SUPPLEMENT EFFECTS ON HYPERTROPHY AND STRENGTH

EFFECTS OF A MULTI-INGREDIENT SUPPLEMENT ON MUSCLE STRENGTH AND HYPERTROPHY IN YOUNG MEN AND WOMEN: A DOUBLE-BLINDED RANDOMIZED CONTROLLED TRIAL

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LAY ABSTRACT

Resistance exercise training (RET) is known to augment muscle size, a process known as hypertrophy. Several factors may affect hypertrophy, such as supplementation with protein and amino acids, increasing an individual's potential to maximize muscle hypertrophy beyond RET-induced gains. However, little is known regarding the effectiveness of multi-ingredient supplements and the synergistic effects they may exhibit on hypertrophy and strength gains. The present thesis shows the effect of a specific multi-ingredient supplement that contained: whey protein, creatine, calcium, vitamin D, and leucine, compared to a low-quality collagen-based supplement, on measures of hypertrophy and strength in young adults. Interestingly, the active supplement (SUPP) induced greater gains in lean body mass (LBM), type II muscle fibre CSA, and bicep CSA and thickness compared to the control (CON), but not strength. These findings provide insight into a novel formulation of ingredients on exercise-induced increases in hypertrophy in young adults.

ABSTRACT

Resistance exercise training (RET) is a well-known stimulus for muscle protein synthesis. Protein supplementation, in conjunction with RET, has been shown to yield greater accretion of lean body mass than RET alone. Few studies have compared two multi-ingredient, isonitrogenous supplements of differing quality protein. Therefore, the purpose of the current study was to determine whether there was an augmented effect of a high-quality whey protein multi-ingredient nutritional supplement on hypertrophy in young adults following a RET program. We hypothesized that the multi-ingredient supplement would induce hypertrophy to a greater extent than the control supplement in young adults. Twenty-six (13 male, 13 female) healthy young adults (22 ± 2 years [mean ± SD]) were randomly assigned to either the multi-ingredient nutritional supplement (SUPP, n=12: 20g whey protein, 2g leucine, 2.5g creatine monohydrate, 300mg calcium citrate, 1000IU vitamin D) or control beverage (CON, n=12: 20g collagen protein, 1.4g alanine, 0.6g glycine) groups, ingesting their respective supplements twice daily. Measurements were obtained prior to and after a 10-week linear RET program. Dual-energy X-ray absorptiometry (DXA), ultrasound, one-rep maximum (1RM), and biopsies from the vastus lateralis muscle were performed. A 2-way ANOVA (time by supplement) revealed significantly larger increases in lean body mass (LBM), as assessed via DXA, from the active supplement compared to the control (SUPP: +4.1 ± 1.3kg CON: +2.8 ± 1.7kg, p=0.004). We conclude that the consumption of a multi-ingredient nutritional supplement increased lean body mass to a greater extent than to that observed in the CON group in healthy young adults.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BF%	Body fat percentage
BIA	Bioelectrical impedance analysis
CON	Control group
CSA	Cross-sectional area
DIAAS	Digestible indispensable amino acid score
DXA	Dual energy x-ray absorptiometry
eIF4F	Eukaryotic initiation factor 4F
FFM	Fat-free mass
FM	Fat mass
GAQ	Get active questionnaire
GH	Growth hormone
IR	Insulin receptor
LBM	Lean body mass
LLM	Lean leg mass
MHCI	Myosin heavy chain I
MHCII	Myosin heavy chain II
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MRF	Myogenic regulatory factors
MRI	Magnetic resonance imaging
MT	Muscle thickness
mTOR	Mechanistic target of rapamycin
mTORC1	Mechanistic target of rapamycin protein complex I
P70S6K1	Protein of 70 kDa S6 kinase I
PCr	Phosphocreatine
PDCAAS	Protein digestibility corrected amino acid score
RET	Resistance exercise training
SUPP	Supplement group
TBW	Total body water
VDR	Vitamin D receptor
1RM	1 Repetition Max
4EBP1	4E-binding protein-1

DECLARATION OF ACADEMIC ACHIEVEMENT AND FUNDING

This thesis is prepared in the standard format as outlined in the School of Graduate Studies Guide for the Preparation of Theses. It includes a general introduction, detailed methods, results, and a general discussion. M. Wageh is the principal contributor for conceptualizing the research question, research hypothesis, experimental design, data collection, data analysis and data interpretation. G. Parise and S. Phillips assisted with research question, research hypothesis, experimental design, and data interpretation. S. Fortino assisted with data collection and data analysis. This work was supported by the Natural Sciences and Engineering Research Council of Canada. MW received an NSERC Canada Graduate Scholarship (CGS) during the course of this study.

CHAPTER 1: INTRODUCTION

1.1. Resistance exercise training and human skeletal muscle

Resistance exercise training (RET) is a principal stimulator of hypertrophy, known for its benefits in enhancing skeletal muscle health in several populations (1). Skeletal muscle adaptations are crucial structurally and functionally for the increase and maintenance of muscle mass, improving lifespan, independence, and even "healthspan", or the proportion of one's life in which they remain healthy (2–4). Skeletal muscle hypertrophy is the increase in muscle mass or muscle cross-sectional area (5), leading to increases in muscle strength and power, especially in previously untrained subjects (6). The majority of strength gains in RET have been purported to be derived from neural adaptations (7), later switching to more morphological adaptations (8). Many factors moderate the hypertrophic response to a RET program, such as sex, genetic background, age, specific training variables, or nutrition (9, 10). The mechanism of hypertrophy itself begins with a stressful stimulus, such as resistance training, causing perturbations in myofibers and the extra-cellular matrix (11). This is mechano-chemically transduced into a molecular and cellular response through initiation of a cascade of myogenic events that lead to an increase in sarcomeres and myofibrillar contractile proteins in muscle tissue (5, 11, 12).

Hypertrophy may be mediated by several factors such as mechanical stress, acting through myogenic pathways that ultimately lead to an increase in the size of muscle fibres and subsequently whole muscle. Satellite cells, or myogenic stem cells located between the sarcolemma and basal lamina in muscle cells (13), are quiescent cells that when

activated, proliferate and eventually differentiate and fuse to existing muscle fibres. This process is regulated by myogenic regulatory factors (MRF), which further aid in muscle repair, regeneration and growth (14). Another main pathway involved in muscle growth is the mechanistic target of rapamycin (mTOR) pathway, and activation of the mTOR complex 1 (mTORC1) inducing phosphorylation of 4E Binding Protein 1 (4EBP1), which regulates translation through promotion of eukaryotic initiation factor 4F (eIF4F) assembly (15), and P70 ribosomal s6 kinase 1 (p70S6K1), which regulates several substrates involved in initiation and elongation of protein synthesis (16, 17). Furthermore, it has been postulated that the hormonal response to RET may aid in potentiating skeletal muscle mass gains, through specific hormones such as growth hormone (GH) and testosterone (18). It is important to note, however, that systemic hormone concentrations are not related to RET-induced muscle hypertrophy, but rather that androgen receptor content is positively correlated with hypertrophy, as revealed by a recent investigation from Morton et al. (19). Overall, this network of systems works synchronously to induce a muscle synthetic response that ultimately leads to muscle protein accretion in humans.

1.2. Supplementation

Nutritional supplementation is an effective method for enhancing muscle size and strength gains above and beyond RET alone. Supplementation in the form of whey protein, creatine and amino acids have all been shown to be potent stimuli for muscle hypertrophy, especially when combined with resistance exercise (20–23). Independently, supplementation demonstrates a small effect (24), but may offer greater benefits to specific populations, such as those looking to slow the progression of sarcopenia, or the

degenerative loss of skeletal muscle mass (25). Literature is limited in young adults with respect to the effect of supplementation alone on muscle growth parameters, but there is evidence to suggest that the ingestion of amino acids improves muscle protein synthesis (MPS) rates in younger populations (21), however less is known of its hypertrophic effects long-term.

Supplement dose is important in determining the amount of protein to ingest in supplementation. A recent meta-analysis by Morton et al. (26) concluded that total protein intakes of 1.62g/kg/day were sufficient to augment RET-induced gains in LBM. Furthermore, a review by Stokes et al. (2) conclude that ~20g of high-quality protein, or 0.3-0.4 g protein/kg/meal is sufficient to optimize MPS rates after ingestion, and that these intakes of protein should be no less than 3 hours apart from each other and other protein-rich meals throughout the day. It is important to note, however, that to maintain constant net protein balance, ingesting 0.3g/kg/meal must occur several times each day, which may be difficult to implement. In a 90kg adult, for example, this would mean ~146g of protein per day according to Morton et al. (26), in doses of about 27g per meal as per Stokes et al. (2). The feasibility of this method is questionable as frequent protein ingestion may be difficult to maintain with aging and increasing protein requirements, and thus further investigation as to the possibility of protein consumption to this degree is required.

Supplement ingestion prior to sleep is a widely-discussed method of supplementation as it is able to concurrently stimulate MPS post-RET and is also able to be properly digested and absorbed during sleep (27–29). Research indicates that intra-

gastric protein ingestion during sleep as well as oral protein ingestion prior to sleep induce overnight stimulation of MPS, improving net protein balance (27, 28). More specifically, MPS rates have been shown to be ~22% higher overnight when ingesting a protein supplement before sleep (28). A study by Snijders et al. (29) tested the impact of a 27.5g bolus of casein protein before bed to young men involved in a RET program for 12 weeks and found greater increases in muscle strength and type II muscle fibre size compared to the placebo. Interestingly, resting metabolic rate, lipolysis and fat oxidation have been shown to be elevated overnight with pre-sleep protein consumption, a finding that may also support fat loss with protein ingestion (30, 31). Despite the positive effects reported on pre-sleep protein ingestion, the ingestion of a large quantity of nutrients in the evening is a recurring topic of concern, especially as it has previously been associated with adverse health outcomes such as weight gain or possible cardiometabolic diseases (32–34). However, recent studies have shown that the ingestion of small, nutrient-dense foods such as a protein supplement is not detrimental to an individual's health (27, 31, 35). Protein ingestion prior to sleep may thus prove to be an important strategy to maintaining or augmenting MPS rates, especially in low (~150 kcal) caloric amounts (36, 37).

1.2.1. Whey Protein

Whey protein is a particular type of protein supplement proven to be very effective in increasing muscle mass. It is one of two main bovine milk proteins (38), the other being casein, and typically also contains a higher amount of essential amino acids (EAAs) (45-55g/100g of protein; (39)). It is especially high in the amino acid leucine (12-16g/100g

protein; (40)) than other sources of protein such as casein and soy. The significance of this can be seen in a study by Tang et al. (41) where whey protein was able to promote a larger MPS response during the first three hours post-ingestion after resistance exercise in healthy young males than either casein or soy protein.

Whey protein supplementation is typically available in the form of whey protein concentrates (above 80% protein) or whey protein isolates (above 90% protein) (38). Whey exhibits rapid absorption kinetics, such that blood amino acid peak and stimulation of MPS is much higher than observed with the ingestion of other protein sources (42–44). Studies estimate the absorption rate of whey to be ~10g every hour (45), which seems beneficial as it allows for greater amounts to be ingested over time. However, there is existing literature that challenges the rapid absorption of protein, indicating that it may not be as beneficial compared to protein sources that are absorbed slowly such as casein, which also increases protein synthesis but is able to more strongly inhibit proteolysis (46). Despite this, several studies have indicated a greater anabolic effect both at rest and after RET using whey as compared to other protein sources with slower digestion kinetics (41, 47, 48). A study by Yang et al. (49) detected lower MPS rates using soy as compared to whey protein under both rested and post-exercise conditions. Additionally, Tang et al. (41) showed that whey protein independent of RET was able to induce ~93% higher MPS rates compared to casein and ~18% higher compared to soy protein, and with RET induced ~122% higher MPS rates than casein and ~31% higher MPS than soy. Whey protein thus appears to be effective at stimulating MPS, at least in part due to its digestibility, and subsequently is able to induce greater gains in muscle mass. Whey

protein has also been shown to augment the phosphorylation of p70S6K, leading to increased MPS (50). Therefore, whey protein is an extremely important and effective supplement that is proven to be useful to enhance muscle hypertrophy in human skeletal muscle.

Protein quality is an important factor in the ability of a protein source to contribute to muscle protein accretion and mitigating muscle protein breakdown. Typically, protein sources may be evaluated according to the protein digestibility corrected amino acid score (PDCAAS) or the digestible indispensable amino acid score (DIAAS), which derive their scores from the fecal digestibility of amino acids and ileal digestibility of amino acids, respectively (51, 52). DIAAS scoring is a newer method and is considered to be a better method of evaluating protein quality, as it more accurately reflects protein metabolism before colonic amino acid metabolism, unlike the PDCAAS (51). Both methods are only able to score complete protein sources, or protein sources that contain all essential amino acids (EAAs), such that any incomplete protein lacking one or more EAAs results in a score of 0. Whey protein achieves a score of 1.09 in the DIAAS, classifying it as a high-quality protein (52).

Collagen protein, on the other hand, is considered low-quality due to its lack of tryptophan, an essential amino acid (EAA), giving it both a PDCAAS and DIAAS score of 0. Collagen has been claimed to benefit skin, hair and nail health (53–56), as well as aid in the relief of joint pain (57), but there has been considerable debate on its effectiveness in supplementation to augment muscle health. Certain investigations proclaim significant benefits of collagen protein, such as an increase in fat-free mass and

handgrip strength (58), and even preservation of LBM in older women (59), however this has been questioned (60) and further contested in other studies (61, 62). A study by Zdzieblik et al. (63) reported a considerable (4.2kg) increase in fat-free mass (FFM), which is truly unusual in that collagen protein contains approximately 0.8g leucine per 30g collagen (64) and was still able to induce larger increases than studies conducted using whey protein that detected increases in FFM of about 0.30kg, despite whey protein consisting of approximately 4.3g leucine per 30g of whey (65). Furthermore, a study by Impey et al. (61) administered either 22g of collagen or whey protein prior to, during, and immediately following exercise, and found that whey protein significantly increased p70S6K1, an important downstream target of mTOR, in the whey condition as compared to the collagen condition. More recent studies by Oikawa et al. (65) detected an increase in lean leg mass (LLM) with whey supplementation compared to collagen. Thus, collagen protein is considered a low-quality protein source, the effects of which remain controversial although unlikely to effectively stimulate MPS and subsequently induce hypertrophy in healthy adults.

1.2.2. Amino Acids

Protein is composed of amino acids. There are 20 amino acids, of which 11 are non-essential, meaning they can be formed in the body, and 9 of which are essential and must be obtained through the diet. Of these EAAs, three are branched-chain amino acids (BCAAs), which are a group of indispensable amino acids popular in the supplement market, and known to exert anabolic effects when ingested (66). Arguably the most important of these three amino acids is leucine, a critical amino acid for increasing

muscle protein synthesis and suppressing muscle protein breakdown (40). Leucine is also a key amino acid in whey protein, argued to be the primary agonist of MPS stimulation in response to hyperaminoacidemia (2, 67, 68).

