# STRATEGIES TO IMPROVE OVERALL HEALTH IN AGING

# MULTI-FACTORIAL EXERCISE AND NUTRITION STRATEGIES TO IMPROVE STRENGTH AND OTHER MEASURES OF MUSCLE FUNCTION AND HEALTH IN OLDER ADULTS

By

KIRSTEN E. BELL, B.Sc. (HONS); M.Sc.

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AUTHOR: Kirsten E. Bell
B.Sc. (McMaster University)
M.Sc. (University of Waterloo)

SUPERVISOR: Dr. Stuart M. Phillips, Ph.D.

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## LAY ABSTRACT

Aging is associated with a variety of deleterious physiological changes including loss of skeletal muscle mass and strength, reduced aerobic fitness, dyslipidemia, impaired glycemic control, and increased systemic inflammation. Broadly, this thesis explores how multiple exercise modalities and nutritional supplements can be used in combination to simultaneously alleviate several of these negative aspects of aging. This series of studies demonstrates that, in older men, consumption of a multi-ingredient nutritional supplement containing whey protein, creatine, vitamin D, calcium, and fish oil: A) stimulates significant improvements in lean mass, strength, plasma lipids, and systemic inflammation over a relatively short period of time (6 weeks) in the absence of exercise training; and B) enhances exercise training-induced gains in strength and glycemic control, as well as reductions in systemic inflammation. The findings of this thesis challenge the relatively common practice of targeting individual facets of aging with singular exercise or nutrition interventions.

## ABSTRACT

Resistance exercise training (RET) and protein supplementation are potent non-pharmacological countermeasures against sarcopenic muscle and strength loss, however other exercise modalities and isolated nutritional supplements are effective in combating additional deleterious age-related changes, such as reduced cardiometabolic health. Accordingly, in Study 1 we assessed the 48-hour integrated muscle protein synthesis (MPS) response to a single session of RE, aerobic exercise, or high-intensity interval exercise (HIIE) in a group of healthy older men using the novel heavy water method. The results of Study 1 indicated that both RE and HIIE were capable of significantly elevating myofibrillar MPS above resting rates, with the most substantial effect observed following RE. In Studies 2 and 3 we evaluated whether daily consumption of a nutritional supplement which comprised whey protein, creatine, vitamin D/calcium, and omega-3 polyunsaturated fatty acids could: augment strength, physical function, and lean tissue mass (Study 2), and also improve glycemic control, lipidemia, and systemic inflammation (Study 3) in healthy older men following 6 weeks of supplementation in the absence of exercise; and enhance exercise training-induced improvements in the same outcomes following a 12-week RET + HIIT program. Six weeks of multi-ingredient nutritional supplementation stimulated gains in strength (~6%) and lean mass (~1%), roughly equivalent to one year's worth of age-related decline, as well as reduced circulating concentrations of lipids and inflammatory markers. Twelve weeks of combined RET + HIIT simultaneously improved strength, aerobic fitness, and glucose handling in the same group of older men. Further improvements in systemic inflammation and glucose

handling were observed when multi-ingredient nutritional supplementation was combined with exercise training. Collectively, these studies demonstrate that multiple exercise modalities and nutritional supplements can be employed concurrently to alleviate various aspects age-related physiological decline.

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| | |
|---|---|
| 1RM | One repetition maximum |
| 10RM | Ten repetition maximum |
| $^{2}H$ | Deuterium |
| AE | Aerobic exercise |
| AET | Aerobic exercise training |
| AUC | Area under the curve |
| CON | Control |
| Cmax | Maximal concentration |
| CRP | C-reactive protein |
| $D_2O$ | Deuterium oxide |
| DXA | Dual-energy X-ray absorptiometry |
| FSR | Fractional synthesis rate |
| HDL-c | High density lipoprotein cholesterol |
| HIIE | High-intensity interval exercise |
| HIIT | High-intensity interval training |
| HOMA-IR | Homeostatic model assessment of Insulin resistance |
| HR | Heart rate |
| IL | Interleukin |
| LDL-c | Low density lipoprotein cholesterol |
| LBM | Lean body mass |
| MCID | Minimum clinically important difference |
| MitoPS | Mitochondrial protein synthesis |
| MPB | Muscle protein breakdown |
| MPS | Muscle protein synthesis |
| MyoPS | Myofibrillar protein synthesis |
| n-3 | Omega-3 |
| OGIS | Oral glucose tolerance test-based index of insulin sensitivity |
| OGTT | Oral glucose tolerance test |
| PUFA | Polyunsaturated fatty acids |
| RE | Resistance exercise |
| RET | Resistance exercise training |
| SarcPS | Sarcoplasmic protein synthesis |
| SUPP | Supplement |
| TG | Triglyceride |
| Tmax | Time of maximal concentration |
| TNF-α | Tumour necrosis factor α |
| Total-c | Total cholesterol |
| TUG | Timed up-and-go |
| $VO_2peak$ | Peak oxygen uptake |

# PREFACE
# DECLARATION OF ACADEMIC ACHIEVEMENT

## FORMAT AND ORGANIZATION OF THESIS

This thesis is prepared in the "sandwich" format as outlined in the School of Graduate Studies' Guide for the Preparation of Theses. It includes a general introduction, three original research papers prepared in journal article format, and a general discussion. The candidate is the first author on all of the manuscripts. At the time of the thesis preparation, Chapter 2 was published in a peer-reviewed journal, Chapter 3 had been recently submitted for publication, and Chapter 4 was in preparation for submission.

## CONTRIBUTION TO PAPERS WITH MULTIPLE AUTHORSHIPS

**Chapter 2 (Study 1):**

**Bell K.E., S**éguin C., Parise G., Baker S.K. & Phillips S.M. (2015) Day-to-day changes in muscle protein synthesis in recovery from resistance, aerobic, and high-intensity interval exercise in older men. *J Gerontol A Biol Sci Med Sci* 70(8): 1024-9.

**Contribution:**

K.E. Bell, C.Séguin, G. Parise, and S.M. Phillips designed the study; K.E. Bell, C.Séguin and S.M. Phillips collected data; K.E. Bell completed analysis; K.E. Bell, and S.M. Phillips interpreted results; K.E. Bell prepared figures and drafted the manuscript with input from S.M. Phillips and all other authors.

**Chapter 3 (Study 2):**

**Bell K.E.,** Snijders T., Zulyniak M., Kumbhare D., Parise G., Chabowski, A. & Phillips S.M. (2017) A whey protein-based multi-ingredient nutritional supplement stimulates gains in lean body mass and strength in healthy older men. *Submitted to the American Journal of Clinical Nutrition.*

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**Chapter 4 (Study 3):**

**Bell K.E.,** Snijders T., Zulyniak M., Kumbhare D., Parise G., Chabowski, A. & Phillips S.M. (2017) A A A whey protein-based multi-ingredient nutritional supplement enhances exercise training-related reductions in glycemic and markers of systemic inflammation, in healthy older men. *In preparation.*

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# CHAPTER 1:

# INTRODUCTION

## 1.1 Introduction

Life expectancies have risen dramatically in the last two centuries and continue to increase. In 2017, a 15 year old has a 22% chance of living to be 100 years old; by comparison, a 30 year old has a roughly 16% chance of reaching 100 (1). Viewed alternatively, in 2011 there were 17 centenarians per 100,000 people in Canada (2) and it is projected that by 2050 the prevalence of centenarians will be more than double (2). Clearly, this demographic shift will have a substantial impact on individuals and also on society as a whole.

### *1.1.1 Aging demographics in Canada and worldwide*

The population of Canadians over the age of 65 is growing. Between 1971 and 2011 the proportion of people aged 65 years or older increased from 8% to 14% of the population, and by the year 2036 seniors are expected to represent 23-25% of the population (or 9.9-10.9 million people) (3). This demographic shift to an older population is also observed on a global scale: the number of people in the world 60 years of age or older tripled between 1950 and 2000 (from 205 to 606 million people), and by the year 2050 the number is expected to triple once again (to nearly 2 billion people) (4). The considerable growth of the senior population in Canada and around the world is largely driven by an increase in lifespan that is not accompanied by a proportional reduction in morbidity (5); people are living longer, but are also living with a greater burden of chronic disease than ever before. In Canada in 2009, 25% of seniors reported living with at least 4 chronic conditions (such as diabetes, cardiovascular disease, and cancer) compared to only 6% of

middle-aged adults (40-65 years) (3). Further, the global burden of non-communicable chronic disease in individuals 60 years of age or older increased 33% between 1990 and 2010 (6). The consequences of this demographic shift towards an older population whose constituents are encumbered with, often times more than one, chronic disease are far-reaching and include an alarming rise in healthcare expenditure (7) as well as an increasingly frail and disabled proportion of population.

### *1.2.1 Sarcopenia*

In general terms, sarcopenia is the age-associated loss of skeletal muscle mass (myopenia) and strength (dynapenia) (8). This condition affects approximately 40% of people over the age of 70 (3); however, since no consensus definition exists at the present time reports on prevalence vary. The European Working Group on Sarcopenia in Older People (EWGSOP) defines sarcopenia as the occurrence of low muscularity (skeletal muscle mass index [measured by dividing whole body muscle mass by height in meters squared] < 9.2 kg/$m^2$ or 7.4 kg/$m^2$ in men and women, respectively; or calf circumference < 33 cm in either sex) combined with low muscle function (grip strength < 32 kg in men or 22 kg in women; or gait speed < 0.8 m/s in either sex) (9). Perhaps unsurprisingly, the prevalence of sarcopenia is greater among institutionalized (14-33%) compared to community-dwelling (1-29%) older adults (8). The consequences of sarcopenia are numerous and include an increased risk of falls and fractures, frailty and disability, as well as all-cause mortality (3). Sarcopenic individuals are more likely to develop metabolic disorders like type 2 diabetes (3), and if admitted to hospital tend to have

greater lengths of stay, higher complication rates, and a greater chance of in-hospital mortality (8).

The etiology of sarcopenia has not been fully elucidated but it appears to be influenced, at least in part, by the normal aging program. Beginning around the fifth the decade of life muscle mass is lost at rates of ~0.8% annually (estimates range from 0.6-1.2% per year) and strength at a rate of 1-3% per year, respectively (10). Compared to their younger counterparts, older adults produce less force per unit of muscle cross-sectional area, which is indicative of reduced muscle quality (11). Thus, older adults are required to perform certain activities of daily living (ADL) at a higher percentage of their maximal muscle force, suggesting that they perceive a greater degree of effort while completing everyday tasks such as lifting bags of groceries and getting up off the toilet compared to younger adults (12). Reductions in the number and size of type 2 muscle fibers (which are primarily responsible for the generation of high force and power) may underpin, in part, the observed decrements in strength and function with age (13, 14). The mechanisms contributing to age-related muscle loss are less clear but likely include: reduced satellite cell number (particularly type 2 muscle fiber-specific satellite cells) (13), reinnervation of type 2 muscle fibers by type 1 motor neurons (15), as well as a blunting of the muscle protein synthetic response to anabolic stimuli such as protein and mechanical loading (16). Although loss of muscle mass and strength is unavoidable during the aging process, the magnitude of loss can be mitigated by regular physical activity (in particular resistive exercise) and proper nutrition (17).

Engaging in regular resistance exercise training (RET) and/or consuming an adequate amount of high quality dietary protein are the two of the most potent non-pharmacological strategies for preserving muscle mass and strength in adults of all ages. The combination of RET and adequate protein can offset a certain degree of age-related muscle mass and strength loss. Previously sedentary older adults who engage in relatively short-term (~12 week) progressive RET programs can improve their strength by ~25-35% (18) and increase their whole body lean tissue mass by ~1.1 kg (19). The addition of ~42 g of protein per day can augment lean tissue mass gains during RET by ~0.48 kg (20). In essence, the combination of RET and protein supplementation can, within a 12 week period, recover age-related lean/muscle mass and strength losses that would typically occur over 5-10 years. Furthermore, RE and protein supplementation are safe and effective in both healthy and more frail older populations (21). As such, these interventions are attractive strategies towards preserving health in older age.

*1.1.3 Age-associated physiological changes*

In addition to sarcopenia, the aging process is characterized by an increased risk of cardiovascular disease (22) and insulin resistance/impaired glycemic regulation (23, 24). The increase in cardiovascular disease risk is related to declines in maximal aerobic capacity (25), undesirable partitioning of blood lipids (i.e. increased triglyceride concentrations and depressed high-density lipoprotein [HDL] cholesterol concentrations) (26), and decreased arterial elasticity (27) with age. As a consequence of insulin resistance/impaired glucose tolerance, the incidence of metabolic syndrome (28) and type

2 diabetes (29) is greater among older versus younger adults. These cardiometabolic changes are thought to be associated with and/or exacerbated by chronic low-grade systemic inflammation which also accompanies aging (26). This inflammation and ensuing progression towards chronic disease/disability may be partially due to the accumulation of senescent cells with age. In fact, rodent studies have shown that the ablation of senescent cells results in an increase in lifespan (30) and a reduction in disease burden (31). These results, while striking, have yet to be reproduced in humans, however. Although the prevention of sarcopenia is of great importance, particularly as the population ages, the contribution of reduced cardiometabolic health (and increased systemic inflammation) to frailty and loss of independence in older adults should not be ignored. Intervention strategies that can simultaneously improve muscularity and strength, and also improve cardiovascular and glycemic health, are therefore particularly important.

### *1.1.4 Improving healthspan and disability-free years*

The healthspan paradigm divides the modern human lifespan into two phases: an initial period of relatively healthy aging (the healthspan) followed by a period of age-associated dysfunction and disability (the morbidity stage) (5). Until the latter half of the 20th century infectious disease was a major determinant of lifespan; however, relatively recent medical advances have shifted the most common causes of death to non-communicable chronic diseases such as cancer (the leading cause of mortality in Canada), cardiovascular disease, and type 2 diabetes (4). In developed nations, advances in the treatment of

chronic diseases has allowed an extension of overall lifespan, driven primarily by an expansion of the morbidity period of life, which is characterized by age-associated disease. In other words, we are living longer – but not necessarily healthier – lives. In order to effectively mitigate the negative personal and societal impacts of global population aging on healthcare and improve quality of life for older adults, we must simultaneously increase the healthspan and shorten the morbidity stage of a person's life. During the healthy years of life, normal physiological changes related to aging accumulate at a subclinical level (see **Figure 1**). Such changes include (but are not limited to): sarcopenic muscle and strength loss, reductions in glycemic control, impairments in mitochondria and stem cell function, as well as increased systemic inflammation and oxidative stress (26). At a certain age, the magnitude of these age-related physiological changes, interacting with lifestyle and genetics, would be enough to cause overt disease or disability. A diverse body of literature supports the use of regular physical activity to delay the onset and reduce the incidence of chronic disease in individuals of all ages (primary prevention) (32-35), as well as to slow the progression of disease in older adults who already have some degree of functional impairment (secondary prevention) (36-38). The beneficial effects of regular physical activity are broad, and target of variety of the physiological mechanisms which contribute to the development of common chronic diseases. The widespread, lifelong adoption of regular physical activity is therefore predicted to increase the healthspan and quality of life, compress the morbidity stage to the later years of life (Figure 1B), and alleviate a

substantial portion of the healthcare costs associated with the global shift to an older population (5).

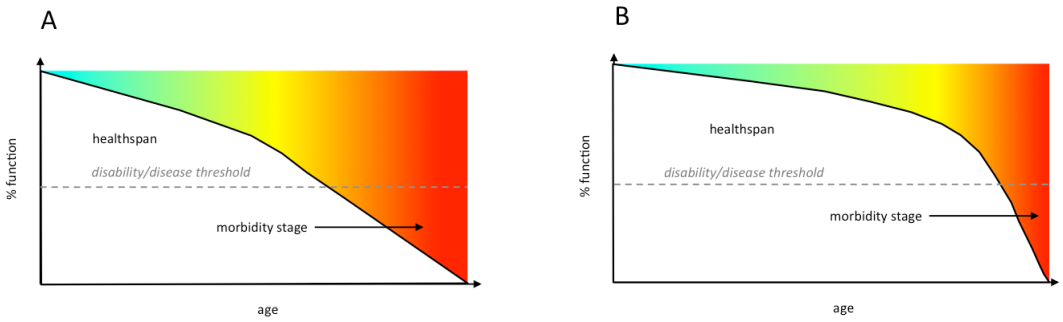


**Figure 1. The healthspan paradigm.** (A) The modern human lifespan (represented by the black curve) generally begins in a period of good health and a high degree of physical function. Over the course of normal biological aging muscularity and strength are gradually reduced, insulin sensitivity and aerobic capacity decline, and systemic inflammation slowly increases (represented by the transition from blue/green [good health] to red [poor health]). These, and other, age-related changes serve to reduce physical function but are sub-clinical during the healthspan (the ages during which the lifespan curve is above the disability/disease threshold). Once physical function is reduced to a point where the lifespan curve crosses the disability/disease threshold the morbidity stage of life begins. This latter stage of life is characterized by overt chronic disease (ie. cardiovascular disease, diabetes, cancer etc.), frailty, and loss of independence. (B) Shifting the lifespan curve to the right would simultaneously increase the healthspan and compress the morbidity stage of life. Regular physical activity combats several age-related changes that contribute to loss of physical function, and ultimately, the manifestation of chronic diseases. Lifelong exercise may therefore help shift the lifespan curve to the right and improve quality of life in older age. *Redrawn from Seals et al. (5).*

### 1.2 Muscle protein synthesis

Whole body skeletal muscle mass is in part determined by the ability to consistently and repeatedly stimulate muscle protein synthesis. Skeletal muscle (the majority of which is composed of myofibrillar proteins) exists in a constant state of turnover, which is driven by a dynamic balance between the opposing processes of muscle protein synthesis (MPS; the creation of new muscle proteins) and muscle protein breakdown (MPB; the

dissolution of existing muscle proteins) (39). Rates of MPS are more responsive to feeding and exercise compared to rates of MPB, and consequently fluctuate 3-5 times more over the course of a day (40). Net muscle protein balance, therefore, depends greatly on the degree to which MPS is stimulated.

Resting, postabsorptive rates of myofibrillar protein synthesis (myoPS) are ~0.03-0.04% per hour and increase 2 to 3-fold for several hours with the ingestion of anabolic stimuli such as protein (41), essential amino acids (42), and leucine (43). Acute RE can sensitize the muscle protein synthetic machinery to the anabolic effects of protein and amino acids for up to 48 hours in younger adults (44, 45) resulting in a superior stimulation of myoPS above resting, postabsorptive rates compared to protein ingestion alone (46-49). Since myofibrillar proteins, such as the contractile proteins actin and myosin, comprise the bulk (60-70%) of skeletal muscle proteins, repeated stimulation of myoPS is required for muscle hypertrophy to occur. Conversely, insufficient stimulation of this protein fraction, such as during disuse, can result in muscle atrophy. The remaining 30-40% of muscle proteins (the non-myofibrillar or sarcoplasmic fraction) include cytosolic, sarcoplasmic reticular, and mitochondrial proteins. Sarcoplasmic protein synthesis (sarcPS) is stimulated by aerobic exercise (AE) (50, 51), possibly due to increased synthesis of sarcoplasmic reticular and/or mitochondrial proteins to support the greater demand on oxidative metabolism, and does not appear to be a major determinant of muscle hypertrophy or atrophy (50, 51). It remains to be established whether differences in rates of sarcPS exist between older and younger adults, however the stimulation of myoPS in response to anabolic stimuli appears to be dampened with age

(16, 46). This impairment is hypothesized to cause chronic net negative muscle protein balance thereby contributing to age-related muscle loss.

*1.2.1 Anabolic resistance of muscle protein synthesis*

Resting, postabsorptive rates of MPS do not appear to differ between younger and older adults (52). In addition, skeletal muscle of older adults retains the ability to respond acutely to anabolic stimuli such as protein ingestion (and the resultant hyperaminoacidemia) and RE (46), as well as to undergo hypertrophy following prolonged protein supplementation and/or RET (19). However, the acute rise in MPS in response to protein ingestion or RE is blunted in older adults compared to the same dose of protein in younger adults (16). Likewise, lean mass gains following chronic protein supplementation and/or RET are smaller in older versus younger adults (11, 20). This blunted response is termed anabolic resistance, and is shown schematically in **Figure 2**. Briefly, failure to stimulate MPS to the same degree as is observed in younger adults can result in a greater amount of time spent in net negative muscle protein balance at the end of the day. Over time, this chronic imbalance of MPS and MPB can translate into detectable muscle loss.

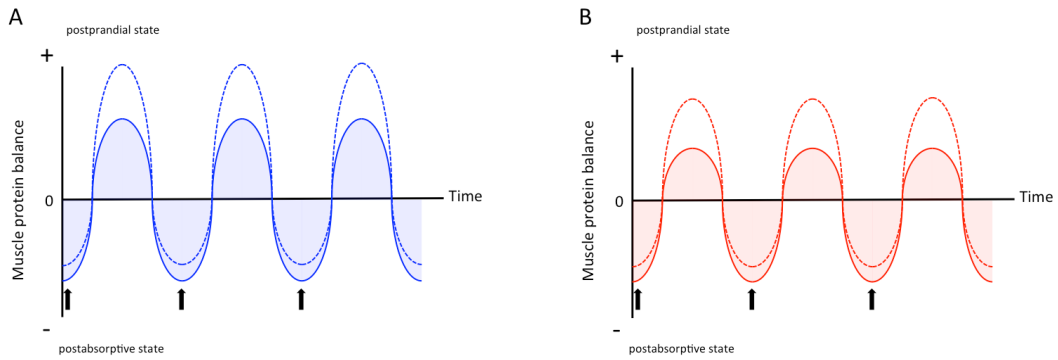


**Figure 2. The dynamic balance between MPS and MPB over the course of the single day in (A) younger and (B) older adults.** In the postabsorptive state (solid lines) at rest, rates of MPB exceed rates of MPS and muscle protein balance is negative. When a protein-containing meal (indicated by the arrows) is consumed, MPS is stimulated and muscle protein balance becomes positive. This transient anabolic state lasts ~3-4 hours, after which point rates of the MPS return to postabsorptive levels and muscle protein balance becomes negative prior to consumption of the next meal. In younger adults, muscle protein accretion (shaded area *above* the x-axis) and muscle protein loss (shaded area *below* the x-axis) are roughly equal, so that net muscle protein balance at the end of the day is approximately zero. In older adults, a meal containing the same amount of protein will not stimulate MPS to the same extent as in the young, which can result in negative net muscle protein balance at the end of the day, and contribute to muscle atrophy overtime. In both younger and older adults, a single session of RE (dashed lines) can augment muscle protein accretion in the postprandial state and reduce muscle protein loss in the postabsorptive state. The acute anabolic effects of RE are more pronounced in younger adults, and persist for up to 48 hours post-exercise in both age groups. Over the course of several weeks to months, regular RE can lead to muscle hypertrophy. MPS, muscle protein synthesis; MPB, muscle protein breakdown; RE, resistance exercise.

