle outcomes in active older adults. Although we have made progress by identifying the interaction of a high-protein dairy milk beverage with resistance training, many gaps in our knowledge remain. The important questions for future research within this study topic include:

1. Would there be any significant differences in skeletal muscle response to a similar intervention if older adults with different sporting backgrounds to those in this study were recruited?
2. How would older athletes (e.g., >65 yrs and >70 yrs) respond to a similar exercise and nutrition intervention? And how would that compare to the middle-aged athletes within this current thesis?

3. How do differences in baseline muscle fibre distribution (e.g., Type I and II muscle fibres) affect an individual's response to exercise/ nutrition interventions?
4. How does lifetime training status affect the individuals' response?
5. What are the potential links between short-chained fatty acids (SCFs) and other relevant gut markers with dairy milk, and could such associations explain the observed increase in IL-10 in this study?
6. How would the skeletal muscle response differ in an active cohort that habitually consumed lower protein intakes (e.g., <1.2 g/kg BM/day)?
7. How would the skeletal muscle response differ in an active cohort that consumed less serves of dairy (e.g., <2.1 serves)?
8. How would the skeletal muscle response differ in a less active (e.g., < 90 mins of physical activity per week) cohort?
9. Can other whole-food products (e.g., yoghurt) achieve a similar result within an active ageing cohort?

7.5 Dairy milk for active older adults; practical implications

Firstly, in **Chapter Two,** it was clear that there was no specific protein dose that was considered "ideal" to further augment changes in SMM, strength and performance in older adults. There were significant effects in these outcomes mentioned above in protein doses from 6-35 g per day. While most of the current research suggests that older adults require ≥25 or ≥0.4 g/kg BM/day of protein per meal to elicit a significant response leading to muscle gain or increased strength, this current study showed in **Chapter Six** that a protein intake of 2x15 g per day at times that are usually inadequate in protein, with resistance training can lead to a significant effect on outcomes of strength. Considering that 28-35% of Australian older adults (50-69 and ≥70 yrs) achieve the recommended daily serve of dairy milk (2.5-4.0 serves), there is a clear gap in the consumption of dairy products within their diets [82]. This was further confirmed in **Chapter Five,** where the

participants in this current thesis had lower intakes of dairy serves (1.3 serves) than what is currently recommended in the Australian Healthy Guidelines recommendations. The additional 2x250 ml of dairy milk would mean that those participants achieve the intake recommended for older adults [84, 85]. Therefore, the recommendation of increasing protein intake with the use of high-protein dairy milk to help maintain SMM is within the current Australian guidelines [85].

Secondly, during this thesis, the partnership with Lion Dairy & Drinks required the generation of industry reports in line with the Food Standards Australia and New Zealand (FSANZ). This has resulted in evidence-based substantiated claims that will be present on the high-protein dairy milk beverage packaging that was used during the clinical trials (Chapter Six). This has practical implications as this thesis' workings have directly influenced the scientific research's food supply to direct-to-consumer.

7.6 Overall conclusion

This current thesis provides novel insight using active older adults, who may be considered the 'ideal' cohort of studying some aspects of age-related sarcopenia. They do not have the confounding factor of sedentary behaviour often seen in sarcopenia research.

Increasing protein intake with resistance training has reported conflicting results on skeletal muscle outcomes, which in part can be explained by the inclusion or frail or community-dwelling older adults. Chapter Two showed mixed results regarding protein supplementation and dairy beverages, with or without exercise interventions on improving skeletal muscle mass, skeletal muscle strength and performance outcomes individuals ≥40 yrs. However, it was clear that active older adults may require higher protein amounts throughout the day, especially when there is little distribution (i.e., breakfast and lunch).

In Chapter Four, it was found that contributions of age, physical activity, daily protein intake, and gender can all contribute to differences in body composition, strength, power, and performance in a cohort of active older adults and should be taken into consideration when implementing nutrition and exercise interventions within an active older cohort.

In Chapter Five, it was found that total protein intake was not shown to be strongly correlated to outcomes of skeletal muscle mass, strength, and power, as the majority already met and exceeded the adequate amount of total protein required for active older adults (>1.2 g/kg BM/day). However, higher protein intakes may offset any negative associations commonly observed from skewed protein intakes. Further comparisons indicate that a minimum of 1 meal containing ≥0.4 g/kg BM of protein may be required to utilise the maximum capacity for muscle protein synthesis, resulting in favourable leg strength outcomes.

Chapter Six found that the daily consumption of two high protein milk beverages at breakfast and lunch (or after PRT), providing 30 g of additional protein per day, significantly enhanced the effects of PRT on skeletal muscle strength outcomes of older adults that already consume higher protein amounts (> 1.2 g/kg BM/day). The consumption of dairy milk did not further enhance FFM outcomes or physical performance or power greater than PRT alone. Additionally, EX+DM led to a significant increase in resting anti-inflammatory cytokine (i.e., IL-10), which all may play a significant role in improving skeletal muscle mass and strength outcomes in active older adults (e.g., inflammaging).

Together, these studies highlight the complex nature of ageing and identify the many cofounding variables (e.g., age, training status, biological sex, and protein intake) that should be considered in future studies and even at a clinical level. Novel data from this thesis provide insight into important

methodological considerations for future research on protein intake and outcomes related to sarcopenia in active older adults, such as; including participants from similar exercise training modalities, standardising baseline diets for all study groups or including participants with reported low habitual intakes (e.g., <1.2 g/kg BM/day), determining lifetime physical activity history, and including other biomarkers that may assist in further understanding of potential mechanisms of sarcopenia.

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APPENDIX A:

Comments and future directions arising from "The Impact of Dairy Protein Intake on Muscle Mass, Muscle Strength, and Physical Performance in Middle-Aged to Older Adults with or without Existing Sarcopenia

The following chapter appears on the next page as published in the Advances in Nutrition

Huschtscha, Z., Porter, J., & JS Costa, R. (2020). Comments and future directions arising from "The Impact of Dairy Protein Intake on Muscle Mass, Muscle Strength, and Physical Performance in Middle-Aged to Older Adults with or without Existing Sarcopenia". *Advances in Nutrition*, *11*(1), 175-176.

Comments and future directions arising from "The Impact of Dairy Protein Intake on Muscle Mass, Muscle Strength, and Physical Performance in Middle-Aged to Older Adults with or without Existing Sarcopenia"

Dear Editor:

We read with interest the systematic literature review by Hanach et al. (1) published in the January issue of *Advances in Nutrition*. We congratulate the authors for writing a high-quality review and meta-analysis that provides valuable insight into the benefits of dairy proteins in the aging population, and the prevention and management of sarcopenia. While we agree with their recommendations suggesting the need for larger scale clinical trials in this area, we also suggest that addressing pre-existing methodological gaps is essential, considering the growing number of recreational and competitive active older adults. This population represents individuals in the ≥5th decade of life that continued, returned, or initiated recreational or competitive sport and exercise activities, and are currently a substantially underrepresented population in the sarcopenia research literature. This significance, for example, is shown by the progressive increase in participation rates of active older adults in endurance-based events over the last 3 decades (2).

