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Microflora derived whey protein compared to dairy-derived whey protein on muscular adaptations to lower limb resistance training in healthy young adults

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Abstract

Background:

Individuals and athletes alike require a higher habitual protein intake to support muscular adaptations in response to resistance training (RT). Many non-animal protein alternatives are typically associated with reduced anabolic potential, compared to animal derived protein sources. With the rising popularity of animal-free diets, researchers are faced with the challenge of investigating novel protein alternatives that are more sustainable. Microflora derived whey protein has a similar composition to traditional dairy, promising a lower carbon footprint and more efficient production process, relative to traditional dairy derived whey. Research to date has not yet revealed whether a novel microflora derived whey protein alternative can comparatively support muscular adaptations to RT.

Aims:

This study aimed to compare muscular adaptations following the ingestion of microflora derived non animal whey protein, against an animal derived whey protein.

Methods:

In a randomised double blinded control trial, 23 healthy young adults (23 ± 5 yr), were allocated to receive either microflora derived whey or a dairy whey supplement. Participants completed a high load (>60% 1RM) lower body focussed resistance training program, three times a week, for 8 weeks. Ultrasonography to evaluate muscle architecture, and muscle strength (isokinetic knee extension and 1RM strength for each exercise) were recorded at baseline, week 5, and week 8 post intervention. Measures of 1-RM and ultrasound were analysed using 2 way repeated measures ANOVA in SPSS (version 29, IBM SPSS, Armonk, NY USA). Mean peak torque of MVC measures across

both visits were analysed using a paired sample T test to evaluate statistically significant differences between test and retest values.

Results:

The results show that 1RM and MVC increased after the 8-week lower body focussed resistance training intervention. There was a statistically significant increase in MVC from baseline (389 ± 132 Nm) in group A, and (464 ± 147 Nm) in group B compared to week 8 (397 ± 101 Nm) and (484 ± 165 Nm; *P<0.01*) in group A and B respectively. Overall, measures of MVC demonstrated a greater increase in group B than group A. There was no significant difference in measures of muscle architecture from baseline to week 8. Measures of pennation angle showed no significant difference in group A (17 ± 3°) and group B (18 ± 3°) at week 8. Fascicle length decreased in both group A (91 ± 18mm), and group B (86 ± 7mm). Muscle thickness remained constant from baseline to week 8 in group A (27 ± 4mm), and group B (28 ± 5mm).

Conclusions:

Amongst a healthy young adult population, ingestion of microflora derived, and standard animal-based whey protein comparatively support increases in muscular strength, facilitating muscular adaptation in response to lower body focussed RT. Overall, there is evidence of a significant time effect on strength in both groups, shown by increases in MVC and 1-RM output following the training protocol for 8 weeks compared to baseline. However, no significant difference in measures of muscle architecture, as a marker of muscle hypertrophy suggests that the extent of muscular hypertrophy achieved during the study remains to be determined.

Keywords: Non animal, resistance training, protein, muscular adaptation, hypertrophy, microflora

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Abbreviations:

θ – pennation angle

4E-BP1 – 4E binding protein

1RM – 1 repetition maximum

AA – amino acid

ACSM – American College of Sports Medicine

BCAA – branched chain amino acid

EAA – essential amino acid

eIF – eukaryotic initiation factor

FAO/WHO – Food and Agricultural Organisation of the United Nations / World Health Organisation

FDA – Food and Drug Administration

ICC – intraclass correlation coefficient

L_f – Fascicle length

MPB – muscle protein breakdown

MPS – muscle protein synthesis

MRI- magnetic resonance imaging

mTORC1 – mechanistic target of rapamycin

MVC – maximum voluntary contraction

MT – muscle thickness

MyoPS – myofibrillar protein synthesis

NAFLD – non alcoholic fatty liver disease

NPB – net protein balance

RDA – recommended daily allowance

RPE – rate of perceived exertion

RT – resistance training

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1. Introduction:

1.1 Overview of skeletal muscle

Skeletal muscle accounts for 40% of human body mass (1). As a major organ for locomotion, structural support, balance, and protecting vital organs in the body, the importance of skeletal muscle is evident for daily life and general health (1). The metabolic capacity of skeletal muscle is also vital as a key regulator of basal metabolic rate in humans. Following diet induced thermogenesis, skeletal muscle can mediate a postprandial increase in metabolic rate (2). As a highly metabolic tissue, skeletal muscle is the largest site for postprandial glucose deposits, where oxidation occurs, and stored as muscle glycogen (2). As an amino acid reservoir, skeletal muscle enables organ-specific protein synthesis to occur throughout the body (3). The amount of dietary protein that can be digested and absorbed by the human body is beyond the threshold that skeletal muscle can utilise towards muscle anabolism (3). Only ~10% of ingested amino acids in circulating plasma contribute towards de novo protein synthesis in skeletal muscle (3). Approximately 50% of ingested amino acids undergo extraction by highly metabolic splanchnic tissues, such as the gut and liver. Another ~40% released into circulating plasma, serves as metabolic substrates for energy production and ureagenesis (3). In the fasted state, circulating amino acids function as precursors for protein synthesis to maintain muscle mass and plasma glucose concentration (4). Therefore, sufficient muscle mass is essential to supply the amino acids for this process. Deficiency in skeletal muscle mass predisposes metabolic diseases including cardiovascular disease, and type II diabetes, and non-alcoholic fatty liver disease (NAFLD) with age (5)(6).

1.2 Protein balance in skeletal muscle tissue

Skeletal muscle is renowned for demonstrating high levels of plasticity (7). Skeletal muscle homeostasis is regulated by the balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB), known as net muscle protein balance (NPB) (8,9). In the absence of a stimulus, either upregulating MPS such as physical activity or downregulation in the context of disease, inadequate protein intake, or ageing, skeletal muscle mass mostly remains constant. The process of protein turnover allows for renewal of old, damaged muscle fibres, to maintain muscle mass and function. These processes governing NPB are highly responsive to resistance training and nutritional intake (23). Following ingestion, proteins are digested into their constituent amino acids. In the fed state, a post prandial increase in amino acids function as precursors to activate adaptive remodelling within skeletal muscle. Subsequently, MPS becomes greater than MPB, shifting NPB to a positive protein balance (8,9). Conversely, when MPB is greater than MPS in the fasted state, a net loss of muscle mass occurs as protein balance remains negative (3). Thus, a catabolic state persists amongst skeletal muscle tissue. Previous research has identified the crucial role of MPB in maintaining plasma amino acid concentrations, in the presence of sufficient levels of muscle mass as a preventative measure towards pathological and chronic disease (4). Heart failure and cancer are linked with cachexia, which is defined as a rapid and immense loss of muscle mass, strength and overall metabolic function is an important factor mediating survival (4).

1.3 Role of the mTOR pathway for muscle protein synthesis

RT stimulates MPS by activating mTORC1, as muscle fibres detect mechanical tension through receptors in their cell membranes (35). The Akt/mTOR pathway is a master regulator of skeletal muscle growth utilising mechano-transduction to trigger a signalling

cascade to activate downstream targets, as a crucial driver of muscle protein synthesis. Akt is an effector of anabolic signalling, and an inhibitor to catabolic signals. Furthermore, mTOR is crucial towards mediating exercise and nutrient induced effects on MPS, demonstrating upregulation in hypertrophic models both in vivo and in vitro (34). Stimuli such as EAAs function as substrates and activate the mechanistic target of rapamycin complex 1 (mTORC1) signalling pathway (34). The targets of this pathway regulate protein synthesis through translation, initiation, and elongation of various proteins (35). Downstream, mTORC1 phosphorylates two well-known proteins S6 kinase 1 (S6K1), and eukaryotic initiation factor 4E binding protein 1(4E-BP1). In line with an increase in MPS, the role of RT as a potent stimulator of MPS is evident through the phosphorylation of S6K1 in the first 1-6 hours following a bout of RT (35). 4E-BP1 is involved in initiating translation by preventing the binding of 4E, which is a eukaryotic initiation factor (eIF) to 4E-BP1, instead enhancing the formation of a translation initiation complex (34). Signalling mTOR positively influences transcription and translation within muscle tissue. The resulting shift in NPB promotes MPS whilst dampening degradation. Therefore, a subsequent increase in muscle fibre size is mediated and hypertrophy is supported within skeletal muscle tissue (10). Although mechanical tension mediated by external mechanical load during RT remains a more prominent MPS stimulus, the importance of AAs provided by dietary protein intake as substrates cannot be ignored. The absence of sufficient dietary protein would lead to negative NPB, as MPB would become greater than MPS (17,36).

1.4 RT and muscle protein turnover

Exercise stimuli, in aerobic and resistive formats are linked to elevated rates of MPS, mediated by changes in muscle cell signalling (10). Of these two variables, the effect of RT dominates, as a potent stimulator of MPS, via activation of mTORC1 (11). Following RT, MPS demonstrates a temporary increase, as skeletal muscle becomes sensitised to a rise in nutrient availability (12). With sufficient exogenous nutrient availability, MPB tends to also increase or remain the same. In instances of increased muscle damage, MPB activity becomes upregulated for removal of damaged proteins, whilst MPS supports regeneration of skeletal muscle (16). In the early stages of RT, MyoPS is largely focussed on attenuating initial muscle damage, rather than solely mediating muscle hypertrophy (16). The number of contractile proteins only gradually increases at around 3 weeks of RT towards mediating MPS (16). Regulation of skeletal muscle mass is highly governed by a combination of mechanical loading and protein intake. An increase in AAs post-exercise drives MPS beyond levels observed with either RT or protein intake alone (13). The latter, stimulates a rise in circulating amino acids, necessary to induce a positive shift in NPB and promote MPS (2)(3). A single bout of RT has the capacity to independently stimulate MPS. However, repeated bouts of RT cause increased MPS following each session, mediating adaptation to strength training (14). The impact of RT towards promoting MPS emerges as quickly as one hour after RT (10). Coupled with adequate protein ingestion, RT can facilitate sensitisation of skeletal muscle to amino acid availability immediately post exercise. This effect of increased MPS remains for up to 24 hours in trained populations but remains for 48 hours amongst untrained individuals (10) (23). The prolonged effect of MPS in untrained groups is likely related to requirements for repair and remodelling of skeletal muscle from damage induced swelling in the early phases of RT, in response to a novel stimulus (16).

1.5 Resistance training and hypertrophy

RT is established as a fundamental anabolic stimulus allowing for increases in skeletal muscle mass. As a key driver of MPS, RT induces mechanical tension, stimulating muscle growth and alterations in muscle architecture that arise from metabolic stress, and muscle damage (17). Hypertrophy is defined as a physiological increase in muscle size that stems from higher muscle fibre volume, linked to greater diameter and length. Heavy mechanical stimulus through RT causes disruption to myofibers and the underpinning extracellular matrix. Thus, synthesising a greater number of myofibrillar contractile proteins (actin and myosin). In line with this, observations of a higher number of total sarcomeres in parallel and series are seen as increases in diameter and length within a muscle fibre respectively (18) (19) (20). In response to mechanical loading, disruption to the extracellular matrix manifests as myogenic activity, causing an increase in size and quantity of contractile proteins actin and myosin, and sarcomeres in parallel. In turn, this also promotes greater actin and myosin cross bridge formation, allowing for matched force production generated with fewer motor units. Subsequently, the changes induced in individual fibres mediate an increase in skeletal muscle cross sectional area (21).