Leucine is unique amongst amino acids in its ability to independently stimulate muscle protein synthesis through activation of the mTOR pathway (69). In a study by Atherton et al. (69), C2C12 (immortalized mouse cell line) myotubes were stimulated with 2mM of each individual amino acid and leucine was found to be unique in its capacity to stimulate mTOR, 4EBP1, p70s6K1, and RPS6 signaling activity. This is significant in that mTORC1, p70S6K1 and RPS6 regulate the translation initiation pathway of MPS (69, 70). Leucine has also been shown to suppress muscle protein degradation, as is shown in a study by Nair et al. (71), where leucine infusion decreased valine and phenylalanine release in healthy young men. Other amino acids confer benefits to muscle protein synthesis through specific signaling pathways. All other essential amino acids have been shown to increase p70S6K1 and RPS6 as well in response to their stimulation of C2C12 cells, albeit to a lesser (yet still significant) degree (69).

Animal model and cell culture studies further demonstrate that only leucine is required to induce protein synthesis (72, 73). An investigation examining at BCAA incorporation of ^{14}C into muscle proteins discovered that leucine alone increased ^{14}C incorporation into proteins by 25% (72). Additionally, leucine has been shown to suppress muscle protein degradation (71, 72). Louard et al. (74) administrated BCAAs via infusion in 10 healthy young adults, using phenylalanine as a tracer and observed a decrease in systemic levels of phenylalanine, suggesting diminished amounts of

proteolysis. Together, these data suggest that leucine is an effective amino acid able to act independently to aid in muscle building and suppressing muscle breakdown.

While whey protein has demonstrated considerable anabolic response to feeding, leucine content may be the primary driver to this response (75). A study conducted by Devries et al. (75) compared the response to either 25g WPI (contains 3g of leucine) to 10g milk protein (also 3g of leucine) for 6 days with unilateral RET, and detected that the lower protein, leucine-matched supplement was able to induce comparable increases in myofibrillar protein synthesis in healthy older women. Another study examining different doses of leucine compared to 25g of whey protein found that regardless of the amount of protein included in the supplement, a high leucine content (~ 5.0g) was able to stimulate similar increases in MPS rates as a high-protein supplement (76). Thus, it can be concluded that low quality protein enriched with leucine has the ability to induce MPS to a similar degree as higher quality protein sources (76).

1.2.3. Creatine

Creatine monohydrate is a popular supplement known for its ability to elevate high energy phosphate re-synthesis in skeletal muscle (77). Over 90% of creatine is stored in skeletal muscle, and has the potential to increase intramuscular water content, which is an indicator of anabolic proliferative signaling in skeletal muscle (78, 79). It is predominantly taken up by skeletal muscle and is enzymatically coupled with phosphate to form phosphocreatine (PCr), which is then able to maintain adenosine triphosphate levels to be used to promote greater work capacity, especially in high-intensity exercise (80, 81). Creatine is also purported to increase skeletal muscle mass through upregulation

of mRNA and protein content of kinases involved in satellite cell proliferation and differentiation (82). Previous work has demonstrated that supplementation with creatine results in an increase in lean tissue mass by an average of 1.37kg in older adults following resistance training for at least 6 weeks (80). In young adults, an increase in satellite cell number and muscle fibre size (83), myosin heavy chain mRNA expression (84), and even decreases in serum levels of myostatin have been reported (85). The primary mechanisms by which creatine supplementation augments muscle hypertrophy involves the simultaneous inhibition of myostatin and activation of myogenic regulatory factors (MRFs), which increases satellite cell activation, leading to increased transcription and subsequently muscle hypertrophy (84, 86). One trial investigating 6g/day of creatine supplementation with 12 weeks of RET detected higher levels of mRNA and protein levels of myogenin and MRF4 (84). Another study looked at 0.3g/kg/day of creatine supplementation during 8 weeks of RET, observing a ~17% decrease in myostatin levels in young men (85). Creatine supplementation has been shown to decrease oxidative stress, meaning that creatine may provide a protective mechanism against mitochondrial DNA damage and subsequently the production of reactive oxygen species (87, 88). Creatine also acts to increase IGF-1 mRNA levels, which works to activate the mTOR pathway that leads to increased protein translation and, in turn, muscle hypertrophy (89). Finally, the creatine-induced increase in PCr that allows for the increase in exercise capacity is able to augment the increase in muscle gains simply due to the increased volume able to be achieved in a bout of exercise training (81). Studies on creatine and

RET also suggest an increase in strength alongside hypertrophy of about 8% in average muscle strength and 14% in weightlifting performance compared to a placebo (90).

Creatine is consumed in the diet from protein sources such as beef, pork and fish (91) and contrary to historical concerns (92) creatine supplementation does not adversely affect kidney and liver function. This is supported by recent data from a randomized controlled trial in humans that directly examined kidney function following creatine ingestion in older adults (93). In younger adults, few case studies have reported adverse effects of creatine supplementation with resistance exercise (94, 95). These studies reported tubular necrosis and interstitial nephritis associated with creatine supplement ingestion, however in both cases the patients fully recovered after ceasing to ingest the supplementation (94, 95). Investigations on both short-term (96) and long-term (97) creatine ingestion report no detrimental effect of creatine supplementation on renal responses in healthy young men.

1.2.4. Calcium Citrate

Dietary calcium is a supplement typically identified as the fundamental nutritional promoter of bone health, however it also has several other biological benefits to the human body, including the regulation of vascular function, water balance, metabolism, nutrient transport, and most notably muscle contraction (98). Current calcium requirements in Canada are approximately 1000mg/d in adults aged 19-50 (99). Requirements may be higher in athletes, as existing literature indicates that regular exercise reduces gastrointestinal absorption, increased sweat (and electrolyte) loss, and increased need for tissue maintenance and repair (100, 101), requiring replenishment of

calcium to offset the losses incurred and prevent deficiencies. The importance of calcium is highlighted in cases of deficiency in humans, whereby energy, muscle protein, and vitamin D levels are negatively affected by insufficient calcium intake, leading to deteriorating bone health and overall muscle weakness (100).

A substantial issue that has long existed with respect to calcium intake is the notion that increasing protein intake is associated with increased calcium loss through urine (102, 103). This is problematic when there is a paradoxical need for increased protein to reinforce muscle protein synthesis, especially in older adults (104). The mechanism typically associated with this issue is the increase in glomerular filtration rate and production of acid, consequently increasing urinary calcium excretion (102, 105). Despite this, meta-analyses investigating the impact of dietary protein on bone health indicate no adverse effects of either soy or animal proteins on measures of bone health such as fracture rate, bone and bone mineral density (102, 106, 107).

Calcium citrate is one of the most bioavailable (108) and one of the most commercially available (98) sources of calcium, able to be ingested on an empty stomach (109). The main limitation of calcium citrate, compared to calcium carbonate or calcium lactate for example, is its absorption rate; calcium citrate contains 21% calcium and is approximately 27-33% absorbed while calcium carbonate contains 40% calcium and is 36-42% absorbed (109). Standard calcium dosage as a nutritional intervention to augment RET falls between 200-1200mg daily (110–112), and is typically combined with vitamin D supplementation to ensure optimal absorption (110, 113–116). In a study by Pfeifer et al. (113), 200mg of calcium citrate (as part of a multi-ingredient supplement) resulted in

an improvement of 8% in quadriceps strength after 12 months of supplementation in older men. Literature is sparse as to the effect of calcium supplementation alone on muscle, however several mechanisms exist as to calcium's purported effect on hypertrophy. Calcineurin (Cn), a calcium-regulated phosphatase, mediates myocyte enhancing factor 2, GATA transcription factors, and nuclear factor of activated T cells (117), linked to muscle fibre hypertrophy and subsequent muscle growth (118, 119). Overall, there appears to be evidence to indicate that calcium does have an effect on muscle parameters, although small and likely of a predominantly permissive role.

1.2.5. Vitamin D

Vitamin D is an essential part of human metabolism, obtained in two main forms: Vitamin D2 (ergocalciferol), which originates from plant sources, or Vitamin D3 (cholecalciferol), which comes from animal sources and sunlight exposure (120). Both forms are converted to the biologically active form, calcitriol ($1,25[OH]_2D$), whose functions are mediated upon ligand binding to the ubiquitously expressed vitamin D receptors (VDRs) (120). Production of vitamin D is affected by skin colour, amount of sun exposure, latitude and altitude, season, time of day, use of sunscreen, type and amount of clothing (120). Vitamin D is a regulator of approximately 1000 genes involved in cell growth and protein synthesis (121), which equates to approximately 5% of the entire protein encoding genome (122), rendering it an important micronutrient for vital bodily function. Both forms of vitamin D are metabolized similarly and have similar functions in the body, despite their difference in structure (120). It should be noted, however, that similar functions in the body do not equate to similar phenotypical changes

such as strength. Evidence suggests that vitamin D is of great importance to the human body, yet vitamin D deficiency is prevalent worldwide (122–124). The general consensus on vitamin D deficiency appears to be a serum level of less than 20ng/ml (122).

Vitamin D provides several benefits, including bone health, immune function, inflammatory modulation, cardiovascular health, and importantly muscle mass and strength (125–128). As previously mentioned, the importance of Vitamin D in bone health is primarily linked to its ability to increase calcium absorption, leading to preservation of bone mass and reduction of fracture risk, as well as some small but still important positive effects on muscle mass (126, 127, 129, 130). Previous literature has shown that vitamin D is able to improve bone health independent of calcium as well, through the induction of mechanical stress via muscle that may further improve bone mass (131, 132). Outside of bone health, recent evidence on vitamin D supplementation has shown an unambiguous positive relation in muscle health (133). Muscle pain, weakness, and diminished functional measures of muscle have all been linked to vitamin D deficiencies (131, 134–137). A serum 25-OHD level below 20 ng/ml predicted lower physical performance scores and subsequent declines in physical performance after three years in a study conducted in older adults (137). In younger adults, a study by Gilsanz et al. (138) identified the relation between muscle adiposity, vitamin D and muscle strength, indicating that muscle adiposity influences muscle strength, and this relationship may be moderated by vitamin D status. Younger women who were found to be deficient in vitamin D had approximately 24% greater muscle fat infiltration (138), which may explain their subsequent decrease in muscle power, force, velocity and jump height (139).

The recommended dietary allowance of vitamin D is 600IU (or 15mcg) daily (99). Most supplementation studies administer a range of 400-2000IU daily, which still falls below the tolerable upper intake level per day (99) and is thus considered safe. One outlier of this, however, is a study conducted by Gupta et al. (114), where young adults were administered 60,000IU Vitamin D per week (~8,500IU per day) for 8 weeks then 60,000IU per month (~2000IU per day) for 4 months, detecting significant increases in handgrip strength, gastro-soleus and pinch grip strength, and six-minute walk test distance. While these increases are significant and clearly indicate a benefit to vitamin D supplementation, they also present an issue with feasibility in the level of intake daily. Other studies maintaining a 2000IU/day intake also found positive effects; Carrillo et al. (140) detected an increase in peak power of a seated row exercise, as well as reduced waist-to-hip ratio, however no significant differences were detected for lean body mass or fat mass (FM) after 12 weeks. Wyon et al. (129) administered 2000IU/day to elite classical ballet dancers for 4 months and found significant increases in isometric strength of the quadriceps as well as vertical jump height. An informative study looking at vitamin D with or without calcium and with or without exercise concluded that vitamin D supplementation was important in improving gait speed and body sway, more so in the vitamin D and calcium group, and that exercise training was the true stimulator of increases in muscle strength (141). A recent meta-analysis by Beaudart et al. (133) detected a small but positive significant effect of vitamin D supplementation on muscle strength in younger and older populations. These effects were more pronounced in the lower body, which may provide further support for the conceptual premise of fall risk in

older adults mediated by quadriceps strength (142). It also appears to be the case that the effect of vitamin D on younger adult muscle strength and hypertrophy is quite limited. This may be due to a lack of deficiency, especially in younger men (143). The increase in muscle strength but not lean body mass with vitamin D supplementation may also indicate specificity in pathways related to calcium signaling and muscle contractility. The implications of these findings are important for not only muscle strength, but also functional mobility and quality of life in the transition to older adulthood.

The mechanisms by which vitamin D acts on muscle are linked to several metabolic factors. Muscle cell calcium uptake appears to be influenced by vitamin D intake (144, 145). Preliminary studies on myoblast and myocyte calcium metabolism report an increase in cell calcium uptake up to 140% higher than baseline over 24 hours (144, 146). Furthermore, animal studies in vitamin D-deficient chicks revealed increases in calcium transport by sarcoplasmic reticulum as well as an increase in mitochondrial calcium after vitamin D treatment for 2 weeks (145). VDRs have also been identified on human muscle fibers and have been shown to translocate from the nucleus to the plasma upon Vitamin D3 administration, as well as form a complex with TRCP3, the protein involved with calcium entry into the muscle, increasing contractility (147). A decrease in VDR with age has been identified (148), however it remains unknown whether this decrease is mediated by the lack of physical activity and calcium reaching the receptors with age, decreasing its need for functionality, or vice versa. Another proposed mechanism involves insulin signaling, whereby optimal vitamin D intake may reduce insulin resistance (132, 149). The proposed effects of vitamin D on insulin sensitivity are

twofold; increased vitamin D levels reduces the presence of inflammatory cytokines released from adipose tissue, which are associated with both obesity and insulin resistance (149). Other studies are in agreement with this theory – Vitamin D is known for its ability to dose-dependently suppress TNF-a and IL-6 inflammatory cytokines and subsequently increase anti-inflammatory IL-10 production (150–152). Secondly, vitamin D has been suggested to activate transcription of the human insulin receptor (IR) gene, stimulating an increase in glucose oxidation and transport, leading to improvement in insulin regulation pathways in the body (153–156). This could have further implications for insulin signaling in the mTOR pathway and the initiation of muscle protein synthesis.

1.2.6. Compound and multi-ingredient supplementation

Compound, multi-ingredient supplements are proposed to have a larger effect on muscle hypertrophy than individual components, due to their variety of nutritional benefits that may promote or allow for increased gains in skeletal muscle mass and strength. The effect of this combination of ingredients may arise as a result of synergetic effects, producing a total effect greater than the sum of individual elements. One particular example of this comes from vitamin D and calcium supplementation. Vitamin D and calcium citrate have been shown to enhance increases in muscle strength and size, albeit in a permissive rather than stimulatory manner in humans (114, 140, 157–161). Adults typically do not achieve adequate levels of vitamin D, which is imperative for calcium absorption, and thus supplementing with vitamin D may allow for the absorption of calcium and thus support skeletal muscle metabolism to efficiently build muscle (98). These two supplements are typically studied in conjunction with one another, as their

synergistic effects surpass their singular effects (113, 114, 157). They distinctly benefit one another; calcium helps build and maintain strong bones while vitamin D acts as the extender/amplifier of the ability to effectively absorb calcium, with its own independent capabilities in building muscle (120, 162).

Whey protein is one of the most common components in multi-ingredient supplements, along with creatine and vitamin D (163). Burke et al. (20) reported that males who supplemented with a combination of whey protein and creatine had greater increases in lean tissue mass and strength than those who supplemented with only whey protein or placebo (20). Furthermore, the combination of leucine and whey protein has been shown to promote significant increases in muscle protein synthesis and cross-sectional area of the *quadriceps femoris* via magnetic resonance imaging (MRI) (164–166). The co-ingestion of leucine has been shown to increase myofibrillar protein synthetic rates up to 19% more than whey protein alone following acute resistance exercise in older men (166).