The WHO generally recommend that older adults should participate in at least 150 min/wk of light- [e.g., 3–5 metabolic equivalents (METs)] to moderate-intensity (e.g., 6–9 METs) physical activity or 75 min/wk of vigorous-intensity (e.g., >9 METs) physical activity (3). It is evident that active older adults can exercise >60 min/d recreationally to >4 h/d in ultraendurance competitive events (2, 3). In comparison to sedentary or low activity adhering older adult populations (e.g., community dwelling or institutionalized), recreational and competitive active older adults show that high levels of exercise adherence can effectively countermeasure against normal age-related muscle wasting and dysfunction (4). However, adults who undertake regular physical activity and exceed physical activity guidelines (e.g., endurance- and power-trained individuals) still show signs of decreasing muscle mass, strength, and performance with increasing age. For example, Pearson et al. (5) compared the muscle function of competitive masters weightlifters to age-matched untrained controls and found similar rates of decline in peak power (1.2% and 1.3%, respectively) and isometric strength (0.6% and 0.5%, respectively) with increasing age. This is supported by similar reductions in metabolic muscle function, as observed by Bagley et al. (6) who found a 7% to 14% decline in aerobic power (assessed through $\dot{V}O_{2peak}$ and relative fat oxidation, assessed by a single and 2-legged incremental bike test), per decade of life, in endurance- and power-trained masters athletes (aged 37–90 y). Such observations suggest that with increasing age there is still a decline in muscle function and strength, irrespective of exercise stress load. Despite active older adults still showing signs of sarcopenia progression, they represent an ideal population to study the aging process as they do not have the confounding factor of muscle disuse seen in sedentary or low activity individuals commonly recruited in experimental models (7).

It is proposed that no significant effects were reported in the systematic literature review with the use of dairy protein intake on performance (1), possibly because the performance measures used within the included studies [e.g., Short Physical Performance Battery (SPPB) and gait speed] may not be sensitive, and therefore, may not be suitable to measure performance in the population group that are considered active or even healthy. The SPPB assessment reportedly has a ceiling effect on a cohort of active older adults, whereby the majority (55%) of the physically active participants are reported to receive the top score of 12 points (8). Moreover, measurements such as gait speed are found to present a nonlinear relation between leg strength, resulting in a wide population variance (e.g., 22%) (9). The potential risk of outcome misinterpretation explains how small changes in physiological capacity produce large effects on performance in frail adults, whereas large changes in capacity have little or no effect on daily function in healthy adults (9). Therefore, the use of gait speed and SPPB in an active older cohort may not be sensitive enough to detect any clinically relevant change in response to exercise and/or nutritional interventions. Considering that in healthy older people, muscle power declines faster than strength, measuring power output may be a better predictor of certain functional activities (10). Evaluation techniques of muscle power output that have been validated and reliability checked include vertical jump on a force platform, unloaded leg extensor power, and isokinetic dynamometry (11). These measures have been incorporated into multiple studies of males and females, athletes and nonathletes, with correlations between vertical jumping power and chair stand-up power with age evident (12). Reid and Fielding's (11) synthesis of physical function in older adults concludes that muscle power rather than muscle strength better predicts

 Adv Nutr 2020;11:175–176; doi: https://doi.org/10.1093/advances/nmz067.

functional performance in older adults. Hence, the inclusion of these methodological approaches in sarcopenia research, irrespective of whether they include dairy milk supplementation, will ensure that markers of physical performance align with all study cohorts. The inclusion of these measures will enable more discretion in the determination of sarcopenia status, overcoming the test sensitivity that may exist in the measurement techniques that currently predominate. Therefore, vertical jump, specific muscle power output, and/or isokinetic dynamometry should be considered in future studies assessing skeletal muscle performance in healthy older adult populations, especially if cohorts are described as "active".

In addition, we note that some included studies provided participants with resistance training but did not assess potential confounding changes in cardiorespiratory fitness (CRF). Improvements in maximal ($\dot{V}O_{2max}$) and submaximal (aerobic economy) oxygen consumption are seen as a result of regular aerobic training. However, growing evidence suggests that strength training may improve the metabolic efficiency of the muscle, which in turn increases aerobic capacity (13). This was observed in a study by Lovell et al. (13) that reported a significant, but modest, $\dot{V}O_2$ improvement (+0.110 L/min; $P < 0.05$) in response to cycle exercise at 70% $\dot{V}O_{2max}$ in healthy older adult males who weight trained for 16 wk. Longitudinal evaluation of $\dot{V}O_{2max}$ has shown linear decline with age (14), in both male and female participants, irrespective of exercise stress status, with declines further influenced by fitness status (i.e., trained compared with untrained) (15). Moreover, there is also evidence that daily lifestyle determined CRF is positively correlated with $\dot{V}O_{2peak}$ ($r = 0.597$, $P < 0.001$), based on findings from a recent study that recruited a cohort of community-dwelling elderly men (16). Therefore, in longer intervention trials that include a resistance-training element, this may result in an increased physical activity adherence outside the exercise protocol (e.g., increased daily steps and daily activities), which is likely and/or will inadvertently affect cardiovascular adaptations and subsequently total aerobic capacity and aerobic efficiency. Controlling for change in CRF or measuring the activity levels along the experimental timeline is essential for accurate result interpretation. For example, an increase in CRF due to increased daily activity may inevitably influence muscle mass, power, strength, and performance outcomes, especially in proportion to baseline starting values in frail and sedentary muscle.

Overall, we concur with Hanach et al. (1) for the need for larger high-quality randomized clinical trials investigating the effects of dairy proteins on standardized outcomes (i.e., muscle mass, power, strength, and performance). However, as we have highlighted, an entirely unexplored area is the effect of dairy proteins and exercise on an active older cohort. We suggest that outcomes of future studies should include appropriate markers of physical performance and quality of life to match the exercise outputs of the active older adults.

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The authors declared no funding and no financial support received for this letter.
Author disclosures: ZH, JP, and RJSC have received industry funding to conduct trials on the effect of milk products on the active aging population.

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APPENDIX B:

Search strategy
(Chapter Two)

Web of science search strategy

1. Exp Older OR elderly
2. Age*
3. 1 OR 2
4. Exp Exp. Amino Acid
5. Dietary protein*
6. Leucine
7. Casein
8. Dietary supplement/
9. Whey
10. Vitamins/
11. Dietary supplements*
12. Milk protein//
13. Whey*. mp
14. Casein*. Mp
15. 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14
16. Skeletal muscle mass
17. Hypertrophy
18. Muscle strength.
19. Exp hand strength
20. Grip strength
21. Physical performance
22. Physical performance. mp.
23. Muscle power
24. 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23

APPENDIX C:

Ethics approval

MONASH University

Monash University Human Research Ethics Committee

Approval Certificate

This is to certify that the project below was considered by the Monash University Human Research Ethics Committee. The Committee was satisfied that the proposal meets the requirements of the *National Statement on Ethical Conduct in Human Research* and has granted approval.