### *1.2.2 Stable isotope tracers*

Rates of MPS have traditionally been measured using the intravenous infusion of amino acids labeled with a stable isotope. For stable isotope tracers to be valid the labeled amino acid, for example $^{13}C_6$ phenylalanine, must behave identically to the unlabeled amino acid (in this case unlabeled phenylalanine) upon entering the body pool (53). One fate of such a labeled amino acid is transportation into the free amino acid pool in a myofiber, becoming covalently bonded to a molecule of transfer RNA, and subsequently

incorporated into muscle proteins. By measuring the ratio of labeled to unlabeled amino acid in the plasma (via blood draws), intracellular free amino acid pool, and bound muscle proteins (via muscle biopsies) we can assess the rate of muscle protein accretion (MPS) in various physiological situations.

For decades stable isotope infusions have been invaluable in the assessment of basic muscle protein metabolism. However, this method has several disadvantages. Firstly, infusion trials involve multiple venous cannulations which require technical skill and also confine participants to the laboratory for the duration of the infusion. As such, measurements of MPS are only made over the course of (typically) ~2-5 hours (43, 46, 54). A valid criticism of this method is that it reflects an artificial setting where participants lie supine, oftentimes in the postabsorptive state, for the majority of the day (except for trips to the bathroom and any planned exercise) and normally consume liquid meals (or have continuous nutrient infusion) instead of their habitual diet. The recent refinement of the heavy water method has circumvented some of the drawbacks associated with acute infusion trials (55, 56). With this method, deuterium oxide ($D_2O$) is administered orally and rapidly equilibrates (over ~2 hours) with the total body water pool, labeling amino acids. Upon entering myofibers and the intracellular free amino acid pool, this tracer is incorporated into muscle proteins (56). Body fluids such as saliva or urine may be obtained in place of plasma for the measurement of precursor enrichment, negating the need for cannulation as well as confinement to the laboratory. Participants are thus "free-living" and integrated measurements of MPS can be made over the course of days to weeks. The assessment of MPS using heavy water is arguably more reflective

of a real-world setting, since habitual diet and physical activity are taken into account. Furthermore, the $D_2O$ method has been validated against the stable isotope infusion technique (57), and has been used to successfully measure resting and post-exercise rates of MPS (58, 59).

## 1.3 Additional metabolic perturbations associated with aging

Impairments in glucose handling/insulin sensitivity (24, 60-62) and lipid partitioning (63) accompany older age. As with anabolic resistance to protein ingestion and RE (discussed in more detail in section 1.2.1), low levels of physical activity and poor diet (notably excess energy intake and an increase in refined carbohydrate consumption) can exacerbate these metabolic perturbations (64, 65). However, age is independently associated with these deleterious metabolic changes even when confounding lifestyle factors, such as physical activity level and adiposity, are controlled (66). Chronic low-grade systemic inflammation may underpin the independent influence of biological aging on alterations to glucose metabolism and lipid concentrations.

### *1.3.1 Glucose dysregulation*

Whole body insulin sensitivity (assessed using the hyperinsulinemic-euglycemic clamp) (67, 68) and rates of intracellular glucose oxidation (69) are depressed in older versus younger adults. As well, fasting and 2 hour plasma glucose and insulin concentrations in response to an oral glucose tolerance test are elevated with age (70). Importantly, the

occurrence of impaired glucose tolerance (2 hour plasma glucose between 7.8-11.1 mM) in response to a 75 g glucose challenge is more common than the occurrence of elevated (5.6-5.9 mM) or impaired (6.0-6.9 mM) fasting glucose in older adults (71). This is more suggestive of decrements in the ability of skeletal muscle to effectively take up excess circulating glucose postprandially, compared to increased glucose output by the liver in the postabsorptive state. Type 2 diabetes is twice as prevalent among older adults compared to middle aged adults (60), and older diabetic adults have lower muscularity, strength (72), and physical function (73-75) compared to healthy older adults. Earlier identification of impairments in glucose metabolism using tools like the oral glucose tolerance test, rather than relying entirely on static measures such as fasting blood samples, may help reduce the incidence a type 2 diabetes among older adults.

*1.3.2 Dyslipidemia*

Dyslipidemia (defined as increased triglyceride and low-density lipoprotein [LDL] cholesterol concentrations and/or depressed HDL cholesterol concentrations) is more prevalent among older compared to younger adults (76-78), and is a significant risk factor for cardiovascular disease. Elevated LDL cholesterol concentrations, in particular, are required for other traditional cardiovascular risk factors such as smoking, diabetes, and hypertension to exert a significant effect (79), and a 1 mM reduction and LDL cholesterol concentrations is associated with a 20% reduction in risk for a clinical cardiovascular events (80). Cardiovascular disease is the leading cause of death in developed countries (81) and 85% cardiovascular disease patients are over 65 years of age (82). The

mechanisms underlying the age-related disturbance in lipid concentrations is unclear, but it may be related to low-grade systemic inflammation.

*1.3.3 Inflammation*

Aging is accompanied, independent of comorbidities, by a modest yet significant increase in circulating concentrations of inflammatory markers (83), such as c-reactive protein (CRP), interleukin (IL) 1β, IL-6, IL-1Ra, and tumour necrosis factor alpha (TNF-α). This chronic low-grade systemic inflammation (often referred to as "inflammaging") is inversely associated with overall survival (84, 85), and positively associated with multi-morbidity (86) as well as the incidence of type 2 diabetes (87) and all other age-related chronic diseases (88). Specifically, elevated serum concentrations of CRP, IL-6, and IL-Ra are correlated with declines in physical function (89, 90) and higher frailty scores (91, 92). In skeletal muscle, IL-6 and TNF-α may contribute to the development of insulin resistance (93) by inhibiting the activation of insulin receptor substrate 1 (IRS-1) and its downstream signaling cascade, thereby preventing the translocation of glucose transporter 4 (GLUT4) to the sarcolemma. This may help explain the association between inflammaging and impaired glucose tolerance/diabetes.

**1.4 Physical activity considerations for older adults**

The majority of older adults do not engage in sufficient physical activity to meet recommendations set out by either the Canadian Society for Exercise Physiology (CSEP) (94) or the American College of Sports Medicine (ACSM) (95). The ACSM recommends

that healthy adults over the age of 65 years perform 150-300 minutes per week of moderate intensity AE in bouts of at least 10 minutes each, or 75-100 minutes per week of vigorous intensity AE (95). They also recommend that older adults engage in resistance-type exercises a minimum of 2 days per week at a moderate intensity of 60-70% one repetition maximum (1RM; or 40-50% 1RM for individuals who are new to resistance training) for 10-15 repetitions for at least one set (95). These recommendations are virtually identical to the ACSM recommendations for healthy adults 18-65 years of age except that older adults with balance or mobility issues are cautioned against certain modalities of AE such as running, road cycling, and stair climbing. During bouts of vigorous intensity AE older adults are discouraged from working at "all out" intensities of 9-10/10 on the Borg perceived exertion scale, although similar censure is not given to healthy adults of 18-65 years. Lastly, strength training intensity is not specified for younger and middle-aged adults, whereas older adults are instructed to train at 70% 1RM or below. Given the inherent physiological differences in older versus younger/middle-aged adults, such as reduced strength and muscularity, further refinement of the physical activity recommendations for older adults is warranted. RE and AE prescriptions should be tailored to reflect the current state of the literature, namely: strength gains should be prioritized in an effort to combat the development of sarcopenia; and secondly, in light of the growing body of evidence supporting the use of high-intensity interval exercise (HIIE) in older adults, the warning against vigorous intensity AE should perhaps be reevaluated.

*1.4.1 Resistance exercise training*

In older age RET should take priority over AET due to its potency as a countermeasure against sarcopenic muscle loss, as well as muscle power and strength loss. Training for lower body strength, in particular, should be emphasized given that: there is a strong association between strength and survival in older adults (96, 97), and also that the majority of age-related strength (and muscle) loss occurs in the anterior compartment of the thigh (i.e. the quadriceps muscles) (98, 99). Strength improvements are directly related to training load, such that heavier loads (> 75% 1RM) result in greater strength gains compared to lighter loads (< 50% 1RM) (100). Contrary to popular belief, strength training with heavier loads is effective and safe in most healthy older populations, provided that sufficient guidance is given and proper technique is consistently practiced. Progressive RET studies in older adults typically report strength gains of 25-35% (11, 101-103), as well as improvements in assessments of physical performance such as gait speed, timed up-and-go, time to complete 5 chair stands, time to climb 15 stairs, 6 minute walk test, and single leg balance (104-106). These measures of physical function provide an indication that an older adult's ability to perform certain daily tasks, such as putting away groceries and getting up off the toilet, would likely be improved. Improvements in these abilities increases independence, quality of life, and would, it is posited, contribute to an expansion of the healthspan.

Given that RET is highly effective in stimulating myofibrillar protein synthesis acutely, and skeletal muscle hypertrophy longer-term, it is a key strategy towards

combating the insidious age-related loss of muscle mass. The magnitude of skeletal muscle hypertrophy in response to RET decreases with age, however older adults on average gain ~1 kg of whole body lean mass (of which skeletal muscle is a major constituent) following 12 weeks of progressive RET (19). Similar to strength, muscle mass is preferentially lost in the anterior thigh compartment during the aging process (98, 99), reinforcing the importance of lower body RE. Unlike strength, however, muscle hypertrophy appears to depend more on training volume (19) as well as a high degree of a muscular effort (performing enough repetitions to induce muscle fatigue) rather than load (amount of weight lifted). As such, training with low loads and high volume (~30% 1RM for 25-30 repetitions) has been shown to result in a similar degree of hypertrophy as training with high loads and low volume (~80% 1RM for 6-8 repetitions), provided that – regardless of load – repetitions are performed to the point of voluntary fatigue (100, 107). It is important to note, however, that strength gains are superior in the high load/ low volume model due mostly to neuromuscular adaptation. Muscle hypertrophy is desirable, but considering the central role that strength (and power) plays in maintaining independence and improving physical function, RET with high loads (> 70% 1RM) should be prioritized for older adults.

Improvements in aerobic capacity (108) and insulin sensitivity (109), as well as a reduction in overall risk of cardiovascular disease (110), have been observed following RET in older adults. Beneficial changes in aerobic capacity and insulin sensitivity are likely related to increases in quantity and quality of muscle mass (73, 111, 112). Since skeletal muscle is the largest glucose storage depot and a major site of oxygen

consumption during exercise, an increase in this tissue mass would provide a greater reservoir for circulating glucose as well as increase metabolic demand. Nevertheless, the degree of improvement in aerobic capacity and cardiovascular disease risk is markedly greater following AET.

*1.4.2 Aerobic exercise training*

Despite the potent anti-sarcopenic effects of RET, aerobic exercise (primarily walking) remains the most popular form of exercise amongst older adults (113). AE generally refers to lower effort, repetitive activities that are performed continuously for extended periods of time, and which require increased oxygen uptake (30-70% $VO_2$peak or 50-85% HRmax). In adults of all ages, AE can augment peak oxygen consumption (114), muscle oxidative capacity, and insulin sensitivity. Other classic adaptations to AE can include reductions in blood pressure, adiposity, improvements in blood lipid profile (i.e., increased circulating HDL cholesterol and decreased triglyceride concentrations), and a reduction in overall risk of cardiovascular disease. These adaptations are particularly valuable to older adults because, similar to low strength and muscularity, reduced aerobic capacity and insulin sensitivity (among other deleterious changes) are associated with older age. Therefore, older adults who regularly engage in AET are more likely to extend their healthspan, decrease morbidity, and reduce their risk of developing one or more chronic diseases.

Although AE training effectively counters several age-related physiological impairments, there is little-to-no stimulation of muscle mass and strength gains compared

to RET. Studies have reported greater strength and muscle mass in lifelong endurance exercisers compared to sedentary age-matched individuals (115, 116), but to date, no studies have evaluated the effect of aerobic exercise training on muscle/lean mass gains in healthy older adults. In clinical populations, however, meta-analysis has identified RET as superior to AET from the perspective of lean mass and strength gains (117). As such, it is appears that AE is a valid countermeasure against age-related physiological decline but likely far less potent than RET from the perspective of the prevention and treatment of sarcopenia.

*1.4.3 High-intensity interval training*

High-intensity interval exercise (HIIE) consists of repeated bouts of vigorous intensity aerobic efforts (80-100% $VO_2$peak or 85-95% HRmax) interspersed with periods of rest or lower intensity activity. Despite the low time commitment required compared to traditional low-moderate intensity continuous exercise, high-intensity interval training (HIIT) has been shown to induce aerobic-type adaptations in a variety of populations. Significant improvements in $VO_2$peak, muscle oxidative capacity, and glycemic control have been observed following HIIT in younger adults (118), healthy older adults (119), and clinical populations (120). It is currently unclear whether HIIT is associated with favourable body composition changes such as reduced fat mass and accretion of muscle mass.

During HIIE muscles are exposed to loads greater than low-moderate continuous AE, but lower than RE, leading some researchers to speculate that regular HIIT may be

capable of stimulating increases in muscle mass and even strength (121-123). However, few studies have been conducted to evaluate the influence of HIIT on these outcomes (124-128). The data in younger adults is it equivocal, with some studies reporting increases in lean body mass, quadriceps cross-sectional area, and peak power output (suggestive of increased muscle strength) (124, 127), and another reporting no change (128). Only two HIIT studies in older adults have evaluated strength and/or body composition changes with exercise training; both report improvements in strength (125, 126), and one reported an increase in quadriceps cross-sectional area and thigh volume (125). Acute stimulation of myofibrillar protein synthesis is required for skeletal muscle hypertrophy. Yet to date, no studies have examined the effect of acute HIIE on myofibrillar protein synthesis in younger or older adults. Investigation into the acute effects of HIIE on MPS may help tease out whether this modality is in fact hypertrophic. This information would be pertinent for exercise prescription in an older population. Specifically, HIIT might be a useful adjunct to RET programs in older adults since it may convey important aerobic adaptations while continuing to provide a stimulus for increases in strength and muscle mass.

### 1.5 Nutritional considerations for older adults

Various isolated nutritional supplements are supportive for skeletal muscle anabolism when ingested independently over the course of several weeks-months in older adults. The supplements supported by the strongest basis of evidence are: protein (in particular proteins with a high leucine content such as whey), creatine, vitamin D (with calcium),

and n-3 PUFA. Each compound targets a different signaling pathway within muscle and also differing aspects of sarcopenia (muscle mass, balance, gait speed) and thus could help mitigate age-related physiological decline. Whey protein helps to build and maintain muscle mass (20); creatine increases muscular strength (129); vitamin D and calcium reduce the incidence of falls/fractures and improve balance (130, 131); and n-3 PUFAs have been shown to augment muscle mass and strength (132), as well as reduce cardiovascular disease risk and system inflammation (133), and improve glycemic control (134, 135). The majority of nutrition intervention trials support the use of these supplements in older adults (20, 129, 132, 136-140), although some report no beneficial effect (141-144). Admittedly, the efficacy of the aforementioned supplements in augmenting lean mass and strength/function is not uniform. This is perhaps not surprising considering they have most commonly been administered as monotherapies. Non-significant results in certain studies are likely due to small sample sizes, short-duration of supplementation, heterogeneity of participants' responses, and differences in the supplement dose. Rationale for the use of, and evidence of efficacy for, the previously mentioned supplements is summarized below.

### *1.5.1 Whey protein*

Dietary protein intake enhances the effects of RET-induced adaptations in lean mass and strength and thus is important in older age. Protein not only supplies amino acids (the substrate for MPS) to skeletal muscle, but also provides an anabolic stimulus for the process of MPS (145). In younger and older adults, the acute rise in MPS following

ingestion of protein is smaller in both amplitude and duration compared to a single session of RE (46-49). Protein ingestion elevates MPS above resting values for ~3-5 hours, whereas the effects of RE can last up to 48 hours (44, 45). Several factors that can alter the effectiveness of dietary protein intake and/or supplementation in older adults are: quantity/dose, quality/source, and timing/distribution.

Due to the reduction in sensitivity of older skeletal muscle to the anabolic effects of protein (16, 46, 146, 147), increased protein quantity (versus the RDA for protein) is arguably the most important dietary consideration for older adults (148). Accordingly, recent recommendations suggest that an optimal protein intake for older adults would be 1.2-1.5 g per kilogram body mass per day (149, 150), which is 50-88% higher than the protein RDA of 0.8 g per kilogram body mass per day. The amount of protein consumed per serving (meal) may also be important. In a retrospective breakpoint analysis Moore et al. (16) determined that older adults require 0.40 g of protein per kilogram body mass per serving (~34 g per serving for a 85 kg older adult) to maximally stimulate myofibrillar protein synthesis, compared to 0.24 g of protein per kilogram body mass per serving in the young (~20 g per serving for a 85 kg younger adult). In a typical Western-style diet, the majority of dietary protein is consumed with the evening meal. Since older adults require ~30-40 g of protein per serving to maximally stimulate MPS, they may only be stimulating muscle mass gains once per day, in the evening. As such, protein supplementation might be most effective if the breakfast and lunchtime meals were targeted to allow for repeated maximal stimulation of MPS over the course of the single day. Furthermore, quickly digested leucine-rich proteins, such as whey protein, would

provide the rapid aminoacidemia and leucinemia necessary to stimulate MPS (151, 152). The rapid aminoacidemia following whey protein ingestion is higher and it occurs more rapidly compared to casein (152) or soy protein (151), strengthening the case for the use of this protein source as a nutritional supplement for older adults.

Whether protein supplementation can also enhance exercise training-mediated gains in lean mass in older adults remains to be definitively determined. Data from acute studies support this thesis because when protein is consumed in close temporal proximity to RE, MPS is increased to a greater extent compared to either stimulus alone (153). Similar to resting conditions though, older adults require greater doses of protein to maximally stimulate MPS following RE compared to young individuals (40 versus 20 g) (46). There is, however, conflicting evidence from meta-analyses on the ability of protein supplementation to enhance RET-related lean mass and strength gains in older adults, with some studies supporting the use of protein and amino acid supplements (20, 138, 139) and another showing no effect (141). Importantly, there are no studies showing that protein is ergolytic for enhancing RET-induced adaptation.

### *1.5.2 Creatine*

In older adults, creatine supplementation enhances muscular strength, physical function, lean/muscle mass, and bone density with (129) and without (140) concomitant RET, although the beneficial effects of creatine appear to be potentiated, similarly to protein, when combined with exercise. In a recent meta-analysis, Devries et al. (129) demonstrated that studies which combined creatine supplementation and RET in older

adults reported superior gains in fat-free mass (which are indicative of increased muscle mass; + 1.33 kg), chest press and leg press 1RM (+ 1.74 kg and + 3.25 kg, respectively), and 30 second chair stand performance (+ 1.93 stands) compared to RET alone. The average duration of exercise training in this meta-analysis was 12.6 weeks, and the average maintenance dose a creatine was 5.0 g per day (range: 3.0-8.6 g per day). The co-ingestion of carbohydrate with creatine may be important since studies have shown when consumed together carbohydrate can increase muscle creatine stores and reduce urinary creatine excretion (154, 155). However, since only 10 studies were included in this meta-analysis, it was not possible to examine whether carbohydrate co-ingestion offered any advantage. The mechanism by which creatine exerts its effects is not fully understood, but it is hypothesized that supplementation increases intramuscular phosphocreatine (PCr) energy stores, enhances the rate of PCr resynthesis, and decreases muscle damage (156). These changes may allow the performance of incrased contractile intensity and/or volume of exercise thereby increasing hypertrophy and strength (156). Creatine supplementation does not appear to directly stimulate MPS, however, hypertrophic gains may result from the performance of a greater training volume (19).

### *1.5.3 Vitamin D and calcium*

Supplementation with vitamin D and calcium reduces the incidence of falls in older adults by 30-40% (131), and reduces the risk of overall fracture and hip fracture by 15% and 30%, respectively, in community-dwelling and institutionalized older adults (137). Bone mineral density decreases with age (157), increasing the risk of fracture in older adults.

As the dominant mineral in bone, calcium supplementation may help to prevent and treat low bone density and prevent subsequent fractures. Vitamin D is important for the efficient absorption of calcium, and may also exert positive effects on skeletal muscle. Improvements in balance (158, 159) and neuromuscular function (130) with vitamin D supplementation may underpin the reductions in the incidence of falls amongst seniors; however, it may be that restoration of vitamin D from deficient/insufficient levels to sufficient is an important mediator here. Increased strength and muscle mass as a result of vitamin D supplementation may also contribute to reduction in fall risk, however data from human clinical trials are lacking. In a cross-sectional study of 419 individuals between the ages of 20-76 years, serum $25(OH)D_3$ concentrations were positively associated with isometric and isokinetic muscle strength, even when age, sex, and BMI were controlled for (160), suggesting that greater amounts of circulating vitamin D may enhance strength. Further, vitamin D receptor knockout mice are 20% less muscular than wild type mice (161), which suggests that vitamin D and its receptor are important for the maintenance of muscle mass. In support of these studies, vitamin D deficiency in humans appears to be associated with age-related sarcopenia, a condition characterized by low muscularity and strength (162). Still, whether vitamin D supplementation is effective in increasing muscle mass and strength in humans remains to be confirmed. Regardless of the potential enhancement of muscle mass and strength with vitamin D/calcium supplementation, the evidence for its ability to protect against falls and fractures is consistent. Doses of 700-1000 IU of vitamin D per day are most effective, and the most

dramatic benefits of supplementation are observed in older adults with low serum $25(OH)D_3$ concentrations that are corrected by supplementation (163).