The remodelling response of skeletal muscle to training stimuli (RT) can be determined by measuring muscle architecture using B-mode ultrasound, which is a well-known medical imaging technique. Muscle architecture is comprised of muscle thickness (MT), pennation angle (θ), and fascicle length (L_f). θ angle and L_f are considered as useful markers of force output from muscle contractions, whilst MT is an indirect assessment of muscle cross-sectional area (29). The linear relationship established between θ, and strength suggests that a greater θ is linked with a larger muscle cross sectional area, and subsequently enhanced force production. More importantly, RT induced muscle hypertrophy may be

linked with an increase in muscle fibre θ, as Kawakami et al demonstrated that a greater cross-sectional area and θ was evident amongst bodybuilders, in comparison with age matched sedentary subjects (70). However, it is unclear whether RT is linked with greater θ in all muscle groups, or whether this effect is muscle group dependent, θ increases appear to be site specific. For instance, there was an increase in triceps θ after 16 weeks of RT, but no significant difference was seen in VL of the quadriceps after 12 weeks of RT (70).

RT variables including, but not limited to, volume, exercise order, tempo, and rest periods between sets can affect the acute stimulation of MPS. Stimulation of MPS can be achieved with loads at lower and higher intensities, with failure especially important at lower intensities (7, 20, 23). To allow sustained increases in muscle hypertrophy, and elevated MPS, the loading stimulus must continue to remain challenging throughout training, termed progressive overload (24). The principle underpinning progressive overload is the presence of an adequate stimulus, such as a greater number of repetitions or heavier loads to induce greater neuromuscular demand (24). This provides greater tension and increased stress on the body during RT, promoting muscular adaptation (24,25,26). The result of progressive overload during RT is a greater number of Type II fibres, approximately 50% more than that of Type I fibres (27). The importance of volume as a hypertrophic variable is evident from greater muscle hypertrophy achieved with higher volume (28-30 sets), than lower volume, (6-10 sets) per week for a given muscle group with RT (28). In agreement, combining high volume with increased load when performing a given exercise can potentiate a prolonged myofibrillar protein synthetic rate, towards mediating a hypertrophic response within a young male population (20). However, much variability remains surrounding optimal loading ranges towards muscle hypertrophy, as a

wide range of repetition ranges between five to thirty at performed at high effort have elicited comparable hypertrophic outcomes (29).

1.5.1 Physiological factors affecting muscle hypertrophy

The extent of muscle hypertrophy in response to a training protocol are dependent on factors such as an individual's training status, genetics, age, and sex (4). Sex has an impact on strength gains and hypertrophy from a morphological and functional perspective, due to naturally larger muscle size and androgens in men, relative to women (30). In previous training studies, the variable levels of muscle hypertrophy achieved between participants is evident, despite following identical training protocols (31). However, in response to RT protocols the proportion of high responders with changes in overall strength and muscle size largely outweighs low responders (30). Although Hubal et al demonstrated that a normal distribution accounts for the muscle hypertrophic response amongst a large sample of participants, this fails to account for genetic predisposition, that can affect interindividual gains in muscle mass (30). Other participant variables during training sessions such as maximal effort, which is a voluntary attribute, differences in endurance to fatigue which can influence the number of repetitions completed with a given mechanical load, and utilisation of a full range of motion can also modulate hypertrophic outcomes (26). Meanwhile, untrained participants show a more sensitive response to RT, manifesting as early strength gains arising due to neural adaptations (32). These neural adaptations are greatest during the first two months of RT in untrained individuals (32,33). In contrast, a trained population exhibits a dampened response to RT, and experienced lifters face challenges, as advances in lean muscle mass become harder to attain. Hence, designing a progressive training plan, utilising appropriate programming of volume (sets and repetitions) and weight is essential.

1.6 Amino acids and activation of mTOR

Following ingestion, AAs are rapidly incorporated into new skeletal muscle proteins, replenishing the protein supply utilised during the fasted state (4). After a bout of RT, ingestion of AAs promotes an enhanced MPS response through independent activation of mTORC1, stimulating protein translation, cell growth, and reducing catabolic factors (37,38,39). Functioning as both a signal and substrate, leucine is an example of crucial EAA considered to have the most potent effect on MPS with the capacity to activate the mTOR pathway independently (40). Previous work has demonstrated that the MPS response following leucine ingestion is dose-dependent, known as the ‘leucine threshold’, indicating a plateau in MPS at around 2.5g leucine ingestion (3).

1.7 The importance of protein

Adequate protein intake is crucial for maintaining general health, as well as supporting growth and development throughout life (3). More importantly, higher habitual protein intake is essential for supporting maintenance of muscle mass, remodelling in response to exercise stimuli, and muscle protein accretion amongst RT trained individuals (17). Proteins consist of 20 AAs, nine of which cannot be produced endogenously by the human body. These exogenous EAAs amplify the anabolic effect of RT on skeletal muscle. Dietary protein ingestion immediately after a bout of exercise and during periods of prolonged recovery the day after exercise contributes towards enhanced MPS rates and mediates further hypertrophy than the effect of feeding alone (41). In addition, increased plasma availability of BCAAs (leucine, valine, and isoleucine) comprising approximately 33% of skeletal muscle, are a key nutritional stimulus governing postprandial MPS rates (42). This is reiterated where, ingestion of 5.6g BCAAs in a young male population immediately after RT successfully stimulates muscle myofibrillar protein synthesis (8, 9,

43). Furthermore, BCAAs undergo metabolism in muscle tissue during exercise, allowing them to function as an immediate energy reservoir (42). Leucine is recognised as the most potent AA for post prandial MPS stimulation, as a key contributor to the muscle protein remodelling response. Following protein ingestion, gradual increases in intracellular and extracellular leucine concentration are responsible for mediating the MPS response. The leucine content within a protein source is a useful tool to predict its ability to stimulate post prandial MPS. Although leucine supplementation is a favourable strategy towards increasing MPS rate, activating the mTOR signalling pathway, leucine may not be as crucial as once thought. In a young population, supplementation with leucine alone did not lead to any hypertrophic response amongst an RT cohort consuming a high protein diet. In the presence of a high protein intake, leucine also had no impact on factors involved in recovery following completion of a lower limb RT protocol, including muscle damage induced from RT volume, which is a key determinant towards muscular adaptations to training, and subsequently hypertrophy (43).

1.7.1 Protein RDA and source

The current RDA for adults is 0.8g protein. kg bm^{-1} dm^{-1} according to the minimum amounts of each EAA to prevent deficiency and sufficiently meet metabolic demands (44). However, the RDA fails to account for greater requirements to support muscular adaptations to training, such as increases in muscle mass (23). The rationale for these recommendations is supported by training induced gains seen following adherence to an animal based high protein diet. From these studies, it can be inferred that protein intake of 1.6 $g \cdot kg^{-1} \cdot d^{-1}$ optimally mediates muscular protein synthesis rates and remodelling (40). Early studies by Friedman & Lemon suggested that the recommended intake for trained groups is double that of untrained individuals (45). In support of this, the American College

of Sports Medicine (ACSM) endorse a protein intake of 1.3–1.8 g · kg^{-1} · d^{-1} evenly distributed across 3-4 isonitrogenous meals throughout the day towards maximally stimulating MPS (3, 23). Nevertheless, the question remains around the validity of guidelines upheld by animal-based data amongst individuals consuming a non-habitually omnivorous diet. Currently, most studies assessing the protein synthetic response to protein intake draw comparisons between protein isolates or blends. In contrast, daily protein intake typically consists of whole food sources that happen to be plant or animal based. Within the western diet, a single serving of meat typically functions as the key protein source in a meal. In the context of a plant-based diet, legumes, nuts, and meat alternatives are the primary protein source.

Following RT, stimulation of MPS appears to induce a graded response to small increases in AA availability. Skeletal muscle demonstrates a refractory response to feeding, even throughout extended periods with high concentrations of circulating amino acids. In young adults, ingestion of 20-25g protein is considered an optimal stimulus towards inducing a maximal post RT MPS response (35,47). Similarly, much of the current body of literature shows that a protein intake above 20g induces a plateau in MPS response. In support of this, data exists to suggest that intake of differing quantities of lean beef with a higher overall protein content (30g v 90g) did not exude any additional benefit towards stimulating MPS, suggesting that a significantly higher dietary protein intake may not always be necessary towards achieving gains in muscle mass (23). Instead, downstream phosphorylation of mTORC1 signalling proteins remained unchanged at higher protein intake (12). Chronically elevated protein consumption is also proposed to lead to desensitisation of the muscle in instances of suboptimal AA intake below 20g (12). Moreover, protein ingestion above this threshold leads to a significant rise in AA lost by oxidation, due to oversaturation of skeletal muscle by AAs (23). This aligns with the

‘muscle full’ effect, where MPS rises in response to increasing EAA supply, until a certain point before decreasing back to basal levels, despite continued amino acid availability (23). Meanwhile, in response to a higher protein intake, which was previously concluded to be above 40g, oxidation of excess EAAs offered no additional benefit towards the muscle synthetic response (13). On the other hand, Macnaughton et al observed the need for a higher protein intake within a young population partaking in RT to initiate a plateau in the dose response curve of MPS (48). As the demand for increased protein intake appears correlated with an individual’s total muscle mass, it is feasible that a higher protein intake would apply to those with greater muscle mass, relative to those with decreased muscle mass to maximally stimulate MPS (47,48).

1.7.2 Protein quality and assessment

Protein quality is a measure of dietary EAA content, absorption kinetics, and digestibility. These factors determine the post-prandial capacity of a given protein source and subsequent delivery of AAs to tissues throughout the body for effective utilisation towards skeletal muscle anabolism (3). Across sources, variations in protein quality are attributed to differences in digestibility, EAA bioavailability and content, and amounts of non-essential nitrogen (49). Furthermore, effects on appetite, satiety, and overall caloric content are also highly variable between protein sources (3, 50,51).

Previously, protein quality was assessed using the protein digestibility-corrected amino acid score (PDCAAS), introduced in 1991 by the FAO/WHO and FDA (3). The PDCAAS rating of protein quality evaluated amino acid absorption, and in vivo protein synthesis. A limitation of PDCAAS, was the inability to quantify differences between faecal and ileal protein digestibility. In 2013, the digestible indispensable amino acid score (DIAAS) was reinstated as an assessment of protein quality. The DIAAS accounts for ileal digestion,

EAA digestion, and absorption. Hence, it is regarded a more accurate evaluation of protein quality. The amount of digestible dietary indispensable amino acids found in a protein source are compared against a reference protein. A score is generated according to the minimum dietary requirements for each EAA (51). The main difference distinguishing these two assessments of protein quality is sample origin. PDCAAS samples are obtained from faeces which often includes microbiota metabolism, whereas DIAAS samples are from the ileum. As ileal digestibility is derived from both colonic protein and nitrogen metabolism, it is a more accurate measure of protein quality (51)(52). Although both the PDCAAS and DIAAS can evaluate protein quality by considering nitrogen levels and EAA content to avoid deficiency, they fail to assess the anabolic potential of a given protein source.

1.8 Animal vs Non-Animal Protein

The effect of a protein source on postprandial MPS stimulation can differ according to variations in EAA profile and digestibility. Whilst both animal and plant protein sources have successfully supported an increase in overall fat free mass, animal-based protein sources appear to be the favoured source towards delivering this outcome (51, 53). These findings are explained by a higher EAA concentration and improved digestibility in animal-derived protein that can rapidly stimulate aminoacidemia postprandially to support muscular adaptation to RT (54). The most studied animal protein sources including beef, eggs, and whey all exhibit high absorbability of around 85-95%, and are all complete sources of protein, with a full EAA profile that closely matches bodily requirements (52). Therefore, they are considered potent stimulators of MPS.