Project Number:	12812
Project Title:	Effect of a high protein milk drink in combination with resistance training on skeletal muscle mass, muscle strength and functional performance in healthy active older adults
Chief Investigator:	Dr Ricardo Da Costa
Approval Date:	17/05/2018
Expiry Date:	17/05/2023

Terms of approval - failure to comply with the terms below is in breach of your approval and the *Australian Code for the Responsible Conduct of Research*.

1. The Chief Investigator is responsible for ensuring that permission letters are obtained, if relevant, before any data collection can occur at the specified organisation.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash letterhead and the Monash University complaints clause must include your project number.
6. Amendments to approved projects including changes to personnel must not commence without written approval from MHUREC.
7. Annual Report - continued approval of this project is dependent on the submission of an Annual Report.
8. Final Report - should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected completion date.
9. Monitoring - project may be subject to an audit or any other form of monitoring by MUHREC at any time.
10. Retention and storage of data - The Chief Investigator is responsible for the storage and retention of the original data pertaining to the project for a minimum period of five years.

Thank you for your assistance.

Professor Nip Thomson

Chair, MUHREC

CC: Ms Zoya Huschtscha, Dr Judi Porter, Assoc Professor David Kannar

List of approved documents:

Document Type	File Name	Date	Version
Explanatory Statement	explanatory-statement-template LION	10/03/2018	1
Consent Form	Consent form - Lion	10/03/2018	1
Supporting Documentation	Consent form - Lion	11/03/2018	1
Supporting Documentation	PRE-EXERCISE QUESTIONNAIRE	11/03/2018	1
Supporting Documentation	PRE-EXERCISE QUESTIONNAIRE	11/03/2018	1
Supporting Documentation	Participant recruitment advertisement (Active aging)	11/03/2018	1
Supporting Documentation	venepuncture SWF	11/03/2018	1
Supporting Documentation	Dairy active aging protocol (ZH 050318)	11/03/2018	1
Supporting Documentation	Medical Physicist Report - Monash University 11 Mar 2018	15/03/2018	1
Ionising Radiation Form	Victorian Specific 15.03	06/04/2018	pdf
Supporting Documentation	DXA Questionnaire	06/04/2018	1

Page 1 of 2

Supporting Documentation	iDXA risk-management-worksheet	06/04/2018	1
Supporting Documentation	iDXA Safe Working Procedure	06/04/2018	1
Supporting Documentation	iDXA SOPs 2013	06/04/2018	1
Consent Form	Consent form - Lion EDIT 070518	07/05/2018	2
Explanatory Statement	explanatory-statement-template LION updated 070518	07/05/2018	2
Supporting Documentation	Food and Drink Intake Chart (1)	11/05/2018	1
Explanatory Statement	Explanatory-statement-lion	17/05/2018	3

APPENDIX D:

Participation information sheet
(Chapter Three)

Monash University Department of Nutrition & Dietetics

PARTICIPANT INFORMATION SHEET- 8th March 2018
Explanatory Statement

Effect of a high protein milk drink in combination with resistance training on skeletal muscle mass, muscle strength and functional performance in healthy active older adults: Pilot study.

Project ID and title it appears on your MUHREC application form.

Dr Ricardo Costa
Department of Nutrition and Dietetics
Phone: 03 99056861
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Zoya Huschtscha
Phone : 03 99056861
email: zoya.huschtscha@monash.edu

You are invited to take part in this study because you are aged 50 yrs and older and belong to an important target group for investing the effects of different protein drinks, with or without exercise on muscle mass and strength.
Please read this Explanatory Statement in full before deciding whether or not to participate in this research. If you would like further information regarding any aspect of this project, you are encouraged to contact the researchers via the phone numbers or email addresses listed above.

Aim/ purpose of the research

Muscle loss occurs naturally as we age and we know that after the age of 40, adults can lose 3-8% of their total muscle mass per decade with more noticeable declines evident after the age of 50. Maintaining muscle mass and strength as we get older is important for preserving activities of daily living (physical performance) and reducing our risk of falls and co-morbidities associated with muscle loss. We know that nutrition, in particularly protein is important to help us maintain muscle throughout our life. As such, research has lead to the development of dietary and exercise focused guidelines and recommendations for protein intake for the aging population. As dairy milk has similar nutritional properties to these proposed guidelines and recommendations, there is emerging evidence suggesting dairy milk has the potential to provide a nutrient source to accompany exercise-stress in the maintenance of muscle mass and strength, especially as we age. The aim of this study is to determine if a high protein milk drink, with or without exercise will help people over 50 yrs old maintain/ gain muscle mass and strength.

What does the research involve?

Prior to commencing the experimental trials, you will be expected to:

Screening (~30minutes): Initial screening will take place via telephone. This screening will comprise of medical history (including current medications), age, self-reported activity levels, completion of Exercise and Sports Science Australia exercise pre-screening tool to assess readiness to exercise. If this tool designates you to be at high-risk for engaging in resistance training exercise, then you will be asked to visit your GP to attain a clearance to perform the study measures and training.

Consent form: If you decide to participate in this study and meet the selection criteria, a consent form will need to be signed prior to any assessments being performed. Prior to signing, you will be given the opportunity to ask any questions and have any concerns clarified.

Visit 1: Baseline measurements (~3 hours)
Once identified as eligible you will be required to attend the Be Active Sleep Eat (BASE) facility at Notting Hill for baseline measurements. You will be asked to participate in the following measures:

1. Body Composition assessment: A dual-energy x-ray absorptiometry scan (DXA) is a specialised x-ray technique to provide a measure of body composition. This is similar to a normal x-ray, with no pain involved and will obtain a measure of total body mass, fat mass and lean mass. Participants are required to lay supine on the scanning bed for the duration of the scan, which is approximately 15 minutes and will be one to two scans depending on your body shape. The machine uses small doses (<1% of your yearly radiation dose) of radiation to estimate tissue density. This test requires you to be fasted with no food, fluid or exercise/ activity prior to the test and requires you to wear light clothing

with no metal items (i.e. zips, clips, underwire etc) and remove all jeweler. Please check with research staff if you are unsure.