*1.5.4 Omega-3 polyunsaturated fatty acids*

Increased supplemental dietary intake of omega-3 polyunsaturated fatty acids (n-3 PUFA), in particular eicosapentanoic acid [EPA] and docosahexanoic acid [DHA], which are long-chain PUFA that are abundant in some fish oils, is positively associated with grip strength (164) and functional performance in older adults (165). In accordance with these observations, Smith et al. (132) have shown that not only did 6 months of n-3 PUFA supplementation improve grip strength (+ 2.3 kg) and 1RM muscle strength (+ 4%), but thigh muscle volume was also increased (+ 3.6%). This increase in muscle mass may be driven by the ability of n-3 PUFA to support increased rates of MPS. For example, supplementation with 1.86 g EPA/ 1.50 g DHA per day for 8wk, potentiated rates of MPS in response to simulated feeding (hyperaminoacidemic-hyperinsulinemic clamp) in older adults (166, 167). When combined with RET, n-3 PUFA supplementation results in greater gains in strength measured as peak torque (136, 168), the rate of torque development (168), muscle quality (136), and muscle mass (169) compared to placebo. The mechanism underlying the improvements in strength and muscle mass with n-3 PUFA supplementation, with and without exercise training, is poorly understood. It is possible that supplementation enriches the n-3 PUFA content of the sarcolemma (170), leading to changes in membrane fluidity and muscle cell function, enhancing the benefits of strength training (168) and the content and functionality of anabolic signaling

molecules such as the mechanistic target of rapamycin (mTOR) and focal adhesion kinase (FAK) (170). In addition to the anti-sarcopenic effects in older adults, n-3 PUFA are associated with improvements in cardiovascular health (171, 172), especially the reduction of plasma triglyceride concentrations (136, 173), increased glycemic control (particularly in rodent studies) (135), and reductions in systemic inflammation (134, 135). Since the positive effects of n-3 PUFA supplementation target numerous facets of biological aging, it is an attractive supplement to recommend for older persons.

### 1.6 The use of multi-factorial exercise and nutrition strategies towards optimizing health in aging

Despite the fact that several isolated nutritional supplements and exercise modalities have each been shown alleviate different aspects of age-related physiological decline, very few studies have employed comprehensive interventions that combine multiple nutritional supplements and/or multi-modal exercise training (174-179). Of these trials, 4 were conducted on frail, malnourished, or sarcopenic older adults (174, 176, 177, 179); one was conducted on obese older adults during energy restriction (175); and one was conducted on generally healthy older adults (178). All studies except for one included a RET component (no studies included AET), and the most common supplement ingredients were whey protein, leucine, and vitamin D. All six trials reported improvements in lean body mass (range: + 0.4-1.7 kg), strength (+ 25%), and/or physical function (reductions in the number of fall incidents, and reduced time to complete 5 chair stands) compared to placebo. Importantly, the multi-ingredient supplements demonstrated

a clear advantage over placebo even though none of these studies provided an optimal dose of protein (30-40 g per serving) or vitamin D (1000 IU per day) to participants. The degree of improvement in lean body mass, strength, and physical function in these comprehensive, multi-factorial studies could potentially be increased by optimizing the dose of each ingredient. Further, overall health in older adults might be enhanced with the addition of AET or HIIT. To date, however, no study has evaluated the combined effects of whey protein, creatine, vitamin D/calcium, and n-3 PUFA along with combined RET and HIIT.

### 1.7 Studies and hypotheses

The overall aim of the studies in thesis was to discover mechanisms underlying and determine the efficacy of interventions that employed HIIT and RET as exercise stimuli. A second main aim was to combine these exercise modalities with supplementation with a multi-ingredient supplement containing whey, creatine, calcium, vitamin D, and DHA- and EPA-rich n-3 PUFA.

In Study 1 we examined the effect of a single session of RE, AE, or HIIE on rates of MPS in healthy older men. We used orally administered $D_2O$ to assess the 2-day integrated myofibrillar and sarcoplasmic protein synthesis response to each exercise mode. We hypothesized that RE and HIIT would stimulate significant increases in myofibrillar protein synthesis, and that HIIT and AE when stimulate significant increases in sarcoplasmic protein synthesis.

In Study 2 we evaluated whether daily consumption of a nutritional supplement which comprised whey protein, creatine, calcium, vitamin D, and n-3 PUFA could: augment strength, physical function, and lean tissue mass in a group of healthy older men following 6 weeks of supplementation in the absence of exercise; and enhance exercise training-induced improvements in the same outcomes following a 12-week RET + HIIT program. In this double-blind proof-of-principle efficacy study, we randomly allocated 49 healthy older men to either 20 weeks of nutritional supplementation or a carbohydrate-based control drink. For the first 6 weeks of the study participants consumed their beverages at home without altering their dietary or physical activity habits, which allowed us to evaluate the effect of the multi-ingredient supplement alone. Following this 6-week supplement-only period, participants completed a 12-week exercise training program (RET twice weekly and HIIT once per week) while continuing to consume their study beverages, allowing us to evaluate the effect of supplementation combined with exercise training. We hypothesized that our multi-ingredient supplement would stimulate beneficial changes in our outcomes of interest independently of exercise, and that we would observe an additive effect of the supplement when consumed in combination with exercise training.

Study 3 utilized the same participants and exercise/nutrition intervention as the Study 2. The objective was to determine whether our protein-based multi-ingredient nutrition supplement was able to improve glycemic control during an oral glucose tolerance test, fasting lipid panel (total, HDL and LDL cholesterol, and triglyceride concentrations), and markers of inflammation (circulating concentrations of CRP, TNF-α,

IL-6). We hypothesized that our novel supplement would improve glucose handling and fasting lipid profile, and reduce systemic inflammation, independently of exercise training. We further hypothesized that a greater degree of improvement in these outcomes would be observed when nutrition supplementation was combined with exercise training.

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# CHAPTER 2:

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Research Article

# Day-to-Day Changes in Muscle Protein Synthesis in Recovery From Resistance, Aerobic, and High-Intensity Interval Exercise in Older Men

**Kirsten E. Bell,[1] Christopher Séguin,[1] Gianni Parise,[1] Steven K. Baker,[2] and Stuart M. Phillips[1]**

[1]Department of Kinesiology, McMaster University, Hamilton, Ontario, Canada. [2]Department of Neurology, School of Medicine, McMaster University, Hamilton, Ontario, Canada.

Address correspondence to Stuart M. Phillips, PhD, Department of Kinesiology, IWC E210, McMaster University, 1280 Main St. W, Hamilton, Ontario L8S 4K1, Canada. Email: phillis@mcmaster.ca

## Abstract

**Background.** Resistance exercise (RE) and aerobic exercise are recommended for older adults for fitness and strength. High-intensity interval exercise (HIIT) is an understudied but potent potential alternative to aerobic exercise. This study aimed to determine how each mode of exercise affected the integrated day-to-day response of muscle protein synthesis.

**Methods.** Sedentary men ($n$ = 22; 67 ± 4 years; body mass index: 27.0 ± 2.6 kg m$^{-2}$ [mean ± *SEM*]) were randomly assigned to perform RE, aerobic exercise, or HIIT. Participants consumed a stable isotope tracer ($D_2O$) for 9 days. Daily saliva samples were taken to measure tracer incorporation in body water. Muscle biopsies were obtained on Days 5–8 of $D_2O$ consumption to measure tracer incorporation into muscle at rest, 24 hours, and 48 hours following each exercise bout: RE (3 × 10 repetitions: leg extensor and press, 95% 10RM), HIIT (10 × 1 minute, 95% maximal heart rate [$HR_{max}$]), or aerobic exercise (30 minutes, 55%–60% $HR_{max}$).

**Results.** Myofibrillar protein fractional synthetic rate was elevated, relative to rest, at 24 and 48 hours following RE and HIIT. The increase in myofibrillar fractional synthetic rate was greater following RE versus HIIT at both time points. HIIT was the only mode of exercise to increase sarcoplasmic protein fractional synthetic rate 24-hour postexercise (2.30 ± 0.34% d$^{-1}$ vs 1.83 ± 0.21% d$^{-1}$).

**Conclusions.** This study shows that in older men, changes in muscle protein synthesis in response to certain exercises are long lasting and that HIIT significantly increases myofibrillar and sarcoplasmic fractional synthetic rate in this population.

**Key Words:** Exercise—Metabolism—Muscle—Sarcopenia.

**Decision Editor:** Stephen Kritchevsky, PhD

The global increase in the population of older adults demands solutions to reduce the risk of age-related disability associated with inactivity. Sarcopenia, the age-associated decline in muscle mass and strength, is measurable beginning in the fifth decade of life (1) and is accompanied by an increased risk of disability, falls, and fractures (2) and a higher incidence of metabolic diseases, such as type 2 diabetes (3). Exercise currently stands as the most viable strategy to counteract age-related declines in skeletal muscle mass and function and to alleviate the risk of disability (1).

Resistance exercise (RE) effectively bolsters muscle mass and strength and is commonly recommended for older adults to counteract sarcopenia (1). Physical activity recommendations for older

persons also include the regular practice of aerobic exercise (AE), which can improve fitness and insulin sensitivity; however, AE is not as effective as RE in eliciting strength gains or increasing muscle mass (4). High-intensity interval exercise (HIIT) is a time-efficient alternative to AE but is understudied in the elderly persons; nonetheless, in younger persons, HIIT is a potent stimulus for gains in aerobic fitness, muscle oxidative capacity (5), and improvements in insulin sensitivity (6).

Changes in muscle mass are largely determined by changes in the rates of muscle protein synthesis (MPS). Characterization of the acute MPS responses to RE, AE, and HIIT would be important in determining the potential of these modalities to alleviate declines in muscle mass in older persons. Oftentimes, MPS is measured using intravenous infusion over a 3- to 5-hour period. In contrast, oral ingestion of deuterium oxide ($D_2O$) can be used to measure MPS over the course of days to weeks, incorporating the influence of habitual diet and physical activity patterns (7–10). The ability of $D_2O$ to measure MPS over this length of time is valuable because it would require multiple infusion trials and yet is too short to detect changes in muscle mass.

Therefore, the aim of this study was to determine how RE, AE, or HIIT affected daily integrated MPS in older men. We hypothesized that RE and HIIT would stimulate an increase in rates of myofibrillar MPS, whereas AE and HIIT would increase rates of sarcoplasmic MPS.

## Methods

### Participants

The study was approved by the Hamilton Health Sciences Integrated Research Ethics Board. We screened and recruited 22 healthy men aged 60–75 years, all of whom gave their written and informed consent to participate. All participants had a body mass index in the normal–overweight range and resting blood pressure < 140/90 mmHg. No participants reported engaging in structured exercise training in the last 6 months, and all participants demonstrated normal cardiac function during a maximal exercise stress test. Exclusion criteria included smoking, diabetes, regular use of nonsteroidal anti-inflammatory drugs, use of statins, and history of chronic illness that would affect the results of the investigation.

### Experimental Design

Approximately 1 week prior to beginning the study protocol, participants underwent a whole body dual-energy x-ray absorptiometry scan (DXA; QDR-4500A Hologic, software version 12.31; Bedford, MA), a peak oxygen consumption ($\dot{V}O_{2peak}$) test to assess cardiovascular fitness, and 10RM testing to assess strength. $\dot{V}O_{2peak}$ tests were conducted on a bicycle ergometer (Ergoline er800s; Ergoline, Bitz, Germany) using the Jones protocol (11) and a breath-by-breath system (SensorMedics, Yorba Linda, CA). Tests lasted 10–12 minutes, and heart rate and function were monitored throughout using a 12-lead electrocardiogram.

Weight machines were used to assess 10RM for leg extension (Atlantis Precision Series Leg Extension C-105; Laval QC) and leg press (HUR 3545 Leg Press Incline; HUR, Northbrook, IL). Following a demonstration of proper technique, participants performed a warm-up of 10 repetitions at a light load (~40%–60% 1RM or 60%–70% 10RM). The weight was then increased, and participants completed up to 10 repetitions. This process was repeated until participants could complete no more than 10 repetitions (ie, 10RM). Participants rested for 3 minutes between attempts, and no more than 3 attempts were required to determine 10RM. If participants were randomized to AE or HIIT, they also completed a familiarization session on a bicycle ergometer to determine the power output necessary to elicit the intensities prescribed (~55%–60% $\dot{V}O_{2peak}$ or ~70% maximal heart rate [$HR_{max}$] for AE and ~90% $\dot{V}O_{2peak}$ or ~95% $HR_{max}$ for HIIT).

One week following baseline testing and familiarization, participants returned to the laboratory on Days 5, 6, 7, and 8 of the study (Figure 1). The morning of each day, after an overnight fast, participants had a muscle biopsy (~100 mg) from the *vastus lateralis* muscle using a custom-modified 5-mm Bergstrom biopsy needle as described elsewhere (12). Biopsies were taken alternately from the left and right legs, so that each leg received a total of two biopsies that were at least 5 cm apart beginning distally and moving in a proximal direction with successive biopsies. Directly following their second muscle biopsy (on study Day 6), participants completed a single session of either RE, HIIT, or AE (Figure 1).

To standardize dietary conditions, participants were prescribed a weight maintenance diet that provided ~1.1 g protein kg body mass$^{-1}$ d$^{-1}$ (macronutrient distribution: ~55% carbohydrate, ~30% fat, and ~15% protein). Diets began 2 days prior to participants' first muscle biopsy (Figure 1) and were continued throughout the rest of the study. Energy intake was estimated for each participant using the Harris–Benedict equation (13), and meal plans were created using NutriBase11 Pro v11.5 (Cybersoft, Phoenix, AZ). Meals were frozen, prepackaged, and could be reheated and consumed directly (Copper County Foods, Cambridge, Ontario).

Participants wore a pedometer during three nonbiopsy days as well as during 3 days where they received a biopsy to verify that they did not change their habitual activity levels as a result of the muscle biopsies.

### Exercise Protocols

Following a warm-up of 10 repetitions at 35% 10RM, participants randomized to RE completed three sets of leg extension and leg press at loads equal to ~95% of their predetermined 10RM. The last set of each exercise was completed to failure.

Participants in the HIIT group completed a single bout of HIIT, which consisted of 10 × 1 minute intervals on a bicycle ergometer cycling at a workload that was determined during familiarization to elicit ~95% $HR_{max}$ (~90% $\dot{V}O_{2peak}$). Participants maintained a cadence of at least 90 rpm during these intervals.

The AE protocol consisted of 30-minute continuous cycling at ~70% $HR_{max}$ (55%–60% $\dot{V}O_{2peak}$). Heart rate was measured throughout each AE and HIIT session.

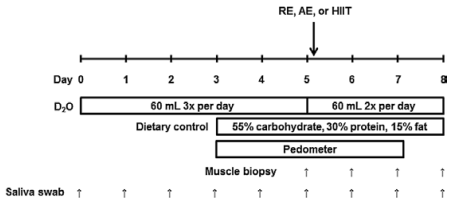


**Figure 1.** Overview of experimental design. AE = aerobic exercise; HIIT = high-intensity interval exercise; RE = resistance exercise.

The volume and intensity of the RE and AE protocols were based on the physical activity recommendations set out by the American College of Sports Medicine (14). We selected a modified low-volume HIIT model, which we considered preferable to Wingate-based HIIT (5), because it does not require specialized equipment and represents a feasible, time-efficient option for the general population (6).

### Isotope Protocol

To increase deuterium ($^2$H) enrichment in total body water to ~1%, participants consumed 3 × 60 mL oral doses of 70% $D_2O$ (Cambridge Isotope Laboratories, Tewksbury, MA) per day during the 5 days prior to their first muscle biopsy (Figure 1). To maintain ~1% enrichment, the dosage was reduced to 2 × 60 mL during the 4 days when participants received muscle biopsies (7,8). All 60 mL doses were consumed at least 3 hours apart. Participants also provided a saliva sample each morning during the days they consumed $D_2O$ to allow for the measurement of $^2$H enrichment in total body water. Total body water $^2$H enrichment was used as a surrogate for plasma alanine $^2$H labeling (7–9).

### Muscle Protein Synthesis

Muscle samples (~40–50 mg) were separated into myofibrillar and sarcoplasmic fractions and processed as previously described (12) for analysis by gas chromatography combustion isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH).

Muscle preparations were analyzed for deuterated-alanine ($^2$H-alanine) with a Thermo Finnigan Delta V isotope ratio mass spectrometry coupled to a Thermo Trace GC Ultra with a gas chromatography combustion interface III and Conflow IV. The *N*-acetyl-*n*-propyl ester of alanine was analyzed using a splitless injection and a Zebron ZB-5 column of 30 m × 0.25 mm × 0.50 μm film thickness (Phenomenex, Torrance, CA). The gas chromatography oven was programmed with an initial column temperature of 80°C with a 2-minute hold, followed by a ramp of 30°C min$^{-1}$ to 330°C. Eluents were directed into the pyrolysis reactor, heated at 1450°C, and converted to hydrogen gas (Metabolic Solutions).

Saliva samples were analyzed for $^2$H enrichment by cavity ring-down spectroscopy by Metabolic Solutions using a Liquid Water Isotope Analyzer with automated injection system (Los Gatos Research, Mountain View, CA). The water phase of the saliva was injected six times, and the average of the last three measurements was used for data analysis. The intrarun precision of this instrument is less than 2.0 $\delta^2$H‰, and the interrun precision is less than 3.5 $\delta^2$H‰. The $^2$H isotopic enrichments for muscle and saliva initially expressed as $\delta^2$H‰ were converted to atom percent excess using standard equations as previously described (9).

### Calculations

The fractional synthetic rate (FSR) of myofibrillar and sarcoplasmic proteins were calculated using the standard precursor-product method as described previously (9). In brief:

$$\mathrm{FSR}\,(\%\,\mathrm{d}^{-1}) = \left[\frac{(E_{\mathrm{Ala2}} - E_{\mathrm{Ala1}})}{E_{\mathrm{BW}} \times t}\right] \times 3.7 \times 100$$

Where $E_{\mathrm{AlaX}}$ is the protein-bound enrichment (in atom percent excess) from muscle biopsies at time X. Thus, the difference between times points is the change in protein-bound alanine enrichment between two time points with appropriate correction for $^2$H incorporation into alanine (9,10). $E_{\mathrm{BW}}$ is the mean $^2$H enrichment (in atom percent excess) in total body water between the time points. Hence, resting FSR (0-hour time point) was calculated using the difference in $^2$H enrichments between Days 5 and 6; FSR at 24- and 48-hour postexercise was calculated using the difference between Days 6–7 and 7–8, respectively. Lastly, $t$ is the tracer incorporation time in days. Multiplication by 3.7 adjusts for the average number of $^2$H atoms that can become incorporated into alanine, and multiplication by 100 converts the values to percentages.

### Statistics

Baseline means and salivary enrichment were compared using a one-way analysis of variance. Other data were compared using a two-way repeated measures analysis of variance with between (exercise type) and within (time) factors. Significant $F$ ratios were further scrutinized using Tukey's post hoc test. Statistical significance was accepted at $p < 0.05$. All statistical analysis was completed using Sigma Plot software (Systat Software, San Jose, CA). Data are mean ± *SEM*.

## Results

### Participants

All measured anthropometric and strength variables were similar between RE, HIIT, and AE groups (Table 1). Participants did not alter their day-to-day activity as a result of the muscle biopsies; participants performed the same number of steps per day on biopsy days as they did on nonbiopsy days (6,227 ± 679 vs 6,774 ± 647, $p = 0.56$). On average, the weight maintenance diets prescribed to participants provided 2,646 ± 51 kcal d$^{-1}$, consisted of 104 ± 5 g d$^{-1}$ protein, 364 ± 7 g d$^{-1}$ carbohydrate, and 88 ± 2 g d$^{-1}$ fat, and maintained participants' body weights. Diets did not differ between exercise groups (data not shown).

### $^2$H Enrichment

Saliva $^2$H enrichment increased significantly following the initiation of $D_2O$ consumption (Figure 2). The elevation was sustained throughout the remainder of the study and reached ~1%–1.2% by Day 5, an enrichment that was not statistically different from that observed at Day 8 (Figure 2). The responses were similar between exercise groups (data not shown).

### Myofibrillar Protein FSR

Prior to exercise, myofibrillar FSR was similar across all groups (Figure 3A). Myofibrillar FSR was significantly increased 24 hours following both HIIT and RE and remained elevated relative to baseline 48-hour postexercise. Although both RE and HIIT resulted in an elevation of myofibrillar FSR, the response was greatest following

**Table 1.** Physical and Exercise Characteristics of Participants in Each Exercise Group

| | RE ($n$ = 7) | HIIT ($n$ = 8) | AE ($n$ = 7) |
|---|---|---|---|
| Age (y) | 66 ± 1 | 67 ± 2 | 68 ± 1 |
| BMI (kg m$^{-2}$) | 26.7 ± 1.3 | 27.1 ± 1.0 | 27.3 ± 0.7 |
| % body fat | 23.9 ± 1.9 | 23.9 ± 2.1 | 25.6 ± 1.6 |
| Lean tissue mass (kg) | 60.3 ± 2.5 | 63.4 ± 2.3 | 60.1 ± 1.8 |
| $\dot{V}O_{2peak}$ (mL kg$^{-1}$ min$^{-1}$) | 27.3 ± 2.4 | 31.9 ± 2.4 | 30.7 ± 2.1 |
| Leg press 10RM (kg) | 49 ± 7 | 60 ± 3 | 61 ± 3 |
| Leg extension 10RM (kg) | 52 ± 6 | 54 ± 5 | 52 ± 2 |

*Notes:* Values are means ± *SEM*. No significant differences exist between groups for any variable. 10RM = 10 repetition maximum; BMI = body mass index; $\dot{V}O_{2peak}$ = peak oxygen consumption.

RE compared with HIIT. Myofibrillar FSR was not different compared with baseline at any time point following AE.

### Sarcoplasmic Protein FSR

Sarcoplasmic protein FSR increased by ~25% following the performance of HIIT (Figure 3B) but returned to baseline by 48-hour postexercise. Neither AE nor RE stimulated a significant increase in sarcoplasmic protein FSR.

## Discussion

The main finding from this study was that performing RE or HIIT resulted in an increase in myofibrillar protein FSR in older men, 24- and 48-hour postexercise. The magnitude of this increase was greater following RE compared with HIIT at both time points. Interestingly, only the performance of HIIT stimulated an increase in sarcoplasmic protein FSR at 24-hour postexercise. Considering these results represent changes in protein synthetic rates that are integrated over a 24-hour period, we propose they may be of greater relevance from an application standpoint compared with the results of acute infusion trials. Specifically, integrated FSR responses may better reflect the potential of these exercise modes to induce phenotypic adaptations (ie, muscle mass changes) over longer-term interventions.

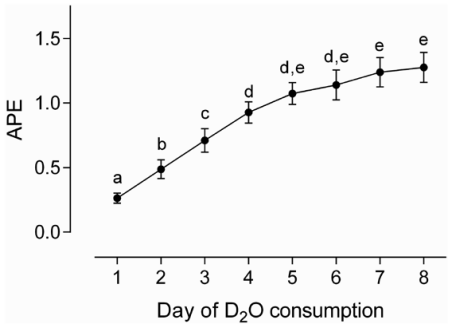


**Figure 2.** Saliva $^{2}H$ enrichment during Days 1–8 of the study. Means with different letters are significantly different ($p < 0.05$). Data are means ± *SEM*. APE = atom percent excess.