Multi-ingredient supplements are commonly composed of protein with creatine or vitamin D (22, 163), and promote net gains in FFM and 1RM strength during prolonged resistance training (≥6 weeks) (163). A recent meta-analysis by O'Bryan et al (163) showed that the consumption of a multi-ingredient protein supplement augmented resistance training-induced benefits by, on average, 0.80kg of lean body mass and 4.22kg in lower body 1RM strength compared to non-multi-ingredient protein supplements. However, it should be noted that this meta-analysis found that multi-ingredient

supplementation was not to superior to protein-only supplementation in improving FFM and strength, but rather induced a greater increase in FM (163).

Differing combinations of multi-ingredient supplements have been shown to induce positive effects on body composition, and not limited to muscle (22, 167, 168). A study by Atherton et al. (169) tested the effects of 10g of protein (a mixture of whey, soy, and casein) with and without leucine in young and older men, and found an augmented rate of MPS and p70S6K1 phosphorylation. Similarly, Churchward-Venne et al. (76) tested different doses of leucine in conjunction with whey protein, ranging from 0.75-5.0g. The authors found that regardless of the protein content, MPS rates were elevated when supplemented with a high (5.0g) amount of leucine (76). The addition of vitamin D to whey protein and leucine indicates possible benefits to muscle mass and lower extremity function (170), as well as decreased chronic low-grade inflammation in older adults (168). A noteworthy study conducted by Kraemer et al. (112) utilized a supplement with several ingredients including whey protein, calcium, and creatine, as well as other macro- and micro-nutrients such as vitamins A, C, and B12, magnesium, zinc, green tea extract, and many more. This supplement, despite being tested on a sample size of only 9 young healthy males and for only one week, showed significant improvements in vertical jump power output and squat 1RM strength (112). This may be an important indicator of the synergistic effects of combined ingredients.

Like exercise, there are those that respond more robustly to individual nutritional supplements than others (171–176). Multi-ingredient supplementation may thus present a method of maximizing biological responses to supplementation (173). In addition,

previous investigations typically compared protein supplements to carbohydrate-based placebo counterparts (20, 177–185), and rarely compared two supplements with equivalent amounts of protein and amino acids (179, 183, 185, 186), or have combined multiple beneficial ingredients to form a multicomponent supplement to promote greater gains in lean body mass and strength (22, 187).

1.3. Supplementation and RET to enhance muscle hypertrophy and strength

RET, an anabolic stimulus on its own, is said to produce enhanced gains with the aid of supplementation. There is ongoing debate around the importance of supplementation with RET, with arguments suggesting a net gain in muscle mass acutely only with the aid of protein ingestion (188), while others report no such effects (3, 189–193). The increase in net muscle protein accretion depends on many factors, including population age, dosage of protein, and duration of the intervention (194). The training status of the individual may also affect gains in skeletal muscle mass, such that untrained individuals benefit more from training while trained individuals benefit more from supplementation (26). However, a recent meta-analysis by O'Bryan et al. (163) reported a greater effect of multi-ingredient supplements and RET on untrained participants compared to trained. This may be explained by the greater increase in skeletal muscle gains from RET rather than supplementation, such that the benefit of RET on untrained individuals overshadows the benefit of supplementation on trained individuals.

Data from supplement studies suggests that the consumption of protein and other specific ingredients (amino acids, creatine) may reduce the muscle protein breakdown (MPB)/catabolic response to muscle damage from exercise (195). Supplementation may

also improve recovery from muscle damage incurred during RET (196). Thus, supplementation in conjunction with RET may be important to optimize skeletal muscle adaptation following RET.

Several reviews and meta-analyses have confirmed the added benefit of protein supplementation to RET (26, 40, 194). Cermak et al. (194) found that supplementation with RET significantly augmented FFM, type I and II muscle fibre cross-sectional area (CSA), and 1RM leg press strength compared to RET alone. They detected a weighted mean increase of 0.69kg of FFM in older and younger adults, 212 μm^2 and 291μm^2, respectively, for type I and II CSA, and about 13.5kg in leg press 1RM (194). A meta-analysis by Morton et al. (26) found an average increase of 0.30kg in FFM, 310μm^2 in fibre CSA, and 2.49kg in 1RM strength in studies consisting of at least 6 weeks of RET. Studies examining whey protein pre- and post- exercise have been shown to also increase skeletal muscle hypertrophy in the *vastus lateralis* as measured by MRI in younger men (3), however the ingestion of the supplement preceded and followed RET, which may not be feasible in most populations due to possible restrictions in time or supplement availability.

The most recent meta-analysis on multi-ingredient protein supplements and RET comes from O'Bryan et al. (163), who showed an increase in FFM and strength with RET greater than 6 weeks. Leucine was said to be one of the main components of multi-ingredient supplementation and was found to be effective in stimulating MPS. Other studies, however, report that leucine on its own may not be as effective as with RET. This can be seen in studies in older men, where supplementation of 7.5g/day was not able to

increase muscle mass or strength (197). It is important to note however that this study did not include RET and was in an older population, who may be resistant to anabolic stimuli in some capacity (198). It is also important to note that leucine as an addition to or a part of multi-ingredient supplementation with RET is only effective when the original protein dose administered is "suboptimal", meaning in the form of either low whey protein doses or other sources of protein with less leucine content (199). Multi-ingredient supplements may be the cohesive link to optimizing the skeletal muscle response to RET, but further research is warranted on the synergistic effects of supplement ingredients on each other and their role in RET-activated pathways in skeletal muscle hypertrophy.

1.4. Study Objectives and Hypotheses

The primary aim of this study was to determine whether a multi-component nutritional supplement could augment the degree of hypertrophy and strength induced by RET in young male and female adults. To date, no study has investigated this specific set of ingredients and their synergistic effects on muscle hypertrophy and strength measures. Few studies have also compared two isoenergetic, isonitrogenous supplements in healthy young adults in conjunction with a 10-week RET program. Based on previous literature (20, 113, 170, 200, 201), we hypothesized that the combination of whey protein, leucine, creatine, calcium citrate and vitamin D, in addition to resistance training, would induce hypertrophy and strength increases to a larger degree than resistance training with an isonitrogenous control supplement in healthy young adults.

CHAPTER 2: METHODS

Participants. 26 (13 male, 13 female) recreationally active (defined as partaking in exercise no more than twice per week) young adults participated in this study. Participants were non-smokers, free of any acute or chronic illness and prescription/non-prescription medication except for birth control, as well as medications known to affect protein metabolism (i.e. corticosteroids, non-steroidal anti-inflammatory drugs), and any form of protein or creatine supplementation. Participants were classified as healthy and eligible for exercise according to the Get Active Questionnaire (GAQ). Of the 27 participants recruited, one dropped out as a result of injury sustained in a non-intervention related event, leaving us with n=13 per group. See **Figure 1** for flowchart of subject recruitment and group allocation. Our sample size calculation was based on previous multi-ingredient supplement studies that investigated lean body mass as their main outcome (22, 168, 169, 202). Participants were informed of the purpose, experimental procedure and risks of the study before providing written and verbal consent. The study was approved by the Hamilton Integrated Research Ethics Board (Reference No. 4449) and was registered at https://clinicaltrials.gov/ct2/show/NCT03525197?term=srct&rank=1, and conformed to the Declaration of Helsinki on research involving human subjects.

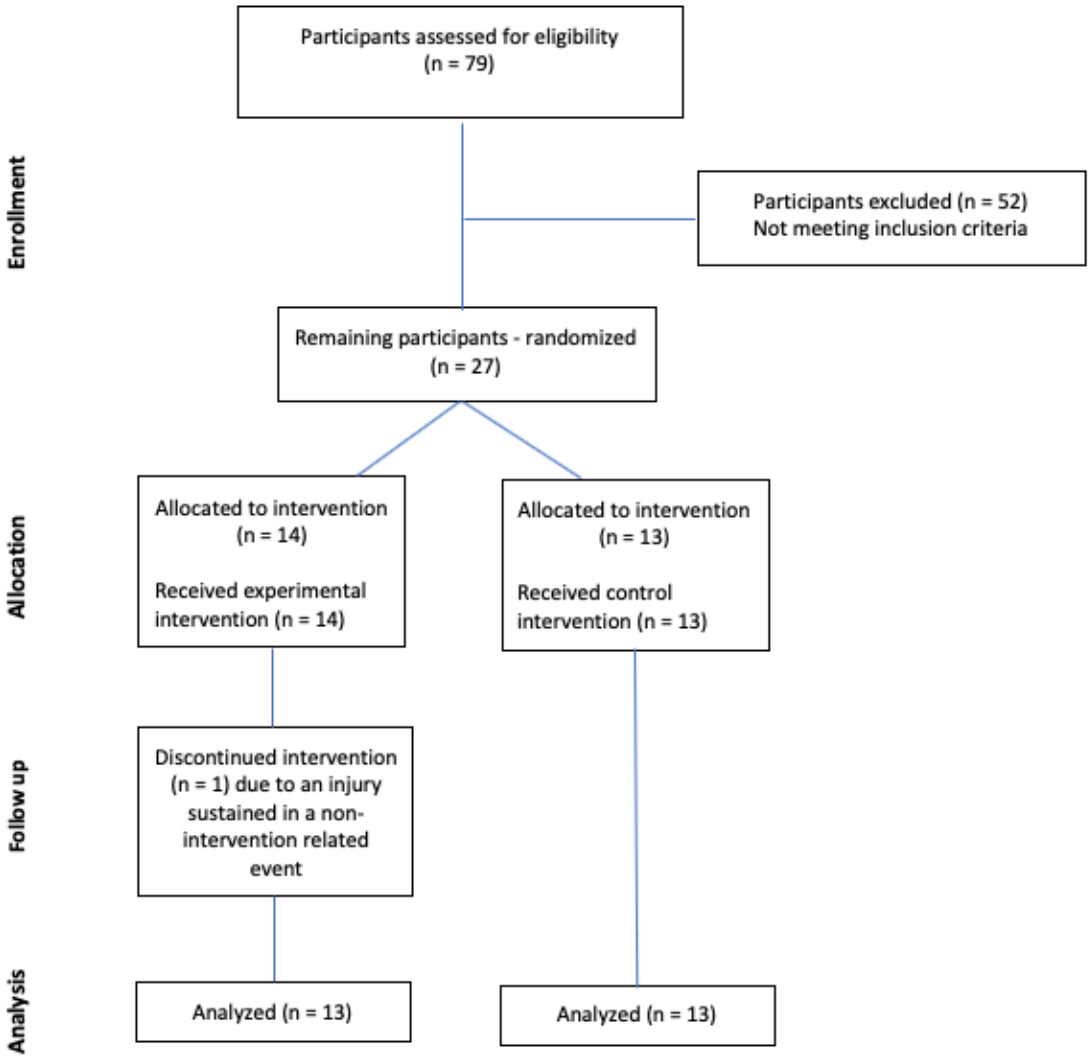

Figure 1. CONSORT flow diagram illustrating the movement of participants through the study, which was conducted between June 2018 and June 2019.

Study design. This study was a randomized, double-blind controlled study, with a between-subject and within-subject design. Participants were randomized to either ingest: the active supplement, consisting of whey protein isolate (20g, Fonterra), creatine monohydrate (2.5g), Leucine (2g, Ajinimoto), calcium citrate (300mg), and vitamin D (1000IU), (Whey; n=13), or the control supplement, consisting of collagen protein (20g,

BodyBalance), as well as non-essential amino acids alanine (1.4g, Ajinimoto) and glycine (0.6g, Ajinimoto) (Collagen/Control; n=13), twice daily in conjunction with a 10-week, linear resistance training program. See **Table 1** for the full list of ingredients in each supplement. Supplements were formulated by Infinit Nutrition (Windsor, ON, Canada), and were matched for flavour (citrus) as well as consistency in powdered form, to be dissolved into 250 mL of water. On training days, supplements were consumed following each training session and prior to sleep. On non-training days, supplements were consumed once in the morning and once prior to sleep. A schematic overview of the study design is depicted in **Figure 2**.

Table 1. Composition of active and control protein supplements.

Ingredient	**SUPP**	**CON**
Whey Protein Isolate	20 g	0g
Collagen	0 g	20g
Leucine	2 g	0 g
Creatine	2.5 g	0 g
Alanine	0	1.4 g
Glycine	0	0.6 g
Calcium Citrate	300 mg	0 g
Vitamin D	1000 IU	0 g

SUPP, active supplementation group; CON, control group.

Familiarization and 1RM strength testing. One week prior to training, participants attended exercise familiarization sessions and received supporting material to start the study (e.g. training schedules and testing schedules). 1RM strength tests were performed at baseline, as well as the midpoint (week 6) and post-training (week 12). The 1RM tests were performed in this order: isolated quadriceps strength (Biodex), leg press, bench

press and *latissimus dorsi* pull-down (Lat Pulldown), and followed the guidelines established by the National Strength and Conditioning Association (NSCA). Strength tests began with a 5-min warm-up on a cycle ergometer, followed by 5-7 repetitions at 50% of participants' predicted maximal strength. Participants rested for 3 minutes then attempted 1 repetition at 100% of their predicted 1RM load. Following another 3 minutes of seated rest, the 1RM load was modified by 10-20% until 1RM was achieved. Total 1RM strength was calculated as the maximal torque achieved (isolated quadriceps kick, Biodex), or the sum of the mass lifted (kilograms, kg) for 1RM of leg press, bench press and *latissimus dorsi* pull-down.

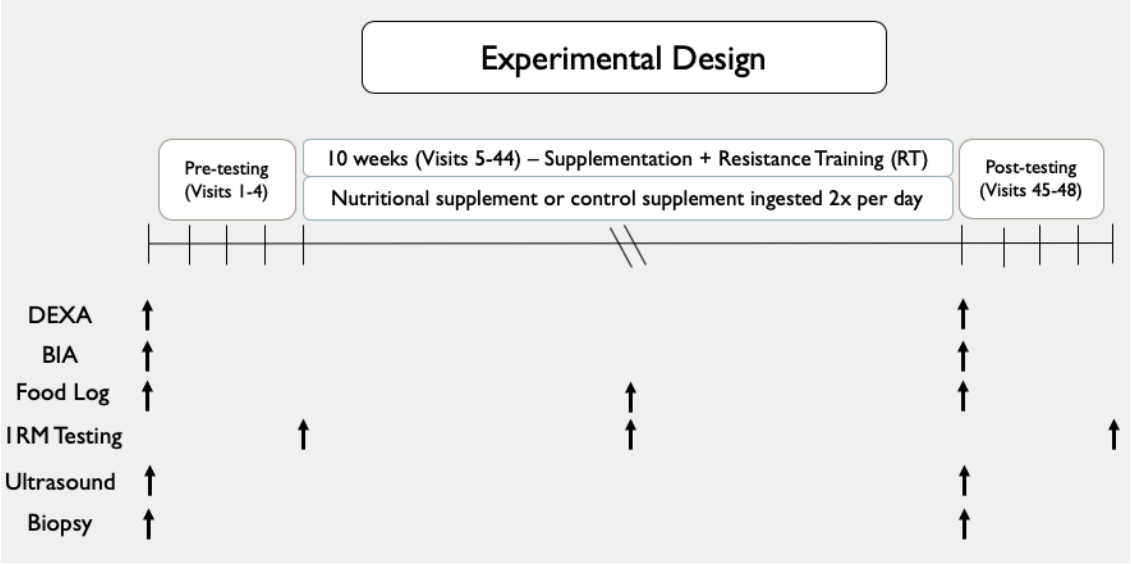

Figure 2. Schematic of study design.