2. Resting Metabolic Rate: This assessment also involves you being fasted and therefore will be completed immediately after the DXA scan. For 25 minutes you will be lying in a quiet, dim-lit room where we can measure your resting metabolic rate using an automated gas analyser. There is a 10-minute rest period followed by a 15-minute period where the data will be collected. The information from this test will also be used to give an assessment of estimated energy requirements which will be used to provide you with individual dietary requirements for the meals provided during the course of the trial.
3. Anthropometric measurements: Height will be measured using a wall mounted stadiometer. Weight and hydration status will be measured using a bioelectrical impedance analysis (BIA). The BIA device passes a low level electrical; current through your body and measures the level of resistance to the flow of electricity, which can then be used to estimate total body water, which can be used to estimate fat-free body mass and hydration status.
4. Strength testing: Maximum muscular strength will be determined during a series of one repetition maximum (1RM) attempts. The 1RM attempts will be performed on the leg press, leg extension, Lateral pull down and bench press exercises. You will be shown correct and safe technique for each exercise by a qualified exercise scientist and will subsequently be provided with appropriate time to safely perform each technique until competent and comfortable. Following an appropriate warm-up for each selected exercise, a single exercise repetition will then be performed with 5 minutes of recovery between each attempt until the repetition load that can be completed once but not a second time is determined. These measures will be supervised by a qualified exercise physiologist or exercise trainer.
5. Maximum effort cycling exercise test [VO2 peak]: Aerobic capacity will be assessed by measuring the maximum volume of oxygen (VO2) you can breathe in and transport to your working muscles while exercising at maximal intensity. After a light 5- minute warm-up on a cycling ergometer (exercise bike), you will pedal at a fixed intensity (watts) that will increase every 1-2.5 min until volitional fatigue. During this test you will wear a mouthpiece and headpiece that is connected to a tube for the collection of expired air. Following the warm-up, the test will last for 10-15 minutes where the last few minutes should be perceived as exhaustive. After the test has finished, you will be given time to rest and recover.
6. Performance test (20-minute bike test): This test requires you to ride on a stationary bike for 20 minutes at the highest intensity you can sustain for that time. During this test we will be measuring your heart rate, watt (power output) and the distance you are able to cover in that time.
7. Urine sample: When you first arrive at the BASE facility, you will need to provide a urine sample in a container provided to you.
8. Blood sample: A blood sample will be drawn (30ml) from a trained phlebotomist.

Completing the experimental trials (3- months)

After completing the baseline measurements you will then be randomly allocated to one of four groups;

1. Dairy group

   Will be provided with a dairy product, standardised food provisions and Actigraph monitor.
2. Resistance training group

   Resistance training intervention, Ad libitum food + Actigraph monitor.
3. Dairy group plus resistance training intervention

   Dairy product and resistance training intervention, standardised food provisions and Actigraph monitor.
4. Control group

   Ad libitum food + Actigraph monitor.

Dairy group:

In the dairy trials (groups 1 and 2) will be provided with a weeks worth of dairy milk and we asked to consume 250ml twice a day, using a measuring cup provided. You will be required to return your empty milk containers to test for compliance.

Meals will be provided (1 -week rotation), 100% of total daily estimate energy requirements. These will be ready made frozen meals and snacks that will be personalized to your energy requirements. You will be asked to fill in a food and drink journal during this time, to record how much of the meals and beverages you consumed and

any additional foods or fluids. Meals will only be provided to the dairy trial groups (groups 1 and 2). To further test compliance, you will be asked to bring back your empty food drink packaging back to the lab.

Resistance training group (~1 hour, 3 x a week)
Those allocated in the exercise trials (groups 2 and 3) will be required to attend 3 supervised sessions a week. The duration of the exercise session, including adequate warm-ups, will be ~60 minutes. The following exercises have been selected as they are low risk and easy for untrained individuals to complete. The following exercises have been selected based on previous established protocols as they are low risk and the most basic exercises for untrained individuals to complete. The following exercises have been used in previous studies thus deemed safe for older adults to perform.
Acute Resistance Exercise Session:

1. Leg Press
2. Leg Extensions
3. Machine Chest
4. Lateral pull down
5. Bicep curls
6. Tricep extensions
7. Shoulder raises

Criteria for Exclusion

To ensure that only suitable participants are included, we have a number of criteria that, if met, will exclude you from participating. Please advise the researcher if you do not meet any of the following criteria:

1. Milk protein intolerance or allergies
2. Subjects currently using natural health products such as whey protein or vitamins. Or will need to disuse during the course of the intervention?
3. Major surgery in the past 12 months
4. Acute coronary (e.g. myocardial infarction) or vascular event in the last year as well as uncontrolled coronary heart disease.
5. Stroke in the past 2 yrs
6. Orthopedic limitations that limit the participant in the exercise program
7. Diagnosed with or taking medication for thyroid condition
8. Weight loss or more than 5% of body weight over the last 6 months.
9. All medications that can interfere with muscle mass such as corticosteroids, testosterone replacement or anabolic drugs
10. Subjects currently undergoing immunosuppressive therapy or hormone replacement therapy.
11. Injuries preventing safe exercise
12. Any chronic diseases such as: Diabetes mellitus, or gastrointestinal disease.
13. Consume more than 2 standard drinks of alcohol per day, or 14 standard drinks of alcohol per week.
14. Are a smoker
15. Exercise less than 3x30 minutes a week (90 minutes)
16. Have a BMI greater than 30 kg.m2

Control group:
The control group trial will receive no intervention and will only be required to attend the laboratory on 3 separate occasions for primary and secondary outcome measurements.

All participants
All participants will be required to Wear an activity tracker (Actigraph) during the course of the 3-month trial. Additionally, all participants are required to attend the laboratory on 3 separate occasions for baseline measurements. This will be pre-trial, 6 weeks and 12 weeks (end of trial). You will be provided with a $200 gift voucher at the conclusion of your trial. However, if you lose or break the Actigraph monitor you will not receive this voucher as this equates the worth of the monitors.

Possible risks to participants

The discomforts and risks of taking part in this study, which you will probably be most concerned about are:.

1. Anthropometrical measures: Height will be measured by a stadiometer and body mass by calibrated weighing scales. These measures are similar to those consistently measured for health monitoring in the GP setting, which pose no direct risk.

2. Blood sampling: There may be mild discomfort from the venous blood sample, but no other discomfort is anticipated. To minimise discomfort, the tests will be performed by a researcher trained in phlebotomy. It is unlikely that participants will experience an adverse reaction to the supplement. But in the event of an adverse reaction, the participant's experience will be documented by the research team, and if necessary, the participant will be advised to seek medical advice.

3. Dual-energy x-ray absorptiometry: This research study will involve exposure to a very small amount of radiation from the iDXA scans. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this study is about 0.03mSv. At this dose level, no harmful effects of radiation have been demonstrated, as any effect is too small to measure.

4. One-repetition maximum (1RM) testing: This test will require maximal exertion and therefore can result in some discomfort. Following an appropriate warm-up for each selected exercise, a single exercise repetition will then be performed with 3 min recovery periods between each attempt until the repetition load that can be completed once but not a second time is determined. The 1RM attempts will be performed on the leg press, leg extension, bench press, seated row and shoulder press exercises.

5. Exercise training: If allocated to the resistance training group you may have some discomfort if you are not accustomed to weight training. These may include cardiorespiratory stress (e.g. shortness of breath) or a musculoskeletal injury (e.g. strained muscle, tendons or ligaments) however it is important to note that the risk of injury associated with this study is the same as with a typical resistance or aerobic exercise session. Importantly, the loads used for each training session in this study will be tailored to your strength capabilities.

6. Incremental and 20-minute bike test: This test will require maximal exertion and therefore can result in some discomfort. The Exercise and Sport Science Australia Pre-Screening Tool will be completed beforehand to ensure that you are suitable to undertake the test. If you are not cleared to participate by the screening tool you will need to obtain approval form a general practitioner to participate. In the unlikely event that you experience discomfort beyond what is expected with exercise, please notify research staff. You are able to end the test at any time.

7. Vertical jump: This test will require you to stand on a force plate and you will jump as high as you can. You will attempt this test, 3 times.

What are the benefits of the research project?