There are no previous data comparing acute RE, HIIT, and AE using an integrated measure of FSR in older men. In fact, few studies have used $D_2O$ to measure FSR in response to acute exercise in any population, so it is difficult to make comparisons of our data. Under fasted conditions at rest, traditional tracer infusion trials have reported sarcoplasmic FSR as exceeding myofibrillar FSR by approximately twofold (~0.050% $h^{-1}$ vs ~0.025% $h^{-1}$) in both younger and older individuals. A multitude of infusion trials have observed increases in mixed-muscle FSR with acute bouts of both RE and AE although these appear to be driven primarily by myofibrillar FSR in the case of RE (12) and by mitochondrial FSR in the case of AE (15). To our knowledge, the synthetic rate of the larger sarcoplasmic subfraction has not yet been fully characterized using stable isotope infusions following acute AE. Nonetheless, based on the stimulation of mitochondrial FSR with AE, we hypothesized that sarcoplasmic FSR would also be elevated in response to AE.

Recently, Wilkinson and coworkers (9) used bolus $D_2O$ ingestion to assess the effect of RE on MPS in young men. Myofibrillar and sarcoplasmic FSR measurements by our group and by Wilkinson and coworkers were similar, both at baseline and following acute RE. In comparison to tracer infusion trials, our FSR measurements are slightly higher, even on a daily basis, although this difference may be explained by integrative nature of $D_2O$. In the present study, we observed a mean baseline myofibrillar FSR of 1.59 ± 0.03% $d^{-1}$ for all participants combined (fasted state), which translates to ~0.07% $h^{-1}$. For men of a similar age, tracer infusion trials have reported baseline myofibrillar FSR values of ~0.03–0.05% $h^{-1}$ in the postabsorptive state and ~0.06–0.09% $h^{-1}$ in the postprandial state (16,17). In the present study, we observed an increase in myofibrillar FSR to 3.10 ± 0.25% $d^{-1}$ (~0.13% $h^{-1}$) 24-hour post-RE, whereas tracer infusion studies have reported values of ~0.07–0.10% $h^{-1}$ for older men following acute RE (16,18). It may be that the exercise in the present study resulted in an increased sensitivity to meal feeding, which we have observed previously (19), thus allowing for a greater net FSR to be observed in the fed state over the course of a day. Even in the fasted state, an acute bout of RE has been shown to stimulate FSR for up to 48 hours (20). Thus, a daily rate of 0.1% $h^{-1}$ at 24-hour post-RE is not unrealistic. The discrepancies in FSR between our study and infusion trials are more likely due to the fact that $D_2O$ allows for the measurement of an integrated MPS response. Therefore, direct comparisons of FSR determined using tracer infusions and in a free-living environment are not possible. It is also likely that our exercise naive participants would show

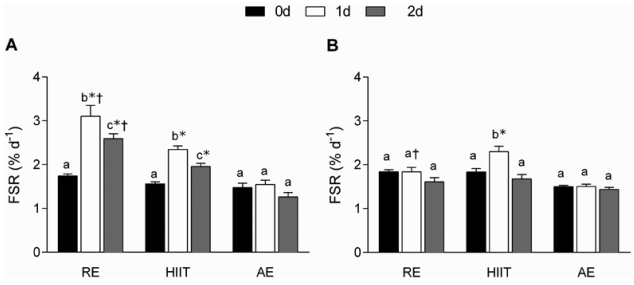


**Figure 3.** Myofibrillar (A) and sarcoplasmic (B) protein fractional synthesis rate (FSR) at baseline and postexercise. Data are means ± *SEM*. Bars bearing different letters are significantly different within each exercise group. *Significantly different ($p < 0.05$) from AE within that time point; †significantly different ($p < 0.05$) from HIIT within that time point. AE = aerobic exercise; HIIT = high-intensity interval exercise; RE = resistance exercise.

an uncharacteristically high FSR response to acute RE that would undoubtedly be attenuated with training (21,22).

To our knowledge, we are the first to report an increase in myofibrillar and sarcoplasmic FSR following acute HIIT in older men. Our results are supported by Scalzo and coworkers (7), who observed an increase in sarcoplasmic FSR using $D_2O$ following 4 weeks of Wingate-based HIIT in young adults. In that study, mixed-muscle and mitochondrial FSR were also elevated post-training (7). Similar to traditional AE, participants who have undergone HIIT typically demonstrate an increased muscle oxidative capacity (5,6). Mixed-muscle FSR has been shown to be elevated following acute AE, and evidence suggests that this is driven by increases in mitochondrial FSR (15). Following the separation of specific muscle subfractions for isotope ratio mass spectrometry, the majority of skeletal muscle mitochondria are located in the sarcoplasmic (cytosolic) fraction. Hence, it follows that increases in mitochondrial FSR may be partially responsible for the observed increase in sarcoplasmic FSR 24-hour post-HIIT in the present study. However, because we did not directly measure mitochondrial FSR, we can only speculate that increases in sarcoplasmic FSR with HIIT are driven by increased synthesis of mitochondrial proteins. The lack of mitochondrial FSR measurement is a result of tissue constraints during data collection and is a limitation of our study; nonetheless, we believe the sarcoplasmic FSR data offer novel insight into the MPS response to HIIT in older adults.

It remains unclear whether HIIT can induce hypertrophy, but lower intensity aerobic training can induce hypertrophy in older men (23). Thus, it seems reasonable to speculate that HIIT would, with training, induce some degree of hypertrophy in older participants. The increase in myofibrillar FSR following acute HIIT in the present study lends strong credence to this hypothesis. It is thought that damage to the contractile apparatus with RE contributes to the stimulation of myofibrillar protein synthesis and subsequent muscle hypertrophy (24). The relatively high-intensity muscle contractions inherent to HIIT may induce muscle damage in a manner similar to RE, increase the need for the repair of actin, myosin, and associated contractile proteins and, therefore, stimulate myofibrillar protein synthesis. Thus, as an exercise modality that can induce improvements in aerobic fitness, mitochondrial content, insulin sensitivity, and potentially hypertrophy, we view HIIT for older persons as an interesting avenue for further study.

The American College of Sports Medicine recommends that older adults accumulate 30–60 minutes of moderate intensity AE per day (150–300 minutes per week) or 20–30 minutes of vigorous AE per day (75–150 minutes per week), as well as moderate to vigorous RE of each major muscle group at least 2 days per week (14). We selected our AE and RE exercise protocols based on these recommendations. We view HIIT as being somewhere between RE and AE on the "exercise spectrum" because it stimulates aerobic adaptations; however, it involves higher intensity muscle contractions compared with AE. Further, a single session of HIIT demands a greater volume of exercise, in terms of number of muscle contractions, compared with a session of RE, but other studies have shown that the HIIT model we employed in the present study demands a considerably lower exercise volume than a session of traditional continuous AE (5). We appreciate that it is not possible to "equate" the exercise bouts in the present study because they require differing patterns of muscle activation, different forces, different muscle fiber recruitment, and involve different energy expenditures. Our choice to compare these three modes of exercise was based on current guidelines for older persons to engage in both RE and AE, but with HIIT being less well understood but effective alternative mode of exercise.

Contrary to our hypothesis, we did not observe an increase in sarcoplasmic FSR with AE. Sarcoplasmic FSR was possibly increased transiently in the hours immediately following exercise. This increase may have been washed out over 48 hours in the $D_2O$ protocol we used, whereas a short-term stable isotope infusion trial may have picked it up. This should be noted as a potential limitation of the $D_2O$ method for assessing MPS and rationale for the continued use of infusion trials to answer specific questions. Another possibility is that our AE protocol was not vigorous enough to stimulate sarcoplasmic protein synthesis. Previous work demonstrating an increase in mitochondrial FSR with AE employed exercise protocols that were more intense (>65% $\dot{V}O_{2peak}$) (25) or longer (~45 minutes) (15). Given that our AE protocol was based on current exercise recommendations, these recommendations may need to be revised to encourage participation in higher intensity aerobic activity that is capable of stimulating MPS.

The novel findings we observed were that although RE induced the greatest increases in myofibrillar protein FSR at both 24- and 48-hour postexercise, HIIT was also able to induce significant increases in myofibrillar protein FSR albeit not to the same degree. Despite this, HIIT was the only mode of exercise that resulted in a stimulation of sarcoplasmic protein FSR 24-hour postexercise. An important limitation of this study was the exclusion of women. The study was restricted to men to reduce variability in the strength and body composition measurements and to improve the homogeneity of our sample, but we acknowledge that the exclusion of one sex may limit the generalizability of our findings. Considering that HIIT is also a potent stimulator of aerobic fitness, muscle oxidative capacity, and insulin sensitivity, it may be beneficial to incorporate HIIT into exercise recommendations for older adults. Future research should examine the effect of a training program combining HIIT and RE on changes in muscle mass and metabolic and functional improvements as well as the effect of a single bout of RE, AE, or HIIT on mitochondrial protein synthesis in older men using the $D_2O$ method.

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# CHAPTER 3:

**A whey protein-based multi-ingredient nutritional supplement stimulates gains in lean body mass and strength in healthy older men.** *Submitted to the American Journal of Clinical Nutrition.*

**Title:** A whey protein-based multi-ingredient nutritional supplement stimulates gains in lean body mass and strength in healthy older men

**Authors:** Kirsten E. Bell, Tim Snijders, Michael Zulyniak, Dinesh Kumbhare, Gianni Parise, Adrian Chabowski, and Stuart M. Phillips

**Author affiliations:** Exercise Metabolism Research Group, Department of Kinesiology (KEB, TS, SMP, GP); Department of Medicine (MZ, DK), McMaster University, Hamilton, ON, Canada; and Department of Physiology, Medical University of Bialystok, Bialystok, Poland (AC)

**Authors' last names:** Bell, Snijders, Zulyniak, Kumbhare, Parise, Chabowski, Phillips

**Short running head:** Multi-ingredient protein-based supplement in aging

**Changed affiliations:** TS is now affiliated with Department of Human Biology and Movement Sciences, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center, The Netherlands

**Disclaimers:** SMP reports receipt of competitive grant support, travel expenses, and honoraria for speaking from the US National Dairy Council

**Corresponding author:** S.M. Phillips, Department of Kinesiology, McMaster University, Hamilton, ON L8S 4K1 905-525-9140 x24465, phillis@mcmaster.ca

**Clinical trials registry number:** NCT02281331 at ClinicalTrials.gov

**Abbreviations:** 1RM, one repetition maximum; CON, control; DXA, dual-energy X-ray absorptiometry; HIIT, high-intensity interval training; HR, heart rate; LBM, lean body mass; n-3, omega-3; RET, resistance exercise training; SUPP, supplement; TG, triglyceride; TUG, timed up-and-go; $VO_{2\ peak}$, peak oxygen uptake

**ABSTRACT**

**Background:** Protein and other compounds can exert anabolic effects on skeletal muscle, but combinations of ingredients, particularly in conjunction with exercise, may be more effective. **Objective:** To evaluate the efficacy of twice daily consumption of a protein-based, multi-ingredient nutritional supplement to increase strength and lean mass independent of, and in combination with, exercise in healthy older men. **Design:** Forty-nine men (age: 73±6 years [mean±SEM]; BMI: 28.5±3.6 kg/m$^2$) were randomly allocated to 20wk of twice daily consumption of either a nutritional supplement (SUPP; n=25; 30g whey protein, 2.5g creatine, 500IU vitamin D, 400mg calcium, and 1500mg n-3 PUFA with 700mg as eicosapentanoic acid [EPA] and 445mg as docosahexanoic acid [DHA]); or a control (n=24; CON; 22g of maltodextrin). The study had two phases. Phase 1 was 6wk of SUPP or CON alone. Phase 2 was a 12wk exercise training program in combination with SUPP or CON. Isotonic strength (one repetition maximum [1RM]) and lean body mass (LBM) were evaluated pre-intervention, at 6wk (Phase 1), and 19wk (Phase 2). **Results:** In Phase 1 only the SUPP group gained strength (Σ1RM: 206±34 vs. 212±33kg, $P$<0.001) and lean mass (LBM: 54.0±5.4 vs. 54.7±5.7kg, $P$<0.01). Following 12wk of exercise training (Phase 2: SUPP/CON + EX) the sum of upper body 1RM at was greater in the SUPP group compared to the CON group (119±4 vs. 109±5kg, $P$<0.05). **Conclusion:** Twice daily consumption of a multi-ingredient nutritional supplement increased muscle strength and lean mass in older men. Increases in strength were enhanced further with exercise training.

**Keywords:** high-intensity interval training, resistance exercise training, creatine, n-3 PUFA, EPA, DHA

## INTRODUCTION

Sarcopenia, which includes loss of muscle mass and function, contributes to various negative health outcomes including falls, metabolic disorders like type 2 diabetes mellitus, and progression to frailty (1). Muscular strength is a strong and independent predictor of all-cause mortality in older adults (2); hence, solutions to attenuate declines in physical function associated with sarcopenia are imperative (3). Resistance exercise training (RET), particularly when combined with nutritional supplements such as protein (4) and creatine (5), is an effective strategy to counter muscle mass and strength loss. In older adults, RET has also been shown to induce modest yet significant reductions in cardiovascular disease risk (6), improvements in metabolic health (7) and aerobic capacity (8), and a decrease in fall risk (9).

The combination of RET with protein and creatine supplementation is a potent stimulus for increases in strength and lean mass. However, recent studies suggest that supplementation with vitamin D (10) and omega-3 (n-3) PUFA (11) may also be effective in augmenting strength and hypertrophy. Additionally, evidence that high-intensity interval training (HIIT) may stimulate muscle protein synthesis (12), improve glycemic regulation (13), and aerobic capacity in older persons (14) is accumulating. Surprisingly few studies have employed a multicomponent supplement with a combination of exercise modalities to optimize health in older populations (15, 16). Such an approach may be

prudent because the individual response to nutritional supplementation and RET, as well as HIIT, is highly heterogeneous. Therefore, a comprehensive nutritional strategy, especially when combined with exercise training, may target a greater proportion of older persons compared to isolated supplements.

The primary objective of this study was to determine whether twice daily consumption of a supplement containing whey protein, creatine, calcium, vitamin D, and n-3 PUFA could stimulate gains in strength, physical function, and lean body mass in a group of healthy older men following 6 weeks of supplementation. We also determined if the addition of exercise would potentiate any supplement-mediated gains in strength, physical function, and lean body mass following a 12-week combined RET + HIIT program. We hypothesized that our multi-ingredient supplement would induce improvements in these outcomes independent of exercise, and that we would observe an additive effect of the supplement when combined with exercise training.

## METHODS

*Screening and recruitment.* Forty-nine healthy older men took part in this randomized, double-blind, placebo-controlled parallel group trial which took place between December 2014 and September 2016. Potential participants responded to advertisements placed in local newspapers and around the community, and were screened first by telephone to ensure they were non-smokers ≥ 65 years old, had a BMI in the normal-overweight range (between 18.5 and 30.0 kg/m$^2$), and had not participated in any structured resistance or aerobic exercise training program in the past 6 months. Exclusion criteria included:

regular consumption of whey protein, creatine, calcium, vitamin D, or n-3 PUFA supplements in the past 5 years; significant weight loss or gain in the past 6 months; regular use of non-steroidal anti-inflammatory drugs, simvastatin, blood thinners; injuries preventing safe participation in an exercise program; diabetes mellitus; cancer; infectious disease; and cardiac or gastrointestinal problems.

To confirm eligibility, subjects were required to be non-diabetic based on an oral glucose tolerance test (OGTT; fasting blood glucose < 7.0 mM; 2 h blood glucose < 11.1 mM) and demonstrate normal cardiac function during a maximal exercise stress test on a cycle ergometer. This trial was approved by the Hamilton Integrated Research Ethics Board and complied with the guidelines set out in the Tri-Council policy statement on ethical conduct for research involving humans (http://www.pre.ethics.gc.ca/pdf/eng/tcps2/TCPS_2_FINAL_Web.pdf). All participants were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. This study ended in September 2016 upon completion of post testing by the last group of participants to begin the protocol. Full details concerning the flow of participants through this study can be found in **Figure 1**.

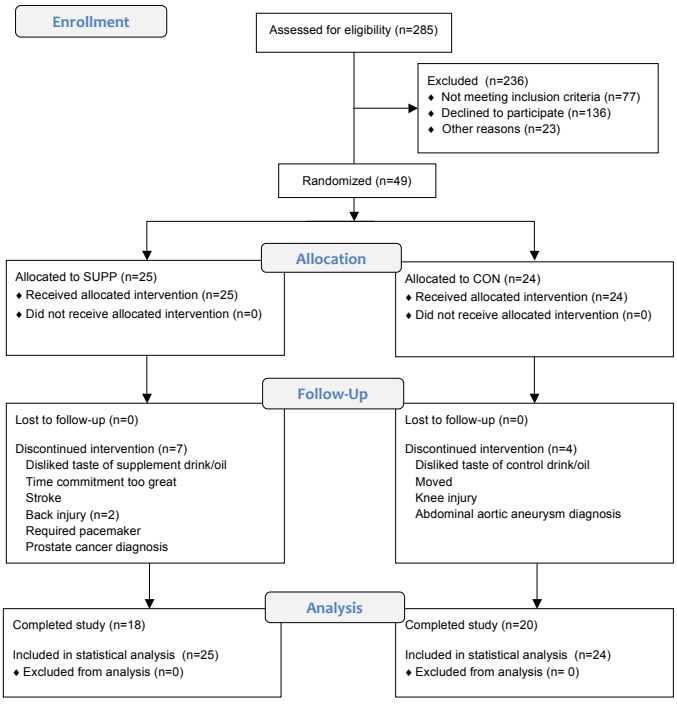


**Figure 1.** CONSORT flow diagram illustrating the movement of participants through the study, which was conducted between December 2014 and September 2016.

Sample sizes were calculated based on detection of differences in strength seen with creatine supplementation in older persons (5). Assuming similar response variance in our subjects and setting power to 80% with alpha at 0.05 yielded an estimate of 19 subjects per group; to be conservative and protect power we aimed for 25 subjects per group.

*Experimental design.* Eligible subjects were randomly assigned to receive either a multi-ingredient nutritional supplement (SUPP) or a control (CON) drink for 20 weeks (**Figure 2**). We employed a coded (group A versus group B) block randomization scheme (block size: 10 participants) generated using http://www.randomization.com/ to sequentially

allocate subjects to groups in order of enrolment. A key to the randomization code was held by an investigator who was not directly involved with subject recruitment, training, or testing. Subjects, as well as investigators who were responsible for recruiting, training, and/or testing subjects, were blind to the individual group assignments.

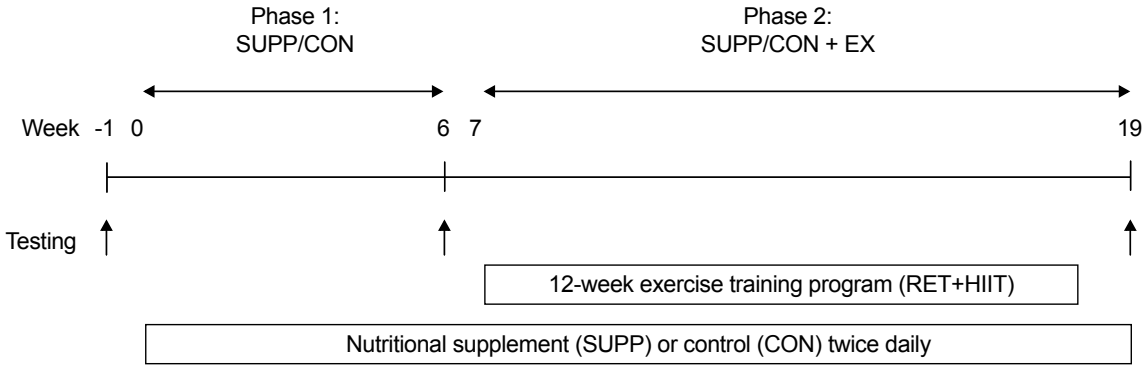


**Figure 2.** Schematic of study design. Participants consumed either a multi-ingredient protein-based nutritional supplement (SUPP) or control (CON) drink for 20 weeks total (from weeks 0-19, inclusive), and completed a 12-week exercise training program (RET twice per week and HIIT once per week) between weeks 7-18. Testing occurred at weeks -1 (baseline), 6, and 19, and included the following assessments: muscular strength (1RM), aerobic fitness ($VO_2$peak), physical function, and body composition (DXA). Phase 1: SUPP/CON took place between weeks 0-6, and Phase 2: SUPP/CON + EX took place between weeks 7-19.

After 6 weeks of consuming their study beverages (Phase 1: SUPP or CON), subjects completed a 12-week supervised exercise training program while continuing to consume their assigned beverages (Phase 2: SUPP + EX or CON + EX). Testing occurred at weeks -1 (pre-intervention), 6, and 19. The following assessments were conducted over the course of each testing week: isotonic strength (one repetition maximum [1RM]), aerobic fitness (peak oxygen uptake [$VO_2$peak]), physical function,

body composition by dual-energy X-ray absorptiometry (DXA), dietary intake (3-day food records), and habitual physical activity (accelerometer).

*Nutritional supplements.* The experimental supplement was composed of whey protein, creatine, calcium, vitamin D, and n-3 PUFA. All ingredients except for the n-3 PUFA (and control oil) were packaged in powder form in individual sachets. Subjects prepared the beverages at home by mixing the contents of 1 sachet with 425 mL water and consumed two drinks daily: the first 1 h after breakfast, and the second 1h prior to retiring to bed. Subjects measured out and consumed 10 mL of oil once per day (SUPP was 3000 mg n-3 PUFA with 1400 mg eicosapentanoic acid [EPA] and 890 mg docosahexaenoic acid [DHA]; CON was safflower oil) with their morning study beverage. The powder and oil provided to the CON group were matched in flavor to the active forms. The exact composition of and nutrition information for the supplement and control drinks and oils can be found in **Table 1**. All study beverages and oils were labelled in a blinded manner and prepared by Infinit Nutrition (Windsor, ON). Participants were instructed not to alter their habitual dietary or physical activity habits for the duration of the study. To encourage compliance we telephoned subjects every 2 weeks during Phase 1 of the study to ask whether they were consuming the study beverages and oils regularly, as a well as whether they had any comments or complaints.

*Exercise training.* The 12-week progressive exercise training program took place at McMaster University. Subjects completed twice weekly RET (Mon and Fri) and once weekly HIIT (Wed).