Plant based proteins contributes to the majority of global protein intake (58%), compared to animal-based protein (42%) intake. In western societies, plant-based diets are

increasing in popularity. More recently, trends indicate that non-animal protein sources have been chosen more often over animal-based sources (52). Plant-derived proteins tend to exhibit lesser anabolic potential, with inferior quality to animal-based sources attributed to reduced EAA profile (due to deficiencies commonly seen in lysine, methionine and leucine), digestion, and absorption kinetics (3). A consequence of decreased absorption kinetics is slower initial post-prandial AA absorption (52). This is evident in plant-based legumes such as chickpeas and yellow peas with lower absorbability (50-70%) (52). Accompanying this, reduced digestibility in plant-based sources originates from their distinct protein structure (31). Several variables including anti-nutritional factors, insoluble fibre, and polyphenolic tannins that are typically either endogenous or develop during processing can impact the absorbability of plant-based sources, leading to less efficient EAA uptake in muscle tissue below the levels seen in a dose matched animal protein source (49). Through purification and extraction of nutritional factors, comparable absorbability to levels observed in animal-based sources have been achieved (52). Cooking plant-based foods is a purification technique that can increase protein digestibility and post prandial plasma availability to a similar extent seen in animal-based protein sources (>90%). Another technique to enhance protein quality involves combining two protein blends, both of which contain the appropriate EAA to replenish deficiencies, resulting in an optimum blend with an improved EAA composition. Lastly, consuming more of a plant-based protein source is a helpful method to increase protein intake (3).

However, emerging evidence is indicating that the gap between animal based, and plant-based protein sources towards supporting muscle anabolism may not be as significant as previously thought. In a group of young males, there was no significant difference in anabolic response following ingestion of plant-based protein isolates and blends, in

comparison to milk protein (52). It is also evident that RT individuals following a high protein vegan diet (1.8 g·kg bm−1·d−1) can still achieve an adequate protein intake to sufficiently supports RT induced skeletal muscle remodelling in a young population, showing similar rates of MyoPS to those consuming an omnivorous diet (63). In response to high volume RT, no significant difference in strength outcomes were observed between vegan and omnivorous groups, reinforcing that vegan and omnivorous diets can both exhibit benefits towards achieving gains in muscle mass (52).

To date, soy protein is the most widely studied plant-based protein where the MPS response has been investigated. The findings indicate that soy remains less effective compared to isonitrogenous quantities of whey, milk or beef in response to exercise (31,52). These differences may be explained by greater splanchnic extraction of EAA following intake of soy protein, causing a shift in EAA supply towards urea synthesis, rather than muscle building (31). Previously, Tang et al demonstrated that ingestion of whey and soy protein offered a faster onset of circulating leucine comparison to ingestion of casein protein. Furthermore, soy protein resulted in greater MPS than animal derived casein protein, due to the slower digestibility of casein protein. In turn, this caused a lower rate of plasma aminoacidemia and thus lowering the rate of MPS in comparison to intake of soy protein. These findings indicate that leucine concentration of a given protein source is the primary factor towards mediating post prandial increases in MPS, rather than the origin of ingested protein.

1.8.1 Environmental concerns and novel protein sources:

With the world population forecasted to reach 9.6 billion by 2050, the need to maintain food security to meet increased caloric demand, and adequate nutrition is continuously

heightened (52). The rising cost of protein, coupled with limited resources has created barriers towards accessing protein-rich food sources in some parts of the world (52).

Whilst most data regarding dietary protein intake and resulting muscle protein turnover has been concluded from studies examining animal-derived protein sources, the ethical and environmental concerns surrounding the production of animal production means there is a renewed focus towards the use of alternative protein sources, such as insects, algae, and mycoprotein (56, 57,58). These alternatives have been recognised for their high protein quality, and promising EAA and BCAA profile. Furthermore, comparable anabolic potential has been seen to classical animal-based proteins such as eggs, milk, and meat (3). More recently, mycoprotein is a novel fungal derived protein, shown to provide a more sensitive muscle synthetic response compared to ingestion of leucine-matched milk protein in a young population under resting and RT conditions (59). This development is a shift from much of the current body of evidence suggesting that animal protein remains a superior protein source towards stimulating MPS compared with non-animal-based sources (59). The hypertrophic response to mycoprotein ingestion is in line with its high protein quality, mediating elevated postprandial AA and leucine concentrations to a similar level seen in milk protein, subsequently driving a higher rate of MPS (59). However, amongst two leucine matched beverages, a higher systemic AA content from ingestion of milk protein than mycoprotein continues to highlight the superior protein quality in animal-based sources, accompanied by rapid digestibility and absorbability (59).

1.8.2 Introduction to Microflora derived protein, a novel non animal protein source

Microflora derived protein is produced by precision fermentation, following a four-step production process (60). Microflorae are the tiny microorganisms containing the exact DNA

sequence required to produce a dairy identical whey protein to that obtained from cows. Prior to fermentation, these microorganisms are fed simple plant sugars, before creating a final product that contains a dairy identical non animal whey protein**.** Traditional agricultural processes underpinning animal and plant protein sources depend on large amounts of land and potable water from surface and groundwater resources, such as freshwater, lakes, rivers and aquifers (60). In turn, this causes reduced biodiversity, driving a net loss of habitat and species. By implementing a sustainable production process, microflora derived protein uses 99% less water, 97% reduction in greenhouse gases, and up to 60% less non-renewable energy, resulting in a lower carbon footprint (60). Aside from being time efficient, with bacteria and yeasts only requiring a few months to generate a protein source, the resulting protein contains a high protein to dry mass ratio at around 50-80%, maintaining high nutritional value and yield (60). Hence, making the most of protein alternatives could also lower the costs associated with food production, as demand increases in response to population growth.

Microflora derived whey protein is a high-quality alternative protein source, containing all nine EAAs, with the same taste and texture found in traditional dairy based products. Furthermore, the resulting supplement is hormone, lactose, and antibiotic free. The dietary profile of each 28.2g serving of Future Whey provides 10g (35% of total amino acids) EAAs, 2.15g leucine, closely matching 3g present in traditional whey protein, and 19% other EAAs, detailed in Table 2. The high leucine content indicates the feasibility of microflora derived protein as a viable protein source towards stimulating muscle protein synthesis.

Currently, no existing study has investigated the anabolic properties of a novel microflora derived whey protein, towards influencing muscular adaptations and stimulating in response to RT in vivo. Most of the literature has only focussed on drawing comparisons between popular non-animal alternatives such as soy, pea, and rice protein against animal derived whey protein.

1.9 Aims and hypothesis:

The primary aim of this study is to compare the effects of a microflora derived whey protein against an animal-based whey protein on muscular adaptations in response to RT in young adults. Based on the dairy identical milk protein in both supplements, β-Lactoglobulin, we hypothesised that ingesting microflora derived whey protein would elicit comparable increases in muscle strength and architecture, compared with animal derived whey protein, during a progressive 8-week lower body focussed resistance. No significant difference in 1RM and MVC strength measures, or observations of muscle architecture were expected amongst groups.

2. Methods:

2.1 Participants

Twenty-three recreationally active participants aged 18-40 years, BMI (18.5-29.9 kg·m^2) volunteered to participate in this double blind randomised controlled trial. Participant characteristics are displayed in Table 1. To qualify for inclusion in the trial, participants had to fulfil the following inclusion criteria: (1) be aged between 18-40 with a BMI between 18.5-29.9 kg/m^2 , (2) at least four months resistance training experience, performing 2-3 exercise sessions per week, prior to beginning the study, (3) not pregnant or breastfeeding, (4) in the absence of adverse health conditions, (5) free from injury, neuromuscular problems or arthritic conditions, (6) not habitual smokers, elite athletes or sports people competing nationally and internationally. Participants were excluded if they indicated any allergies to milk, intolerances to study materials, taking medication which may interfere with muscle anabolism (e.g beta blockers, androgenic anabolic steroids, corticosteroids, non-steroidal anti-inflammatory drugs, or prescription strength acne medication), and creatine supplementation. Participants completed a general health questionnaire to ensure the absence of any health issues listed in the exclusion criteria. All participants were informed about the purpose, potential benefits and risks associated with the study, before providing written informed consent. Participants were recruited between December 2023 and April 2024. This study was part of a research project funded by 'The Hut Group'. Ethical approval was obtained from NHS Health Research Authority Research Ethics Committee (23/YH/0233). The procedures in the study were conducted in accordance with the Declaration of Helsinki of 1975, revised in October 2013. Data collection was carried out between January 2024 and June 2024 at the School of Sport and Exercise Science, at University of Birmingham.

Table 1. Baseline participants' characteristics

	GROUP A	***P***	**GROUP B**
	(n = 11)		**(n = 12)**
	6 f 5 m		7 f 5 m
Age (y)	23±5	0.979	23±6
Body mass (kg)	75±14	0.994	75±6
Height (cm)	173±12	0.577	171±12
BMI kg/m2	25±3	0.559	26±3
Fat (% body mass)	30±4	0.911	30±4
Lean mass (kg)	49±13	0.979	49±11

Values represent mean ± SD. No significant difference between groups (P >0.05). Participant characteristics across groups were compared using independent sample t-tests. f,female; m, male

2.2 Pretesting:

Following screening and enrolment in the study, all participants underwent preliminary testing one week prior to beginning RT sessions. Participants were asked to refrain from vigorous physical activity prior to baseline testing. Baseline testing consisted of DXA scan, ultrasound, and familiarisation with the exercise equipment involved in the training sessions. Participants completed 1RM tests for leg press and reverse lunge. All participants in both groups completed the leg press. Participants in group A completed the 1RM testing for reverse lunges, and all but one participant who was excluded due to injury in group B completed 1RM testing. To prevent injury, participants completed as few repetitions as possible, as a low intensity warm up prior to 1RM testing. Rest periods of 3

minutes between each rep were implemented, and the weight on each exercise was increased until the participant could no longer complete a controlled rep with full range of motion at a given weight. This testing was repeated at week 5 and week 8 upon completion of the intervention. Participants were provided with a supply of their allocated protein supplement for the study.

2.2.1 Muscle function tests

Using isokinetic dynamometry (Biodex Medical Systems, Shirley, New York, USA), participants attended the laboratory to perform a maximal isokinetic knee extension of their dominant leg. Evaluation of unilateral peak isokinetic torque, as a measure of quadricep strength was completed using the Biodex Medical System 3 (Biodex Medical Systems, Shirley, NY, USA). Participants remained seated throughout the test, with their dominant leg secured with a strap. The lateral epicondyle was referenced as an anatomical landmark for the knee joint's axis of rotation and aligned with the dynamometer axis. Prior to conducting the 1RM test, participants performed a repetition at 50% of perceived 1RM as warm up. To assess quadricep strength, three consecutive maximal isokinetic leg extension contractions of the dominant leg were recorded, at 60° range of motion. Verbal encouragement was given, allowing participants to push with maximal strength for approximately 4 seconds. Participants had a 40 second break between each concentric contraction. All participants in group A underwent MVC testing. In group B, 11 out of 12 participants completed MVC testing.

2.2.2 Muscle architecture

To quantify superficial muscle architecture, ultrasound images of the vastus lateralis (VL) were recorded using a portable ultrasound machine (GE Logiq S8), using a 45mm,

12.0MHz linear array probe and transmission gel. Whilst the participant remained supine, measurements were carried out on the dominant leg, following a 10 min rest period. The transducer was orientated perpendicular to the vastus lateralis (VL). The medial and lateral borders of the muscle were identified, using the ultrasound probe to locate the mid sagittal axis of the VL. Scans were obtained at 50% of the distance between the greater trochanter and the midpoint of the patella. MT, L_f, and θ were measured at baseline and at 8 weeks.