Dietary records. Protein intake was assessed at weeks 0, 6, and 12, using a 3-day food diary (2 weekdays, 1 weekend day), and was analyzed using the NutriBase dietary analysis software (Nutribase11 Professional Edition, version 11.5, Cybersoft Inc., Phoenix, AZ, USA).

Resistance training intervention. Participants underwent a 10-week, linear resistance training program. Loads were increased (~5%) between weeks to ensure a progressive load. Each week consisted of four days of training, with alternating days (Monday, Thursday) focused on the lower body (squat, deadlifts, lunges, hamstring curl, leg press, calf raise, and leg extension), and the remaining two (Tuesday, Friday) focused on the upper body (bench press, standing military barbell press, incline dumbbell press, single-arm row, seated row, pronated and supinated *latissimus dorsi* pulldowns, and supinated incline bicep curls). See **Table 2** for complete RET protocol.

Table 2. RET Protocol, weeks 1-10.

RET Protocol			
Monday	Tuesday	Thursday	Friday
1. Barbell Back Squat 2. Walking Weighted Lunges 4. Leg Extension x Hamstring Curl 4. Close-stance Leg Press 5. Calf Raises	1. Barbell Bench Press 2. Standing Military Barbell Press 3. Incline Dumbbell Press 4. Skull Crushers x Assisted Dips 5. Close-grip press 6. Sit-ups	1. Deadlift 2. Leg press (neutral stance) 3. Single-Leg Press 4. Leg Extension x Hamstring Curl 5. Calf Raises	1. Pronated Lat Pulldown (wide) 2. Single-Arm Row (neutral grip) 3. Seated row (neutral grip) x Supinated Lat Pulldown 4. Supinated Incline Dumbbell Curls 5. Plank (30s)
Repetition, set schema	Repetition, set schema	Repetition, set schema	Repetition, set schema
5 sets: #1 4 sets: #2-4 3 sets: #5	4 sets: #1-3 3 sets: #4-6	5 sets: #1 4 sets: #2-4 3 sets: #5	4 sets: #1-2, 4 3 sets: #3,5
8-12 RM Loads: #1-3 15 RM Loads: #4-5	8-12 RM Loads	8-12 RM Loads	8-12 RM Loads
90s rest: #1-4 45s: rest: #5	60s	90s rest: #1-3 45s: rest: #4-5	60s rest
80% 1RM	80% 1RM	80% 1RM	80% 1RM
Total time = ~ 1hr	Total time = 50min	Total time = 1h	Total time = ~ 50min

Body composition. Body composition [LBM, FM, and body fat percentage (BF%)] was determined using a Lunar Prodigy dual-energy x-ray absorptiometry (DXA) apparatus (GE Lunar iDXA total body scanner, GE Medical Systems, Madison, WI, USA) as well as Bioelectrical Impedance Analysis (BIA; BioScan 920-II, Maltron International Ltd.,

Essex, UK) to determine total body water (TBW) between 06:00-08:00 following a >8 hour fast. Participants refrained from any exercise or alcohol consumption for >24 hours. TBW was assessed using a BIA apparatus (BIA-101A; RJL Systems, Mt. Ckemens, MI). All body composition assessments were performed by the same technician to minimize variability.

Ultrasound muscle thickness and cross-sectional area. Muscle thickness (MT) of the *vastus lateralis* and *biceps brachii* were assessed by the same investigator using a B-mode ultrasound (gE logiq e 2008, Wauwatosa, WI, USA) and a 50mm, 12.5 linear-array probe. Ultrasound testing was performed fasted and within the same hour as body composition testing. Participants laid supine for 20 minutes with their leg or arm (side randomized) in full extension in a custom mount for the limb. Thickness of the *vastus lateralis* was assessed at 50 percent of the distance between the greater trochanter and the lateral epicondyle of the femur, and, for the biceps, at 30 percent of the distance between the acromioclavicular joint and the centre of the antecubital fossa. Lines were drawn in permanent ink to record the reference point, and the probe was placed transversally on the leg or arm. Water-based gel was then applied to enable good transfer of acoustic energy between the transducer and the leg or arm. No pressure was applied to the skin to avoid any compression of muscle in the ultrasound video. A second investigator ensured the images were clear and void of any shadows or blurred lines, as well as that identifiable aponeuroses were visible, and the ultrasound video was encrypted and stored. To assess muscle CSA, ultrasound videos were converted to individual framed cross-sectional images of the *vastus lateralis* and *biceps brachii* and compiled into one panoramic image

using Autostitch (Version 2.2, http://matthewalunbrown.com/autostitch/autostitch.html). The stitched images were analyzed using ImageJ and manually traced to encompass the entire muscle. Muscle thickness of the *vastus lateralis* was determined from the border of the rectus femoris to the border of the biceps femoris, and for the *biceps brachii* was determined from the border of the brachialis to the border adjacent to the brachial artery and median nerve.

Muscle fiber type and cross-sectional area (CSA). Skeletal muscle biopsies were taken at baseline and post-training from the *vastus lateralis*. Participants arrived following an overnight fast and having refrained from resistance exercise for ≥72 hours. Muscle biopsies from the *vastus lateralis* were performed under local anesthesia (2% xylocaine) using a 5-mm Bergström needle. The muscle sample was cleared of any connective tissue or fat, and oriented longitudinally in an optimal cutting temperature medium, then frozen in liquid isopentane, cooled by liquid nitrogen and stored at -80°C for analysis. Cross-sectional muscle sections of 5 μm thickness were prepared from each sample using a Microm HM550 Cryostat (Thermo Fisher scientific, Waltham, MA, USA), mounted on glass slides and stored at -80°C. Immunofluorescent staining procedures were used to assess fiber type and CSA of each sample cut. Samples were fixed to the slide using 4% PFA, incubated overnight at 4˚C using primary antibodies for laminin (1:500), MHCI (1:1) and MHCII (1:1000) then incubated for 2-h at room temperature using secondary fluorescent antibodies for laminin (Alexa Fluor 488-R, 1:500), MHCI (Alexa Fluor 488-M, 1:500), and MHCII (Alexa Fluor 647-R, 1:500). Slides were mounted using Dako Fluorescence Mounting Medium (Dako, Burlington, ON, Canada) and imaged the next

day. Images were acquired from a Nikon Eclipse Ti microscope at 20x magnification and captured with a Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY). Images were captured and analyzed manually using the Nikon NIS Elements AR 3.0 software, with the same image capture settings for each subject (Nikon Instruments). Muscle fiber type was assessed by counting an average of ~400 fibers per subject per time point, and CSA was assessed through tracing the periphery of approximately 50 fibers each of fibre types I and II for each sample.

Statistical analyses. Baseline participant characteristics were assessed using independent t-tests. A 2-way analysis of variance (ANOVA) (time by group) was used to assess morphological body composition measures (LBM, FM, BF%), as well as fiber type distribution, fiber CSA, muscle thickness, and 1RM strength measures. Where significance was detected, a Tukey's HSD was performed. All analysis was performed using SPSS statistical package version 23 (IBM SPSS Statistics for Mac, Version 23.0, Armonk, NY), as well as GraphPad Prism (version 6.0, San Diego, CA). All graphs were also designed in GraphPad Prism. For all statistical analyses, significance was set to $p<0.05$. Values are presented as means ± standard deviation (SD) or using box and whisker plots.

CHAPTER 3: RESULTS

3.1. Participant characteristics.

27 healthy young adults were randomized: 26 completed the study and 1 dropped out from the SUPP group, due to injuries sustained outside of the study. Participants' baseline anthropometric characteristics are provided in **Table 3**. There were no significant differences between groups at baseline ($p>0.05$) except for age ($p=0.03$). There was no significant difference in dietary protein intake (g/kg/day) between groups at baseline or week 12 ($p>0.05$).

Table 3. Participant Characteristics.

	SUPP (n=13)		CON (n=13)		p (SUPP vs. CON)
	Male (n=7)	Female (n=6)	Male (n=6)	Female (n=7)	
Age, years	23 ± 1.8	23 ± 2.7	21 ± 2.1	21 ± 1.4	0.03
Height, m	1.8 ± 0.04	1.6 ± 0.10	1.8 ± 0.10	1.6 ± 0.05	0.77
BMI, kg/m^2	24 ± 1.9	24 ± 2.6	23 ± 3.4	23 ± 3.7	0.38
Body mass, kg	76 ± 3.3	63 ± 9.1	73 ± 8.7	62 ± 12.9	0.35
LBM, kg	56 ± 5.1	42 ± 7.6	52 ± 4.8	39 ± 6.0	0.44
FM, kg	17 ± 4.2	21 ± 5.3	17 ± 6.0	20 ± 9.2	0.86
BF%	24 ± 5.8	33 ± 5.9	24 ± 6.5	33 ± 9.4	0.98
Leg Press 1RM, kg†	4.1 ± 1.0	3.2 ± 0.5	3.3 ± 0.7	2.7 ± 0.7	0.06
Lat Pulldown 1RM, kg†	2.6 ± 0.4	2.4 ± 0.4	2.6 ± 0.3	2.2 ± 0.2	0.39
Bench press 1RM, kg†	1.3 ± 0.3	0.8 ± 0.2	1.1 ± 0.1	0.8 ± 0.2	0.27
Isometric unilateral leg extension 1RM, Nm*†	3.5 ± 1.0	2.9 ± 0.9	3.4 ± 1.0	2.5 ± 0.9	0.53

Values are mean±SD. SUPP, active supplementation group; CON, control group; BMI, body mass index; LBM, lean body mass; FM, fat mass; BF%, body fat %; 1RM, 1 repetition maximum. †Values normalized to lean body mass. *Note: Isometric unilateral leg press n=11 per group (SUPP, CON) due to technical issues.

3.2. Body composition.

No significant differences in TBW were detected in either group between measurement time points (SUPP: $p=0.58$; CON: $p=0.76$). A significant group-by-time effect was observed for LBM ($p<0.05$). In the SUPP group, LBM increased by 4.1 ± 1.3kg ($p<0.0001$), while in the CON group LBM increased by 2.8 ± 1.7kg ($p<0.0001$; **Figures 3A, 3B**). No differences were observed between groups for changes in fat mass (SUPP: -1.0 ± 1.1 kg, CON: -0.7 ± 1.5 kg; $p=0.33$; **Figures 3C, 3D**) or BF% (SUPP: -2.8 ± 1.4 kg, CON: -1.8 ± 1.7 kg; $p=0.099$; **Figures 3E, 3F**).

3.3. Maximal strength.

Maximal strength measures were significantly increased over time in both groups (see **Table 4**), but no significant differences were detected between conditions. Leg press strength increased by 1.9 ± 0.6 kg/kg LBM in the SUPP group, and by 2.0 ± 0.6 kg/kg LBM in the CON group ($p=0.69$). Lat Pulldown strength increased in the SUPP group by 0.5 ± 0.2 kg/kg LBM and in the CON group by 0.5 ± 0.3 kg/kg LBM ($p=0.92$). Bench Press strength increased by 0.2 ± 0.1 kg/kg LBM in both SUPP and CON groups ($p>0.99$), and Isometric Unilateral Leg Extension strength increased by 1.7 ± 1.3 Nm/kg LBM and 1.6 ± 0.8 Nm/kg LBM in the SUPP and CON groups, respectively ($p=0.65$). Technical issues with the isometric unilateral leg extension resulted in the inability to obtain values for 4 participants, resulting in 11 participants per group.

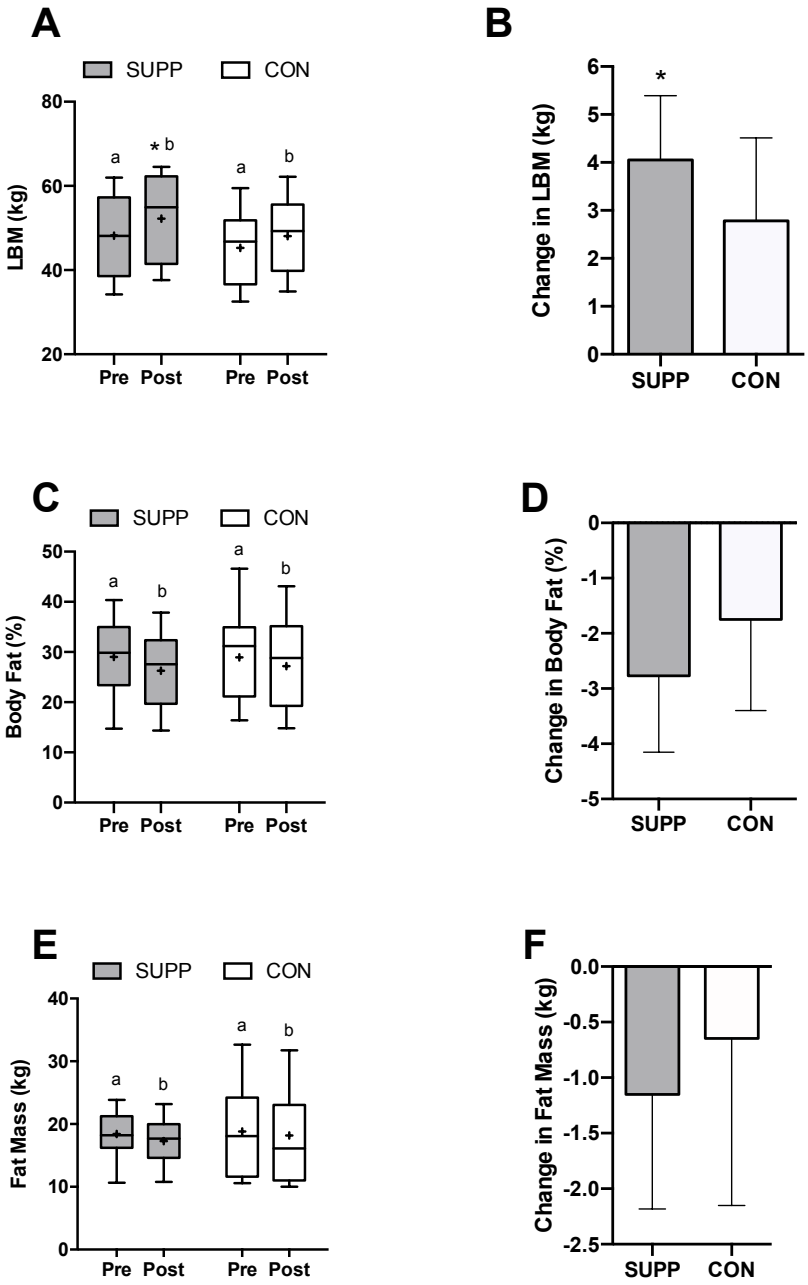

Figure 3. Absolute A) lean body mass (LBM), C) Body fat percentage (BF%) and E) Fat mass (FM) values pre- and post-training and change in B) LBM, D) BF%, and E) FM for SUPP (n=13, shaded box) and CON (n=13, open box) following 10 weeks of resistance exercise training. Graphs A, C, E: Values are presented as median lines and interquartile range (boxes) ± maximum and minimum values (whiskers), with + representing the mean. Means that do not share a letter are significantly different within the group, $p<0.05$. Graphs B, D, F: Values are presented as means + SD. *Significantly different ($p<0.05$) from CON group.

Table 4. Changes in Leg Press, Lat Pulldown, Bench Press, and Isometric Unilateral Leg Extension 1RM by group.