Table 1. Nutritional composition of the study drinks and oils. The quantities listed below represent a single drink and a single serving of oil. Participants consumed two drinks per day and one serving of oil per day.

| | SUPP | CON |
|---|---|---|
| | Whey-based supplement | |
| Whey protein (g) | 30 | 0 |
| Creatine (g) | 2.5 | 0 |
| Calcium (mg) | 400 | 0 |
| Vitamin D (IU) | 500 | 0 |
| Carbohydrate (g) | 1 | 22 |
| Energy (kcal) | 105 | 56 |
| | Oil | |
| n-3 PUFA (mg) | 3000 | 0 |
| EPA (mg) | 1400 | 0 |
| DHA (mg) | 890 | 0 |

SUPP, supplement; CON, control; n-3, omega-3.

After a 5 min warm-up on a cycle ergometer, subjects performed 3 sets of 4 separate exercises in the following order: leg press, chest press or lateral pull-down, horizontal row or shoulder press, and leg extension. Chest press and horizontal row were only performed on Monday, and lateral pull-down and shoulder press were only performed on Friday (leg press and leg extension were performed at every RET session). RET sessions were concluded with a 5 min cool-down on a cycle ergometer. During the first 3 weeks of exercise training, workload was gradually increased from 65% 1RM (10-12 repetitions) to 80% 1RM (6-8 repetitions). The third set of each exercise was always completed until volitional fatigue, which we defined as the inability to complete an addition repetition with proper form. Loads were adjusted based on 1RM strength tests or when subjects could complete ≥ 12 repetitions during the third set of each exercise.

Participants performed HIIT on a cycle ergometer (ISO1000 Upright Bike; SCIFIT, Tulsa, OK) while wearing a heart rate (HR) monitor (H7 Heart Rate Sensor; Polar Electro Canada, Lachine, QC). Following a 3 min warm-up at 25 W, subjects completed 10 x 60 s intervals at a workload which elicited ~90% maximal HR (HRmax), while maintaining a cadence of ≥ 90 rpm. Workload was adjusted by 3-5 W as needed to maintain an average HR of ~90% HRmax over the 10 intervals. Intervals were interspersed with 60 s of rest where subjects cycled at a self-selected pace against 25 W. HIIT sessions were concluded with a 5 min cool-down at 25 W.

*Strength Assessments.* At baseline, proper lifting technique was demonstrated and practiced by participants during a familiarization session. Muscle strength was assessed using 1RM strength tests for the following exercises: leg press, chest press, lateral pull-down, horizontal row, shoulder press, and leg extension (HUR; Northbrook, IL). The 1RM load was reassessed four days after the initial assessment as previously described (17). Strength is reported as individual 1RMs for each of the six exercises, as well as the sum of upper body 1RMs (horizontal row, chest press, lateral pull-down, and shoulder press), the sum of lower body 1RMs (leg extension and leg press), and the sum of all 1RMs.

*Aerobic fitness testing.* Subjects performed a $VO_2$peak test on an electronically braked cycle ergometer (Lode Excalibur Sport V 2.0; Groningen, The Netherlands) while wearing a chest-strap HR monitor. A metabolic cart and online gas collection system (MOXUS Modular Oxygen Uptake System; AEI Technologies, Pittsburgh, PA) were used to quantify respiratory gases. Following a 1 min warm-up at 30 W, the load was

increased by 1 W every 4 s. Participants were instructed to maintain a cadence of 60-90 rpm, and tests were terminated if the cadence dropped below 55 rpm for > 10 s, or if volitional fatigue was attained.

*Physical function.* Subjects completed 3 assessments to measure physical function. The 30 s chair stand required subjects to rise from a chair without the use of their arms as many times as possible in 30 s (18). For the timed up-and-go (TUG), subjects were instructed to rise from the same chair, walk to and from a clearly marked point a distance of 3 m away, and sit back down in the shortest amount of time possible. Subjects were given a practice trial before both the 30 s chair stand and the TUG, and the average of 3 trials (with 3 min rest allowed between trials) was recorded for each outcome. Lastly, the 6 min walk test was performed on a 200 m indoor track. Subjects were instructed to attempt to cover as much distance as possible within 6 min while walking in a safe manner at their usual walking speed.

*Body composition.* Whole body and regional lean soft tissue mass (i.e. fat-free and bone-free mass), fat mass and bone mineral content were measured using DXA (GE-LUNAR iDXA; GE, Mississauga, ON) following a 10-12 h overnight fast. Regional body compartment analysis was performed in batches by a single investigator who was blinded to group assignment. Waist and hip circumferences were measured at the top of the iliac crests and at the widest portion of the hips, respectively, using a tape measure while participants stood with their arms relaxed and feet together.

*Dietary intake.* Weighted 3-day food records (2 weekdays and 1 weekend day) were analyzed using ESHA (Food Processor Nutrition Analysis Software; Salem, OR).

Subjects were instructed by research staff on how to record the types and quantities of food, beverages, study drinks/oil, and other nutrition supplements or vitamins that they consumed during this period. Baseline 3-day food records were completed prior to commencing the study protocol.

*Biochemical analysis.* Plasma 25(OH)$D_3$ concentrations were measured by radioimmunoassay using a commercially available kit (DiaSorin Canada; Mississauga, ON), and plasma cystatin C concentrations were measured with a BN100 Nephelometer (Dade Behring; Deerfield IL) using a particle-enhanced immunonephelometric assay. Erythrocyte membrane phospholipid composition was measured as described previously (19). Briefly, total lipids from the samples were extracted (20), and thin layer chromatography was used to separate individual classes of phospholipids (phosphatidylcholine, PC; phosphatidylethanolamine, PE; phosphatidylinositol, PI; phosphatidylserine, PS; and sphingomyelin, SM). Once isolated, phospholipids were methylated with 1 M methanolic sodium methoxide at room temperature for 10 min (21), and the fatty acid composition of each class of phospholipids was analyzed by gas chromatography (Hewlett-Packard 5890 Series II System, equipped with a double flame ionization detector, and Agilent CP-Sil 88 capillary column, 100 m, internal diameter of 0.25 mm) (22, 23). Fatty acids were identified by comparing retention times to those of a known standard, and absolute amounts of individual fatty acids were calculated with the aid of an internal standard (pentadecanoic acid), which was added to samples before the methylation process. Total amounts of each phospholipid were determined from the sum of fatty acids in each fraction.

*Statistical analysis.* Statistical analysis was completed using SPSS (IBM SPSS Statistics for Windows, version 23.0; IBM Corp., Armonk, NY). We conducted an intention-to-treat analysis using a linear mixed model with an unstructured covariance matrix including group (SUPP or CON) and time (weeks -1, 6, and 19) as factors, and respective baseline values as covariates. Significant differences were identified using post hoc test t-tests with a Bonferroni correction. Based on recommendations for human clinical trials with missing data (24), all participants (completers as well as participants who withdrew prior to week 6 or week 19 testing) were included in the final analysis, and missing values were not replaced. Statistical significance was accepted as $P < 0.05$. Data are presented as mean ± SEM.

**RESULTS**

*Participants.* Forty-nine older men were randomized: 38 completed the study and 11 dropped out (n=7 and n=4 dropouts in the SUPP and CON groups, respectively). Of the participants who dropped out, 4 withdrew prior to week 6 testing (during Phase 1: SUPP/CON), and 7 withdrew partway through the exercise training program and prior to week 19 testing (during Phase 2: SUPP/CON + EX). Reasons for withdrawal from the study are provided in Figure 1. Participants' baseline characteristics are presented in **Table 2.**

Table 2. Baseline characteristics of participants

| | SUPP (n=25) | CON (n=24) |
|---|---|---|
| Age (years) | 71 ± 1 | 74 ± 1 |
| Systolic BP (mm Hg) | 138 ± 4 | 138 ± 3 |
| Diastolic BP (mm Hg) | 78 ± 2 | 78 ± 2 |
| Body mass (kg) | 85.3 ± 2.4 | 84.5 ± 2.5 |
| Height (m) | 1.72 ± 0.01 | 1.73 ± 0.01 |
| BMI ($kg/m^2$) | 28.9 ± 0.8 | 28.1 ± 0.7 |
| Whole body lean mass (kg) | 54.0 ± 1.1 | 54.5 ± 1.4 |
| Whole body fat mass (kg) | 28.2 ± 1.7 | 26.8 ± 1.4 |
| % body fat | 33.6 ± 1.3 | 32.6 ± 1.0 |
| Waist:hip ratio | 0.99 ± 0.01 | 0.99 ± 0.01 |
| Leg extension 1RM (kg) | 27 ± 1 | 27 ± 2 |
| Leg press 1RM (kg) | 77 ± 3 | 69 ± 4 |
| $VO_{2peak}$ (mL/kg/min) | 23.8 ± 0.8 | 24.4 ± 0.9 |
| Peak power (watts) | 154 ± 5 | 158 ± 7 |
| Fasting glucose (mM) | 5.6 ± 0.1 | 5.8 ± 0.1 |
| 2h glucose (mM) | 6.8 ± 0.4 | 7.2 ± 0.4 |
| HOMA-IR | 2.1 ± 0.1 | 2.2 ± 0.1 |
| Total-c (mM) | 4.69 ± 0.22 | 4.83 ± 0.19 |
| LDL-c (mM) | 2.74 ± 0.21 | 2.87 ± 0.18 |
| HDL-c (mM) | 1.27 ± 0.06 | 1.29 ± 0.06 |
| TG (mM) | 1.49 ± 0.19 | 1.50 ± 0.21 |
| 25(OH)D (nM) | 44.3 ± 2.6 | 37.6 ± 2.8 |
| Cystatin C (mg/L) | 0.85 ± 0.03 | 0.83 ± 0.05 |

Data are means ± SEM. 25(OH)D, 25 hydroxy vitamin D; SUPP, supplement; CON, control; BP, blood pressure; 1RM, 1 repetition maximum; $VO_2$peak, peak oxygen uptake; Total-c, total cholesterol; TG, triglycerides.

*Compliance.* Compliance (based on self-report as well as volume of returned sachets) with beverage consumption was: SUPP = 87 ± 2%; CON = 92 ± 2%. A similar degree of compliance was observed with oil consumption: SUPP = 92 ± 2%; CON = 95 ± 2%. Subjects in the SUPP and CON groups attended 95 ± 1% and 94 ± 1% of their prescribed training sessions, respectively. All subjects attended at least 80% of all RET and HIIT sessions.

*Blood.* We assessed plasma 25(OH)D (25 hydroxy vitamin D) concentrations at weeks -1, 6, and 19. Concentrations of $25(OH)D_3$ were 44.3 ± 2.6 nM (SUPP) and 37.6 ± 2.8 nM (CON) at baseline (range: 21.0 – 64.2 nM; Table 2), and increased significantly in the SUPP group (to 50.5 ± 3.1 nM at week 6 and 57.1 ± 3.9 nM at week 19; $P < 0.001$). No change was observed in the CON group over the course of study (37.3 ± 2.6 nM at week 6 and 35.6 ± 2.5 nM at week 19; $P > 0.05$).

Erythrocyte membrane phospholipids were measured as described in detail previously (19), and the content of EPA plus DHA each increased 90% (phosphatidylcholine), 22% (phosphatidylserine), 65% (phosphatidylinositol), and 43% (phosphatidylethanolamine) between baseline and week 6 in the SUPP group, and each increased an additional ~30% by week 19. The EPA plus DHA content of erythrocyte membranes did not change significantly over the course of the study in the CON group.

Cystatin C concentrations were 0.85 ± 0.03 mg/L (SUPP) and 0.83 ± 0.05 mg/L (CON) at baseline (range: 0.60 – 1.08 mg/L; see Table 2), and did not change significantly over the course of the study in either group.

*Isotonic muscle strength.* Significant group-by-time interactions were observed for the sum of all 1RMs ($P < 0.05$), upper body 1RMs ($P < 0.05$), and horizontal row 1RM ($P < 0.01$) muscle strength, so each group was analyzed separately for these measures. In the SUPP group, the sum of all 1RMs increased by 3% during Phase 1 from 206 ± 7 kg at baseline to 212 ± 8 kg at week 6 ($P < 0.001$; **Figure 3A**) and a further 20% during Phase 2 ($P < 0.001$). Broadly, the results were similar in the SUPP group for other lifts (Figure 3B). In the CON group, there was no change in total (Figure 3A), upper body (Figure

3B), or horizontal row (**Table 3**) 1RM muscle strength during Phase 1. However, following Phase 2 the sum of all 1RMs increased by 21% ($P < 0.001$; Figure 3A), the sum of upper body 1RMs increased by 11% ($P < 0.001$; Figure 3B), and horizontal row 1RM increased by 7% (from 27 ± 2 kg at week 6 to 29 ± 1 kg at week 19, $P < 0.001$) in the CON group.

We observed a main effect of time for lateral pulldown ($P < 0.001$), shoulder press ($P < 0.01$), chest press ($P < 0.001$), leg extension ($P < 0.001$), leg press ($P < 0.001$), the sum of lower body 1RMs ($P < 0.001$) whereby, for all exercises except for chest press, no significant changes occurred following Phase 1; however, 1RM muscle strength improved significantly in both the SUPP and CON groups over the course of Phase 2: SUPP/CON + EX (Table 3). Chest press 1RM increased significantly by 1 kg following Phase 1 (SUPP: 0% and CON: +10%, $P < 0.05$), and by an additional 3 kg following Phase 2 (SUPP: +23% and CON: +14%, $P < 0.001$).

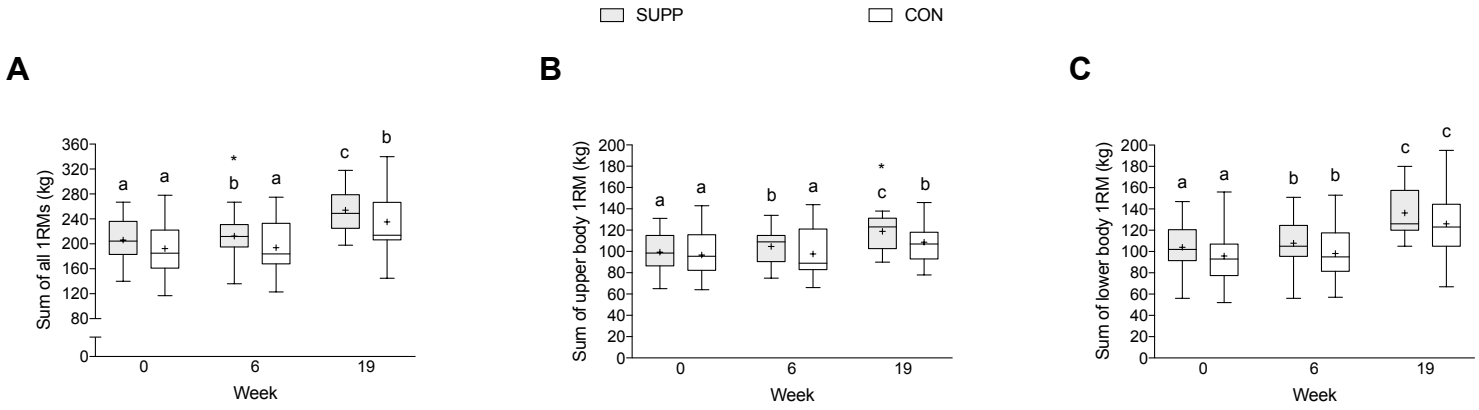


Figure 3. Isotonic strength expressed as the sum of all (A), upper body (B), and lower body (C) 1RMs. Boxes (SUPP: grey; CON: white) represent interquartile ranges, with the horizontal lines indicating the medians. Whiskers represent the maximal and minimal values, and the crosses indicate the means. Data was analyzed using a two-way ANCOVA with baseline values as covariates. We observed significant group-by-time interactions for the sum of all 1RMs and for the sum of upper body 1RMs ($P < 0.05$), and a main effect of time for the sum of lower body 1RMs ($P < 0.001$). Dissimilar letters denote changes over time within a given treatment group (SUPP or CON). * Indicates a significant difference from the CON group at that time. 1RM, one repetition maximum; SUPP, supplement group (n=25); CON, control group (n=24).

Table 3. 1RM muscle strength measurements for individual exercises

| | SUPP | | | CON | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 wk | 19 wk | Baseline | 6 wk | 19 wk |
| Leg extension (kg)[2] | 27 ± 1[a] | 29 ± 2[a] | 39 ± 2[b] | 27 ± 2[a] | 28 ± 2[a] | 36 ± 2[b] |
| Leg press (kg)[2] | 77 ± 3[a] | 80 ± 5[a] | 98 ± 5[b] | 69 ± 4[a] | 70 ± 5[a] | 91 ± 7[b] |
| Chest press (kg)[2] | 22 ± 1[a] | 22 ± 1[b] | 27 ± 1[c] | 19 ± 1[a] | 21 ± 1[b] | 24 ± 2[c] |
| Horizontal row (kg)[1] | 25 ± 1[a] | 27 ± 1[b] | 32 ± 1[c] | 26 ± 1[a] | 27 ± 2[a] | 29 ± 1[b] |
| Lateral pull-down (kg)[2] | 26 ± 1[a] | 27 ± 1[a] | 31 ± 1[b] | 27 ± 2[a] | 27 ± 2[a] | 30 ± 1[b] |
| Shoulder press (kg)[2] | 26 ± 1[a] | 27 ± 2[a] | 29 ± 2[b] | 24 ± 2[a] | 24 ± 2[a] | 26 ± 2[b] |

Values are mean ± SEM and were analyzed using a two-way ANCOVA with baseline values as covariates. [1]Group-by-time interaction ($P < 0.01$). [2]Main effect for time ($P < 0.01$). For each outcome, different letters represent significant differences within each group. Significance accepted as $P < 0.05$. 1RM, one repetition maximum; SUPP, supplement group (n=25); CON, control group (n=24).

*Body composition.* We observed significant time-by-group interactions for whole body lean mass ($P < 0.01$), appendicular lean mass ($P < 0.05$), leg lean mass ($P < 0.05$), and trunk lean mass ($P < 0.01$) over the course of the study. In the SUPP group, whole body lean mass increased by 0.7 kg in response to Phase 1 (from 54.0 ± 1.1 kg at baseline to 54.7 ± 1.2 kg at week 6, $P < 0.001$; **Figure 4A**); however, no further increase was observed during Phase 2. Likewise in the SUPP group at week 6 we observed increases in appendicular lean mass of 0.4 kg ($P < 0.01$; Figure 4B), leg lean mass of 0.3 kg ($P < 0.01$; Figure 4C), and trunk lean mass of 0.4 kg ($P < 0.01$; **Table 4**) compared to baseline. However, no further changes in appendicular, leg, or trunk lean mass were observed during Phase 2. In the CON group, conversely, we did not observe any significant change in whole body lean mass (Figure 4A) or regional measurements of lean mass over the course of the study.

There was a main effect of time for whole body fat mass that increased ($P < 0.01$) by 0.2 kg during Phase 1 (SUPP: -1% and CON: +3%, $P < 0.05$; Table 4) and subsequently decreased by 0.9 kg during Phase 2 (SUPP: -8% and CON: 0%, $P < 0.05$).

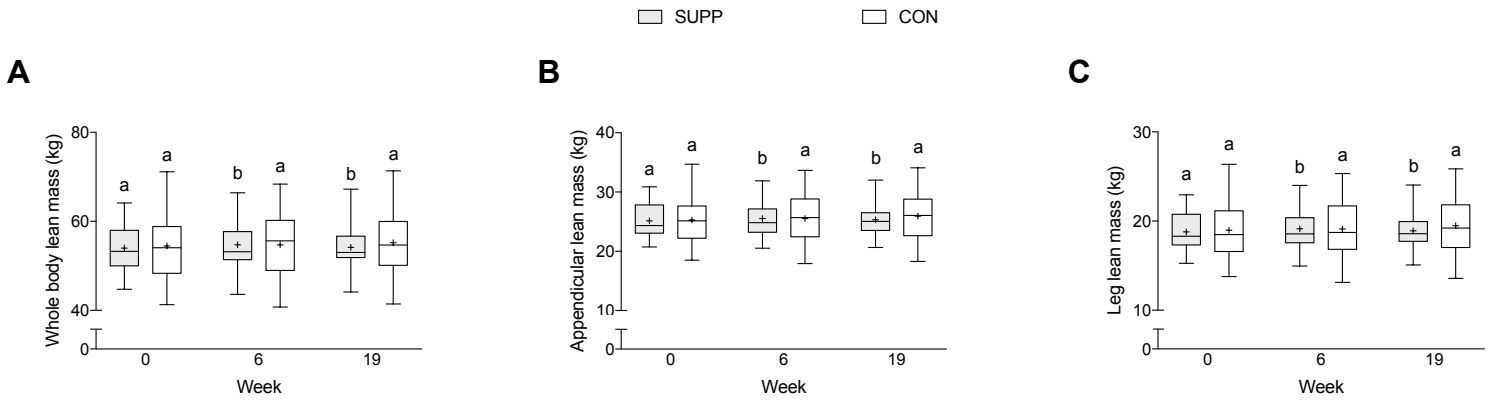


Figure 4. Whole body (A), appendicular (B), and leg (C) lean mass over the course of the study. Boxes (SUPP: grey; CON: white) represent interquartile ranges, with the horizontal lines indicating the medians. Whiskers represent the maximal and minimal values, and the crosses indicate the means. Data was analyzed using a two-way ANCOVA with baseline values as covariates. We observed significant group-by-time interactions ($P < 0.05$) for whole body, appendicular, and leg lean mass. Dissimilar letters denote changes over time within a given treatment group (SUPP or CON). SUPP, supplement group (n=25); CON, control group (n=24).