We cannot guarantee or promise that you will receive any benefits from this research. However, potential personal benefits may include measurements of your blood glucose regulation, body composition, resting metabolic rate, cardiovascular fitness (VO2peak) and strength. Participating in supervised exercise training may also be of benefit.

Consenting to participate in the project and withdrawing from the research

In order to participate in this study the written consent form must be read, signed and returned to Zoya Huschtscha. Participation in this research is voluntary. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Confidentiality

Any information obtained in connection with this project that can identify you will remain confidential. This information will only be disclosed with your permission or as required by law. Your name will be assigned a code, which will be used in discussing data, so that your identity is not disclosed. Only de-identified results will be presented in meetings, conferences, as part of a thesis or published in scientific journals or reports. The

company who are donating the supplement (Marinova Pty Ltd) will not have access to any of the results until they are published in a de-identified, grouped manner.

Storage of data

Data collected will be stored in accordance with Monash University regulations. The collected and coded data will be stored either on a password protected computer or in a locked filing cabinet on University premises for five yrs, after which it will be destroyed.

Use of data for other purposes

In accordance with the National Health and Medical Research Council Statement on Data Sharing, de-identified data should be made available for use by the other researchers unless this is prevented by ethical, privacy or confidentiality matters. This data will be held on secure public repositories and is a requirement of some journals prior to publication. Any shared data will remain anonymous and there will be no way that you can be identified. Blood samples will, with your permission, be retained for possible use in future research examining related outcomes.

Results

If requested, participants will be given their body composition results and fasting blood sugar levels at the end of the study. If you would like to receive a report of the aggregate research findings please contact Zoya Huschtscha, PhD candidate at Monash University, on (03) 9905 5357 or email zoya.huschtscha@monash.edu. The findings are accessible for 6 months following completion of the study.

Funding

This research has received funding from Lion Dairy and Drinks Pty Ltd.

Counselling services

Although we do not expect that this research will cause you any distress and anything more than minor discomfort, it should be noted that the optional screening questionnaires and mood state questionnaires may involve issues of depression, anxiety and cognitive impairment. Although unlikely, should you become concerned about your level of depression, anxiety or cognitive impairment throughout or after the study, please consult the list of services below and speak to your GP about your concerns.

For everybody 24/7

Service	Phone	Hours of operation
Lifeline	13 11 14	24 hours
Suicideline	1300 651 251	24 hours
Suicide Call Back Service	1300 659 467	24 hours

For Monash students & staff

Service	Phone	Hours of operation
Monash counselling	9905 3020	Monday – Friday 9am-5pm

Monash counselling after hours	1800 350 359	5pm-9am weeknights, 24 hours weekends

Complaints

Should you have any concerns or complaints about the conduct of the project, you are welcome to contact the Executive Officer, Monash University Human Research Ethics (MUHREC):

Executive Officer
Monash University Human Research Ethics Committee (MUHREC)
Room 111, Building 3e
Research Office
Monash University VIC 3800
Tel: +61 3 9905 2052 Email: muhrec@monash.edu Fax: +61 3 9905 3831

APPENDIX E:

Consent form
(Chapter Three)

CONSENT FORM

General consent form for exercise testing at the Be Active Eat Sleep (BASE) Facility, Monash University.

Exercise Physiology Lead: Dr Ricardo Costa

I have volunteered to take part in exercise testing at the BASE Facility at Monash University. I participate at my own free will and take full responsibility for my participation. I confirm I have read and/or have been informed, and understood all the relevant test procedures. The nature, demands, and risks have been explained to me. I have had the opportunity to consider the information, ask questions regarding the testing procedures, and have had these answered satisfactorily by the test operator. I also confirm that all the relevant health and safety aspects of the test have been explained to me. I hereby consent to participate in the exercise testing and acknowledge that I am free to withdraw from the test procedure at any time without giving a reason.

I consent to taking part in the following aspects of exercise physiology test procedures:	Yes	No
1. Muscle mass and bone mineral density measured using iDXA	☐	☐
2. Incremental exercise test to voluntary exhaustion (VO_{2max}) (bike).	☐	☐
3. 1 rep maximum (1RM) Strength testing (Leg press, chest press, lateral pull down, and leg curl)	☐	☐
4. Vertical jump test (force plate)	☐	☐
5. General anthropometry (i.e., weight, height, girth, lengths, widths).	☐	☐
6. Bioelectrical impedance analysis (body composition or hydration).	☐	☐
7. Venous blood sampling for biomarker analysis.	☐	☐
8. Gait speed analysis	☐	☐
9. Diet or nutritional intervention	☐	☐
I consent for the data collected during my participant in the exercise testing at BASE Facility at Monash University be used for teaching, education, and/or research purposes.	☐	☐

Name of Participant

APPENDIX F:

Pre-exercise questionnaire
(Chapter Three)

Monash University
Department of Nutrition & Dietetics

PRE-EXERCISE QUESTIONNAIRE

Name of participant…… Date……..

Age……..

Are you in good health ? Yes No If no, explain:

How would you describe your present level of activity?

Tick intensity level and indicate approximate duration.

Vigorous		Moderate		Light	

Duration (minutes) :……

How often?
Tick as appropriate.

< once per month		4-5 times per week	
Once per month		>5 times per week	
2-3 times per week			

Have you suffered from a serious illness or accident? Yes No If yes, explain:

Do you suffer, or ever suffered from the following:
Tick as appropriate.

Asthma		Epilepsy	
Diabetes		High blood pressure	
Bronchitis		Heart conditions	

Are you currently on any medication? Yes No If yes, explain:

Are you currently attending the GP for any condition or have you consulted your doctor in the last three months? Yes No If yes, explain:

Have you, or are you, presently taking part in any other laboratory experiment?

Yes No If yes, explain:

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental/testing exercise task if they:

- Have a fever, cough, cold, or suffer from fainting spells or dizziness.
- Have suspended training due to a joint or muscle injury.
- Have a known history of medical disorders, i.e. high blood pressure, heart or lung disease.
- Have a hyper/hypothermia, heat exhaustion, or any other heat or cold disorder.
- Have anaphylactic shock symptoms with needles, probes, or other medical-type equipment.
- Have chronic or acute symptoms of gastrointestinal bacterial infections.
- Have a history of infectious disease (e.g. HIV, Hepatitis B), and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other conditions of the rectum.

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments / tests during …… (date/s).

My replies to the above questions are correct to the best of my knowledge and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment/test and possible risks involved.

I understand that I may withdraw from the experiment/test at any time and that I am under no obligation to give reasons for withdrawal or to a return for experimentation. I also understand that if I fail to complete a experiment/test, I will not be able to obtain or have access to the results due to absence of results.
Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment/test, will neither be detrimental to, or further, my position as a student.

I undertake to obey laboratory/study regulations and the instructions of the experimenter/tester regarding safety, subject only to my right to withdraw declared above.

Participant signature……

Name of experimenter/tester…….

Signature…… Date…….