	SUPP (n=13)	CON (n=13)	p
ΔLeg Press 1RM, kg†	1.9 ± 0.6	2.0 ± 0.6	0.69
ΔLat Pulldown 1RM, kg†	0.5 ± 0.2	0.5 ± 0.3	0.92
ΔBench press 1RM, kg†	0.2 ± 0.1	0.2 ± 0.1	>0.99
ΔIsometric unilateral leg extension 1RM, Nm*†	1.7 ± 1.3	1.6 ± 0.8	0.65

Values are mean±SD. SUPP, active supplementation group; CON, control group; 1RM, 1 repetition maximum. *Note: Isometric unilateral leg press n=11 per group.

3.4. Fibre type and CSA.

In response to 10 weeks of RET, type 1, 2 and Hybrid fibres (i.e. fibres that co-express myosin heavy chain I and II) did not change significantly in distribution in either group ($p>0.05$). Following training there was no significant change in fibre type distribution for type 1 (SUPP: 1.9 ± 8.5 %, CON: 1.2 ± 7.2 %; $p=0.83$), type 2 (SUPP: -3.9 ± 8.7 %, CON: -1.2 ± 7.6 %; $p=0.41$), and Hybrid (SUPP: 0.3 ± 1.2 %, CON: 0.3 ± 1.7 %; $p=0.61$) fibres. See **Table 5** for distribution changes between groups over time. Fibre type distribution was calculated as the percentage of a given fibre type in a specific muscle sample at a specific time point. Changes in fibre type distribution were calculated as the difference between time points of the average distribution of fibre type in a given group (SUPP vs. CON).

Table 5. Group differences in relative fibre type distribution changes in young adults.

	SUPP (n=13)	CON (n=13)	p
Δ% Type I	1.9 ± 8.5	1.2 ± 7.2	0.83
Δ% Type II	-3.9 ± 8.7	-1.2 ± 7.6	0.41
Δ% Hybrid Fibres	0.3 ± 1.2	0.3 ± 1.7	0.61

Values are mean±SD. SUPP, active supplementation group; CON, control group.

Fibre type CSA changes are outlined in **Figure 4**. Type I fibre CSA increased significantly in both the SUPP (37 ± 25 %) and CON (25 ± 21 %, **Figure 4B**) groups ($p<0.05$) with time. Type II fibre CSA also increased significantly over time; however, only in the SUPP group (47 ± 24 %; $p<0.05$, **Figure 4C**). A group-by-time interaction was detected only in type 2 fibres ($p=0.029$). In type I fibres, no interaction was detected, although it showed a trend towards statistical significance ($p=0.077$).

3.5. Ultrasound muscle thickness and cross-sectional area.

Following the intervention there was a significant increase in *vastus lateralis* CSA in both groups over time (SUPP: 43 ± 23 %; $p=0.0001$ and CON: 26 ± 31 %; $p=0.005$, **Figures 5A, 5B**), however there was only a trend towards significance between groups ($p=0.06$). There was an increase in *vastus lateralis* muscle thickness over time (SUPP: 15 ± 15 %; $p=0.01$ and CON: 14 ± 14 %; $p=0.008$, **Figures 5C, 5D**), with no difference between groups ($p=0.80$). Bicep CSA and thickness increased significantly over time with significant differences between groups ($p<0.05$). Bicep CSA increased by 42 ± 39 % ($p=0.0001$) in the SUPP group and by 14 ± 10 % in the CON group ($p=0.0001$, **Figures 5E, 5F**). Bicep muscle thickness increased significantly in the SUPP group (29 ± 24 %; $p<0.0001$) and remained unchanged in the CON group (5 ± 16 %; $p=0.3$, **Figures 5G, 5H**).

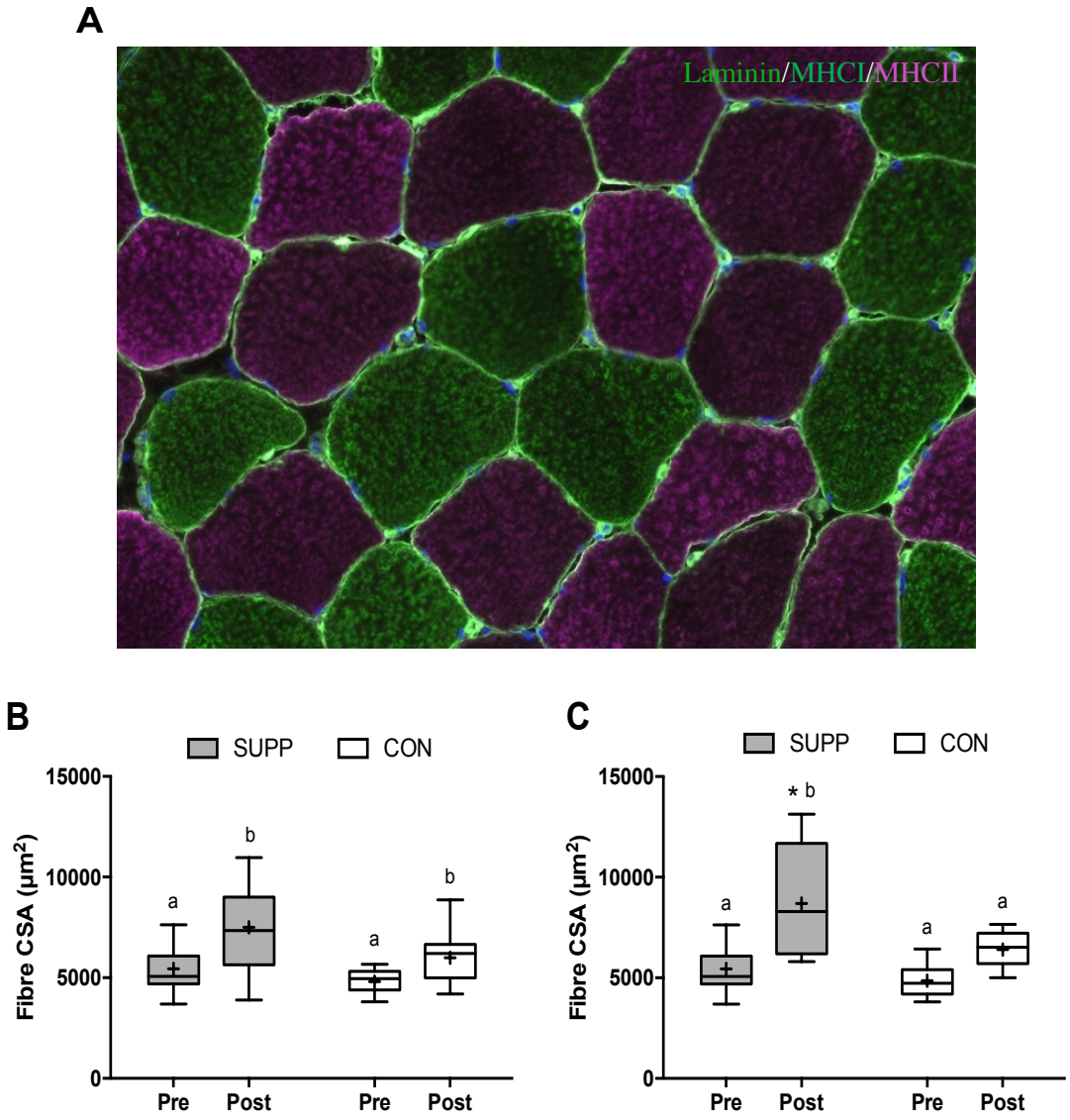

Figure 4. Fibre type-specific staining. A) Representative image of a Laminin/MHCI/MHCII stain of a muscle cross-section. Absolute values of B) Type 1 Fibre and C) Type 2 Fibre CSA pre- and post-training for SUPP (n=13, shaded box) and CON (n=13, open box) following 10 weeks of resistance exercise training. Values are presented as median lines and interquartile range (boxes) ± maximum and minimum values (whiskers), with + representing the mean. Means that do not share a letter are significantly different within the group, $p<0.05$. *Significantly different ($p<0.05$) from CON group.

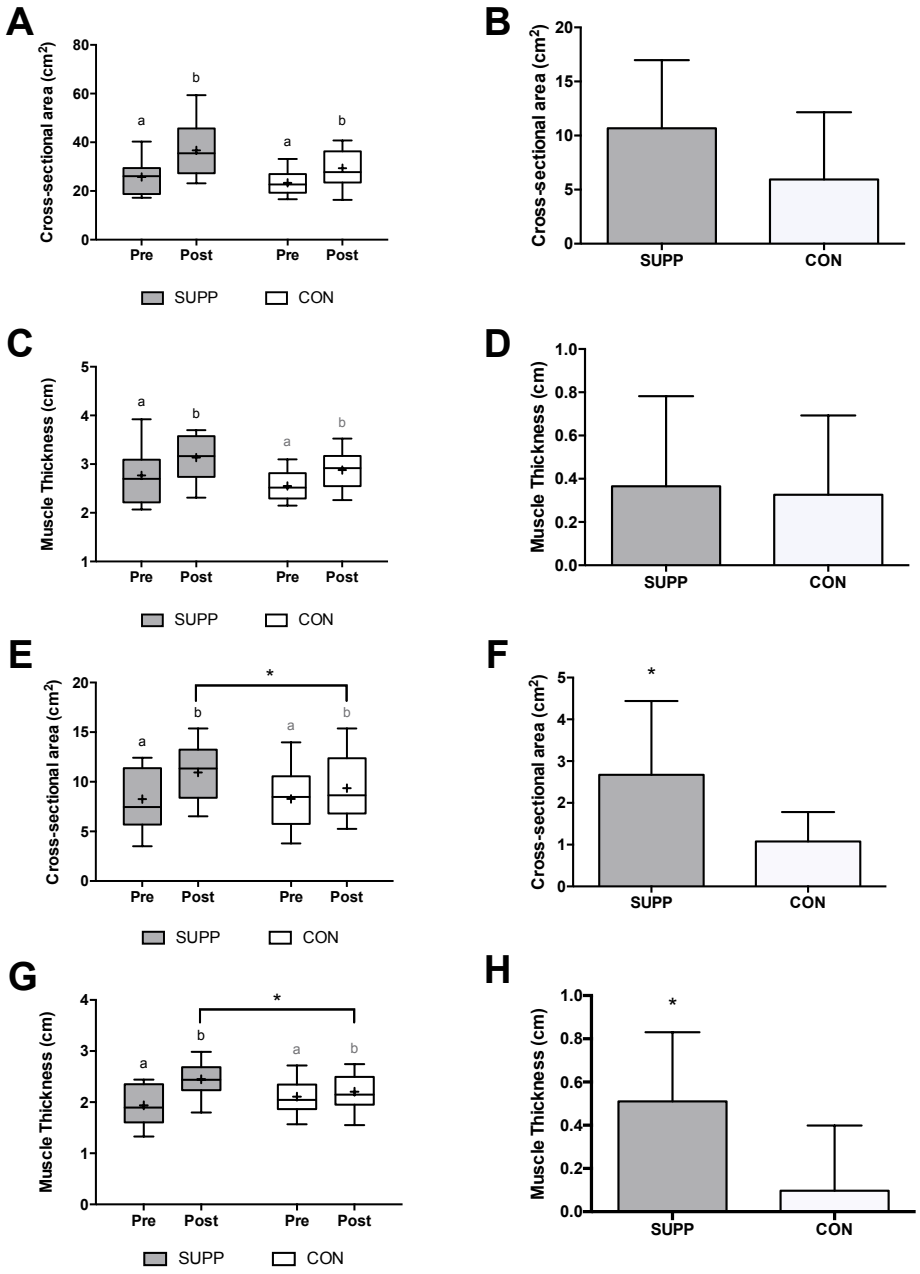

Figure 5. Absolute muscle cross-sectional area (A, E) and muscle thickness (C, G) of the *vastus lateralis* (A, C) and *biceps brachii* (E, G) pre- and post- training, and change in cross-sectional area (B, F) and muscle thickness (D, H) of the *vastus lateralis* (B, D) and *biceps brachii* (F, H) for SUPP (n=13, shaded box) and CON (n=13, open box) following 10 weeks of resistance exercise training. Graphs A, C, E, G: Values are presented as median lines and interquartile range (boxes) ± maximum and minimum values (whiskers), with + representing the mean. Means that do not share a letter are significantly different within the group, $p<0.05$. *Significantly different ($p<0.05$) from CON group. Graphs B, D, F, H: Values are presented as means + SD.

CHAPTER 4: DISCUSSION

We have demonstrated that the ingestion of a multi-ingredient protein supplement consisting of whey protein (20g), Leucine (2g), Creatine (2.5g), Calcium Citrate (300mg) and Vitamin D (1000IU) versus a supplement consisting of collagen (20g) and non-essential amino acids (1.4g Alanine, 0.6g Glycine) ingested twice daily resulted in significant differences in training-induced LBM gains, as well as type II fibre CSA, and bicep CSA and thickness. These results are partially in line with our original hypothesis in that the multi-ingredient supplement induced greater hypertrophy in all measures except for type I fibre CSA and vastus lateralis cross-sectional area and thickness, and furthermore did not augment strength gains in healthy young adults involved in a 10-week resistance training protocol. Our results align with the existing literature on multi-ingredient protein supplements in that the supplement induced greater gains in LBM and bicep muscle size and thickness; however, not upper or lower body strength (163, 167), nor did the supplement induce gains in fat mass (163). It is not possible to isolate each ingredient's individual effect on the outcomes observed in this study, however previous literature has shown that each ingredient is linked to its own benefits to muscle strength and hypertrophy, hence its rationale for inclusion in our supplement. Furthermore, the multi-ingredient nature of this supplement may prove to be beneficial in targeting populations with variability in their response to certain ingredients, thus inducing response heterogeneity. Therefore, we conclude that our multi-ingredient nutritional supplement augments gains in whole-body LBM, upper body muscle size and type II fibre CSA, but not strength, lower body muscle size or type I fibre CSA.

Studies investigating protein supplementation in conjunction with RET report gains in LBM ranging from 0.2-5 kg, with average increases of approximately 0.3-0.8kg (22, 203, 204). Multi-ingredient protein supplements have also been shown to be effective in increasing lean body mass, upper and lower body strength in RET studies that are at least 6 weeks long (20, 163, 167, 205–208). We demonstrate that with either the whey protein-based supplement or the collagen control a greater increase (SUPP: 4.1kg, CON: 2.8kg) in LBM than previously reported in multi-ingredient or protein supplement studies with RET (22, 26, 163, 204, 207, 208). Furthermore, the increases in strength detected in the present study are much greater than those reported previously (26, 163). Recent meta-analyses report an average increase of 4.22kg in lower body 1RM and 2.56kg in upper body 1RM (163), or an average total body strength increase of 2.49kg (26). Meanwhile, our results indicate an average increase of ~8kg in bench press 1RM and ~90kg in leg press 1RM. These differences in strength measures may be attributed to several factors. Firstly, the sample population in this study consisted of young, healthy adults, who have been shown to respond to resistance training to a larger degree than older populations (26). Secondly, our participants were classified as “recreationally active”, indicating physical activity up to 3 times per week. Furthermore, while all participants were classified as recreationally active, the activities performed by each individual were not specified – some participants had previous weight training experience while others had little to none, rendering resistance training a novel exercise. Additionally, participants were instructed to ingest one of their protein supplements daily before bed, which has been shown to increase muscle strength and CSA to a larger degree than a non-caloric

placebo (29), and may thus have offset the negative fluctuations in muscle protein balance and allowed for further muscle accretion.