Table 4. DXA regional body compartment analysis and anthropometry

| | SUPP | | | CON | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 wk | 19 wk | Baseline | 6 wk | 19 wk |
| Body mass (kg)[2] | 85.3 ± 2.4[a] | 85.9 ± 2.6[b] | 83.1 ± 2.6[a,b] | 84.5 ± 2.5[a] | 85.3 ± 2.7[b] | 85.9 ± 2.8[a,b] |
| BMI (kg/m$^2$)[2] | 28.9 ± 0.8[a] | 29.2 ± 0.8[b] | 28.9 ± 0.9[a,b] | 28.1 ± 0.7[a] | 28.4 ± 0.8[b] | 28.5 ± 0.8[a,b] |
| Fat mass (kg)[2] | 28.2 ± 1.7[a] | 28.0 ± 1.9[b] | 25.9 ± 2.0[a] | 26.8 ± 1.4[a] | 27.5 ± 1.5[b] | 27.5 ± 1.6[a] |
| % body fat[2] | 33.6 ± 1.3[a,b] | 33.2 ± 1.4[a] | 31.7 ± 1.6[b] | 32.6 ± 1.0[a,b] | 33.0 ± 1.1[a] | 32.9 ± 1.1[b] |
| Arm lean mass (kg)[2] | 6.3 ± 0.2[a] | 6.4 ± 0.2[b] | 6.4 ± 0.2[b] | 6.3 ± 0.2[a] | 6.4 ± 0.2[b] | 6.5 ± 0.2[b] |
| Trunk lean mass (kg)[1] | 25.1 ± 0.5[a] | 25.5 ± 0.6[b] | 25.2 ± 0.6[b] | 25.5 ± 0.6[a] | 25.4 ± 0.6[a] | 25.5 ± 0.7[a] |
| Waist circumference (cm) | 105.0 ± 1.8 | 105.3 ± 2.0 | 104.3 ± 2.0 | 104.1 ± 1.7 | 104.6 ± 1.9 | 103.8 ± 2.2 |
| Hip circumference (cm) | 106.3 ± 1.1 | 106.6 ± 1.3 | 106.8 ± 1.3 | 105.7 ± 1.4 | 105.7 ± 1.7 | 106.6 ± 1.7 |
| Waist:hip ratio[2] | 0.99 ± 0.01[a,c] | 0.99 ± 0.01[b,c] | 0.97 ± 0.01[a] | 0.99 ± 0.01[a,c] | 0.99 ± 0.01[b,c] | 0.97 ± 0.01[a] |

Values are means±SEM and were analyzed using a two-way ANCOVA with baseline values as covariates. [1]Group-by-time interaction ($P < 0.05$). [2]Main effect for time ($P < 0.05$). For each outcome, different letters represent significant differences within each group. Significance accepted as $P < 0.05$. DXA, dual-energy X-ray absorptiometry; SUPP, supplement group (n=25); CON, control group (n=24).

*Physical function and aerobic fitness.* We observed a main effect of time for the TUG ($P < 0.001$; **Table 5**), the 6 min walk test ($P < 0.001$), $VO_2$peak ($P < 0.001$), and peak power ($P < 0.001$). There were no significant changes occurred as a result of Phase 1; however, significant improvements were made following Phase 2. Between weeks 6 and 19, the time taken to complete the TUG decreased by 0.3 s (SUPP: -7% and CON: -3%; $P < 0.01$), and the distance covered in the 6 min walk test increased by 25 m ($P < 0.001$). In addition, relative $VO_2$peak increased overall by 1.8 mL/kg/min, and peak power increased by 13 W ($P < 0.001$). We did not observe any significant changes in the 30 s chair stand test over the course of study.

*Dietary intake.* We observed, as expected, significant time-by-group interactions for protein intake (expressed as g, g/kg body mass, and % energy; $P < 0.001$; **Table 6**), vitamin D ($P < 0.001$), calcium ($P < 0.001$), and n-3 PUFA ($P < 0.01$). In the CON group, we did not observe any significant change in macro- or micronutrient intake over the course of the intervention. A significant main effect of time was observed for daily energy intake ($P < 0.01$). Total energy intake was significantly higher at week 6 compared to baseline (SUPP: +12%; CON: +9%, $P < 0.01$), with no further change following Phase 2.

Table 5. Physical function and aerobic fitness assessments

| | SUPP | | | CON | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 wk | 19 wk | Baseline | 6 wk | 19 wk |
| 30s chair stand (stands)[1] | 12 ± 1[a] | 13 ± 1[a,b] | 13 ± 1[b] | 13 ± 1[a] | 13 ± 1[a,b] | 13 ± 1[b] |
| Timed up-and-go (s)[1] | 7.07 ± 0.25[a] | 6.89 ± 0.26[a] | 6.44 ± 0.19[b] | 7.61 ± 0.33[a] | 6.99 ± 0.26[a] | 6.80 ± 0.32[b] |
| 6 min walk (m)[1] | 576 ± 14[a] | 585 ± 15[a] | 616 ± 19[b] | 593 ± 17[a] | 621 ± 16[a] | 639 ± 23[b] |
| Relative $VO_{2peak}$ (mL/kg/min)[1] | 23.8 ± 0.8[a] | 24.6 ± 0.9[a] | 26.2 ± 1.2[b] | 24.4 ± 0.9[a] | 24.4 ± 1.1[a] | 26.4 ± 1.4[b] |
| Absolute $VO_{2peak}$ (L/min)[1] | 2.0 ± 0.1[a] | 2.1 ± 0.1[a] | 2.1 ± 0.1[b] | 2.1 ± 0.1[a] | 2.1 ± 0.1[a] | 2.3 ± 0.1[b] |
| Peak power (W)[1] | 154 ± 5[a] | 157 ± 5[a] | 164 ± 7[b] | 158 ± 7[a] | 158 ± 7[a] | 178 ± 10[b] |

Values are mean ± SEM and were analyzed using a two-way ANCOVA with baseline values as covariates. [1]Main effect for time ($P < 0.05$). For each outcome, different letters represent significant differences within each group. Significance accepted as $P < 0.05$. SUPP, supplement group (n=25); CON, control group (n=24); TUG, timed up-and-go; $VO_2$peak, peak oxygen uptake.

Table 6. Daily dietary intakes (via self-report from 3-day food records) and habitual physical activity (using arm-mounted accelerometers)

| | SUPP | | | CON | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 wk | 19 wk | Baseline | 6 wk | 19 wk |
| Dietary intakes | | | | | | |
| Energy (kcal)[2] | 2146 ± 488[a] | 2405 ± 701[b] | 2375 ± 153[a,b] | 2336 ± 553[a] | 2541 ± 590[b] | 2417 ± 757[a,b] |
| Protein | | | | | | |
| g[1] | 89 ± 24[a] | 142 ± 34[b*] | 130 ± 25[b*] | 97 ± 27[a] | 100 ± 35[a] | 99 ± 41[a] |
| g/kg body mass[1] | 1.1 ± 0.3[a] | 1.7 ± 0.5[b*] | 1.6 ± 0.4[b*] | 1.2 ± 0.3[a] | 1.2 ± 0.4[a] | 1.2 ± 0.5[a] |
| %[1] | 17 ± 3[a] | 24 ± 5[b*] | 26 ± 5[b*] | 17 ± 4[a] | 16 ± 4[a] | 17 ± 5[a] |
| Carbohydrate | | | | | | |
| g | 265 ± 65 | 257 ± 87 | 223 ± 63 | 272 ± 102 | 309 ± 96 | 304 ± 121 |
| %[1] | 50 ± 6[a] | 43 ± 6[b*] | 43 ± 6[b*] | 46 ±12[a] | 49 ± 10[a] | 51 ± 13[a] |
| Fat | | | | | | |
| g | 71 ± 22 | 82 ± 34 | 68 ± 32 | 86 ± 30 | 89 ± 35 | 80 ± 34 |
| % | 30 ± 6 | 30 ± 6 | 28 ± 6 | 33 ± 8 | 31 ± 8 | 29 ± 8 |
| Vitamin D (IU)[1] | 161 ± 128[a] | 1061 ± 220[b*] | 1086 ± 168[b*] | 175 ± 140[a] | 193 ± 205[a] | 148 ± 169[a] |
| Calcium (mg)[1] | 775 ± 354[a] | 1491 ± 617[b*] | 1423 ± 305[b*] | 944 ± 553[a] | 919 ± 517[a] | 856 ± 499[a] |
| n-3 PUFA (g)[1] | 1.2 ± 1.4[a] | 2.5 ± 1.4[b*] | 2.3 ± 0.8[b*] | 0.9 ± 0.7[a] | 1.2 ± 1.0[a] | 1.1 ± 1.3[a] |
| Habitual physical activity | | | | | | |
| TEE (kcal)[2] | 2153 ± 318[a] | 2143 ± 303[a] | 1892 ± 467[b] | 2157 ± 627[a] | 2347 ± 567[a] | 1856 ± 397[b] |
| AEE (kcal) | 394 ± 258 | 378 ± 231 | 324 ± 201 | 374 ± 348 | 424 ± 325 | 293 ± 278 |
| Average METs | 1.3 ± 0.1 | 1.4 ± 0.2 | 1.4 ± 0.1 | 1.2 ± 0.3 | 1.3 ± 0.2 | 1.3 ± 0.2 |

Values are mean ± SEM and were analyzed using a two-way ANCOVA with baseline values as covariates. [1]Group-by-time interaction ($P < 0.01$). [2]Main effect for time ($P < 0.05$). For each outcome, different letters represent significant differences within each group. Significance accepted as $P < 0.05$. SUPP, supplement group (n=25); CON, control group (n=24); n-3 PUFA, omega-3 polyunsaturated fatty acids; TEE, total energy expenditure; AEE, active energy expenditure; MET, metabolic equivalent.

## DISCUSSION

We discovered that twice daily consumption of a whey protein-based, multi-ingredient supplement resulted in significant gains in muscle strength and lean mass. In addition, muscle strength, physical function, and aerobic capacity were further improved in response to the 12-week combined exercise training program and to a greater extent by the supplement for some outcomes. While it is not possible to isolate which compounds in the supplement were responsible for the outcomes observed, each has been shown to independently affect aspects of sarcopenia and thus has a rational basis for inclusion. Notably, whey protein supplementation can variably enhance lean mass and strength (4, 25-28), creatine can improve strength (5, 29) vitamin D supplementation can reduce the risk of falls (30) and fractures (31), and n-3 PUFA has been shown it to improve muscle quality (32), mass (11), and function (11, 32) in older adults. However, the response heterogeneity to the individual compounds would seem to dictate that a combination would be more efficacious than either compound alone.

Some studies (11, 33, 34) have demonstrated a beneficial effect of nutritional supplementation independent of exercise on muscle strength, physical function, and lean mass in older adults, but others have not (35, 36). This discrepancy may, in part, be explained by a heterogeneity of response to supplementation in older adults. By employing a multi-ingredient approach to dietary supplementation we hypothesized that we would be more likely to affect at least one independent potential pathway/mechanism through which older persons would experience an anti-sarcopenic effect. The present study clearly showed increases in total muscle strength and lean mass in response to a

multi-ingredient nutritional supplement in the absence of exercise training. Other trials using a combination of ingredients (whey protein and vitamin D) similar to those we employed in our supplement have shown preservation of lean mass during weight loss (15), enhanced strength (16), increased lean body mass (16), and enhanced physical function (in sarcopenic older persons) (16). Such changes in lean body mass and strength are clinically relevant given that strength and muscle mass decrease at respective rates of ~1-3% and ~0.5-1% annually, likely commencing in or around the fifth decade of life (37). Notably, the gains in strength and lean body mass we observed would be the equivalent of offsetting one year of age-related decline, suggesting that this relatively short-term nutritional intervention could attenuate the progression of sarcopenia in older adults.

Exercise training is another highly effective intervention to counter sarcopenia. Resistance exercise, in particular, acutely elevates myofibrillar protein synthesis for several days (12), resulting in long-term gains in muscle mass and strength in older adults when practiced regularly (38, 39). Previously, we have shown that a single bout of high intensity interval exercise was also capable of stimulating myofibrillar protein synthesis in older adults (12). High intensity interval exercise offers numerous other health benefits in both younger and older populations, such as improvements in glycemic regulation (13) and aerobic capacity (14). Knowing that aging is associated with reduced cadiovascular health, as well as low muscularity and strength, we designed an exercise training program that combined exercise modalities for optimal benefit in older adults. Our data show that RET and HIIT can be safely used in combination to elicit significant gains in lean mass,

strength, and physical function. Additionally, markers of cardiovascular health, such as aerobic capacity and blood pressure, also improved in this sample of older men. The lack of a significant increase in lean mass over the course of the exercise training program is likely due to the relatively low volume of resistance exercise performed by participants in the current study compared to previous studies (40), and/or a potential antagonizing effect of concurrent aerobic exercise (i.e. HIIT) on traditional RET adaptations (41). Nonetheless, the significant gains in muscle strength and function that we observed following exercise training may be important for physical function and mobility and a quality of life in older age (42).

We show that consumption of the multi-ingredient supplement during the exercise training program did not result superior gains in muscle strength, lean mass, or physical function compared to participants on the control drink. Several previous studies have failed to identify an additional beneficial effect of nutritional supplementation on exercise-mediated gains in lean mass and strength (23-26). Conversely, other studies have reported a positive effect (20-22). Sample size may have limited our ability to detect a difference between the supplement and control group over the course of exercise training; post hoc calculations have revealed that we would have required 80 participants per group to detect a difference in the magnitude of overall strength. The relative good health of the participants included in the present study also may have prevented us from observing such an effect. Indeed, if the same exercise and nutrition intervention were applied to a more frail/functionally impaired group of older adults (16), it is possible that the same multi-ingredient supplement would be even more efficacious.

The current results demonstrate the potential of a multi-ingredient nutritional strategy as a practical intervention to mitigate the development of sarcopenia. Our multi-ingredient supplement may be beneficial in patients for whom structured exercise is not possible or who are undergoing periods of muscle disuse. This is of particular importance since the relatively slow and steady decline in strength and muscle mass with age is often punctuated by brief periods of muscle disuse (e.g. during hospitalization) where losses are accelerated (43, 44). Furthermore, structured exercise may not be possible or feasible during hospitalization, recovery from surgery, or convalescence from illness.

In conclusion, we have demonstrated that twice daily consumption of a whey protein-based supplement containing creatine, vitamin D, calcium, and n-3 PUFA was effective in stimulating strength and lean body mass gains in the absence of exercise in a group of healthy older men. Future trials with a larger sample size, for a greater duration, and including women, would confirm if this supplement represented a viable anti-sarcopenic intervention with a broader potential for use.

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# CHAPTER 4:

**A whey protein-based multi-ingredient nutritional supplement enhances exercise training-related reductions in glycemia and markers of systemic inflammation, in healthy older men.** *In preparation.*

**Title: A whey protein-based multi-ingredient nutritional supplement enhances exercise training-related reductions in glycemia and markers of systemic inflammation, in healthy older men**

**Authors:** Kirsten E. Bell, Tim Snijders, Michael Zulyniak, Dinesh Kumbhare, Gianni Parise, Adrian Chabowski and Stuart M. Phillips

**Author affiliations:** Exercise Metabolism Research Group, Department of Kinesiology (KEB, TS, SMP, GP); Department of Medicine (MZ, DK), McMaster University, Hamilton, Ontario, Canada; and Department of Physiology, Medical University of Bialystok, Bialystok, Poland (AC)

**Authors' last names:** Bell, Snijders, Zulyniak, Kumbhare, Parise, Chabowski, Phillips

**Short running head**: Nutritional supplement improves glucose handling

**Changed affiliations:** TS is now affiliated with Department of Human Biology and Movement Sciences, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center+, The Netherlands

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**Corresponding author:**

S.M. Phillips, Department of Kinesiology, McMaster University, Hamilton, ON L8S 4K1

905-525-9140 x24465, phillis@mcmaster.ca

**Clinical trials registry number:** NCT02281331 at ClinicalTrials.gov

**ABSTRACT**

**Background:** Aging is associated with reduced glycemic control and increased systemic inflammation. Exercise and nutraceutical strategies are independently effective in improving glycemia and inflammation. **Objective:** We evaluated whether daily consumption of a whey protein-based, multi-ingredient nutritional supplement would improve glycemic regulation and systemic inflammatory markers independent of, and when combined with, exercise in healthy older men. **Design:** Forty-nine men (age: 73±1 years [means±SEM]; BMI: 28.5±0.5 kg/m$^2$) were randomly allocated to 20wk of twice daily consumption of a supplement (SUPP; n=25), which contained 30g whey protein, 2.5g creatine, 500IU vitamin D, 400mg calcium, and 1500mg omega-3 (n-3) PUFA; or a control (CON: n=24) beverage, which contained 22g carbohydrate. Phase 1 of the study was 6wk of SUPP or CON. Phase 2 of the study was SUPP or CON plus a 12wk exercise training program. Circulating cytokines, and glucose and insulin concentrations in response to an oral glucose tolerance test (OGTT) were evaluated at wks -1 (pre-intervention), 6 (Phase 1), and 19 (Phase 2). **Results:** In Phase 1, tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) concentrations were significantly reduced (both $P<0.05$) in the SUPP group only. In Phase 2, reductions in OGTT-induced glucose and insulin area under the curve were observed in both groups. Importantly, significant reductions in pro-inflammatory cytokines, as well as maximal and mean glucose concentrations, were only observed in the SUPP group. **Conclusion:** Twice daily consumption of a multi-ingredient nutritional supplement reduced systemic inflammation

independent of exercise training, and enhanced exercise-related improvements in glucose handling, in older men.

## INTRODUCTION

Aging is associated with impairments in glycemic control (1-4). In comparison to younger adults, older adults have: higher glucose and insulin concentrations during an oral glucose tolerance test (OGTT) (5); reduced insulin sensitivity during a hyperinsulinemic-euglycemic clamp (6, 7); and depressed intracellular glucose oxidation rates (8). Such observations are in part explained by age-related increases in abdominal adiposity, decreased physical activity levels, and sarcopenic muscle loss (9). Age-associated low-grade systemic inflammation may also play a role in the development of impaired glucose tolerance (10).

Resistance exercise training (RET) and high-intensity interval training (HIIT) have previously been shown to improve insulin-stimulated glucose uptake (11) and reduce HOMA-IR (12), respectively, in older adults. Given that low appendicular muscle mass and abdominal adiposity are associated with insulin resistance in older adults (13), it is possible that exercise training-induced increases in muscle mass and metabolic 'quality' (i.e., enhanced capacity of skeletal muscle to take up and store or oxidize glucose), as well as reductions in fat mass, may underpin improvements in insulin sensitivity.

Previous studies have investigated the independent effects of whey protein (14-17), vitamin D (18-21) and omega-3 (n-3) PUFA (22-29) supplements in reducing

systemic inflammation in older adults, an outcome which may be conducive to improvements in glycemic health and lipid partitioning. A limited number of studies has investigated whether creatine supplementation – which stimulates lean mass gains in older adults (30) – is associated with improvements in cardiometabolic health (31-33). We propose that a comprehensive multi-themed intervention combining RET and HIIT, along with whey protein, creatine, vitamin D/calcium, and n-3 PUFA supplementation may represent a superior strategy towards improving cardiometabolic health in older adults.

The objectives of this study were to determine whether a protein-based multi-ingredient nutritional supplement, which has previously been shown to elicit lean body mass gains in older men independent of exercise (Chapter 3), would improve glycemic regulation, improve lipid profile, and reduce markers of systemic inflammation in a group of sedentary older men following 6 weeks of twice daily consumption. Additionally, we aimed to determine whether the same supplement would enhance exercise training-related improvements in these outcomes following 12 weeks of a combined RET and HIIT program. We hypothesized that supplementation would induce favourable changes in glucose handling and lipid profile, as well as a reduction in systemic inflammation, independent of exercise, and that these improvements would be enhanced with the addition of exercise training.

## METHODS

*Screening and recruitment.* Forty-nine healthy older men from Hamilton, Ontario and its surrounding area took part in this study. Participants were non-smokers ≥ 65 years old, had a BMI in the normal-overweight range (between 18.5 and 30.0 kg/m$^2$), and had not engaged in any structured resistance or aerobic exercise training program in the past 6 months. Subjects were also non-diabetic based on a 2 h, 75 g oral glucose tolerance test (OGTT; fasting blood glucose < 7.0 mM; 2 h blood glucose < 11.1 mM) and demonstrated normal cardiac function during a maximal exercise stress test on a cycle ergometer. Exclusion criteria included: regular consumption of whey protein, creatine, calcium, vitamin D, or n-3 PUFA supplements in the past 5 years; significant weight loss or gain in the past 6 months; use of non-steroidal anti-inflammatory drugs, simvastatin, or anticoagulants; injuries preventing safe participation in an exercise program; diabetes mellitus; cancer; infectious disease; and cardiac or gastrointestinal problems.

This trial was approved by the Hamilton Integrated Research Ethics Board and complied with the guidelines set out in the Tri-Council policy statement on ethical conduct for research involving humans (http://www.pre.ethics.gc.ca/pdf/eng/tcps2/TCPS_2_FINAL_Web.pdf). All participants were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained.

*Experimental design.* Details of the study protocol and testing procedures have previously been described (Chapter 3). Briefly, eligible subjects were randomly assigned to receive either a multi-ingredient nutritional supplement (SUPP) or a control (CON) drink for 20

weeks. After 6 weeks of consuming their study beverages at home (Phase 1: SUPP/CON), subjects completed a 12-week supervised exercise training program at McMaster University while continuing to consume their assigned beverages (Phase 2: SUPP + EX and CON + EX). Glucose handling was evaluated using a 75 g OGTT at weeks -1 (pre-intervention), 6, and 19.

*Nutritional supplements.* Participants in the SUPP group consumed a multi-ingredient beverage containing: 30 g whey protein, 2.5 g creatine, 400 mg calcium, 500 IU vitamin D, and 1500 mg n-3 PUFA (which delivered 700 mg eicosapentaenoic acid [EPA] and 445 mg docosahexaenoic acid [DHA]) twice daily. Participants in the CON group consumed a control beverage containing 22 g of carbohydrate twice daily. The exact composition of the supplement and control drinks has been previously outlined (Chapter 3). Subjects consumed their first daily beverage within the hour after breakfast, and the second 1h prior to retiring to bed. The control beverages were matched in volume and flavour to the active blend. All study beverages were prepared and labeled in a blinded manner by Infinit Nutrition (Windsor, ON), and both subjects and researchers were blind to individual group assignments. Participants were instructed not to alter their habitual dietary or physical activity habits for the duration of the study.

Compliance with the study beverages was high (SUPP group: 87 ± 2%; CON group: 92 ± 2%), and was verified via: 1) self-report in journals; 2) based on the volume of unused study beverages returned upon completion or withdrawal; 3) EPA/DHA enrichment of erythrocyte plasma membranes; as well as 4) plasma 25-hydroxyvitamin D (25[OH]D) concentrations.

*Exercise training.* From weeks 7 to 18, subjects engaged in a 12-week progressive exercise training program at the Physical Activity Centre of Excellence (PACE) at McMaster University. In brief, subjects completed three exercise sessions per week: whole body RET twice per week (Mondays and Fridays) and HIIT on a cycle ergometer once per week (Wednesdays). At every RET session, participants completed two upper body (chest press and horizontal row on Mondays; lateral pulldown and shoulder press on Fridays) and two lower body exercises (leg press and leg extension on both Mondays and Fridays). Training was performed at 80% 1RM (6-8 repetitions) for three sets, with the last set completed until volitional fatigue. Training intensity was adjusted based on 1RM strength tests conducted at weeks 11 and 15. Additionally, to ensure an adequate training stimulus, workload was increased outside of these weeks when subjects could complete ≥ 12 repetitions during the third set of each exercise.