WHEN COMPLETED- ONE COPY TO RESEARCHER FILE

APPENDIX G:

3-day food diary
(Chapter Three)

Completing your 3-day Food & Fluid Diary

As part of your clinical trial visit, we ask that you keep a record of everything you eat and drink for 3 days. A sample journal has been provided for you on page 2. This journal may be completed at any time as long as it is prior to your initial visit to the laboratory.

<u>To complete your food journal, please follow the guidelines below</u>

- Select days that you will be making typical food choices and try not to change your eating habits. Holidays and special days may not represent usual eating behaviours.
- Try to include 2 weekdays (Monday-Friday) and 1 weekend day (Saturday/Sunday) for a total of 3 days (they do not have to be consecutive).
- Carry the food journal with you during the day so that items can be recorded immediately after they are eaten.
- Record EVERYTHING you eat and drink. Please be as specific as possible.
- List the type of food you ate including all condiments and extras (sauces, gravy, butter, tomato sauce, mayo, etc.)
- Describe combination foods, such as what toppings came on the pizza or what was included in the sandwich.
- Mention how the food was prepared (grilled, baked, fried, steamed, roasted, etc.)
- List a brand name or restaurant name when possible.
- Include portion sizes for all items, estimating to the best of your ability. In grams or millilitres (please use kitchen scales or measuring jug to determine food/fluid portion consumed).

Sample diary		
Date: 12/02/2018	Circle one: Weekday Weekend	
Day 1	**Food & fluid type**	**Food & fluid quantity**
Breakfast	Scrambled eggs with salt a pepper Whole wheat toast with margarine Coffee with skim milk and sugar	2 eggs 1 slice/1 tablespoon 1 cup (250ml)/1/4 cup/ 1 teaspoon
Mid-morning snack	Apple Uncle toby's choc chip muesli	1 medium 1 bar
Lunch	Bought at café: Toasted sandwich: whole meal bread, tasty cheese, tomato, ham and margarine	1 sandwich (1 cheese slice, 3 slices of ham, 3 slices of tomato)
Mid-afternoon snack	latte with skim milk	Medium (275ml)
Evening meal	Grilled chicken breast (without skin) Baked potato (with skin) topped with butter Cauliflower and broccoli steamed Red wine	200g (palm size) 1 medium potato/ 1 tablespoon 1 cup 2 glasses (125ml each)
Supper	Ben and Jerry's vanilla ice cream with Cottees chocolate topping	1 cup/ 2 tablespoons
***Additional supplements**	Blackmores muscle magnesium	2 tablets
Estimated daily water intake		

Participants were provided 3-blank food diary sheets

Day 1

Date: Circle one: Weekday Weekend

	Food & fluid type	Food & fluid quantity
Breakfast		
Mid-morning snack		
Lunch		
Mid-afternoon snack		
Evening meal		
Supper		
*Additional supplements		
Estimated daily water intake		

APPENDIX H:

Borg Scale

(Chapter Three)

Rating of perceived exertion (RPE) Scale

Borg Scale

6	
7	Very very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very very hard
20	

Borg, G.A.V., (1982). Psychophysical bases of perceived exertion. MSSE, 14(5):377-381.

APPENDIX I:

Data collection sheet
(Chapter Three)

Monash University
Department of Nutrition & Dietetics
Sport & Exercise Dietetic Clinic

Active Aging Study

Trial: Pre-trial/ 6 weeks/ 12 weeks

Name:				**Test date:**	
DOB:		**Age:**		**Contact number:**	
BW:		**Height:**		**BMI:**	
FM:		**SMM:**		**FFM:**	
TBW (L/%):		**ECW: (L/%):**			

ICW Litres (L)= (TBW in L - ECW in L)- 0.5L for third space water volume
ICW %= (ICW L / body weight) *100

1. Estimation of Energy requirements:

A. Resting metabolic rate (RMR):
(Make sure this is performed in the morning with the subjects fasted. participant should have withdrawn from physical activity, alcohol and caffeine 24 hr prior to measurements. participant should be in the supine position with absence of movements and producing a normal breathing pattern.

Temp=_____ Time =_____. Verify heart rate monitor is on.

10mins lying in supine position. Put canopy on participants.

a) 0-10 mins. Measurement from 5-10mins.

VE(STPD)=_____ VO2(L·min^{-1})=_____ VCO2=_____ RQ=_____ REE_____(record last minute only)

	½ min	1min	1 ½mins	2mins	2 ½ mins	3mins	3 ½ mins	4mins	4 ½ mins	5mins
HR (bpm)										

b) 10-20 mins. Measurement from 15-20mins.

VE(STPD)=_____ VO2(L·min^{-1})=_____ VCO2=_____ RQ=_____ REE_____ (record last minute only)

	½ min	1min	1 ½mins	2mins	2 ½ mins	3mins	3 ½ mins	4mins	4 ½ mins	5mins
HR (bpm)										

c) 20-30 mins. Measurement from 25-30mins.

VE(STPD)=_____ VO2(L·min^{-1})=_____ VCO2=_____ RQ=_____ REE_____(record last minute only)

	½ min	1min	1 ½mins	2mins	2 ½ mins	3mins	3 ½ mins	4mins	4 ½ mins	5mins
HR (bpm)										

Estimated RMR:_____ (from the Vmax directly)

B. RMR+SPA/DIT:
Estimated REE X 1.2 (Schofield activity factor) = _____

C. Add physical activity factor (see last page): _____

D. Estimated total daily energy expenditure:
TDEE=B_____+ C_____=_____kcal.day-1

1

Monash University
Department of Nutrition & Dietetics
Sport & Exercise Dietetic Clinic

Active Aging Study

Performance outcome

A. Sub max Vo2 max - Incremental bike test (RPE 15-17)

Minute	Watt	Watt/kg (FFM)	HR (bpm)	RPE	VO_2 (ml/kg/min)	VCO2	RQ	VE
3 minute warm up	0.5w/kg of FFM		X	X	X	X	X	X
3min	1 W/kg of FFM							
6min	0.5W/ kg of FFM							
9min	0.5W/ kg of FFM							
12min	0.5W/ kg of FFM							
15min	0.5W/ kg of FFM							
18min	0.5W/ kg of FFM							
21min	0.5W/ kg of FFM							
24min	0.5W/ kg of FFM							
27min	0.5W/ kg of FFM							

*Participant needs to maintain RPM >60

B. Counter movement (vertical jump)

1. Ask participant to stand on the plate with hands on their hips and feet shoulder width apart.
2. Start test and count 3-2-1- and get participant to squat down (self-selected depth) and jump as high as they can with hands on their hips. Get participant to stand up and keep still for 2-3 seconds before stepping down.

.

Attempt	Peak velocity (m/s)	Peak force (N)	Peak power	Minimum velocity	Flight time	Flight to contraction ratio
1						
2						
3						
*flight = time in the air contraction is the sum of eccentric and concentric phases						
Jump height= = 9.81 * (flight time seconds) ^ 2 / 8 Jump Height = peak velocity ^ 2 / (2 * 9.81)						

Name:
Date:
Trial: Pre-trial/ 6 weeks/ 12 weeks

C. **GAIT SPEED- 4m**

Instructions:

1. Ask participant to walk their usual walking speed
2. Time from the first foot movement
3. Stop the timer as soon as the first foot crosses the 4m mark.