2.3 Study design

Following pretesting, participants were randomly assigned to two groups: group A (n=11) or group B (n=12), differing according to the provided supplement, either microflora derived whey or standard whey protein. Randomisation was carried out by the lead investigator with a computerised randomiser. Participants completed a preliminary visit, followed by three structured progressive lower body resistance training exercise sessions three times a week for 8 weeks, detailed in figure 1. The preliminary visit one week prior to the first resistance training session, consisted of eligibility screening, measurements of height and body mass, and a general health questionnaire.

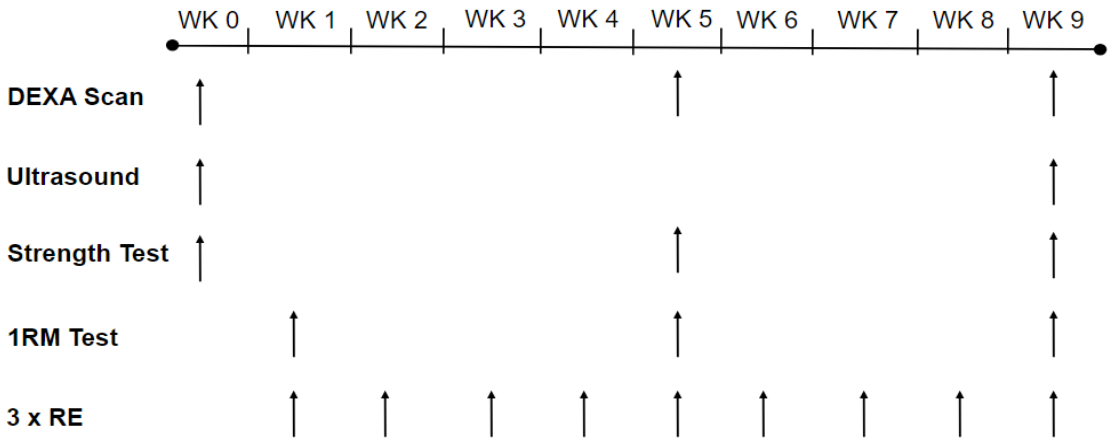

Figure 1: Schematic of study protocol. 23 healthy adults completed lower body focussed RT sessions x3 a week for 8 weeks, alongside ingestion of either standard whey protein or microflora derived whey protein. Participants underwent DXA, ultrasound scans to measure muscle architecture, and strength testing at

regular intervals to determine muscular adaptations to resistance training. 'WK0', 'WK1' refers to week 0, week 1, 1RM, 1 Repetition maximum; RE, resistance exercise.

2.4 Experimental procedures

Resistance training exercise program. The RT protocol was structured to utilise progressive overload within five exercises: leg press, leg extension, leg curl, calf raises, and reverse lunges to maximise muscle hypertrophy over the 8-week period. A lower body focussed program was selected, as changes in muscle architecture were assessed using ultrasound focussed on the *vastus lateralis* muscle. Participants were asked to refrain from strenuous lower body exercise before training sessions and throughout the duration of the study, to prevent interference with outcomes following our protocol. Participants completed three training sessions a week, lasting approximately 45-60 minutes over an 8-week period at the SportExR gym. Implementing high frequency training (3 or more sessions per week) can be beneficial towards increasing training volume towards maximising overall muscular strength and hypertrophy. Training sessions were scheduled on non-consecutive days, allowing for rest and optimal recovery between bouts from RT induced mechanical stress. Prior to each session, participants had the option to complete a warmup, which was not mandatory as part of the study protocol. In accordance with the ACSM guidelines, each exercise involved 3-4 sets of 8-12 repetitions, to enhance muscular strength and hypertrophy (3). To regulate effort across sessions, participants received verbal encouragement, ensuring each repetition was carried out in close proximity to complete failure, followed by a 2-minute rest between sets. After completing a set of a given exercise, participants were asked by the researcher to rate the overall effort required to perform the set, using the Borg CR_{10} RPE scale. The effort ratings were considered, allowing for appropriate adjustment of load to maintain the progressive nature of the

training program. Each session was supervised by at least two members of the research team, ensuring repetitions were performed in a controlled manner with proper technique and a suitable load of 60-80% according to one repetition maximum was utilised for each exercise. One of the researchers ensured that participant training data, including repetitions, load, and RPE for each exercise were recorded on a confidential spreadsheet and securely stored on the University cloud. All 1RM testing was repeated halfway at week 5, and week 8 after completion of the intervention. In instances where multiple participants were training together, the order of exercises was determined by the availability of equipment.

Dietary intervention. Throughout the duration of the study, there was no prescribed meal plan designed for participants. To maintain minimal dietary influences on the results, participants were instructed to continue their habitual diet, avoiding additional supplementation that was not included in the study.

DXA. Whole-body dual energy X-ray absorptiometry (DXA) scan to assess body composition (QDR Discovery W, Hologic, UK) was conducted prior to testing, at week 5 and week 8 after completion of training to understand changes in body composition. Body composition measurements detailed fat free mass (FFM), and fat mass (FM). Participants were scanned after an overnight fast (10-12h) with no fluid intake to minimise the impact of altered fluid balance. The participants were scanned using standard mode in a supine position with their feet approximately 10cm apart. Body position was recorded on the scanner to ensure consistent positioning between scans.

2.5 Supplemental beverages:

The nutritional composition of the microflora derived whey protein and the standard animal whey protein were analysed via third party company Premier Analytical Services. The macronutrient, micronutrient, and amino acid composition of the experimental beverages are presented in table 2. The beverages had similar protein content, and participant characteristics taken during preliminary testing were used to determine individual protein requirements for each participant, according to body mass (kg). The dosage of each beverage was also determined according to amount required to optimally stimulate MPS in a RT population, according to existing literature to allow for maximal anabolic effect (35). Both the microflora derived whey protein and animal whey protein were commercially available supplements, obtained from MyProtein™ THG plc, Manchester, UK. Participants ingested 0.3g·kg bm^{1}·d^{1} of either non animal whey protein or animal whey, depending on whether they were assigned to group A or group B. Across both protein sources, the participants consumed 31.4 ± 6.1g (range 16.4-27.8g) per serving of their allocated protein supplement. The supplement was provided in a dry powder format, and participants were instructed to dissolve the supplement with 300ml cold water, prior to consumption. On training days, one beverage was consumed one hour after the training session and one beverage an hour before sleep, in line with previous literature, indicating that protein consumption at these timings were effective at increasing muscle mass and strength. Following exercise, protein intake between 45 minutes to 1 hour following a workout is considered crucial to promote the muscle adaptive response and support muscular repair and remodelling, therefore maximising MPS and enhancing hypertrophy related adaptations (29). On non-training days, participants were instructed to consume one beverage mid-morning and one beverage 1h before sleep. Double blinding of the given protein supplements was conducted by an independent researcher and using the same flavour across both supplements.

Table 2: Nutritional composition of provided protein supplements

g/100 g	Recombinant bovine β-lactoglobulin (rBLG)	Dairy-derived whey protein (WHEY)
Energy (kcal)	360.00	367.00
Protein	71.00	74.00
Fat	3.10	3.20
Carbohydrate	7.70	8.00
Aspartic acid	7.72	8.67
Serine	2.77	4.13
Glutamic acid	13.4	13.00
Glycine	1.36	1.43
Histidine	0.91	1.17
Arginine	1.52	1.53
Threonine	3.23	5.00
Alanine	4.93	3.95
Proline	3.61	4.63
Cystine	1.88	1.69
Tyrosine	2.18	1.97
Valine	2.49	3.22
Methionine	2.03	1.57
Lysine	7.78	6.90
Isoleucine	2.72	3.39
Leucine	9.13	7.30
Phenylalanine	2.17	2.05
ΣTAA	69.83	71.60
ΣEAA	30.46	30.60
ΣNEAA	39.37	41.00

ΣTAA = summed total of total amino acids; ΣEAA = summed total of essential amino acids (His, Thr, Val, Met, Lys, Iso, Leu, Phe); ΣNEAA = summed total of non-essential amino acids.

2.6 Statistical Analyses

An a priori power analysis was performed using G* power version 3.1 9.7 to estimate a suitable sample size to draw comparisons between muscular adaptations following ingestion of microflora and traditional whey protein. Based on effect sizes estimated from previous literature of muscular adaptation in response to protein supplementation, a sample size of 36 participants per group was calculated, using independent sample T test *(P<0.08,* 80% power, effect size =0.67) (59).

Ultrasound images were analysed using Image J Fiji, SMA script a semi-automated software package (Simple Muscle Architecture Analysis, V1.7; Seynnes & Cronin, 2020), to quantify MT, L_f ,and θ of the VL. Statistical analysis of 1-RM, ultrasound, and MVC reliability were conducted using analysed using 2-way repeated measures ANOVA in SPSS (Version 29, IBM SPSS, Armonk, NY, USA) software. Time, baseline (week 0), and post intervention (week 8) and group A or B representing each supplement were used as factors. The mean peak torque obtained from MVC measures across both visits was calculated, across the three measures taken each session to characterise the sample. A paired sample T test was used to evaluate statistically significant differences between test and retest. Bland-Altman analysis was performed to identify the level of agreement between test and retest values. For ultrasound muscle architecture, means were calculated across the three scans for each parameter. Relative reliability was determined using the intraclass correlation coefficient (ICC). ICC was calculated using a two-way mixed model, and absolute agreement type. ICC value above 0.90 was excellent, good (0.75-0.90), moderate (0.5-0.75), and poor (<0.5). The formula used to calculate standard error of measurement was SEM = SD × $\sqrt{}$ 1 − ICC. The SEM% was concluded from SEM% = SD × $\sqrt{}$ 1 − ICC/Meantest1&test2 × 100. The α level of significance was set at $P < 0.05$. All data was displayed as mean ± SD, and 95% confidence intervals (CI).

3. Results

3.1 Participant characteristics:

Participant characteristics for individuals partaking in the trial are detailed in table 2. In total, 23 young adults, consisting of an even distribution of male and female participants, and a mean age of 23 years completed the study (n=11, group A and n = 12 group B). One participant in group B withdrew from the study, citing personal reasons. The groups

were well balanced for male and female participants. Independent T tests revealed no significant differences between groups concerning age, body mass, height, BMI, fat free mass, and lean mass (P>0.05). There were no reports of adverse events throughout the study.

3.2 1RM measures

Data from all participants in group A (n=11), and group B (n=10) were considered for final analysis. Two participants in group B were excluded due to personal circumstances at week 5, so no data was available to draw progressive strength comparisons, hence group B reverse lunge and leg press data is presented for *n=9.* Over the 8-week training intervention, RT led to increases in 1-RM on reverse lunge, leg press, and greater force output on MVC measures, shown in figure 2**.** Leg press 1-RM increased from baseline in group A by 40% (207 ± 58kg vs 290 ± 84kg) at week 8 (P<0.05) and 35% in group B (212 ± 57kg vs 287 ± 69kg) at week 8 (P<0.05).