During their HIIT sessions, subjects completed 10 x 60 s intervals cycling against a workload predetermined to elicit ~90% maximal HR (HRmax), while maintaining a cadence of ≥ 90 rpm. Workload was adjusted by 3-5 W as needed to maintain an average HR of ~90% HRmax over the 10 intervals. Intervals were interspersed with 60 s of rest where subjects cycled at a self-selected pace against 25 W.

Subjects in the SUPP and CON groups attended 95 ± 1% and 94 ± 1% of training sessions, respectively. All subjects attended at least 80% of all RET and HIIT sessions.

*Oral glucose tolerance test.* After a 10h overnight fast, a 20 G catheter was inserted into an antecubital vein and a blood sample (0 min) was obtained for the measurement of fasting plasma glucose and insulin concentrations, as well as lipid panel (total cholesterol

[c], HDL-c, LDL-c, and triglycerides [TG]) and inflammatory markers (c-reactive protein [CRP], tumour necrosis factor alpha [TNF-α], and interleukin-6 [IL-6]). Subjects then consumed a 75 g dextrose solution (Trutol™; NERL Diagnostics LLC, East Providence, RI) within 5 min. Serial blood samples were obtained at 30, 60, 90 and 120 min post-ingestion of the dextrose solution for the measurement of postprandial plasma glucose and insulin concentrations.

*Biochemical analysis.* Plasma glucose concentrations were measured using the glucose oxidase method (YSI 2300; Yellow Springs, OH). Plasma insulin concentrations were measured using the dual-site chemiluminescent method (Siemens Immulite 2000; Malvern, PA). Cholesterol (total, HDL, and LDL) and TG were analyzed using the Architect Clinical Chemistry Analyzer (Abbott Diagnostics). Plasma TNF-α and IL-6 concentrations were measured using a Bio-Plex system (Bio-Rad Laboratories; Hercules, CA), and plasma CRP concentrations were measured using an Express Plus Autoanalyzer (Chiron Diagnostics Co; Walpole, MA) and a commercially available high-sensitivity CRP latex kit (Pulse Scientific; Burlington, ON).

*Statistical analysis.* Statistical analysis was completed using SPSS (IBM SPSS Statistics for Windows, version 23.0; IBM Corp., Armonk, NY). We conducted an intention-to-treat analysis using a linear mixed model with an unstructured covariance matrix and respective baseline values for each outcome and trunk fat mass as covariates. We adjusted for baseline values due to numerical but non-significant differences between groups prior to beginning the intervention, and trunk fat mass because it is highly correlated with both glycemic health (34, 35) and systemic inflammation (36). Data were analyzed using a

two-way ANCOVA with group (SUPP or CON) and time (-1, 6, and 19) as factors. Significant differences were identified using a using post hoc t-tests with a Bonferroni correction. Based on recommendations for human clinical trials with missing data (37), all participants (completers as well as participants who withdrew prior to week 6 or week 19 testing) were included in the final analyses, and missing values were not replaced. Statistical significance was accepted as $P < 0.05$. Data are presented as mean ± SEM.

**RESULTS**

*Participants.* At baseline, participants were 73 ± 1 years of age, overweight according to BMI (28.5 ± 0.5 kg/m$^2$), and pre-hypertensive (SBP: 138 ± 2 mmHg; DBP: 78 ± 1 mmHg) with elevated fasting blood glucose (5.7 ± 0.1 mM) based on the definition set out by the International Diabetes Federation (IDF) (38).

*Glucose tolerance.* No significant differences in any measures of glucose tolerance existed between the SUPP and CON groups at baseline. We observed significant group-by-time interactions for maximal (Cmax) ($P < 0.05$) and mean plasma glucose concentrations ($P < 0.05$) during an OGTT, so each group was analyzed to separately for these outcomes. In the SUPP group, glucose Cmax and mean plasma glucose concentrations decreased ~11% and ~7%, respectively, from baseline to week 19 (**Table 1**), whereas no change in either outcome was observed in the CON group over the course of the study.

Table 1. Insulin sensitivity, glucose- and insulin-related variables

| | SUPP | | | CON | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 wk | 19 wk | Baseline | 6 wk | 19 wk |
| HOMA-IR[2] | 2.1 ± 0.1[a] | 2.1 ± 0.1[a] | 1.7 ± 0.1[b] | 2.2 ± 0.1[a] | 2.0 ± 0.1[a] | 1.7 ± 0.1[b] |
| Matsuda index[2] | 5.1 ± 0.7[a] | 5.3 ± 0.6[a] | 6.4 ± 0.8[b] | 5.1 ± 0.6[a] | 5.4 ± 0.8[a] | 6.4 ± 0.8[b] |
| Mean plasma glucose (mM)[1] | 7.2 ± 0.1[a] | 7.0 ± 0.1[a,b] | 6.7 ± 0.1[b] | 7.0 ± 0.1[a] | 7.1 ± 0.1[a] | 6.9 ± 0.1[a] |
| Plasma glucose Cmax (mM)[1] | 9.6 ± 0.3[a] | 9.1 ± 0.2[a,b] | 8.5 ± 0.2[b] | 8.9 ± 0.2[a] | 9.1 ± 0.2[a] | 9.1 ± 0.2[a] |
| Plasma insulin Cmax (μIU/mL)[2] | 63.7 ± 1.6[a] | 64.7 ± 1.7[a] | 53.0 ± 1.8[b] | 64.8 ± 1.5[a] | 60.8 ± 2.3[a] | 52.0 ± 1.5[b] |

Values are mean ± SEM and were analyzed using two-way ANCOVA with respective baseline values for each outcome and trunk fat mass as covariates. [1]Group-by-time interaction ($P < 0.05$). [2]Main effect for time ($P < 0.01$). For each outcome, different letters represent significant differences within each group. Significance accepted as $P < 0.05$. SUPP, supplement group (n=25); CON, control group (n=24); Cmax, maximal concentration.

We observed a main effect of time for glucose AUC ($P < 0.01$), insulin AUC ($P < 0.001$), HOMA-IR ($P < 0.001$), the Matsuda Index of insulin sensitivity ($P < 0.001$), and insulin Cmax ($P < 0.001$). We did not detect significant changes in any of these measures following Phase 1; however, significant differences were apparent during Phase 2. Between weeks 6 and 19, we observed a reduction in glucose AUC (from 895 ± 8 to 857 ± 10 mM•120 min; $P < 0.05$; **Figure 1**), insulin AUC (from 3951 ± 60 to 3460 ± 47 µIU/mL•120 min; $P < 0.001$; Figure 1), HOMA-IR (SUPP: -20% and CON: -15%; $P < 0.001$; Table 1) and insulin Cmax (SUPP: -18% and CON: -14%; $P < 0.001$), as well an increase in the Matsuda Index (SUPP: +20% and CON: +19%; $P < 0.001$).

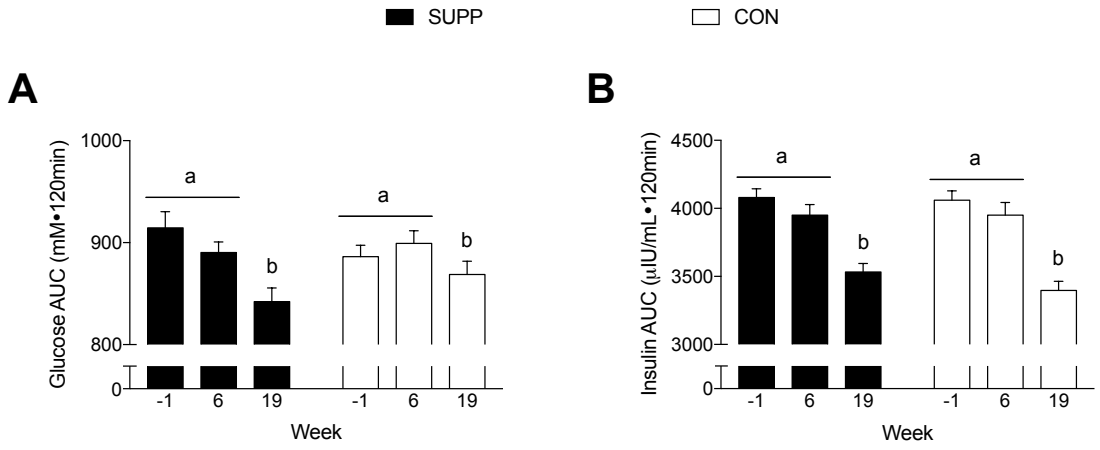


**Figure 1**. Plasma glucose (A) and insulin (B) AUC during an OGTT in the SUPP (black bars) and CON (white bars) groups. Values are mean ± SEM and were analyzed using a two-way ANCOVA with respective baseline values for each outcome and trunk fat mass as covariates. We observed significant main effects of time for glucose ($P < 0.01$) and insulin ($P < 0.001$) AUC. Different letters represent significant differences across time. Significance accepted as $P < 0.05$. OGTT, oral glucose tolerance test; SUPP, supplement group (n=25); CON, control group (n=24).

Of note, although we did not observe a significant group-by-time interaction for glucose AUC, this effect approach significance ($P = 0.056$).

*Fasting lipids.* Fasting TG and cholesterol (total, LDL, and HDL) were not significantly different between the SUPP and CON groups at baseline (**Table 2**). We observed significant group-by-time interactions for TG ($P < 0.05$) and total cholesterol ($P < 0.05$). In the SUPP group, fasting TG (-35%; $P < 0.01$) and total cholesterol (-7%; $P < 0.01$) were reduced in Phase 1 with no further reduction following Phase 2. In the CON group no change in fasting TG was observed over the course of the study, however, total cholesterol decreased significantly (-3%; $P < 0.05$) following Phase 2.

Table 2. Fasting blood lipids

| | SUPP | | | CON | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 wk | 19 wk | Baseline | 6 wk | 19 wk |
| Total-c (mM)[1] | 4.69 ± 0.22[a] | 4.34 ± 0.23[b] | 4.56 ± 0.30[a,b] | 4.83 ± 0.19[a,b] | 4.86 ± 0.19[a] | 4.73 ± 0.19[b] |
| LDL-c (mM) | 2.74 ± 0.21 | 2.63 ± 0.19 | 2.77 ± 0.25 | 2.87 ± 0.18 | 2.87 ± 0.20 | 2.85 ± 0.19 |
| HDL-c (mM) | 1.27 ± 0.06 | 1.27 ± 0.08 | 1.28 ± 0.09 | 1.29 ± 0.06 | 1.29 ± 0.06 | 1.26 ± 0.07 |
| TG (mM)[1] | 1.49 ± 0.19[a] | 0.97 ± 0.09*[b] | 1.10 ± 0.14[b] | 1.50 ± 0.21[a] | 1.52 ± 0.18[a] | 1.35 ± 0.16[a] |

Values are mean ± SEM and were analyzed using two-way ANCOVA with respective baseline values for each outcome and trunk fat mass as covariates. [1]Group-by-time interaction ($P < 0.05$). For each outcome, different letters represent significant differences within each group. *Significantly different from CON at that week. Significance accepted as $P < 0.05$. SUPP, supplement group (n=25); CON, control group (n=24); c, cholesterol; TG, triglycerides.

*Inflammatory markers.* At baseline, no differences in inflammatory markers existed between the groups. We observed significant group-by-time interactions for CRP ($P < 0.001$), TNF-α ($P < 0.001$), and IL-6 ($P < 0.001$). In both the SUPP and CON groups, plasma CRP concentrations did not change in Phase 1; however, we observed a significant reduction following Phase 2 (SUPP: -10% and CON: -1%; $P < 0.05$; **Table 3**). In the SUPP group, plasma TNF-α and IL-6 concentrations each decreased ~1-3% during Phase 1 and a further ~11-12% in response to Phase 2. In the CON group, plasma TNF-α and IL-6 concentrations did not change significantly over the course of the study. Plasma CRP, TNF-α, and IL-6 concentrations were significantly lower in the SUPP group compared to the CON group at week 19.

Table 3. Inflammatory markers

| | SUPP | | | CON | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 wk | 19 wk | Baseline | 6 wk | 19 wk |
| CRP (mg/L)[1] | 9.6 ± 0.6[a] | 9.6 ± 0.6[a] | 8.7 ± 0.7[b*] | 11.1 ± 0.6[a] | 10.8 ± 0.7[a] | 10.7 ± 0.7[b] |
| TNF-α (pg/mL)[1] | 16.1 ± 0.7[a] | 15.6 ± 0.7[b] | 13.7 ± 0.6[c*] | 15.8 ± 0.7[a] | 16.0 ± 0.7[b] | 16.1 ± 0.7[b] |
| IL-6 (pg/mL)[1] | 7.0 ± 0.4[a] | 6.9 ± 0.4[b] | 6.2 ± 0.4[c*] | 6.9 ± 0.3[a] | 7.1 ± 0.3[a] | 7.0 ± 0.4[a] |

Values are mean ± SEM and were analyzed using two-way ANCOVA with respective baseline values for each outcome and trunk fat mass as covariates. [1]Group-by-time interaction ($P < 0.001$). For each outcome, different letters represent significant differences within each group. *Represents a significant difference from CON at that week. Significance accepted as $P < 0.05$. SUPP, supplement group (n=25); CON, control group (n=24); CRP, c-reactive protein; TNF, tumour necrosis factor; IL, interleukin.

## DISCUSSION

Six weeks of twice daily consumption of a protein-based multi-ingredient nutritional supplement elicited significant improvements in fasting lipids and systemic inflammatory markers in the absence of exercise in healthy older men. The addition of 12 weeks of combined RET + HIIT resulted in improvements in glucose control in both groups, however, participants in the SUPP group showed a greater degree of improvement in glucose handling and superior reductions in systemic inflammation compared to participants in the CON group.

Low-grade systemic inflammation is associated with the development cardiovascular disease (39) and insulin resistance (40) in older adults. Accordingly, in the present study we employed a multi-ingredient supplement – in the absence of exercise – with components aimed improving glucose handling, lipidemia, as well as reducing concentrations of pro-inflammatory cytokines. Our inflammation and lipid panel results in the SUPP group following Phase 1 are consistent with a decreased cardiovascular disease risk profile (39). Improvements in glucose handling, however, were not different ($P = 0.056$) between groups, which may have been a type 2 error. Post hoc calculations revealed that we would have required roughly twice as many participants per group to detect a difference in the magnitude of improvement in glucose AUC during Phase 1 of this study. It is worth noting, however, that following supplementary analysis the proportion of participants who met the IDF criteria for metabolic syndrome (38) decreased in the SUPP group (from 9/25 at baseline to 6/23 at week 6) but remained unchanged in the CON group (from 11/24 at baseline to 11/22 at week 6). Low-grade

inflammation due to increased oxidative stress (41) and/or central (particularly visceral) adiposity (41, 42) has been suggested as a mechanism by which cardiometabolic disease develops in older age, and is therefore a worthwhile target for intervention. Previous studies have investigated the independent ability of whey protein (14-17), vitamin D (18-21), and n-3 PUFA (22-29) to reduce various markers of systemic inflammation in older adults, and by extension improve other health outcomes. In a recent review Ticinesi et al. (43) concluced that sufficient evidence existed only for support of n-3 PUFA supplementation to reduce inflammation in older adults. However, the authors postulated that nutrition interventions which combine whey protein, vitamin D, and n-3 PUFA may be especially effective since the anti-inflammatory activity of one isolated nutrient could be influenced by the intake of another. The multi-ingredient nature of the supplement used in the present study prevents us from determining what ingredient(s) was (were) responsible for the anti-inflammatory effects that we observed. As Ticinesi et al. (43) suggest, the interaction between the various ingredients in the supplement may have been important for the observed reduction in systemic inflammation and the improvement in lipidemia.

Although aging per se is independently associated with impairments in glucose handling (44), both physical inactivity (42) and abdominal adiposity (45, 46) often accompany aging and likely contribute to dysglycemia. We discovered that 12 weeks of combined RET + HIIT independently improved glucose tolerance in this group of healthy older men, possibly due to increased physical activity levels and/or reduced abdominal adiposity. Consistent with our findings of a ~20% improvement in HOMA-IR and

Matsuda Index, both aerobic exercise training (AET) and RET have been shown to stimulate a similar degree of improvement in insulin sensitivity (20-30%) in older adults (47-49). Low-moderate intensity continuous AET and HIIT are known to increase glucose transporter 4 (GLUT4) protein content in skeletal muscle (50, 51), which would enhance clearance of glucose in response to an OGTT. Another mechanism by which the RET + HIIT program employed in this study may have sensitized participants to the effects of insulin is via reductions in abdominal adiposity. In Chapter 3 we observed a significant 0.7 kg decrease ($P < 0.01$) in trunk fat mass over the course of exercise training, which may be indicative of decreased visceral adipose tissue. Visceral adipose tissue content is an independent risk factor for glucose intolerance (34) and insulin resistance (35) even after adjusting for whole body fat mass, possibly as a result of heightened pro-inflammatory cytokine production in visceral compared to subcutaneous adipose tissue depots (41). Differential reductions in visceral adipose tissue content may therefore help explain the greater relative improvement in maximal and mean postprandial glucose concentrations in the SUPP versus CON group over the course of exercise training.

Twice daily consumption a protein-based multi-ingredient nutritional supplement enhanced exercise-related reductions in maximal and mean glucose concentrations during an OGTT compared to a control drink. This potentiation was accompanied by reductions in circulating concentrations of pro-inflammatory cytokines in the SUPP group only. Both TNF-α and IL-6 interfere with insulin signaling in skeletal muscle primarily via inhibition of phosphoinositide-3 (PI3) kinase (52-54) and subsequent GLUT4 translocation to the

plasma membrane. Decreased circulating concentrations of TNF-α and IL-6 may therefore have permitted greater GLUT4 vesicle trafficking to the sarcolemma, and enhanced insulin-stimulated glucose uptake following an oral glucose challenge in the SUPP group. The aforementioned decrease in trunk fat mass with exercise training (main effect of time, $P < 0.01$; Chapter 3), may have been predominantly driven by the -8% change in the SUPP group, with the 0% change in the CON group constituting a relatively small contribution (data not shown). Visceral adipose tissue is a source of pro-inflammatory cytokines (36), and in overweight and obese individuals this tissue can become infiltrated with macrophages which also secrete inflammatory markers (55). As such, preferential reductions in trunk fat mass (which is a proxy of visceral adipose tissue) during exercise training with multi-ingredient nutritional supplementation may therefore explain improvements in both systemic inflammation as well as glucose handling; however, this speculation remains to be confirmed in future with adequately powered studies.

In the present study, we have demonstrated that consumption of a multi-ingredient nutritional supplement during 12 weeks of combined RET + HIIT stimulated greater improvements in glucose handling and systemic inflammation compared to a carbohydrate-based control drink in a group of healthy older men. The prevalence of type 2 diabetes (1) and cardiovascular disease (39) is greater among older compared to middle-aged and younger adults. Improvements in insulin sensitivity and lipid partitioning may prevent the progression to overt disease in older individuals who already have some degree of metabolic impairment. In this proof-of-principle study we show that multi-

ingredient nutritional supplementation can independently improve lipid partitioning and markers of inflammation, which may be relevant for older adults who are unable to perform structured exercise. The improvements in glucose handling that we observed following exercise training, with and without nutritional support, have important implications in the prevention of sarcopenia. Hyperglycemia and insulin resistance are associated with low muscularity and strength (56, 57), as well as impaired lower extremity function in older adults with and without diabetes (57-59). Further, improved insulin sensitivity may also play a role in the anabolic resistance to protein ingestion and acute RE (60). Thus, improved insulin sensitivity may improve anabolic responses and reduce the risk of frailty and sarcopenia.

In conclusion, 6 weeks of twice daily consumption of a protein-based multi-ingredient nutritional supplement, which has previously been shown to independently elicit lean mass gains, resulted in significant improvements in lipidemia and systemic inflammation in a group of older men. Further, this nutritional intervention potentiated exercise-related improvements and glucose control.

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# CHAPTER 5:

# GENERAL DISCUSSION

## 5.1 Introduction

Beginning in or around the fifth decade of life, muscle mass and strength are lost at rates of ~1% and ~3% annually, respectively (1). Low muscularity and strength in older age contribute to: physical frailty, the development of metabolic disorders like type 2 diabetes, an increased risk of falls and fractures, an increased risk for loss of independence, and reduced quality of life (2). Strength loss, in particular, is concerning because strength is an independent predictor of mortality risk in older adults (3, 4). In addition to sarcopenia, aging is associated with reduced aerobic fitness (5), dyslipidemia (6), and impaired glycemic regulation (7). Cardiometabolic risk factors such as dyslipidemia and dysglycemia may be mediated by low-grade systemic inflammation (8). Exercise and nutritional strategies that target a range of these age-related changes would be welcome as they would intervene against a broad range of age-related chronic disease and potentially extend healthspan during aging.

The broad aim of this thesis was to explore how multiple exercise modalities and nutritional/nutraceutical ingredients could be used in combination to alleviate several deleterious physiological changes related to aging. One novel aspect of this work is our use of the recently reintroduced deuterated water method to evaluate the longer-term day-to-day integrated muscle protein synthesis (MPS) response to acute exercise. Our findings build upon the results of previous stable isotope infusion trials, and are more reflective of "real-world" changes. Further, despite the wealth of research on high-intensity interval exercise (HIIE), Study 1 (Chapter 2) was the first study to assess whether this exercise modality is capable of acutely stimulating rates of myofibrillar protein synthesis (myoPS)

in adults of any age. Although the effects of HIIE on certain aspects of skeletal muscle metabolism (oxidative metabolism, in particular) are well described (9, 10), there is a paucity of data on its potential influence on muscle protein turnover. Also unique to the current thesis was the design and composition of the multi-ingredient nutritional supplement used in Studies 2 and 3 (Chapters 3 and 4, respectively). Previous studies have shown that the individual components of this supplement (whey protein, creatine, vitamin D/calcium, and omega-3 (n-3) polyunsaturated fatty acids [PUFA]) are effective as stand-alone ingredients in ameliorating sarcopenic lean mass and strength loss and/or other negative aspects of aging, and several studies have combined subsets of these same ingredients (11-16). However, to our knowledge, our study is the first to combine all of these ingredients into a single supplement, as well as to utilize it in conjunction with a resistance exercise training (RET) + HIIT exercise training program in older men.