Supplement studies have long displayed mixed results with respect to fat mass and BF% in humans, although this may be moderated by variables such as population, specific ingredients in supplements, or even the type of RET undertaken. Certain studies detect small but significant differences between supplemented and control groups, such that the supplemented group exhibits greater decreases in BF% (209). Others find no differences between groups, despite existing decreases in fat mass (22). In the present study, we detected decreases in %BF, specifically 2.8% in the SUPP group and 1.8% in the CON group. The difference between groups, however, remained non-significant, and this may be attributed to factors such as small sample size. A power calculation revealed a requirement of 82 subjects to detect a between-group difference over 10 weeks of resistance training. The absolute decrease in fat mass is less than the decrease in BF%, and this is due to fat mass being a measure obtained directly from the DXA scan, whereas BF% relies on an equation that factors in both fat mass and total body mass to derive its value. BF% is equal to fat mass divided by total body mass, and thus the substantial increases in total body mass inversely affected BF%, while fat mass directly affected BF%, leading to an overall larger decrease in BF% than fat mass.

All strength outcomes in this study were not significantly different between groups. Between the upper and lower body, lower body strength achieved greater gains. Of the 4 days per week spent training, 2 were dedicated to the lower body, including multiple variations of leg press utilized within single training sessions. The remaining two

days were inherently different to both the lower body and to each other with respect to target muscle groups, such that they consisted of opposing motions – namely "push" and "pull" exercises. Upper body routines typically isolated specific muscles while lower body exercises remained general, working groups of muscles instead. Thus, the volume of training was much greater for the lower body than upper body, encouraging greater gains and potentially allowing for greater sensitization of the muscle to the effect of protein ingestion (23, 199). Despite this, there remains a lack of significance between groups in all measures of strength. This may be attributed to a small sample size, but may also simply be due to the absence of an effect over an extended period of time. This is supported by Morton et al.'s finding that supplementation contributed to ~9% of strength gains while RET was responsible for the remaining 91% (203). As such, the ability to tease out the differences between supplements and their effects may be more difficult and possibly washed out by the amount of variability within and between groups.

The leg press and isometric unilateral leg extension exercises both target the *quadriceps femoris*, however, the leg press uses both the knee and hip joints, making it a compound exercises that also recruits the hamstring muscles as well. The isometric unilateral leg extension is a more isolated exercise for the quadriceps and uses only the knee joint. Thus, we would expect a greater increase in leg extension strength, however this was not the case. The two exercises induced similar increases in muscle strength in both groups, with similar differences between groups detected as well ($p=0.69$ and $p=0.65$ for leg press and leg extension, respectively). The reason this result turned out as such is likely attributed to our smaller than normal sample size in this specific exercise, which

came about due to mechanical and technical issues and thus left us with a sample size of approximately 11 subjects per group, compared to the original 13. We acknowledge this as a limitation with respect to this strength measure.

We assessed TBW before and after 12 weeks of training and found no differences within participants or between groups that would have altered LBM changes. It has been suggested that creatine imparts its effect on muscle mass by increasing intramuscular water content, which has been postulated to result in anabolic signaling in skeletal muscle (78, 79). This mechanism is likely not at play to a degree that would induce significant differences in changes between SUPP and CON groups, despite small increases in TBW in both groups (and more so in the SUPP group). Creatine supplementation studies often used creatine at doses around 0.3g/kg/day (85), which in a population similar to our subject pool would equate to approximately 21g of creatine daily in adults who have a total mass of ~70kg. In this study, the amount of creatine supplemented was 5g daily, and thus it is unlikely that we would detect similar changes, although we still observed a minor trend towards increased TBW. We also collected food logs prior to, at the halfway point, and at the end of this study to examine protein intake in the normal diet. Participants in either group did not differ with respect to habitual protein intake over the course of the study, which suggests that any group differences detected in any outcome measures were a result of supplementation and not changes in the diet.

Fibre CSA increased significantly over time in type I and II fibres with RET, as has been shown in previous literature (83, 194), however there was also a group-by-time interaction detected in type II fibre CSA ($p=0.03$). Type I fibres exhibited a trend towards

an interaction, which is indicative of growth despite not being the main target for this specific mode of exercise training. The training protocol was strictly resistance-focused, targeting type II fibres to a greater extent than type I, which may suggest specificity of the supplement towards more functional type II fibres in this kind of exercise. Ultrasound measures of *biceps brachii* CSA and thickness parallels this outcome, as we observe significant increases in both bicep CSA and thickness that are significant both over time and between SUPP and CON groups, however we only observe a trend towards an interaction for the *vastus lateralis*. Both muscle groups were trained to failure during each session, and thus we would expect similar increases as well as similar differences between groups. We propose that this difference in significance may be attributed to the limitations in ultrasound use leading to an increase in variability. Firstly, ultrasound presents a major limitation when dealing with different populations (i.e., males compared to females), as detecting muscle becomes increasingly difficult with increasing fat mass surrounding the muscle (as is evident in females compared to males) (210). There is inherent difficulty in differentiating muscle from subcutaneous fat which may lead to incorrect muscle parameter identification. Secondly, certain tissues are more compressible than others (211), such as fat, and thus individuals with greater fat mass may also be susceptible to increased compression and thus a distortion of muscle size. Furthermore, the level of hydration of the muscle may also provide another source of variability within and between subjects. Some literature suggests a possible solution to counter this issue being the use of full compression of the ultrasound probe on the body segment (212), the obvious caveat being the distortion of the muscle image, and

subsequently the lack of a true measure of cross-sectional area and thickness. Thus, these sources of error and variability may be contributing factors to the lack of significant (albeit meaningful) differences between groups in *vastus lateralis* cross-sectional area and muscle thickness.

With respect to differences between supplement and control groups, it should be noted that in previous work the difference observed between groups is typically much larger than in the current investigation. This may be attributed to a number of factors, most notably that supplement studies typically compare the active supplement to a carbohydrate-based control (22, 193, 206, 213–215). In this study we used an isonitrogenous, isoenergetic protein supplement consisting of collagen peptides and non-essential amino acids. The benefits of whey are evident and well-known, and it has been identified as a high-quality source of protein able to induce a greater anabolic effect compared to other protein sources such as soy, casein or collagen proteins (6, 48, 177, 204, 216, 217). The literature is mixed with respect to the benefits of collagen protein, with some groups suggesting it is an ineffective stimulant of muscle protein synthesis and subsequently, hypertrophy (61, 218, 219). Conversely, other literature suggests a possible benefit to ingesting collagen protein compared to carbohydrate-based controls (63). One study by Zdzieblik et al. showed that the ingestion of 15g of collagen daily during a 12 week RET program induced an ~5kg increase in muscle mass (63). Our study resulted in a 2.8kg increase in LBM with the collagen-based control supplement, which falls in line with several supplement trials (20, 58, 215, 220, 221). Thus, there may be a possibility

that the collagen supplement was able, in part, to aid in RET-induced muscle gains, thus reducing the relative difference between groups.

The novel aspects of this study are primarily associated with the multi-ingredient supplement. This supplement contains a new formulation of ingredients yet to be studied together. Another unique aspect of our study is the comparison of two isoenergetic, isonitrogenous supplements as stimulants for muscle growth, as opposed to the use of carbohydrates as a contrasting control (190, 191, 193, 206, 214, 222, 223). The current results indicate that this multi-ingredient supplement may be beneficial for optimizing muscle growth in healthy young men and women – notably, the main implication being that supplementation in this form is able to enhance muscular LBM and *biceps brachii* CSA and thickness over and above RET alone. We assessed changes in LBM using various methods: DXA, MT, muscle CSA, and fibre CSA. We also included females in this study, which allowed for further insight into the effect of the supplement across the general young adult population. However, the inclusion of women likely also increased the variability in all of the outcome measures. We acknowledge other limitations in our study – notably, that we did not include a control group of just RET participants in this study, only comparing two supplemented groups, limiting our ability to contrast between supplemented and non-supplemented training.

Along with the novel data that the present study illustrates, there are other limitations that we must acknowledge. Firstly, we see a large amount of variability within and between groups, which may be due to a number of factors including response heterogeneity to exercise and/or supplementation, or possible sex-based differences. We

postulate that the effect of the supplement may have also been mitigated by inherent characteristics of the sample population. Both groups consisted of healthy young adults, who typically ingest sufficient amounts of protein in their habitual diet, and are more efficiently able to stimulate muscle protein synthesis with lower protein intakes than, for example, older adults, thus requiring lower relative protein intakes in a single meal (203, 224). Thus, it may be more difficult to tease out the differences between groups when both may already be ingesting optimal amounts of protein. Given that diet was not controlled for the 10-week intervention period, this could further increase variability in day-to-day protein kinetics. We also believe that the habitual activity level of our participants may have differed with respect to the kind of physical activity performed, such that some groups may have experienced resistance training prior to this study while others may have been completely novice, further increasing the amount of variability. Finally, we recognize that protein intake requirements may differ from person to person, especially with recommended intakes that are normalized to body weight rather than on a 'per meal' basis (2, 26). The supplement contained 20g of whey protein, twice daily, which may not have been sufficient for larger individuals, and on the other hand may have been considered unnecessary overfeeding for smaller individuals. Studies that recommend 1.62g/kg/day to optimize RET-induced gains in FFM (26) would require approximately 110g of protein per day in this population of young adults who average ~68kg body mass, an amount that is likely not representative of what our participants ingested daily, even when accounting for other meals ingested throughout the day. Thus, this may lead to lesser gains than fully expected.

The next steps and future directions of this research should focus on identifying the underlying cellular mechanisms that may govern the observed responses. Sex-based response differences should also be investigated to determine if certain supplements may benefit these populations differently. Furthermore, altering the doses of specific ingredients in future investigations may prove to optimize hypertrophy better than in the current study, for example with creatine monohydrate, which is typically supplemented at 0.3g/kg/day (85), or about 21g per day compared to our 5g per day. Tailoring of protein dosage to body mass may standardize the hypertrophic response to RET, better indicating optimal protein intakes in such a diverse population. Longer duration of studies may also allow for this diverse population to reach optimal muscular hypertrophic gains and a greater sample size in males and females may aid us in determining whether this method of supplementation is feasible and able to potentiate muscle protein accretion long-term.

We conclude that the twice-daily consumption of a whey-protein based multi-ingredient supplement containing creatine, leucine, calcium citrate and vitamin D was effective in augmenting lean body mass gains and upper body muscle CSA and thickness as well as type II fibre CSA in conjunction with a 10-week RET program.

CHAPTER 5: REFERENCES

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Appendix A: Raw Data

Table 1. Baseline DXA Characteristics.

ID	Group	Age	Height (cm)	Mass (DXA)	Fat Mass (kg)	Lean Mass (kg)	BF%	BMI (kg/m^2)	Ex Leg
119	1	22	184.1	76	10.655	61.974	14.7	22.42	L
473	1	19	154	52.71	16.01	34.201	31.9	22.23	R
191	1	26	187.1	74.8	14.733	56.661	20.6	21.37	L
106	1	21	177.4	74.2	18.02	52.866	25.4	23.58	R
922	1	26	151.1	60.2	23.532	34.918	40.3	26.37	R
729	1	26	160.5	58.9	17.76	38.98	31.3	22.86	R
612	1	24	177.6	72.4	18.208	51.246	26.2	22.95	L
309	1	23	173.5	81	20.226	57.875	25.9	26.91	L
101	1	21	177	80.6	16.396	60.736	21.3	25.73	L
201	1	22	177.2	74.6	23.838	48.127	33.1	23.76	L
553	1	22	170.3	79.7	19.292	46.633	39.6	27.48	L
621	1	22	177.5	65.1	18.567	43.796	29.8	20.66	R
503	1	23	158.3	62.8	22.265	38.21	36.8	25.06	R
345	2	22	172	85.4	32.66	49.718	39.6	28.87	R
314	2	20	167.8	57.8	10.922	44.15	19.8	20.53	R
224	2	20	188	82.1	19.132	59.479	24.3	23.23	L
227	2	22	181.6	64.6	12.299	49.696	19.8	19.59	R
242	2	21	159.9	52.9	16.174	34.45	31.9	20.69	L
821	2	21	161.8	72.2	22.982	46.798	32.9	27.58	R
109	2	23	162.5	48.9	10.653	35.926	22.9	18.52	R
448	2	22	164	72	32.58	37.32	46.6	26.77	L
273	2	22	155.6	53.6	19.069	32.504	37	22.14	L
928	2	19	158	60.73	18.09	39.937	31.2	24.33	L
413	2	23	180.5	84.9	25.444	55.761	31.3	26.06	R
288	2	18	187	67.57	10.592	53.926	16.4	19.32	R
801	2	19	173	65.97	14.189	48.971	22.5	22.04	R

Group 1, SUPP; Group 2, CON; Ex Leg, exercised leg.

Table 2. Baseline Strength.

ID	Group	Bench Press (kg)	Lat Pulldown (kg)	Leg Press (kg)	Biodex (Nm)
119	1	95.45	177.27	286.36	x
473	1	29.55	81.82	97.73	x
191	1	52.27	113.64	204.55	234.20
106	1	88.64	143.18	265.91	219.70
922	1	25.00	72.73	81.82	69.60
729	1	65.91	138.64	163.64	126.00
612	1	43.18	120.45	134.09	135.00
309	1	97.73	184.09	245.45	217.20
101	1	38.64	106.82	134.09	96.40
201	1	70.45	163.64	327.27	260.60
553	1	38.64	115.91	172.73	183.30
621	1	25.00	86.36	154.55	90.00
503	1	25.00	81.82	115.91	114.70
345	2	52.27	122.73	150.00	x
314	2	43.18	97.73	102.27	x
224	2	75.00	145.45	245.45	226.50
227	2	47.73	134.09	113.64	169.00
242	2	27.27	79.55	90.91	81.20
821	2	27.27	75.00	81.82	51.10
109	2	25.00	75.00	93.18	87.60
448	2	56.82	115.91	159.09	x
273	2	25.00	79.55	65.91	84.80
928	2	25.00	81.82	163.64	157.20
413	2	61.36	122.73	163.64	97.80
288	2	52.27	154.55	165.91	216.60
801	2	52.27	143.18	204.55	203.80

Group 1, SUPP; Group 2, CON. x denotes data points that were not obtained as a result of technical issues.

Table 3. Body Mass as assessed by DXA.

ID	Group	Mass (kg)		Change (kg)
		Pre	Post	
119	1	76	78.6	2.6
473	1	52.71	54.7	1.99
191	1	74.8	78.6	3.8
106	1	74.2	78.8	4.6
922	1	60.2	60.5	0.3
729	1	58.9	61.3	2.4
612	1	72.4	74.3	1.9
309	1	81	84.6	3.6
101	1	80.6	81.8	1.2
201	1	74.6	80.7	6.1
553	1	79.7	84	4.3
621	1	65.1	68.2	3.1
503	1	62.8	65.8	3
345	2	85.4	85.4	0
314	2	57.8	59.6	1.8
224	2	82.1	81.7	-0.4
227	2	64.6	64.4	-0.2
242	2	52.9	54.6	1.7
821	2	72.2	72.7	0.5
109	2	48.9	52.5	3.6
448	2	72	71.8	-0.2
273	2	53.6	57.2	3.6
928	2	60.73	64.77	4.04
413	2	84.9	88.7	3.8
288	2	67.57	75.09	7.52
801	2	65.97	68.05	2.08

Group 1, SUPP; Group 2, CON.