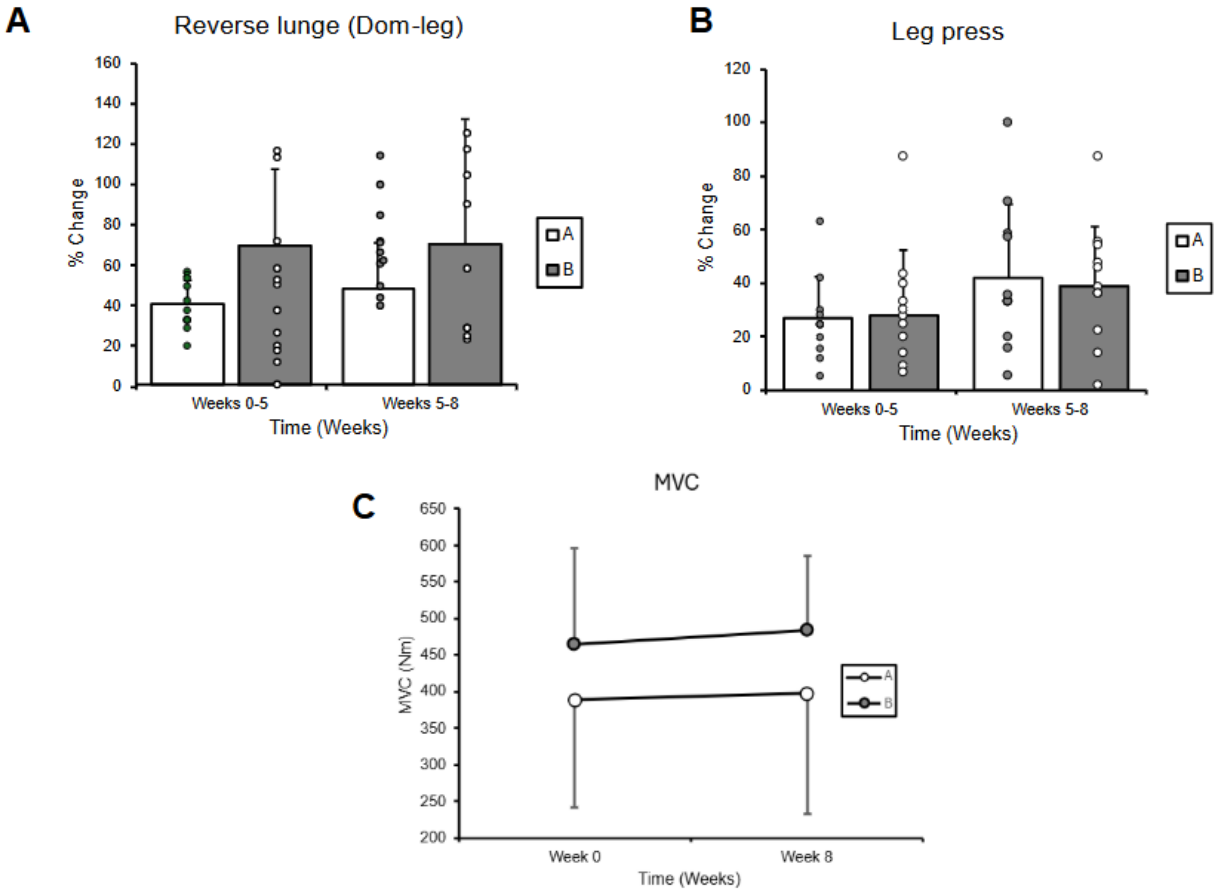

Figure 2: Changes in 1RM in group A and group B from baseline, through to week 5 and week 8. Measures were analysed using a two-way repeated measures ANOVA (time - % change), whilst MVC measures were compared using a paired sample T test. Group A is represented in white and group B is represented in grey, with reverse lunge and leg press measures taken at 3 different time points (Week 0,5, and 8). MVC measures were only recorded at week 0 and week 8 of the intervention. *P<0.05, demonstrates significant difference from baseline, and P < 0.05 indicates a significant difference between time points week 5 and week 8 of the intervention

Reverse lunge increased by 65% from baseline in group A (64 ± 20kg vs 105 ± 24 kg), and 58% in group B (56 ± 20kg vs 89 ± 26kg). Progressive changes in 1RM were reported in both exercises at week 5. In group A, reverse lunge 1RM continued to increase from week 5 to week 8, at (40.5% ± 12). Overall group B also showed an increase in strength from week 5 to week 8 (82% ± 61.97). Leg press 1RM increased from 26.7% ± 16 in group A, and 30.67% ± 23 in group B from baseline to week 5. Further increases were evident from week 5 to week 8 of (42.1% ± 27) in group A and (39% ± 22) and group B. From week 5 to

week 8, leg press increased to 290 ± 84 kg, and 287 ± 69kg, in group A and B respectively.

3.3 MVC measures

Two-way ANOVA analysis, comparing MVC revealed a significant time effect on strength increases amongst both groups (p=0.047). MVC increased from baseline (389 ± 132 Nm) in group A, and (464 ± 147 Nm) in group B compared to week 8 (397 ± 101 Nm) and (484 ± 165 Nm; *P<0.01*) in group A and B respectively. All participants in group A (n=12) completed the MVC. One participant in group B (n=11) did not complete MVC testing. ICC MVC was excellent for pre and post strength measures in both groups (ICC = 0.936, p < 0.001*).* Validity of isokinetic knee extension, as a strength measure was determined by performing intraday test-retest reliability of the dominant leg. This was conducted in a separate population of twelve healthy young adults, across two consecutive days. The test and retest data obtained for is presented in table 3. There was no statistically significant difference between the test and retest mean values for peak torque. The testing protocol mirrored that of the study, detailed above.

Table 3: Summary of Peak Torque in isokinetic knee extension.

Peak Torque (N.m)				
	Test		Re-Test	
Subject	Mean	SD	Mean	SD
1	242.01	6.12	247.47	19.04
2	739.62	6.64	709.82	36.42
3	483.58	21.20	491.35	5.65
4	401.25	23.81	398.30	24.60
5	784.09	9.57	768.17	23.05
6	604.35	11.59	601.15	16.88
7	342.90	3.09	371.21	6.14
8	507.17	12.34	464.61	29.09
9	508.19	25.06	413.41	39.43

10	334.14	8.83	372.14	8.44
11	594.07	24.53	490.00	13.81
12	407.13	8.84	393.30	15.20
P = 0.16				

N.m: Newton meter, SD: Standard Deviation.

Relative reliability (ICC) and standard error measurements of peak torque is presented in table 4. The ICC of peak torque outcome was excellent, for isokinetic knee extension.

Table 4: Relative test-retest reliability and absolute reliability of Peak Torque

Measurement	**ICC (95% CI)**	**SEM (N.m)**	**SEM (%)**
Total (n=12)		Peak Torque (N.m)	
Isokinetic knee extension	0.98 (0.93-0.99)	18.83	16.44

N.m: Newton meter; ICC: Intraclass Correlation Coefficient; CI: Confidence Interval; SEM: Standard Error Measurement

Figure 3 shows the Bland-Altman peak torque analysis for the dominant leg in extension.

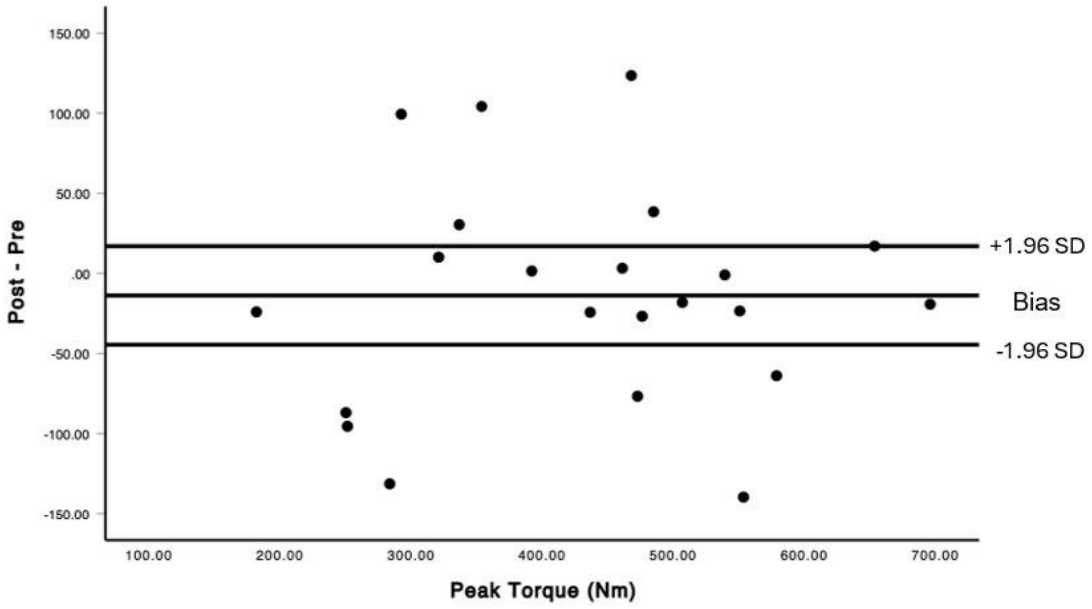

Figure 3: Bland-Altman peak torque analysis for dominant leg at baseline and after 8 weeks of training.

3.3 Ultrasound assessment of muscle architecture:

We were unable to achieve satisfactory ultrasound images from four participants in group B that were suitable for analysis using SMA. As a result, we had to exclude these participants from overall analysis, and group B data denotes *n=6*. At baseline, the coefficient of variation (CV) in group A was 14.81% for MT, 18.75% for L_f, and 12.5% for θ. In group B, baseline CV was 14.04% for MT, 13.33% for L_f, and 15.79% for θ. Mixed effects ANOVA model revealed no statistically significant difference between measures from baseline to week 8 in both group A *(p*=0.57*)* and group B *(p*=0.34*).* In group A, MT remained constant from baseline (2.7± 0.4cm) to week 8. In group B, mean MT remained the same from baseline (2.8 ± 0.4 cm) to week 8 (2.8 ± 0.5 cm). Overall, L_f decreased from baseline (9.6 ± 2.3cm) vs (9.1± 1.8cm) at week 8 in group A. Similarly, decrease was observed in group B (9.0 ± 1.2cm) vs (8.6 ± 0.7cm) at week 8. Baseline θ increased in group A to week 8 (16 ± 2°) vs (17 ± 3°) but decreased in group B (19 ± 3°) vs (18 ± 3°)