	Seconds	m/second
Attempt 1		
Attempt 2		

4. Strength outcomes 1-RM

1-RM testing protocol (National Strength and conditioning association)

	Leg press	Lat pull down	Chest press
Seat position/arm bar			
Warm up (5-10 reps) Rest 1 minute			
Set 1 (3-5 reps) Rest 2 minutes			
Set 2 (2-3 reps) 3-minute rest			
Attempt 1 (1 rep) 3 minute			
Attempt 2 (1 rep) 3 minute			
Attempt 3 (1 rep) 3 minute			
Attempt 4 (1 rep) 3 minute			
MAX 1-RM			

*** increase weight 5-10% upper body (4-9kg)**
10-20% lower body (14-18kg)

If participant failed
if the participant failed, provide a 2- to 4-minute rest period, then decrease the load by subtracting 2-4 kg or 2.5% to 5% for upper body exercise or 7-9 kg or 5% to 10% for lower body exercise AND then go back to 1-RM attempt. The participant should have 3-5 testing sets to test their 1-RM.

5. Dyanometer (handgrip)

Dominant hand (Left/right)			
Dominant	1.	Non-dominant	1.
	2.		2.
	3.		3.
AVG (SD)		AVG (SD)	

Name:
Date:
Trial: Pre-trial/ 6 weeks/ 12 weeks

Blood			
Blood glucose (venous sample)			
Haemoglobin			
Haematocrit			
Plasma osmolality			
WBC			
Neutrophils			
Lymphocytes			
Monocytes			
Heparin x 6 (✔only)			
EDTA x 4 (✔only)			
Serum x 4 (✔only)			

Checklist/ timeline

Period	Time	Samples
Fasted	~8-9am	☐ Consent form ☐ Exercise form ☐ iDXA scan (printout attached) ☐ Height ☐ Fill in QoL ☐ Blood sample ☐ Body weight ☐ TBW (FFM) ☐ RMR
After standardised breakfast	10-11	☐ Incremental bike test ☐ Vertical jump ☐ Strength test ☐ Hand grip ☐ Gait speed

APPENDIX J:

Exercise program
(Chapter Three)

University of Monash
School of Nutrition and Dietetics
Active aging study
Exercise plan

Participant:
Start date:

1 RM	Baseline	Week 6	Week 12	Notes (i.e., position of seat)
Lateral pull down				
Leg press				
Chest press				

Warm up protocol:

- 5 minutes on bike (~25% of maximum effort)
- dynamic stretches (self- selected)

Exercise protocol:

- Participant to perform 1-2 warm up sets on all resistance training equipment. Warm up sets to be 25%-50% of the working weight for the first set and 60-70% for the second set.
- Eccentric and concentric load to be 2 controlled with two counts each way.
- Rest 1-2 minutes' in-between sets. 2-3 minutes' in-between exercises.
- Exercises are to be performed to, or near task failure in the range of repetitions provided. If participants can perform the end range of repetitions for the given sets, then increase weight by 5% and aim for the lower end of the range and work up again.
- Accessory exercises:
  To be performed 10-15 repetitions based on the percentage given. Participants should be pushed as far to fatigue on the accessories.

Cool down protocol:

- 5 minutes on bike (<25% maximum effort)

Week 1-2 (Familiarisation phase) RPE ~5-6

- Warm up (5 minutes)
- Cool down (5-10 minutes)
- Rest 1-2 minutes' in-between sets. 2-3 minutes' in-between exercises.
- Exercises are to be performed to, or near task failure in the range of repetitions provided. If participants can perform the end range of repetitions for the given sets, then increase weight by 5% and aim for the lower end of the range and work up again.

Week 1					Week 2				
Day 1	**Range**	**%**	**Target**	**Actual**	**Day 1**	**Range**	**%**	**Target**	**Actual**
Leg press	3 x10-15	50-60%			Leg press	3 x10-15	50-60%		
Lateral pull down	3 x10-15	50-60%			Lateral pull down	3 x10-15	50-60%		
Chest press	3 x10-15	50-60%			Chest press	3 x10-15	50-60%		
Cable bicep curls (bar)	3x10-15	RPE 5-6			Cable bicep curls (bar)	3x10-15	RPE 5-6		
Triceps extensions (ropes)	3x10-15	RPE 5-6			Triceps extensions (ropes)	3x10-15	RPE 5-6		
Cable high pull (bar)	3x10-15	RPE 5-6			Cable high pull (bar)	3x10-15	RPE 5-6		
Day 2					**Day 2**				
Leg press	3 x10-15	50-60%			Leg press	3 x10-15	50-60%		
Lateral pull down	3 x10-15	50-60%			Lateral pull down	3 x10-15	50-60%		
Chest press	3 x10-15	50-60%			Chest press	3 x10-15	50-60%		
Back row (standing)	3x10-15	RPE 5-6			Back row (standing)	3x10-15	RPE 5-6		
Calf raises	3x10-15	RPE 5-6			Calf raises	3x10-15	RPE 5-6		
Day 3					**Day 3**				
Leg press	3 x10-15	50-60%			Leg press	3 x10-15	50-60%		
Lateral pull down	3 x10-15	50-60%			Lateral pull down	3 x10-15	50-60%		
Chest press	3 x10-15	50-60%			Chest press	3 x10-15	50-60%		
Cable deadlifts	3x10-15	RPE 5-6			Cable deadlifts	3x10-15	RPE 5-6		
Single leg hamstring curl	3x10-15	RPE 5-6			Single leg hamstring curl	3x10-15	RPE 5-6		

Week 3-6 (strength and hypertrophy phase) RPE ~6.9-7.5- moderate intensity

- Warm up (5 minutes)
- Cool down (5-10 minutes)
- Rest 1-2 minutes' in-between sets. 2-3 minutes' in-between exercises.

Exercises are to be performed to, or near task failure in the range of repetitions provided. If participants can perform the end range of repetitions for the given sets, then increase weight by 5% and aim for the lower end of the range and work up again

Week 3					Week 4				
Day 1	**Range**	**%**	**Target**	**Actual**	**Day 1**	**Range**	**%**	**Target**	**Actual**
Leg press	3 x8-12	69-75%			Leg press	3 x8-12	69-75%		
Lateral pull down	3 x8-12	69-75%			Lateral pull down	3 x8-12	69-75%		
Chest press	3 x8-12	69-75%			Chest press	3 x8-12	69-75%		
Cable bicep curls (bar)	3 x10-15	RPE 6.9-7.5			Cable bicep curls (bar)	3 x10-15	RPE 6.9-7.5		
Triceps extensions (ropes)	3 x10-15	RPE 6.9-7.5			Triceps extensions (ropes)	3 x10-15	RPE 6.9-7.5		
Cable high pull (bar)	3 x10-15	RPE 6.9-7.5			Cable high pull (bar)	3 x10-15	RPE 6.9-7.5		
Day 2	3 x8-12				**Day 2**	3 x8-12			
Leg press	3 x8-12	69-75%			Leg press	3 x8-12	69-75%		
Lateral pull down	3 x8-12	69-75%			Lateral pull down	3 x8-12	69-75%		
Chest press	3 x8-12	69-75%			Chest press	3 x8-12	69-75%		
Back row (standing)	3 x10-15	RPE 6.9-7.5			Back row (standing)	3 x10-15	RPE 6.9-7.5		
Calf raises	3 x10-15	RPE 6.9-7.5			Calf raises	3 x10-15	RPE 6.9-7.5		
Day 3	3 x8-12				**Day 3**	3 x8-12			
Leg press	3 x8-12	69-75%			Leg press	3 x8-12	69-75%		
Lateral pull down	3 x8-12	69-75%			Lateral pull down	3 x8-12	69-75%		
Chest press	3 x8-12	69-75%			Chest press	3 x8-12	69-75%		
Cable deadlifts	3 x10-15	RPE 6.9-7.5			Cable deadlifts	3 x10-15	RPE 6.9-7.5		
Single leg hamstring curl	3 x10-15	RPE 6.9-7.5			Single leg hamstring curl	3 x10-15	RPE 6.9-7.5		