Table 4. Fat Mass as assessed by DXA.

ID	Group	Fat Mass (kg)		Change (kg)
		Pre	Post	
119	1	10.655	10.797	0.142
473	1	16.01	14.614	-1.396
191	1	14.733	13.284	-1.449
106	1	18.02	17.333	-0.687
922	1	23.532	20.985	-2.547
729	1	17.76	17.694	-0.066
612	1	18.208	14.646	-3.562
309	1	20.226	19.003	-1.223
101	1	16.396	14.689	-1.707
201	1	23.838	23.178	-0.66
553	1	19.292	18.243	0.268
621	1	18.567	18.031	-0.536
503	1	22.265	22.047	-0.218
345	2	32.66	31.746	-0.914
314	2	10.922	10.031	-0.891
224	2	19.132	16.108	-3.024
227	2	12.299	11.327	-0.972
242	2	16.174	15.079	-1.095
821	2	22.982	21.027	-1.955
109	2	10.653	10.16	-0.493
448	2	32.58	30.063	-2.517
273	2	19.069	20.191	1.122
928	2	18.09	20.891	2.801
413	2	25.444	25.048	-0.396
288	2	10.592	10.675	0.083
801	2	14.189	14.022	-0.167

Group 1, SUPP; Group 2, CON.

Table 5. Lean body mass as assessed by DXA.

ID	Group	Lean Mass (kg)		Change (kg)
		Pre	Post	
119	1	61.974	64.54	2.566
473	1	34.201	37.638	3.437
191	1	56.661	61.881	5.22
106	1	52.866	58.14	5.274
922	1	34.918	37.827	2.909
729	1	38.98	41.448	2.468
612	1	51.246	56.709	5.463
309	1	57.875	62.635	4.76
101	1	60.736	63.553	2.817
201	1	48.127	54.916	6.789
553	1	46.633	50.667	4.034
621	1	43.796	47.49	3.694
503	1	38.21	41.479	3.269
345	2	49.718	50.75	1.032
314	2	44.15	46.79	2.64
224	2	59.479	62.173	2.694
227	2	49.696	50.426	0.73
242	2	34.45	37.229	2.779
821	2	46.798	49.255	2.457
109	2	35.926	40.015	4.089
448	2	37.32	39.631	2.311
273	2	32.504	34.917	2.413
928	2	39.937	41.134	1.197
413	2	55.761	59.948	4.187
288	2	53.926	61.359	7.433
801	2	48.971	51.201	2.23

Group 1, SUPP; Group 2, CON.

Table 6. Body fat percentage as assessed by DXA.

ID	Group	BF%		Change (%)
		Pre	Post	
119	1	14.7	14.3	-0.4
473	1	31.9	28	-3.9
191	1	20.6	17.7	-2.9
106	1	25.4	23	-2.4
922	1	40.3	35.7	-4.6
729	1	31.3	29.9	-1.4
612	1	26.2	20.5	-5.7
309	1	25.9	23.3	-2.6
101	1	21.3	18.8	-2.5
201	1	33.1	29.7	-3.4
553	1	39.6	37.8	-1.8
621	1	29.8	27.5	-2.3
503	1	36.8	34.7	-2.1
345	2	39.6	38.8	-0.8
314	2	19.8	17.7	-2.1
224	2	24.3	20.6	-3.7
227	2	19.8	18.3	-1.5
242	2	31.9	28.8	-3.1
821	2	32.9	29.9	-3
109	2	22.9	20.2	-2.7
448	2	46.6	43.1	-3.5
273	2	37	36.6	-0.4
928	2	31.2	33.7	2.5
413	2	31.3	29.5	-1.8
288	2	16.4	14.8	-1.6
801	2	22.5	21.5	-1

Group 1, SUPP; Group 2, CON.

Table 7. Bench press 1RM (kg).

		Bench Press				
ID	Group	0	5	10	% Change	Delta
119	1	95.5	95.5	95.5	0.0	0
473	1	29.5	31.8	34.1	15.4	4.55
191	1	52.3	61.4	70.5	34.8	18.18
106	1	88.6	88.6	93.2	5.1	4.55
922	1	25.0	27.3	29.5	18.2	4.55
729	1	65.9	70.5	79.5	20.7	13.64
612	1	43.2	43.2	47.7	10.5	4.55
309	1	97.7	95.5	102.3	4.7	4.55
101	1	38.6	38.6	47.7	23.5	9.09
201	1	70.5	77.3	81.8	16.1	11.36
553	1	38.6	40.9	45.5	17.6	6.82
621	1	25.0	34.1	38.6	54.5	13.64
503	1	25.0	27.3	34.1	36.4	9.09
345	2	52.3	52.3	54.5	4.3	2.27
314	2	43.2	47.7	50.0	15.8	6.82
224	2	75.0	70.5	79.5	6.1	4.55
227	2	47.7	43.2	52.3	9.5	4.55
242	2	27.3	34.1	36.4	33.3	9.09
821	2	27.3	27.3	31.8	16.7	4.55
109	2	25.0	27.3	34.1	36.4	9.09
448	2	56.8	52.3	61.4	8.0	4.55
273	2	25.0	20.5	27.3	9.1	2.27
928	2	25.0	36.4	40.9	63.6	15.91
413	2	61.4	70.5	79.5	29.6	18.18
288	2	52.3	52.3	65.9	26.1	13.64
801	2	52.3	54.5	61.4	17.4	9.09

Group 1, SUPP; Group 2, CON.

Table 8. Lat Pulldown 1RM (kg).

		Lat Pulldown				
ID	Group	0	5	10	% Change	Delta
119	1	177.3	181.8	211.4	19.23	34.09
473	1	81.8	95.5	102.3	25.00	20.45
191	1	113.6	122.7	143.2	26.00	29.55
106	1	143.2	159.1	175.0	22.22	31.82
922	1	72.7	84.1	102.3	40.63	29.55
729	1	138.6	154.5	181.8	31.15	43.18
612	1	120.5	102.3	125.0	3.77	4.55
309	1	184.1	184.1	215.9	17.28	31.82
101	1	106.8	113.6	122.7	14.89	15.91
201	1	163.6	184.1	184.1	12.50	20.45
553	1	115.9	102.3	134.1	15.69	18.18
621	1	86.4	88.6	102.3	18.42	15.91
503	1	81.8	72.7	88.6	8.33	6.82
345	2	122.7	122.7	134.1	9.26	11.36
314	2	97.7	106.8	125.0	27.91	27.27
224	2	145.5	179.5	184.1	26.56	38.64
227	2	134.1	138.6	175.0	30.51	40.91
242	2	79.5	79.5	88.6	11.43	9.09
821	2	75.0	75.0	79.5	6.06	4.55
109	2	75.0	65.9	81.8	9.09	6.82
448	2	115.9	134.1	143.2	23.53	27.27
273	2	79.5	65.9	81.8	2.86	2.27
928	2	81.8	102.3	111.4	36.11	29.55
413	2	122.7	154.5	163.6	33.33	40.91
288	2	154.5	154.5	184.1	19.12	29.55
801	2	143.2	143.2	184.1	28.57	40.91

Group 1, SUPP; Group 2, CON.

Table 9. Leg Press 1RM (kg).

		Leg Press				
ID	Group	0	5	10	% Change	Delta
119	1	286.4	327.3	400.0	39.68	113.64
473	1	97.7	175.0	190.9	95.35	93.18
191	1	204.5	277.3	306.8	50.00	102.27
106	1	265.9	338.6	338.6	27.35	72.73
922	1	81.8	150.0	175.0	113.89	93.18
729	1	163.6	236.4	245.5	50.00	81.82
612	1	134.1	170.5	234.1	74.58	100.00
309	1	245.5	318.2	327.3	33.33	81.82
101	1	134.1	195.5	265.9	98.31	131.82
201	1	327.3	420.5	454.5	38.89	127.27
553	1	172.7	215.9	265.9	53.95	93.18
621	1	154.5	184.1	204.5	32.35	50.00
503	1	115.9	163.6	163.6	41.18	47.73
345	2	150.0	236.4	236.4	57.58	86.36
314	2	102.3	172.7	215.9	111.11	113.64
224	2	245.5	286.4	293.2	19.44	47.73
227	2	113.6	200.0	236.4	108.00	122.73
242	2	90.9	115.9	154.5	70.00	63.64
821	2	81.8	138.6	163.6	100.00	81.82
109	2	93.2	145.5	179.5	92.68	86.36
448	2	159.1	204.5	227.3	42.86	68.18
273	2	65.9	136.4	163.6	148.28	97.73
928	2	163.6	225.0	245.5	50.00	81.82
413	2	163.6	245.5	286.4	75.00	122.73
288	2	165.9	245.5	265.9	60.27	100.00
801	2	204.5	265.9	286.4	40.00	81.82

Group 1, SUPP; Group 2, CON.

Table 10. Isometric Unilateral Leg Extension 1RM (Nm).

		Isometric Unilateral Leg Extension				
ID	Group	0	5	10	% Change	Delta
191	1	234.2	352.8	234.9	0.30	0.7
106	1	219.7	287.2	249.4	13.52	29.7
922	1	69.6	168.8	136.3	95.83	66.7
729	1	126	226	350.1	177.86	224.1
612	1	135	128.5	133.4	-1.19	-1.6
309	1	217.2	255.3	385.8	77.62	168.6
101	1	96.4	164.6	227.8	136.31	131.4
201	1	260.6	306.6	318.3	22.14	57.7
553	1	183.3	280.4	261.4	42.61	78.1
621	1	90	152.6	179.1	99.00	89.1
503	1	114.7	154	190	65.65	75.3
224	2	226.5	347.9	295.6	30.51	69.1
227	2	169	253.6	245.4	45.21	76.4
242	2	81.2	182.7	118.2	45.57	37
821	2	51.1	152	130.3	154.99	79.2
109	2	87.6	123.5	120	36.99	32.4
448	2	x	228.9	286.1	x	x
273	2	84.8	123.5	108.1	27.48	23.3
928	2	157.2	192.1	263.2	67.43	106
413	2	97.8	260.4	276.6	182.82	178.8
288	2	216.6	281.5	276.6	27.70	60
801	2	203.8	286.1	259.3	27.23	55.5

Group 1, SUPP; Group 2, CON. x denotes data points that were not obtained as a result of technical issues.

Table 11. Average Fibre Number Pre- and Post-training.

		Type 1		Type 2		Hybrid	
ID	Group	Pre	Post	Pre	Post	Pre	Post
119	1	56.6	41.5	43.0	56.6	0.5	0.0
473	1	30.4	25.2	69.6	73.8	0.0	1.0
191	1	28.8	36.7	71.2	63.2	0.0	0.1
106	1	37.2	28.8	66.2	62.8	0.2	0.0
922	1	33.1	38.2	66.0	61.8	0.9	0.0
729	1	36.5	44.2	63.5	55.8	0.0	0.0
612	1	34.3	35.5	64.7	62.6	1.1	1.9
309	1	33.8	39.7	66.2	60.3	0.0	0.0
101	1	52.7	68.7	47.3	31.3	0.0	0.0
201	1	33.5	31.4	65.9	68.6	0.6	0.0
553	1	46.3	43.9	53.3	56.1	0.4	0.0
621	1	17.7	28.7	82.3	62.2	0.0	1.2
503	1	45.8	48.4	54.2	47.9	0.0	3.7
345	2	47.7	33.9	52.3	65.7	0.0	0.4
314	2	51.1	52.7	48.9	47.3	0.0	0.0
224	2	48.9	50.5	50.9	48.4	0.2	1.0
227	2	18.9	22.8	80.8	77.2	0.2	0.0
242	2	42.9	52.1	56.6	47.5	0.5	0.4
821	2	26.9	39.2	73.1	56.1	0.0	1.0
109	2	28.4	28.5	71.6	71.5	0.0	0.0
448	2	33.2	35.2	65.3	63.1	1.5	1.8
273	2	51.4	45.8	48.6	52.1	0.0	2.1
928	2	28.8	35.3	67.2	63.6	4.0	1.2
413	2	31.9	28.6	67.8	69.8	0.3	1.6
288	2	45.0	52.7	51.7	47.3	3.3	0.0
801	2	43.1	36.5	54.0	63.5	2.8	0.0

Group 1, SUPP; Group 2, CON.

Table 12. Fibre cross-sectional area pre- and post-training (μm^2).

ID	Group	Type 1 Pre	Type 1 Post	Type 2 Pre	Type 2 Post	Delta Type 1	Delta Type 2
119	1	5074.57	8868.37	7231.02	13126.07	3793.80	5895.05
473	1	7627.58	10395.72	9030.73	12795.11	2768.14	3764.39
191	1	5052.86	9060.99	6409.12	12043.34	4008.12	5634.22
106	1	6928.37	10966.26	5898.56	8289.19	4037.89	2390.63
922	1	5685.15	8939.16	4871.01	8464.47	3254.01	3593.47
729	1	4557.30	5999.94	5015.70	6595.79	1442.63	1580.08
612	1	6165.02	8565.92	6563.87	11307.12	2400.90	4743.25
309	1	4886.24	6198.55	4373.10	5800.28	1312.31	1427.19
101	1	4406.90	5280.83	6332.20	7096.22	873.93	764.02
201	1	6005.91	7349.40	6778.68	9072.15	1343.49	2293.47
553	1	4827.88	4893.43	5061.41	6212.35	65.55	1150.94
621	1	3692.20	3892.38	4537.78	6171.24	200.19	1633.46
503	1	5874.81	7180.50	4060.27	6057.47	1305.70	1997.20
345	2	4435.32	4199.07	4736.23	5005.69	-236.25	269.46
314	2	3804.03	6416.76	5409.59	7639.19	2612.74	2229.60
224	2	4472.28	5455.56	6100.64	6517.21	983.28	416.57
227	2	4969.99	6480.81	4006.98	5982.19	1510.82	1975.21
242	2	4364.97	4750.57	3802.64	5032.21	385.60	1229.57
821	2	4989.41	6468.09	4773.15	6900.33	1478.68	2127.17
109	2	4428.19	4589.58	4583.66	5576.03	161.39	992.36
448	2	4962.05	6842.85	4078.89	6706.90	1880.80	2628.01
273	2	5041.48	5247.02	4939.53	5999.47	205.55	1059.94
928	2	5600.39	7069.86	6435.01	5841.44	1469.47	-593.58
413	2	5641.18	8865.45	4611.71	7654.48	3224.27	3042.77
288	2	4166.40	5271.46	4301.49	7494.86	1105.06	3193.37
801	2	5666.85	6202.14	5413.62	6943.21	535.29	1529.59

Group 1, SUPP; Group 2, CON.

Table 13. *Vastus Lateralis* and *Biceps Brachii* cross-sectional area as assessed by ultrasound (cm^2).