upon completion of the training regime

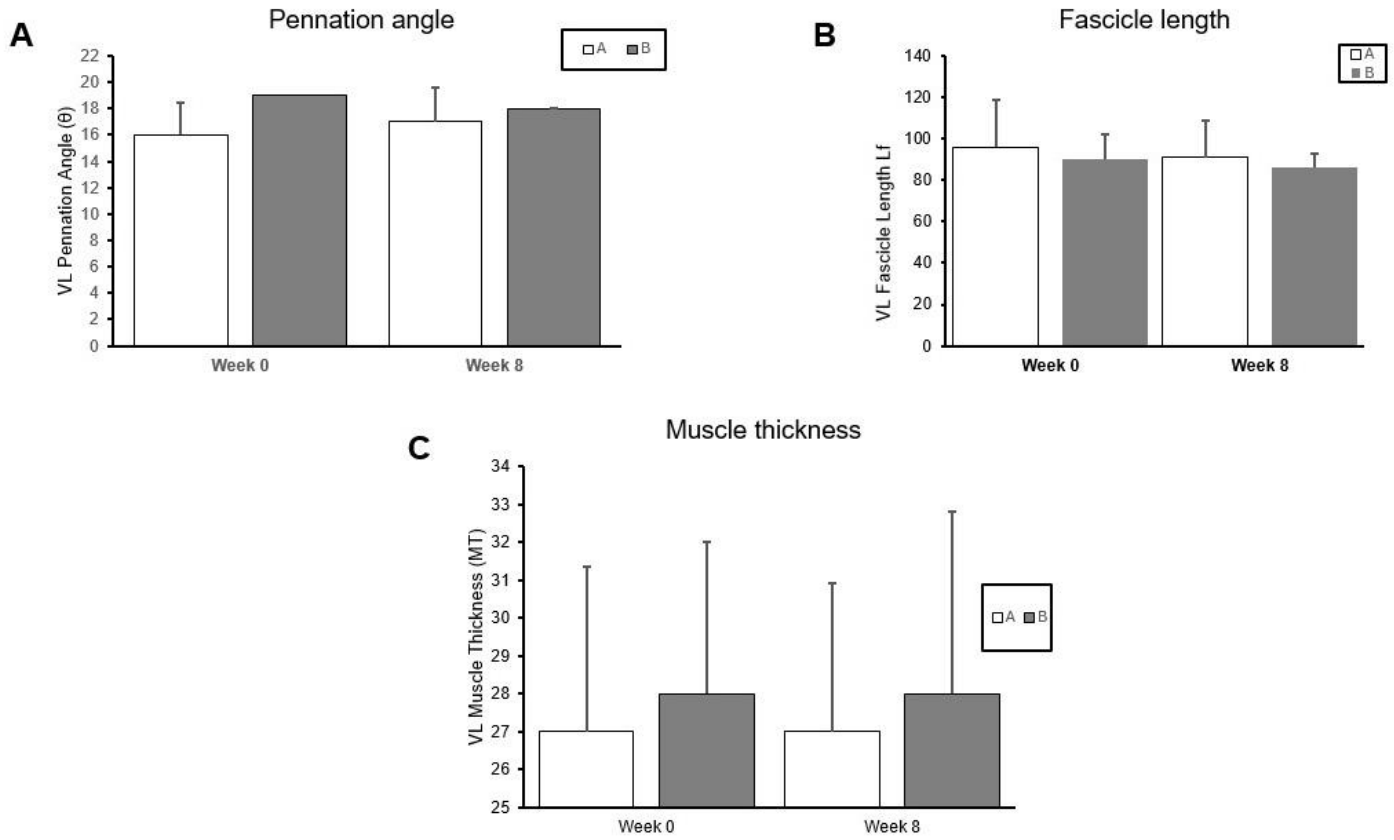

Figure 4: Time course of VL MT (A), L_f (B), and θ (C) in Group A and B, at baseline (week 0), and 8 weeks. Group A (p=0.57), Group B (p=0.34).

Data taken during intra-week reliability of ultrasound measurements of MT, L_f, *and* θ are displayed in table 5. ICC calculated to assess intra-day reliability showed good reliability for θ, moderate reliability for MT, and poor reliability for $L_{f,}$ denoted in table 6. Paired T tests demonstrated no significant difference between θ and $L_{f,}$ measurements during visit 1 and visit 2 (P>0.05), but measures of MT demonstrated a statistically significant difference between visits (P > 0.05), shown in table 6.

Table 5: Intra-day reliability of ultrasound measurements

Parameter	**Day 1**	**Day 2**

θ°	**Mean**	**SD**	**Mean**	**SD**
	13.66	0.29	12.20	0.13
	13.02	0.10	14.68	0.13
	12.81	0.66	14.54	0.11
	14.11	1.55	14.68	1.36
	21.38	1.91	22.53	1.21
	13.83	1.49	13.69	1.15
	21.68	2.38	13.84	1.94
	13.85	0.49	13.34	0.65
	14.92	3.43	13.17	0.27
	11.39	0.97	9.71	0.71
	17.14	1.79	18.77	1.52
	20.58	0.84	15.06	2.46
MT, cm				
	2.19	0.25	1.93	0.05
	1.11	0.06	2.59	0.18
	1.98	0.41	2.05	0.15
	1.68	0.10	2.46	0.48
	1.90	0.08	3.23	0.09
	1.82	0.02	2.07	0.23
	2.43	2.48	2.37	0.12
	1.46	1.46	2.49	0.18
	2.01	1.89	2.16	0.37
	2.30	2.29	2.79	0.71
	3.07	2.96	2.85	0.13
	2.43	2.30	2.24	0.06
L*f, cm*	7.50	0.70	9.46	0.27
	5.64	0.19	7.49	0.15
	9.10	0.80	8.02	1.65
	7.48	0.35	7.67	0.21
	5.97	0.18	8.56	0.18
	8.33	1.73	8.60	0.15
	7.14	0.93	9.58	1.03
	6.16	0.17	8.89	0.33
	7.36	2.13	8.20	0.99
	13.01	0.39	11.35	0.93
	11.19	0.36	8.62	0.37
	6.72	1.23	9.82	2.06

Θ°: pennation angle, MT: muscle thickness; L*f:* fascicle length

Table 6: Relative test-retest reliability of ultrasound parameters

Parameter	ICC	95% CI	*P*-value
θ°	0.759	0.214-0.929	0.26
MT, cm	0.547	-0.339-0.863	0.04
L*f, cm*	0.101	-0.949-0.687	0.13

Θ°: pennation angle, MT: muscle thickness; L*f:* fascicle length, 95% CI: 95% confidence interval

4. Discussion:

4.1 Summary of key findings

In the present study, we demonstrated that participants consuming either microflora derived, or traditional whey protein supplements as part of a high protein diet displayed comparable increases in 1RM (leg press and reverse lunge), and MVC over an 8-week RT period. Our findings also suggested no significant difference in muscle architectural parameters: MT, θ, or L_f after the 8-week training period. Here we have shown that animal-based protein sources, such as whey protein are not necessarily superior towards inducing a muscle synthetic response to RT amongst a young population following a high protein diet. Thus, alongside a high protein intake, microflora derived whey protein can facilitate comparable RT induced strength adaptations to traditional dairy whey protein.

Dietary protein intake and RT are potent stimulators of skeletal muscle hypertrophy. Following RT, increased MPS is a useful towards mediating further gains in strength and muscle mass (29). Amongst individuals partaking in RT, it remains crucial to mediate positive NPB, with protein intake above the RDA proving optimal towards mediating subsequent increases in muscle mass (3). Without sufficient protein intake, NPB becomes negative, and a catabolic state persists amongst skeletal muscle tissue. Many studies evaluating the postprandial MPS response are largely based on cohorts consuming high quality animal-based sources including milk proteins, beef, and egg. Animal based protein

sources contain all EAAs required to prevent deficiency, exhibit good digestibility, and stimulate the mTORC1 pathway to mediate MPS. Previous evidence in a young cohort favours the superior quality of animal-based protein towards stimulating post exercise MPS and promoting an increased positive EAA balance from ingestion of milk protein compared to soy protein following RT (61). Conversely, non-animal derived proteins usually present deficiencies in certain EAAs, namely leucine, lysine, and methionine, which hinders optimal myoPS following RT (3). This raises questions about their efficacy as leucine is recognised as one of the most potent amino acids towards independently stimulating the mTORC1 pathway that upregulates MPS, through promoting three times more phosphorylation of downstream mTORC1 targets ribosomal s6 kinase, and ribosomal s6 in comparison to other EAAs (35). Hence, leucine content of a protein source is considered an important determinant towards their overall effect on MPS, and therefore muscle hypertrophy (33). In addition, there are issues of reduced digestibility within non animal protein sources that are associated with differences in overall protein structure (3). In the present study, we tested a microflora derived protein blend, which is animal free and has a highly similar amino acid composition to traditional whey protein. Moreover, the microflora derived blend had a higher EAA content (leucine, lysine, methionine) than the traditional whey protein supplement tested alongside, highlighting the benefits that the microflora derived blend provides towards maximising the muscle protein synthetic response to RT.

Population growth has increased global demand for protein. Heightened consumer awareness towards sustainability issues involved in animal and plant derived protein production, reinforces the need for protein alternatives. More recently, research is shifting from classical focusses of optimal timing and dosage of protein intake towards the

anabolic potential of novel protein sources. Currently, limited studies have concluded the ability of novel protein sources, such as algae, mycoprotein, and insects to mediate hypertrophy amongst RT individuals (62). Similar to our findings, recent data has supported the anabolic capacity of microalgae-based protein alternatives Spirulina and Chlorella, delivering comparable increases in MyoPS to levels seen with mycoprotein ingestion, in response to RT and at rest. The promising absorption kinetics of Spirulina were also clear when concentrations of plasma EAA following ingestion were deemed comparable with levels seen with mycoprotein ingestion (63). The superior post prandial aminoacidemia observed from dose matched whey protein, versus pea or cricket supplements, indicates the reduced bioavailability of the latter two protein alternatives. Despite this, all three protein sources equally stimulated activation of the mTOR1 signalling pathway following RT amongst a young male population (64, 65).

In concordance with Laang et al, we demonstrated that microflora derived protein potentiates increases in strength in the same manner as traditional animal derived whey protein (64). As microflora derived protein is engineered to contain the identical β-Lactoglobulin protein as traditional whey, we hypothesised that there would be no significant difference in strength changes, measured by 1RM and MVC and muscle architecture between 2 groups of healthy young adults. The nutritional content of the microflora derived protein, detailed in table 2, indicates a 25% greater leucine content per 100g (9.13g versus 7.3g) than traditional whey protein. Indeed, the increased leucine content in the microflora derived protein may have contributed towards activation of the mTORC1 pathway, functioning as an optimal stimulus towards providing comparable rates of MPS (68). In the presence of both optimal protein dosage and exercise protocol towards

achieving muscular hypertrophy, this study is the first to demonstrate the feasibility of an animal free microflora derived protein blend towards optimising MPS in response to RT.

The significant strength increases from our findings are likely associated with experimental design. In line with our hypothesis, both supplements elicited a significant increase for both leg press and reverse lunge 1RM measures from baseline to week 8. Leg press increased by 40% in group A and 35% in group B. Reverse lunge increased by 65% in group A and 58% in group B. One participant in group B was unable to re-test their reverse lunge 1RM at week 8 due to knee pain. Moreover, another participant in group B had a 45% decrease in strength at week 8, which may represent an outlier. Within group A, a 100% increase in strength recorded from week 5 to week 8 on both leg press and reverse lunge may provide reasoning for a higher average increase within the group. Our training protocol included 3 weekly sessions performed on non-consecutive days for 8 weeks. Following the same intervention duration, our observations are in line with strength increases shown by Schoenfeld et al, amongst a RT population (33). Our study cohort also included female participants, consolidating the relevance of previous findings that were mainly observed in a trained male population partaking in RT. We implemented a high protein intake above the RDA and progressive overload utilising high volume and intensity to ensure work was maintained at around 60-80% 1RM to mediate optimal gains in muscle hypertrophy within the study period (66). Progressive overload exhibits the greatest power towards increasing the overall number of sarcomeres in parallel and series by continuously induces stress across multiple muscle groups and mediating adaptations to greater amounts of force. During the early stages (first two weeks) of an RT program, rapid increases to maximal strength arise from neurological, functional, and morphological adaptations (67). This is further evidenced by Brook et al. (2015) showing prominent hypertrophic remodelling

during early stages of RT at 0-3 weeks (32). In support of our findings, a similar study also recorded increases in strength after only 5 weeks of RT (19). Our measures at week 5 showed reverse lunge increased by 40.5% in group A and 48.4% in group B. Leg press increased by 26.7% in group A, and 27.8% in group B.