Week 7-12 (strength and hypertrophy phase)

- Warm up (5 minutes)
- Cool down (5-10 minutes)
- Rest 1-2 minutes' in-between sets. 2-3 minutes' in-between exercises.

Exercises are to be performed to, or near task failure in the range of repetitions provided. If participants can perform the end range of repetitions for the given sets, then increase weight by 5% and aim for the lower end of the range and work up agai

Week 7					Week 8				
Day 1	Range	%	Target	Actual	Day 1	Range	%	Target	Actual
Leg press	3 x6-8	75-80%			Leg press	3 x6-8	75-80%		
Lateral pull down	3 x6-8	75-80%			Lateral pull down	3 x6-8	75-80%		
Chest press	3 x6-8	75-80%			Chest press	3 x6-8	75-80%		
Cable bicep curls (bar)	3 x10-15	RPE 7-8			Cable bicep curls (bar)	3 x10-15	RPE 7-8		
Triceps extensions (ropes)	3 x10-15	RPE 7-8			Triceps extensions (ropes)	3 x10-15	RPE 7-8		
Cable high pull (bar)	3 x10-15	RPE 7-8			Cable high pull (bar)	3 x10-15	RPE 7-8		
Day 2					**Day 2**				
Leg press	3 x6-8	75-80%			Leg press	3 x6-8	75-80%		
Lateral pull down	3 x6-8	75-80%			Lateral pull down	3 x6-8	75-80%		
Chest press	3 x6-8	75-80%			Chest press	3 x6-8	75-80%		
Back row (standing)	3 x8-10	RPE 7-8			Back row (standing)	3 x8-10	RPE 7-8		
Calf raises	3 x8-10	RPE 7-8			Calf raises	3 x8-10	RPE 7-8		
Day 3					**Day 3**				
Leg press	3 x6-8	75-80%			Leg press	3 x6-8	75-80%		
Lateral pull down	3 x6-8	75-80%			Lateral pull down	3 x6-8	75-80%		
Chest press	3 x6-8	75-80%			Chest press	3 x6-8	75-80%		
Cable deadlifts	3 x10-15	RPE 7-8			Cable deadlifts	3 x10-15	RPE 7-8		
Single leg hamstring curl	3 x10-15	RPE 7-8			Single leg hamstring curl	3 x10-15	RPE 7-8		

APPENDIX K:

Amino acid profile

DTS FOOD ASSURANCE
A BUREAU VERITAS ASUREQUALITY JOINT VENTURE

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AsureQuality
BUREAU VERITAS

LABORATORY REPORT
on
LIQUID MILK

Date: 08/06/2017
Our Ref: DTS17041853
Report No: 2204625
Interim

FOR: **CCIC AUSTRALIA PTY LTD**

Suite 275-276, Lvl 11, 7-11 The Avenue
Hurstville NSW 2220

STEVEN

Date received: 12/05/2017
Origin:
Code/Ref: 1. VANMILK WHOLE MILK 3.4% 1 LITRE
Order Number:
No of samples: 8
Package Type:
Temperature on receipt: 4°C

TEST	RESULTS	METHOD N
12MAY17/9899800		
Client ID:	**1. VANMILK WHOLE MILK 3.4% 1LITRE**	
Amino Acid Profile*	See Attached Report	SUBC 11 05.05
Histidine*	0.95 mg/g	SUBC 11 05.05
Serine*	1.86 mg/g	SUBC 11 05.05
Arginine*	1.16 mg/g	SUBC 11 05.05
Glycine*	0.65 mg/g	SUBC 11 05.05
Aspartic Acid*	2.54 mg/g	SUBC 11 05.05
Glutamic Acid*	7.09 mg/g	SUBC 11 05.05
Threonine*	1.45 mg/g	SUBC 11 05.05
Alanine*	1.06 mg/g	SUBC 11 05.05
Proline*	3.22 mg/g	SUBC 11 05.05
Lysine*	2.75 mg/g	SUBC 11 05.05
Tyrosine*	1.54 mg/g	SUBC 11 05.05
Methionine*	0.90 mg/g	SUBC 11 05.05
Valine*	2.07 mg/g	SUBC 11 05.05
Isoleucine*	1.74 mg/g	SUBC 11 05.05
Leucine*	3.25 mg/g	SUBC 11 05.05
Phenylalanine*	1.61 mg/g	SUBC 11 05.05
Tryptophan*	0.39 mg/g	SUBC 11 05.05
Cysteine*	0.30 mg/g	SUBC 11 05.05
12MAY17/9899801		
Client ID:	**1. VANMILK WHOLE MILK 3.4% 1LITRE**	
Vitamin B12*		SUB002 00.199
12MAY17/9899735		
Client ID:	**1. VANMILK WHOLE MILK 3.4% 1LITRE**	
Aerobic Plate Count	620 cfu/mL	PCFD 12 07.13
Coliforms	<1 cfu/mL	COFD 14 07.13
Staphylococcus aureus	Not Detected /25g	STFD 090415
Salmonella	Not Detected /25g	SMFD 02 05.05

NATA ACCREDITED LABORATORY
Number - 345
Sample(s) tested as received

Measurement Uncertainty (MU) data can be found on DTSLIVE at https://lims.dtsfoodassurance.com.au.
Please note that the MU provided is indicative for general matrices and analytes only.

APPENDIX L:

Food diary compliance sheet: 1 day sample

Name: Week:			
Tuesday (non-training)		Amount eaten	Notes (extra foods/ topping etc)
Breakfast	Oats	None ¼ ½ ¾ all	
	Milk drink (250mls)	None ¼ ½ ¾ all	
	Fruit	None ¼ ½ ¾ all	
Snack/s	Cheese and biscuits	None ¼ ½ ¾ all	
Lunch	LC chicken satay with noodles	None ¼ ½ ¾ all	
	Milk drink (250mls)	None ¼ ½ ¾ all	
Snack	Jatz	None ¼ ½ ¾ all	
Dinner	Beef lasagna	None ¼ ½ ¾ all	
Supper	Chocolate mousse	None ¼ ½ ¾ all	
Extra foods			
Extra drinks			