Study 1 demonstrated that, as expected, a single session of RE elevated MPS above resting rates for 48 hours post-exercise in a group of healthy older men. Importantly though, a single session of HIIE also stimulated MPS, albeit to a slightly lesser extent compared to RE. Since regular and repeated stimulation of MPS is required in order for skeletal muscle hypertrophy to occur, the results of Study 1 led us to hypothesize that HIIE might function as a hypertrophic stimulus in older individuals when practiced over several weeks to months. Specifically, we were interested in determining whether HIIT would confer important cardiometabolic adaptations while providing a greater anabolic stimulus than traditional low-moderate continuous intensity aerobic exercise training (AET). In Studies 2 and 3 we report that strength, aerobic

fitness, and glucose handling were improved in a group of healthy older men following 12 weeks of combined RET + HIIT, however lean mass was unchanged. An additional and noteworthy finding of Study 2 was that, in the same group of older men, significant gains in lean mass and muscular strength were seen after 6 weeks of twice daily consumption of a protein-based multi-ingredient nutritional supplement. Finally, in Study 3 we show that the same protein-based multi-ingredient supplement reduces triglyceride concentrations and markers of systemic inflammation independently of exercise training.

Collectively, this thesis has provided evidence to demonstrate that RET and HIIT can be used in conjunction with each other to elicit substantial gains in strength and cardiometabolic health in a group of older men, and that in situations where structured exercise may not be possible, multi-faceted nutritional supplementation might be effective in stimulating significant improvements in lean mass, strength, and systemic inflammation. This chapter integrates the findings of Studies 1, 2, and 3 and places them into context within the current body of literature. This chapter will also discuss the strengths and limitations of the present thesis and expand on future directions for this area of research.

**5.2 Acute response of skeletal muscle to various modalities of exercise in older men**

In agreement with a previous data (17), the results of Study 1 confirm that RE is a more potent stimulator of myoPS compared to AE. To the best of our knowledge, no previous studies have compared RE, AE, and HIIE using an integrated measurement of MPS, which limits our ability to compare our data to the literature. However, the magnitude of

increase in myoPS that we observed 24 hours post-RE (~95%) is in line with infusion trials where older adults were provided with an acute RE stimulus combined with an optimal 40 g dose of whey protein (~167%) (18). Since myoPS is typically measured 3-5 hours following protein ingestion and/or exercise during traditional infusion trials (18-20), and since our measurements in Study 1 integrated all postabsorptive and postprandial periods during the time between biopsies (as well as habitual physical activity), it follows that the peak in myoPS would be slightly lower in our study. Nonetheless, our integrated longer-term measurements of MPS may better reflect the potential of anabolic stimuli such as RE to induce phenotypic adaptations (i.e. skeletal muscle hypertrophy) over the course of weeks to months.

A major contribution of this thesis to the literature is the observation that HIIE significantly elevated myoPS at 24 and 48 hours post-exercise, and sarcoplasmic protein synthesis (sarcPS) at 24 hours post-exercise. The increase in myoPS with HIIE is provocative, as it provides indirect evidence that this form of exercise may induce skeletal muscle hypertrophy when practiced chronically. The increase in sarcPS with HIIE was not unexpected based on previous studies in younger adults: similar to traditional lower-moderate intensity continuous AE, HIIE has well-documented stimulatory effects on muscle oxidative capacity immediately (~3 hours) following exercise (21-23) as well as after several weeks of HIIT (9, 10, 24, 25). The ability of HIIE to augment sarcPS therefore possibly reflects stimulation of the mitochondrial muscle sub-fraction. Due to tissue constraints, we were unable to measure mitochondrial protein synthesis (mitoPS) and confirm this hypothesis, which is a limitation of Study 1. Additionally, special

preparation of the muscle tissue is required in order to accurately measure mitoPS. Specifically, the myofibrillar pellet must be thoroughly dounce-homogenized to ensure that the majority of the intermyofibrillar mitochondria (which constitute 80% of the mitochondria in skeletal muscle (26)) are liberated, and subsequently combined with the subsarcolemmal mitochondria suspended in the aqueous sarcoplasmic fraction (17). Since the muscle tissue in Study 1 was not prepared in this way, the increase in sarcPS that we observed following HIIE may, in fact, be an underestimate. Nevertheless, Study 1 provides the first indication that HIIE modulates MPS in healthy older men.

The increase in myoPS that we observed following an acute bout of HIIE provides equivocal support for this exercise modality as a hypertrophic stimulus. Heightened turnover of myofibrillar proteins could reflect muscle fiber remodeling towards a more oxidative phenotype (i.e. increased synthesis of myosin heavy chain I) rather than skeletal muscle hypertrophy, which – if true – may help explain the lack of increase in whole body lean tissue mass following combined RET + HIIT in a group of healthy older men in Study 2. However, data from acute and chronic studies are conflicting. In response to a single session of Wingate-based HIIE in younger men, protein kinase B (PKB)/Akt phosphorylation was depressed and the activity of its downstream targets p70 and 4E binding protein 1 (4EBP1) were unchanged, suggesting a lack of activation of the muscle protein synthetic machinery (23). Further, AMP-activated protein kinase (AMPK) and p38, which have previously been shown to inhibit MPS, are activated following HIIE (23). Very few studies have assessed changes in lean mass following several weeks of HIIT; however, some support the use of HIIT to induce gains in lean mass and strength in

both younger (27, 28) and older adults (29, 30), although other studies in younger adults report no change in either outcome (31, 32). Nevertheless, we were interested in exploring whether HIIT, when practiced concurrently with RET, would impart additional health benefits (hypertrophic or otherwise) to healthy older men.

### 5.3 The effect of multi-modal exercise training on strength, lean mass, cardiometabolic health, and systemic inflammation in older men

In Studies 2 and 3 we demonstrated that a 12-week exercise training program consisting of RET twice weekly and HIIT once per week resulted in significant improvements in isotonic muscle strength, cardiorespiratory fitness, blood pressure (data not shown in Chapter 3, Study 2), and glycemic control (Study 3). Progressive RET has well-described stimulatory effects on strength and muscle/lean tissue mass in older adults (33, 34), as well as significant (yet somewhat less widely known) positive effects on glucose metabolism and lipid partitioning (35-38). In older adults, RET induces a similar degree of improvement in glucose metabolism and lipid partitioning compared to AET (7, 35) although AET appears to be more effective than RET in enhancing cardiorespiratory fitness and reducing blood pressure (35). We chose HIIT over more traditional low-moderate continuous AET, in part, because it stimulates slightly greater improvements in peak oxygen uptake (~ 1.2 mL/kg/min) (39) without an associated increase in the risk of cardiovascular events (40). For sedentary older adults, with pre-training peak oxygen uptakes between 20-30 mL/kg/min, 1.2 mL/kg/min represents a not insignificant 4-6% change. The 1.8 mL/kg/min (8%) increase in $VO_2$peak that we observed following

exercise training in Study 2 was therefore likely primarily driven by the HIIT component, with minimal contribution from RET. The magnitude of increase that we observed is lower than the 9-13% increase in cardiorespiratory fitness reported by other HIIT studies in older adults (29, 30, 41-44), however participants in these studies trained 3-4 times per week and our subjects trained only once per week. In a meta-analysis, Kodama et al. (45) showed that a 1 metabolic equivalent (MET; equal to 3.5 mL/kg/min) increase in cardiorespiratory fitness is associated with a 13% and 15% reduction in the risk of all-cause mortality and cardiovascular disease, respectively. While the 1.8 mL/kg/min increase in Study 2 falls short of this benchmark, it does not necessarily mean that our participants did not benefit. Kodama et al. (45) included adults of all ages in their meta-analysis, and it is possible that since cardiorespiratory fitness decreases with inactivity and age, a smaller change in $VO_2$peak might be sufficient to induce a substantial reduction in disease risk in previously sedentary older adults. Regardless of the relevance of improved aerobic capacity to overall health, the 7 mmHg reduction in systolic blood pressure that we observed is clinically meaningful, since every 3 mmHg reduction in systolic blood pressure is associated with a 22% reduction in cardiovascular disease-related death, heart attack, and stroke over almost 5 years of follow-up (46). We speculate, based on the degree of change reported in previous studies (35), that the improvements in $VO_2$peak and blood pressure were mainly due to HIIT, and that the ~20% improvement in insulin sensitivity was a product of both HIIT and RET, but this is impossible to determine based on our study design. More important is the fact that we

were able to elicit simultaneous improvements in cardiometabolic health and muscular strength using a combined exercise strategy and healthy older men.

Isotonic muscle strength improved by 20-30% for all exercises following high load (70-80% 1RM) resistance training, which is in line with the results of several meta-analyses in older adults (34, 47-49). Contrary to previous work, however (33), as well as our hypotheses, lean body mass did not increase following 12 weeks of combined RET + HIIT in healthy older men. Furthermore, fasting lipids and systemic inflammation were also unchanged. In a meta-analysis of 49 studies, Peterson et al. (33) demonstrated that older adults can expect to gain 1.1 kg of whole body lean mass (assessed using hydrodensitometry or dual-energy x-ray absorptiometry [DXA]) following ~21 weeks (pooled mean, range: 10-52 weeks) of training at 50-80% 1RM. Importantly, higher training volume and lower age were the most important determinants of the magnitude of lean mass gained. Our participants performed RET at a comparatively lower weekly training volume (6 sets of 4 exercises versus ~9 sets of ~8 exercises), which may not have been sufficient to induce a detectable change when measured using DXA. A more sensitive imaging technique such as magnetic resonance imaging (MRI) or computed tomography (CT), which directly measures muscle mass rather than estimates lean body mass based on bone and fat mass, may have revealed a significant increase in skeletal muscle mass with exercise training. In addition, our participants (73 ± 1 years, range: 65-90 years) were slightly older than participants in the meta-analysis studies (66 ± 7 years, range: 50-83 years), which also may have hindered skeletal muscle hypertrophy. The concurrent performance of HIIT may have also contributed to the lack of increase in lean

body mass following exercise training, due to an interference effect of aerobic-type training on strength and lean mass changes during RET (50, 51). However, not all studies support the training interference concept (52). Furthermore, strength gains in this study appeared to be unaffected by concurrent HIIT, lending more credence to the idea that low training volume and, potentially, sub-optimal imaging methodology played a greater role in our inability to detect an increase in lean body mass following RET + HIIT.

We observed modest yet significant improvements in certain measures of physical function following 12 weeks of RET + HIIT, with no difference between groups. When interpreting these data it is important to recall that – due to the stringent screening measures in this series of studies – our participants were quite healthy compared to the average community dwelling older adult, which may have tempered the degree of improvement that our exercise intervention stimulated. Improvements in physical function are often discussed in the context of the minimal clinically important difference (MCID), or the smallest change in a treatment outcome that would be meaningful to the patient/individual. The MCID of any given outcome does not necessarily align with statistically significant changes observed in research studies because clinically relevant changes that can impact the day-to-day lives of individuals may not achieve statistical significance if sample size is too small. Conversely, statistically significant changes in adequately powered studies may be too small to be of use to older adults. MCIDs for measurements of physical function have been reported to be: 20-30 m in frail older adults (53) and chronic heart failure patients (54) for the 6 min walk test; 0.8-1.2 s for the TUG in hip osteoarthritis patients (55); and 2-3 stands for the 30 s chair stand test in hip

osteoarthritis patients (55). Although the improvements we observed in Study 2 were similar to (+25 m for the 6 min walk test) or smaller than (0 stands for the 30 s chair stand test, and 0.31 s for the TUG) these previously reported MCIDs our participants began with a higher degree of function and, presumably, independence. For instance, the mean distance covered by our participants during the 6 min walk test was ~600 m compared to the ~300-350 m completed by impaired older adults (53, 54). Similarly, our participants completed 13 stands prior to training compared to the 10-11 stands completed by hip osteoarthritis patients (55). Therefore, even though the improvements in physical function we observed likely translate to a reduction in overall risk of falls/fractures and frailty, is difficult to quantify in these comparatively healthy older adults. Future studies should investigate the magnitude of improvement in physical function that can be elicited using a similar RET + HIIT program in more functionally impaired older adults.

### 5.4 The influence of a multi-ingredient nutritional intervention, with and without exercise training, on strength, lean mass, cardiometabolic health, and systemic inflammation in older men

Six weeks of twice daily consumption of a whey protein-based multi-ingredient nutritional supplement stimulated gains in strength and lean tissue mass roughly equivalent to one year's worth of age-related decline (Study 2), and simultaneously improved fasting lipid partitioning and reduced circulating markers of inflammation (Study 3) in the same group of healthy older men. Previous studies support the use of whey protein (56, 57), creatine (58), vitamin D/calcium (59, 60), and n-3 PUFA (61, 62)

as isolated ingredients, in the absence of exercise, to target specific negative health outcomes in older adults. Despite this, Studies 2 and 3 are the first to co-administer these 5 ingredients with the goal of alleviating a range of deleterious age-related physiological changes.

Only within the past 5-10 years have clinical researchers begun evaluating the potential of multi-ingredient nutritional interventions, with and without exercise training, to elicit superior improvements in lean mass and physical function in older individuals. Only two studies (14, 15) have employed a multi-ingredient nutritional intervention independent of exercise training. However, differences in study population, composition and dosage of the supplements administered, and outcome measures make it difficult to compare the findings of these two studies to those described in Studies 2 and 3 of this thesis. In malnourished hospitalized older adults (n=210, 55% women), Neelemaat and colleagues (14) observed a 50% reduction in patients who had a fall as well as a significant decrease in the total number of fall incidents after 12 weeks of consuming a supplement which provided 24 g of protein and 600 IU vitamin D (600 kcal) per day. No change in fat-free mass (assessed using bioelectrical impedance analysis) or measures of physical function (grip strength, timed walk, chair stands, tandem stand) were observed. Important criticisms of this study included a lack of placebo beverage in the control group, and the high degree of heterogeneity of the study participants, who were admitted to hospital for a wide range of reasons (i.e. acute infection, vascular disease, kidney insufficiency, and fractures, etc.). Furthermore, hospital length of stay was not reported even though it may have been relevant to the interpretation of the findings of the study.

Although the 12 week nutrition intervention began after discharge, participants randomized to the supplement group received a specialized hospital menu during their stay which provided additional 750 kcal and 30 g of protein more than the standard hospital menu. An additional 30 g of protein per day may have had a different impact on patients who remained several weeks in hospital compared to patients whose stays lasted several days. This in turn might have affected the ability of patients to respond to the 12 week intervention. In a separate study on sarcopenic older adults (n=302, 65% women), Bauer et al. (15) observed a 0.24 kg increase in appendicular lean mass (assessed using DXA) along with a significant decrease in the time taken to complete 5 chair stands in participants who consumed a supplement containing 20 g of leucine (Leu)-enriched whey protein (which provided a total of 3 g Leu) and 800 IU vitamin D twice per day compared to an isocaloric carbohydrate and fat-based control beverage for 13 weeks. A strength of this study (15) was that the supplement was consumed at breakfast and lunch in an attempt to increase per meal protein (and Leu) ingestion, thereby stimulating MPS and translating to detectable gains in lean/muscle mass. A drawback, however, is that the researchers did not assess muscular strength despite the central role that strength loss plays in the development and consequences of sarcopenia (3, 4). What is more, both Neelemaat et al. (14) and Bauer et al. (15) focused on ameliorating sarcopenic muscle and function losses but did not assess the effects of their respective multi-ingredient nutrition interventions on other aspects of aging such as glycemia, lipidemia or systemic inflammation, which are known to accompany and interact with sarcopenia.

The unique protein-based multi-ingredient nutritional supplement that we utilized in this thesis stimulated superior gains in whole body (+0.7 kg) and appendicular (+0.4 kg) lean mass within a shorter time span (6 versus 12-13 weeks) compared to the two studies discussed above (14, 15). In contrast to these studies, we did not observe any improvements in measures of physical function. We did, however, observe a 6% increase in isotonic muscular strength, and reduced circulating concentrations of triglycerides (TG), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α). The improvements in whole body and appendicular lean mass may be explained by the comparatively higher dose of whey protein and vitamin D provided to participants in Studies 2 and 3. Our participants consumed 30 g whey protein at each serving which likely allowed for greater postprandial stimulation of MPS compared to a bolus dose of 20 g or 24 g of protein (18), as well as a higher total protein intake per day. Doses of 700-1000 IU vitamin D per day have been shown to be most effective in the prevention of falls and fractures in older adults (63), suggesting that the 600 IU per day dose used by Neelemaat et al. (14) may have been sub-optimal. Observational studies support a positive association between vitamin D intake and muscular strength/mass (64, 65), but this remains to be verified with longitudinal studies in humans. Therefore, the hypothesis that the 1000 IU dose of vitamin D in our multi-ingredient supplement may have contributed to the superior gains in lean mass (and strength) compared to Neelemaat et al. (14) and Bauer et al. (15) is speculative.

Another significant contribution of Studies 2 and 3 is that exercise-mediated improvements in strength and glucose handling can be potentiated by the addition of

protein-based multi-ingredient nutrition supplementation. Furthermore, participants who consumed the supplements demonstrated a significant reduction in pro-inflammatory cytokine concentrations during exercise training, whereas no improvement was observed in the control group. Only 3 other studies (11, 12, 16) have investigated the potential of multi-ingredient nutritional supplementation (consisting of some permutation of whey protein, creatine, vitamin D/calcium, and/or n-3 PUFA) to enhance exercise adaptations in older adults, in comparison to a suitable protein-free control beverage. However, much like the exercise-independent studies discussed earlier in this section, our ability to compare our data to these works is limited due to differences in the study population, composition of the active supplement, primary outcomes, and nature of the exercise intervention.

Rondanelli et al. (11) provided either a supplement comprising 22 g whey protein, 8.9 g essential amino acids, and 100 IU vitamin D (in total, the supplement delivered 4 g Leu per drink, and participants ingested one drink per day), or an isocaloric carbohydrate-based control beverage, to sarcopenic older adults over the course of the 12 week resistance-based training program. The supplement group demonstrated a greater increase in fat-free mass (+1.7 kg, 4%) and hand grip strength (+3.7 kg, 22%), as well as a superior reduction in circulating c-reactive protein (CRP) concentrations (-0.63 mg/dL), following exercise training compared to the control group. The relative improvements in these outcomes are greater than what we report in this thesis despite the comparatively lower dose of protein and vitamin D, and the exclusion of creatine and n-3 PUFA, in the nutritional supplement. However, the individuals studied by Rondanelli et al. (11) were

older (~80 years), less muscular (pre-training fat-free mass: ~38 kg), and more functionally impaired at baseline compared to the participants we recruited for Studies 2 and 3, which may explain their heightened responsiveness to the intervention. Further, participants in by Rondanelli et al. (11) trained 5 days per week and thus received a greater exercise stimulus. As previously discussed, training volume is a significant determinant of RET-derived lean mass gains in older adults (33). In a separate study, Verreijen et al. (12) evaluated the effect of a Leu-enriched supplement which contained 20 g whey protein and 800 IU vitamin D (in total, the supplement delivered 2.8 g of Leu per drink, and participants interested 1-2 drinks per day), compared to a carbohydrate- and fat-based control beverage, in a group of obese older adults during 13 weeks of diet-induced weight-loss combined with RET. Participants in the supplement group gained 0.4 kg lean body mass (whereas participants in the control group lost 0.5 kg lean body mass), yet both groups improved muscular strength and physical function to a similar degree. Unlike the multi-ingredient nutritional supplement used in this thesis, the supplement used by Verreijen et al. (12) failed to enhance exercise-induced improvements in muscular strength. It did, however, allow for superior gains in lean body mass in the supplement versus control group. Although not explicitly stated, participants in the study were also sarcopenic (skeletal muscle mass index at baseline: ~7.8 $kg/m^2$) and they trained three days per week. Thus, the subjects in this study (12) were similar to those studied by Rondanelli et al. (11) and had lower pre-training muscularity combined with a greater training stimulus, which likely resulted in detectable increases in lean mass, in contrast to the results reported in this thesis. Lastly, Candow et al. (16) provided a

supplement containing 25 g protein and 9 g creatine, or a carbohydrate-based control beverage, three times per week to healthy older men over the course of 10 weeks of RET. Participants in supplement group gained 2.6 kg lean body mass and 10 kg upper body strength (bench press 1RM) more than the control group. Candow et al. (16) evaluated a similar, healthy population as Studies 2 and 3, but it is noteworthy that their exercise and nutrition intervention resulted in a net gain of 3.2 kg lean body mass in the supplement group. Considering that this is greater (almost 2-fold) than changes typically reported in older men following RET programs of similar duration with protein (66) or creatine (67) supplementation, these data are perhaps atypical and it may be prudent to interpret them with caution.

In summary, a handful of studies have reported on a similar combination of multi-ingredient nutritional supplements provided to older adults during exercise training. The ability of the supplements used in these studies to enhance exercise-induced improvements in strength was similar to the multi-ingredient supplement used in this thesis, however, the lean mass gains reported were generally superior. This may have been due to the sarcopenic and comparatively more impaired nature of participants in these studies and the training volumes/frequency employed. Future studies should evaluate effectiveness of our supplement – which contains a more optimal dose of whey protein and vitamin D, as well as additional ingredients known to alleviate other facets of sarcopenia – in a more mobility compromised and/or frail elderly population. A unique aspect of this thesis is its breadth, which allows us to comment on the influence our multi-ingredient nutrition supplement and exercise intervention on, not only muscular strength,

but glycemic control and systemic inflammation as well.

### 5.5 Conclusions

Effective means of mitigating sarcopenia as well as other age-associated negative health consequences, are essential as the global population of older adults continues to grow. Taken together, the series of studies that comprise this thesis supports the use of multi-factorial exercise and nutrition regimens (both independently as well as in conjunction with one another) towards achieving improvements in various aspects of health in older adults. This is particularly important in reducing the prevalence of chronic diseases such as type 2 diabetes and cardiovascular disease in the later stages of life.

In Study 1, we employed the novel heavy water method to measure the 48-hour MPS response to acute exercise in healthy older men. Since these data integrate postabsorptive and postprandial rates of MPS, as well as habitual physical activity during the period of measurement, they are arguably more predictive of chronic adaptations to exercise training compared to earlier infusion trials. This increases the relevance of our findings. It remains to be determined, however, whether HIIT is in fact a hypertrophic stimulus. Even so, Studies 2 and 3 provide meaningful proof-of-principle data supporting the efficacy of a combined RET + HIIT program as well as broad-spectrum nutritional supplementation, from the perspective of overall health in older men. Although the magnitude of improvement was substantial, the individuals included in these studies were relatively healthy older men, limiting our ability to generalize these findings to women as well as more frail/impaired older adults. Future work should evaluate the capacity of

similar multi-factorial exercise and nutrition interventions to improve health outcomes in more specialized populations.

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