Surprisingly, our reports identifying no significant difference in muscle architecture after 8 weeks of training do not align with a hypothesised increase in response to RT, based on existing studies within the literature, such as significant improvements in VL muscle thickness following RT seen by Schoenfeld et al (33). Additionally, numerous studies have backed the efficacy of ultrasound as a reliable imaging technique to determine changes in quadriceps muscle size, in response to an RT intervention (33). The strength increases evidenced by 1RM and MVC testing provide rationale for predicted increases in MT that correlate with RT mediated hypertrophy. Quantification of MT is a useful indicator of muscular adaptation that corresponds with RT induced changes in skeletal muscle cross sectional area, to a similar extent seen using MRI, which is considered as a gold standard method for muscle imaging. Indeed, VL hypertrophy also appears to favour multi-joint exercises, leading to a greater increase in MT elicited by leg press compared to leg extension, both exercises that were included in the prescribed exercise regime (69). Although there are many benefits to ultrasound quantification of skeletal muscle including convenience, portability, and cost effectiveness, this method presents obstacles amongst certain populations. Firstly, ultrasound images captured can be affected by intermachine and interoperator variability. For example, there are difficulties around recording accurate images for assessment of muscle architecture in obese populations due to excess adipose tissue. Currently, there are no ‘cut off’ threshold values for differing populations and gender, which can offer reassurance in instances of participants that present with low

muscle mass. Furthermore, threshold values can support the recognition of ultrasound an even more reliable reference method for quantifying muscle architecture. Throughout the study, all scans were performed by the same operator to reduce inter-operator variability and minimise measurement errors that were operator dependent.

It is possible that 8 weeks of RT was an insufficient period to elicit significant changes in muscle architecture detectable by ultrasound, even though it is a sufficient period to achieve RT induced hypertrophy and increases in strength (33). There are limits to ultrasound sensitivity, and there may have been a small change in muscle size that the ultrasound was not sensitive enough to pick up, particularly during early stages of muscle growth. During a longer 12-week RT study, Rutherford and Jones also demonstrated no increase in pennation angle within the VL, in the presence of increased muscle CSA. In agreement with our findings, Rutherford and Jones also reported an increase in maximal isometric strength despite no significant changes in muscle architecture. Muscle hypertrophy is believed to occur in a regional specific manner, with greater hypertrophy emerging in proximal to distal aspects of the muscle (33, 70). Despite the shorter duration of our study, previous data has indicated that 14 weeks of RT allowed for detectable training induced alterations in muscle architecture with 14 weeks of heavy RT, evidenced by elevated VL muscle fibre pennation angle, accompanied by enhanced force generating capacity. It is well established that muscle hypertrophy exhibits a dose dependent relationship, where higher training volumes correlate with greater gains in muscle mass. Hence, it is possible that the higher training volume achieved over 14 weeks may have been a key factor towards promoting more marked strength gains and muscular hypertrophy, that would translate into detectable changes upon ultrasound. Thus, it can be inferred that time is a particularly important variable to maximise muscular hypertrophy,

allowing for an extended training period that would enhance the likelihood of ultrasound to measure muscular changes following RT. Therefore, the true morphological effect of RT, following intake of microflora or standard whey protein requires further investigation.

However, Seynnes and colleagues have proven that fascicle length in the VL increased only 10 days into a 5-week training program, ahead of increased pennation angle, which occurred after 5 weeks of training (19). Indeed, the presence of a greater number of sarcomeres in series and parallel, measured by increases fascicle length following 8 weeks of RT in a young cohort illustrates muscle remodelling (19,71). Adding to the unexpected nature of our findings, it has been proposed that pennation angle is a measure likely linked with RT induced muscle hypertrophy, evidenced by greater muscle CSA and muscle fibre pennation angles recorded in the triceps brachii of bodybuilders, relative to untrained individuals (72). As muscle hypertrophy increases, muscle fibre pennation angle is believed to also increase (71). Amongst existing studies using ultrasound to detect changes in muscle mass, heterogeneity is present across methods described which in turn can affect conclusions drawn from studies. As a result, variability in ultrasound methodology reinforces the need to develop a universal approach to standardise measurements obtained from ultrasound.

The present study has several limitations that should be acknowledged. The study had a relatively small sample size and may have been underpowered with an increased risk of type II errors occurring, to detect a significant change related to muscle architecture. Despite subjects being advised not to perform additional exercise throughout the intervention, we do not have solid evidence to conclude that participants adhered to these guidelines outside of the study. Our findings are specific to a young RT cohort. Hence,

whether the same anabolic benefits of microflora derived whey can be postulated amongst other populations including untrained individuals or older adults remains to be determined. During the study, our participants followed a high dietary protein intake (1.6g.kg $bm^{-1}.d^{-1}$) and it is unclear whether these findings would be applicable to those with a lower protein intake, or simply adhering to the RDA (0.8g.kg $bm^{-1}.d^{-1}$). With the significant contribution of protein supplementation towards achieving a high protein diet, it is difficult to determine whether an entirely plant-based diet with limited supplementation would still comparatively support the high protein requirements towards achieving increased muscle mass and strength gains. Nevertheless, there is a well-established dose response relationship between dietary protein ingestion, and rises in MPS, showing no response to excess available amino acids present once a given threshold is reached. Currently, the established figure is ~20g intact protein. Studies conducted around whey protein intake further consolidated that intake of 20g protein offered maximal MPS stimulation, relative to 10g and 40g whey protein respectively. In fact, evidence is indicating that excess protein intake above 40g may not be optimal, as the excess amino acids are no longer incorporated to MPS. Instead, they become re-directed towards whole body AA catabolism through AA oxidation and ureagenesis. In daily life, the majority of protein intake arises from whole food intake, rather than supplementation. The only instance where a higher protein intake would be beneficial towards achieving similar levels of protein synthesis observed in a younger population would be amongst older populations that are perhaps affected by anabolic resistance.

Although maintaining a high protein intake may be challenging outside of study conditions, our findings have proved that supplementation can be implemented as a useful tool towards reaching these goals for individuals with differing dietary requirements. In the

absence of habitual diet data detailing individual caloric and protein intake, it remains speculative that sufficient protein intake amongst participants was maintained by protein supplementation from the intervention, mediating the MPS response.

The present study is the first to evaluate the anabolic potential of a microflora derived whey protein compared to a traditional dairy based whey protein, in response to RT. Our data demonstrate that comparable increases in strength are possible when consuming a novel non animal whey protein, provided that an adequately high protein intake is maintained.

We have demonstrated the adaptability of our findings, as both protein supplements were consumed alongside participant's habitual diet, rather than a controlled diet for the 8-week trial period. Our data also indicate that protein source may have less of an impact on MPS rates when following a high protein diet. Further research should continue to establish whether microflora derived protein alternatives still have the capacity promote strength gains in response to RT, under circumstances where individuals are consuming a suboptimal protein intake within their habitual diet. Meanwhile, the capacity of microflora derived protein to support other athletic populations, such as endurance and hybrid training to the same extent also remains to be determined.

5. Conclusion:

In summary, novel microflora derived protein, and standard whey protein delivered comparable increases in 1RM and MVC measures following 8 weeks of lower limb focussed RT. There was no significant difference in ultrasound measures of MT, L_f, and θ with either supplement in response to the training protocol. These findings indicate that

microflora derived protein can be incorporated into the diet of young RT trained individuals, aiming to achieve muscle hypertrophy and optimise the skeletal muscle adaptive response.

6. References:

1. Morgan J, Partridge T. Skeletal muscle in health and disease. Dis Model Mech. 2020 Feb 6;13(2):dmm042192. doi: 10.1242/dmm.042192. PMID: 32066552; PMCID: PMC7044447.
2. Periasamy M, Herrera JL, Reis FCG. Skeletal Muscle Thermogenesis and Its Role in Whole Body Energy Metabolism. Diabetes Metab J. 2017 Oct;41(5):327-336. doi: 10.4093/dmj.2017.41.5.327. PMID: 29086530; PMCID: PMC5663671.
3. Stokes T, Hector AJ, Morton RW, McGlory C, Phillips SM. Recent Perspectives Regarding the Role of Dietary Protein for the Promotion of Muscle Hypertrophy with Resistance Exercise Training. Nutrients. 2018 Feb 7;10(2):180. doi: 10.3390/nu10020180. PMID: 29414855; PMCID: PMC5852756.
4. Wolfe RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr. 2006 Sep;84(3):475-82. doi: 10.1093/ajcn/84.3.475. PMID: 16960159.
5. Son JW, Lee SS, Kim SR, Yoo SJ, Cha BY, Son HY, Cho NH. Low muscle mass and risk of type 2 diabetes in middle-aged and older adults: findings from the KoGES. Diabetologia. 2017 May;60(5):865-872. doi: 10.1007/s00125-016-4196-9. Epub 2017 Jan 19. PMID: 28102434.
6. Kim G, Kim JH. Impact of Skeletal Muscle Mass on Metabolic Health. Endocrinol Metab (Seoul). 2020 Mar;35(1):1-6. doi: 10.3803/EnM.2020.35.1.1. PMID: 32207258; PMCID: PMC7090295.
7. Kumar V, Atherton P, Smith K, Rennie MJ. Human muscle protein synthesis and breakdown during and after exercise. J Appl Physiol (1985). 2009 Jun;106(6):2026-39. doi: 10.1152/japplphysiol.91481.2008. Epub 2009 Jan 22. PMID: 19164770.
8. López-Martínez MI, Miguel M, Garcés-Rimón M. Protein and Sport: Alternative Sources and Strategies for Bioactive and Sustainable Sports Nutrition. Front Nutr. 2022 Jun 17;9:926043. doi: 10.3389/fnut.2022.926043. PMID: 35782926; PMCID: PMC9247391.
9. Gorissen SHM, Trommelen J, Kouw IWK, Holwerda AM, Pennings B, Groen BBL, Wall BT, Churchward-Venne TA, Horstman AMH, Koopman R, Burd NA, Fuchs CJ, Dirks ML, Res PT, Senden JMG, Steijns JMJM, de Groot LCPGM, Verdijk LB, van Loon LJC. Protein Type, Protein Dose, and Age Modulate Dietary Protein Digestion and Phenylalanine Absorption Kinetics and Plasma Phenylalanine Availability in Humans. J Nutr. 2020 Aug 1;150(8):2041-2050. doi: 10.1093/jn/nxaa024. PMID: 32069356; PMCID: PMC7398787.
10. Dreyer HC, Drummond MJ, Pennings B, Fujita S, Glynn EL, Chinkes DL, Dhanani S, Volpi E, Rasmussen BB. Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. Am J Physiol Endocrinol Metab 294: E392–E400, 2008.
11. Drummond MJ, Dreyer HC, Fry CS, Glynn EL, Rasmussen BB. Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. J Appl Physiol (1985). 2009 Apr;106(4):1374-84. doi: 10.1152/japplphysiol.91397.2008. Epub 2009 Jan 15. PMID: 19150856; PMCID: PMC2698645.
12. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, Phillips SM. Ingested protein dose response of muscle and