ID	Group	T1 VL	T3 VL	Delta	% Change	T1 B	T3 B	Delta	% Change
119	1	40.28	47.58	7.30	18.13	12.03	12.83	0.81	6.70
473	1	17.22	32.22	15.00	87.13	5.03	8.72	3.69	73.33
191	1	23.75	36.60	12.85	54.09	9.99	11.52	1.53	15.30
106	1	34.18	x	7.66	x	11.66	13.09	1.42	12.19
922	1	27.71	34.61	6.90	24.89	3.50	7.49	3.99	113.79
729	1	18.48	25.85	7.37	39.85	5.57	10.44	4.88	87.62
612	1	34.23	59.37	25.13	73.42	12.42	15.37	2.95	23.74
309	1	29.83	40.05	10.22	34.26	11.09	14.66	3.58	32.25
101	1	27.39	36.44	9.06	33.06	10.37	13.40	3.03	29.21
201	1	28.24	49.29	21.04	74.50	5.83	11.34	5.50	94.36
553	1	24.79	31.56	6.77	27.31	6.67	8.15	1.48	22.24
621	1	18.06	23.91	5.85	32.40	7.46	6.52	-0.94	-12.60
503	1	19.50	23.15	3.65	18.73	5.81	8.64	2.83	48.78
345	2	29.03	40.75	11.72	40.36	8.48	8.64	0.16	1.89
314	2	25.94	36.63	10.69	41.19	6.83	6.98	0.15	2.18
224	2	18.43	35.93	17.49	94.91	11.90	13.27	1.37	11.49
227	2	33.19	40.70	7.51	22.62	11.14	13.71	2.57	23.10
242	2	16.62	17.65	1.03	6.20	5.93	6.71	0.79	13.24
821	2	20.16	34.50	14.34	71.12	9.98	10.19	0.21	2.08
109	2	18.12	22.93	4.81	26.55	5.58	6.91	1.33	23.93
448	2	22.69	24.02	1.33	5.87	4.94	5.42	0.48	9.64
273	2	21.39	16.39	-5.01	-23.40	3.79	5.26	1.47	38.79
928	2	22.55	26.91	4.37	19.37	6.69	7.78	1.09	16.25
413	2	25.16	27.76	2.60	10.33	13.97	15.38	1.41	10.08
288	2	27.99	32.97	4.98	17.78	9.93	11.49	1.56	15.74
801	2	22.85	24.27	1.41	6.19	8.49	9.92	1.43	16.84

VL, *vastus lateralis*; B, *biceps brachii*; Group 1, SUPP; Group 2, CON. x denotes data points that were not obtained as a result of technical issues.

Table 14. *Vastus Lateralis* and *Biceps Brachii* muscle thickness as assessed by ultrasound (cm).

ID	Group	T1 VL	T3 VL	Delta	% Change	T1 B	T3 B	Delta	% Change
119	1	3.11	3.63	0.52	16.77	2.44	2.62	0.18	7.21
473	1	2.25	3.09	0.84	37.36	1.61	1.80	0.19	11.87
191	1	2.54	3.32	0.77	30.46	2.23	2.41	0.18	8.25
106	1	2.57	x	x	x	2.41	2.71	0.30	12.40
922	1	2.65	3.07	0.42	15.77	1.33	2.38	1.05	79.35
729	1	2.10	2.31	0.21	10.15	1.46	2.44	0.98	66.60
612	1	2.87	3.42	0.55	19.28	2.36	2.81	0.46	19.39
309	1	3.92	3.24	-0.68	-17.39	2.03	2.66	0.63	31.00
101	1	3.05	3.70	0.65	21.44	2.35	2.99	0.64	27.13
201	1	3.72	3.63	-0.09	-2.34	1.60	2.57	0.98	61.08
553	1	2.75	2.97	0.23	8.19	1.84	2.32	0.48	26.14
621	1	2.20	2.66	0.46	20.75	1.90	2.15	0.25	13.29
503	1	2.07	2.57	0.50	24.20	1.66	1.98	0.32	19.57
345	2	2.36	2.78	0.43	18.22	2.29	2.15	-0.14	-6.16
314	2	2.44	2.81	0.38	15.40	1.96	1.99	0.04	1.79
224	2	2.52	2.92	0.40	15.84	2.40	2.48	0.08	3.24
227	2	3.10	3.53	0.43	13.71	1.86	2.72	0.86	46.44
242	2	2.30	3.22	0.91	39.63	1.87	2.09	0.22	11.74
821	2	2.77	3.32	0.55	19.88	2.51	2.47	-0.04	-1.39
109	2	2.15	2.56	0.41	19.26	1.86	1.91	0.05	2.80
448	2	2.63	2.95	0.32	12.10	1.57	1.67	0.10	6.44
273	2	2.97	2.26	-0.71	-23.95	2.05	1.55	-0.50	-24.29
928	2	2.61	3.12	0.51	19.36	2.01	2.07	0.05	2.58
413	2	2.28	2.46	0.17	7.57	2.72	2.74	0.03	0.96
288	2	2.86	2.96	0.10	3.46	2.26	2.51	0.25	10.98
801	2	2.19	2.53	0.35	15.84	2.04	2.31	0.26	12.77

VL, *vastus lateralis*; B, *biceps brachii*; Group 1, SUPP; Group 2, CON. x denotes data points that were not obtained as a result of technical issues.

Appendix B: Statistical Outputs

1.0. Morphometric Outputs

1.1. Lean Body Mass

	2way ANOVA					
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	0.1144	0.0471	*	Yes	
8	Time	3.317	< 0.0001	****	Yes	
9	Column Factor	3.529	0.3480	ns	No	
10	Subjects (matching)	92.41	< 0.0001	****	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	5.241	1	5.241	F (1, 24) = 4.382	P = 0.0471
14	Time	152.0	1	152.0	F (1, 24) = 127.1	P < 0.0001
15	Column Factor	161.6	1	161.6	F (1, 24) = 0.9164	P = 0.3480
16	Subjects (matching)	4233	24	176.4	F (24, 24) = 147.5	P < 0.0001
17	Residual	28.70	24	1.196		
18						
19	Number of missing values	0				

1.2. Fat Mass

	2way ANOVA					
1	Table Analyzed	Fat Mass_2WayANOVA				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	0.04844	0.3301	ns	No	
8	Time	0.6189	0.0016	**	Yes	
9	Group	0.3315	0.7780	ns	No	
10	Subjects (matching)	97.82	< 0.0001	****	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	0.8225	1	0.8225	F (1, 24) = 0.9882	P = 0.3301
14	Time	10.51	1	10.51	F (1, 24) = 12.63	P = 0.0016
15	Group	5.629	1	5.629	F (1, 24) = 0.08133	P = 0.7780
16	Subjects (matching)	1661	24	69.21	F (24, 24) = 83.15	P < 0.0001
17	Residual	19.98	24	0.8323		
18						
19	Number of missing values	0				

1.3. Body Fat Percentage

	2way ANOVA					
1	Table Analyzed	%BF_2WayANOVA				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	0.1016	0.0997	ns	No	
8	Time	1.979	< 0.0001	****	Yes	
9	Column Factor	0.08135	0.8884	ns	No	
10	Subjects (matching)	97.01	< 0.0001	****	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	3.402	1	3.402	F (1, 24) = 2.933	P = 0.0997
14	Time	66.26	1	66.26	F (1, 24) = 57.12	P < 0.0001
15	Column Factor	2.723	1	2.723	F (1, 24) = 0.02013	P = 0.8884
16	Subjects (matching)	3247	24	135.3	F (24, 24) = 116.6	P < 0.0001
17	Residual	27.84	24	1.160		
18						
19	Number of missing values	0				

2.0. Strength Outputs

2.1. Leg Press

	2way ANOVA					
1	Table Analyzed	Leg Press_2WayANOVA				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	0.01266	0.6927	ns	No	
8	Time	38.00	< 0.0001	****	Yes	
9	Group	7.619	0.0742	ns	No	
10	Subjects (matching)	52.47	< 0.0001	****	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	171.0	1	171.0	F (1, 24) = 0.1600	P = 0.6927
14	Time	513322	1	513322	F (1, 24) = 480.4	P < 0.0001
15	Group	102929	1	102929	F (1, 24) = 3.485	P = 0.0742
16	Subjects (matching)	708854	24	29536	F (24, 24) = 27.64	P < 0.0001
17	Residual	25644	24	1068		
18						
19	Number of missing values	0				

2.2. Lat Pulldown

2way ANOVA						
1	Table Analyzed	Lat Pulldown_2WayANOV				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	0.001113	0.9202	ns	No	
8	Time	8.947	< 0.0001	****	Yes	
9	Column Factor	2.321	0.4292	ns	No	
10	Subjects (matching)	86.12	< 0.0001	****	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	4.327	1	4.327	F (1, 24) = 0.01024	P = 0.9202
14	Time	34789	1	34789	F (1, 24) = 82.31	P < 0.0001
15	Column Factor	9024	1	9024	F (1, 24) = 0.6467	P = 0.4292
16	Subjects (matching)	334875	24	13953	F (24, 24) = 33.01	P < 0.0001
17	Residual	10144	24	422.7		
18						
19	Number of missing values	0				

2.3. Bench Press

2way ANOVA						
1	Table Analyzed	Bench Press_2WayANOVA				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	0.0	> 0.9999	ns	No	
8	Time	3.208	< 0.0001	****	Yes	
9	Group	4.586	0.2824	ns	No	
10	Subjects (matching)	91.02	< 0.0001	****	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	0.0	1	0.0	F (1, 24) = 0.0	P > 0.9999
14	Time	4069	1	4069	F (1, 24) = 64.86	P < 0.0001
15	Group	5817	1	5817	F (1, 24) = 1.209	P = 0.2824
16	Subjects (matching)	115460	24	4811	F (24, 24) = 76.68	P < 0.0001
17	Residual	1506	24	62.74		
18						
19	Number of missing values	0				

2.4. Isometric Unilateral Leg Extension

	2way ANOVA					
1	Table Analyzed	Biodex_2WayANOVA				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	0.1324	0.6497	ns	No	
8	Time	22.77	< 0.0001	****	Yes	
9	Group	2.781	0.3686	ns	No	
10	Subjects (matching)	62.30	0.0003	***	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	367.7	1	367.7	F (1, 19) = 0.2130	P = 0.6497
14	Time	63238	1	63238	F (1, 19) = 36.63	P < 0.0001
15	Group	7725	1	7725	F (1, 19) = 0.8482	P = 0.3686
16	Subjects (matching)	173038	19	9107	F (19, 19) = 5.276	P = 0.0003
17	Residual	32797	19	1726		
18						
19	Number of missing values	0				

3.0. Fibre cross-sectional area

3.1. Type 1 Fibres

	2way ANOVA					
1	Table Analyzed	Type 1 Fibres_2WayANOVA				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	1.745	0.0771	ns	No	
8	Time	23.45	< 0.0001	****	Yes	
9	Group	10.34	0.0392	*	Yes	
10	Subjects (matching)	52.19	0.0004	***	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	2.539e+006	1	2.539e+006	F (1, 24) = 3.412	P = 0.0771
14	Time	3.412e+007	1	3.412e+007	F (1, 24) = 45.86	P < 0.0001
15	Group	1.505e+007	1	1.505e+007	F (1, 24) = 4.756	P = 0.0392
16	Subjects (matching)	7.595e+007	24	3.165e+006	F (24, 24) = 4.253	P = 0.0004
17	Residual	1.786e+007	24	743997		
18						
19	Number of missing values	0				

3.2. Type 2 Fibres

	2way ANOVA					
1	Table Analyzed	Type 2 Fibres_2WayANOVA				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	4.060	0.0291	*	Yes	
8	Time	32.18	< 0.0001	****	Yes	
9	Group	11.54	0.0089	**	Yes	
10	Subjects (matching)	34.12	0.0637	ns	No	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	9.432e+006	1	9.432e+006	F (1, 24) = 5.384	P = 0.0291
14	Time	7.475e+007	1	7.475e+007	F (1, 24) = 42.67	P < 0.0001
15	Group	2.680e+007	1	2.680e+007	F (1, 24) = 8.115	P = 0.0089
16	Subjects (matching)	7.925e+007	24	3.302e+006	F (24, 24) = 1.885	P = 0.0637
17	Residual	4.204e+007	24	1.752e+006		
18						
19	Number of missing values	0				

4.0. Ultrasound measures

4.1. *Vastus lateralis* cross-sectional area

	2way ANOVA					
1	Table Analyzed	CSA_VL				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	1.855	0.0625	ns	No	
8	Time	21.25	< 0.0001	****	Yes	
9	Group	7.133	0.1092	ns	No	
10	Subjects (matching)	59.10	< 0.0001	****	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	77.51	1	77.51	F (1, 23) = 3.833	P = 0.0625
14	Time	888.1	1	888.1	F (1, 23) = 43.91	P < 0.0001
15	Group	298.1	1	298.1	F (1, 23) = 2.776	P = 0.1092
16	Subjects (matching)	2470	23	107.4	F (23, 23) = 5.310	P < 0.0001
17	Residual	465.2	23	20.22		
18						
19	Number of missing values	0				

4.2. *Biceps brachii* cross-sectional area

	2way ANOVA					
1	Table Analyzed	CSA_B				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	1.614	0.0059	**	Yes	
8	Time	8.926	< 0.0001	****	Yes	
9	Group	1.549	0.5114	ns	No	
10	Subjects (matching)	83.67	< 0.0001	****	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	8.265	1	8.265	F (1, 24) = 9.139	P = 0.0059
14	Time	45.71	1	45.71	F (1, 24) = 50.54	P < 0.0001
15	Group	7.930	1	7.930	F (1, 24) = 0.4442	P = 0.5114
16	Subjects (matching)	428.4	24	17.85	F (24, 24) = 19.74	P < 0.0001
17	Residual	21.71	24	0.9044		
18						
19	Number of missing values	0				

4.3. *Vastus lateralis* thickness

	2way ANOVA					
1	Table Analyzed	Length_VL				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	0.04440	0.8021	ns	No	
8	Time	13.44	0.0002	***	Yes	
9	Group	6.257	0.1486	ns	No	
10	Subjects (matching)	64.41	0.0007	***	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	0.004927	1	0.004927	F (1, 23) = 0.06428	P = 0.8021
14	Time	1.492	1	1.492	F (1, 23) = 19.46	P = 0.0002
15	Group	0.6944	1	0.6944	F (1, 23) = 2.234	P = 0.1486
16	Subjects (matching)	7.148	23	0.3108	F (23, 23) = 4.055	P = 0.0007
17	Residual	1.763	23	0.07665		
18						
19	Number of missing values	0				

4.4. *Biceps brachii* thickness

	2way ANOVA					
1	Table Analyzed	Length_B				
2						
3	Two-way RM ANOVA	Matching: Stacked				
4	Alpha	0.05				
5						
6	Source of Variation	% of total variation	P value	P value summary	Significant?	
7	Interaction	7.115	0.0024	**	Yes	
8	Time	15.36	< 0.0001	****	Yes	
9	Group	0.2411	0.7634	ns	No	
10	Subjects (matching)	62.42	0.0004	***	Yes	
11						
12	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
13	Interaction	0.5552	1	0.5552	F (1, 24) = 11.49	P = 0.0024
14	Time	1.199	1	1.199	F (1, 24) = 24.81	P < 0.0001
15	Group	0.01881	1	0.01881	F (1, 24) = 0.09268	P = 0.7634
16	Subjects (matching)	4.871	24	0.2030	F (24, 24) = 4.201	P = 0.0004
17	Residual	1.159	24	0.04831		
18						
19	Number of missing values	0				