albumin protein synthesis after resistance exercise in young men. Am J Clin Nutr. 2009 Jan;89(1):161-8. doi: 10.3945/ajcn.2008.26401. Epub 2008 Dec 3. PMID: 19056590.
13. Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. Am J Clin Nutr. 2014 Jan;99(1):86-95. doi: 10.3945/ajcn.112.055517. Epub 2013 Nov 20. PMID: 24257722.
14. Atherton PJ, Smith K. Muscle protein synthesis in response to nutrition and exercise. J Physiol. 2012 Mar 1;590(5):1049-57. doi: 10.1113/jphysiol.2011.225003. Epub 2012 Jan 30. PMID: 22289911; PMCID: PMC3381813.
15. van Vliet S, Beals JW, Holwerda AM, Emmons RS, Goessens JP, Paluska SA, De Lisio M, van Loon LJC, Burd NA. Time-dependent regulation of postprandial muscle protein synthesis rates after milk protein ingestion in young men. J Appl Physiol (1985). 2019 Dec 1;127(6):1792-1801. doi: 10.1152/japplphysiol.00608.2019. Epub 2019 Nov 14. PMID: 31725358; PMCID: PMC7054632.
16. Damas F, Libardi CA, Ugrinowitsch C. The development of skeletal muscle hypertrophy through resistance training: the role of muscle damage and muscle protein synthesis. Eur J Appl Physiol. 2018 Mar;118(3):485-500. doi: 10.1007/s00421-017-3792-9. Epub 2017 Dec 27. PMID: 29282529.
17. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol. 1997 Jul;273(1 Pt 1):E99-107. doi: 10.1152/ajpendo.1997.273.1.E99. PMID: 9252485.
18. Johnson TL, Klueber KM. Skeletal muscle following tonic overload: functional and structural analysis. Med Sci Sports Exerc. 1991 Jan;23(1):49-55. PMID: 1997813.
19. Seynnes OR, de Boer M, Narici MV. Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training. J Appl Physiol (1985). 2007 Jan;102(1):368-73. doi: 10.1152/japplphysiol.00789.2006. Epub 2006 Oct 19. PMID: 17053104.
20. Burd NA, West DW, Staples AW, Atherton PJ, Baker JM, Moore DR, Holwerda AM, Parise G, Rennie MJ, Baker SK, Phillips SM. Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. PLoS One. 2010 Aug 9;5(8):e12033. doi: 10.1371/journal.pone.0012033. PMID: 20711498; PMCID: PMC2918506.
21. Schoenfeld BJ. The mechanisms of muscle hypertrophy and their application to resistance training. J Strength Cond Res. 2010 Oct;24(10):2857-72. doi: 10.1519/JSC.0b013e3181e840f3. PMID: 20847704.
22. Krzysztofik M, Wilk M, Wojdała G, Gołaś A. Maximizing Muscle Hypertrophy: A Systematic Review of Advanced Resistance Training Techniques and Methods. Int J Environ Res Public Health. 2019 Dec 4;16(24):4897. doi: 10.3390/ijerph16244897. PMID: 31817252; PMCID: PMC6950543.
23. Phillips, S. M., & Van Loon, L. J. C. (2011). Dietary protein for athletes: From requirements to optimum adaptation. *Journal of Sports Sciences*, *29*(sup1), S29–S38. https://doi.org/10.1080/02640414.2011.619204
24. Plotkin D, Coleman M, Van Every D, Maldonado J, Oberlin D, Israetel M, Feather J, Alto A, Vigotsky AD, Schoenfeld BJ. Progressive overload without progressing load? The effects of load or repetition progression on muscular adaptations. PeerJ. 2022 Sep 30;10:e14142. doi: 10.7717/peerj.14142. PMID: 36199287; PMCID: PMC9528903.

25. Kraemer WJ, Ratamess NA, French DN. Resistance training for health and performance. Curr Sports Med Rep. 2002 Jun;1(3):165-71. doi: 10.1249/00149619-200206000-00007. PMID: 12831709.
26. Schoenfeld, Brad PhD, CSCS, CSPS, FNSCA1; Grgic, Jozo MSc2. Evidence-Based Guidelines for Resistance Training Volume to Maximize Muscle Hypertrophy. Strength and Conditioning Journal 40(4):p 107-112, August 2018. | DOI: 10.1519/SSC.0000000000000363
27. Adams GR, Bamman MM. Characterization and regulation of mechanical loading-induced compensatory muscle hypertrophy. Compr Physiol. 2012 Oct;2(4):2829-70. doi: 10.1002/cphy.c110066. PMID: 23720267.
28. American College of Sports Medicine. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Med Sci Sports Exerc. 2009 Mar;41(3):687-708. doi: 10.1249/MSS.0b013e3181915670. PMID: 19204579.
29. Schoenfeld BJ, Grgic J, Ogborn D, Krieger JW. Strength and Hypertrophy Adaptations Between Low- vs. High-Load Resistance Training: A Systematic Review and Meta-analysis. J Strength Cond Res. 2017 Dec;31(12):3508-3523. doi: 10.1519/JSC.0000000000002200. PMID: 28834797.
30. Hubal, monica j.1; gordish-dressman, heather2; thompson, paul d.3; price, thomas b.1,3,4; hoffman, eric p.2; angelopoulos, theodore j.5; gordon, paul m.6; moyna, niall m.7; pescatello, linda s.8; visich, paul s.9; zoeller, robert f.10; seip, richard l.3; clarkson, priscilla m.1. Variability in Muscle Size and Strength Gain after Unilateral Resistance Training. Medicine & Science in Sports & Exercise 37(6):p 964-972, June 2005. | DOI: 10.1249.01.mss.0000170469.90461.5f
31. Phillips SM. Dietary protein requirements and adaptive advantages in athletes. Br J Nutr. 2012 Aug;108 Suppl 2:S158-67. doi: 10.1017/S0007114512002516. PMID: 23107527.
32. Brook MS, Wilkinson DJ, Mitchell WK, Lund JN, Szewczyk NJ, Greenhaff PL, Smith K, Atherton PJ. Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling. FASEB J. 2015 Nov;29(11):4485-96. doi: 10.1096/fj.15-273755. Epub 2015 Jul 13. PMID: 26169934.
33. Schoenfeld BJ, Contreras B, Krieger J, Grgic J, Delcastillo K, Belliard R, Alto A. Resistance Training Volume Enhances Muscle Hypertrophy but Not Strength in Trained Men. Med Sci Sports Exerc. 2019 Jan;51(1):94-103. doi: 10.1249/MSS.0000000000001764. PMID: 30153194; PMCID: PMC6303131.
34. Nader, G.A., & Esser, K.A. (2001). Intracellular signaling specificity in skeletal muscle in response to different modes of exercise. *Journal of applied physiology, 90 5*, 1936-42 .
35. Schoenfeld, B.J., Aragon, A.A. How much protein can the body use in a single meal for muscle-building? Implications for daily protein distribution. *J Int Soc Sports Nutr* 15, 10 (2018). https://doi.org/10.1186/s12970-018-0215-1
36. Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol. 1997 Jul;273(1 Pt 1):E122-9. doi: 10.1152/ajpendo.1997.273.1.E122. PMID: 9252488.
37. Smith K, Reynolds N, Downie S, Patel A, Rennie MJ. Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. Am J

Physiol. 1998 Jul;275(1):E73-8. doi: 10.1152/ajpendo.1998.275.1.E73. PMID: 9688876.
38. Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol. 1999 Apr;276(4):E628-34. doi: 10.1152/ajpendo.1999.276.4.E628. PMID: 10198297.
39. Dyachok J, Earnest S, Iturraran EN, Cobb MH, Ross EM. Amino Acids Regulate mTORC1 by an Obligate Two-step Mechanism. J Biol Chem. 2016 Oct 21;291(43):22414-22426. doi: 10.1074/jbc.M116.732511. Epub 2016 Sep 1. Erratum in: J Biol Chem. 2020 Jun 26;295(26):8868. doi: 10.1074/jbc.AAC120.014360. PMID: 27587390; PMCID: PMC5077182.
40. Biolo, G., S.P. Maggi, B.D. Williams, K.D. Tipton and R.R. Wolfe (1995). Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. Am. J. Physiol. 268:E514-520.
41. Burd NA, Beals JW, Martinez IG, Salvador AF, Skinner SK. Food-First Approach to Enhance the Regulation of Post-exercise Skeletal Muscle Protein Synthesis and Remodeling. Sports Med. 2019 Feb;49(Suppl 1):59-68. doi: 10.1007/s40279-018-1009-y. PMID: 30671904; PMCID: PMC6445816.
42. VanDusseldorp TA, Escobar KA, Johnson KE, Stratton MT, Moriarty T, Cole N, McCormick JJ, Kerksick CM, Vaughan RA, Dokladny K, Kravitz L, Mermier CM. Effect of Branched-Chain Amino Acid Supplementation on Recovery Following Acute Eccentric Exercise. Nutrients. 2018 Oct 1;10(10):1389. doi: 10.3390/nu10101389. PMID: 30275356; PMCID: PMC6212987.
43. Jacinto JL, Nunes JP, Ribeiro AS, Casonatto J, Roveratti MC, Sena BNS, Cyrino ES, DA Silva RA, Aguiar AF. Leucine Supplementation Does Not Improve Muscle Recovery from Resistance Exercise in Young Adults: A Randomized, Double-Blinded, Crossover Study. Int J Exerc Sci. 2021 Apr 1;14(2):486-497. doi: 10.70252/QLQR5371. PMID: 34055154; PMCID: PMC8136571.
44. Harper AE. Evolution of recommended dietary allowances--new directions? Annu Rev Nutr. 1987;7:509-37. doi: 10.1146/annurev.nu.07.070187.002453. PMID: 3300745.
45. Friedman JE, Lemon PW. Effect of chronic endurance exercise on retention of dietary protein. Int J Sports Med. 1989 Apr;10(2):118-23. doi: 10.1055/s-2007-1024886. PMID: 2722324.
46. Bohé J, Low JF, Wolfe RR, Rennie MJ. Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. J Physiol. 2001 Apr 15;532(Pt 2):575-9. doi: 10.1111/j.1469-7793.2001.0575f.x. PMID: 11306673; PMCID: PMC2278544.
47. Morton RW, McGlory C, Phillips SM. Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. Front Physiol. 2015 Sep 3;6:245. doi: 10.3389/fphys.2015.00245. PMID: 26388782; PMCID: PMC4558471.
48. Macnaughton LS, Wardle SL, Witard OC, McGlory C, Hamilton DL, Jeromson S, Lawrence CE, Wallis GA, Tipton KD. The response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein. Physiol Rep. 2016 Aug;4(15):e12893. doi: 10.14814/phy2.12893. PMID: 27511985; PMCID: PMC4985555.
49. Sarwar Gilani G, Wu Xiao C, Cockell KA. Impact of Antinutritional Factors in Food Proteins on the Digestibility of Protein and the Bioavailability of Amino Acids and on

Protein Quality. *British Journal of Nutrition*. 2012;108(S2):S315-S332. doi:10.1017/S0007114512002371
50. Pinckaers PJM, Kouw IWK, Gorissen SHM, Houben LHP, Senden JM, Wodzig WKHW, de Groot LCPGM, Verdijk LB, Snijders T, van Loon LJC. The Muscle Protein Synthetic Response to the Ingestion of a Plant-Derived Protein Blend Does Not Differ from an Equivalent Amount of Milk Protein in Healthy Young Males. J Nutr. 2023 Jan 14;152(12):2734-2743. doi: 10.1093/jn/nxac222. PMID: 36170964; PMCID: PMC9839989.
51. Tang J.E., Moore D.R., Kujbida G.W., Tarnopolsky M.A., Phillips S.M. Ingestion of whey hydrolysate, casein, or soy protein isolate: Effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J. Appl. Physiol. 2009;107:987–992. doi: 10.1152/japplphysiol.00076.2009.
52. Pinckaers PJM, Trommelen J, Snijders T, van Loon LJC. The Anabolic Response to Plant-Based Protein Ingestion. Sports Med. 2021 Sep;51(Suppl 1):59-74. doi: 10.1007/s40279-021-01540-8. Epub 2021 Sep 13. PMID: 34515966; PMCID: PMC8566416.
53. Lim MT, Pan BJ, Toh DWK, Sutanto CN, Kim JE. Animal Protein versus Plant Protein in Supporting Lean Mass and Muscle Strength: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Nutrients. 2021 Feb 18;13(2):661. doi: 10.3390/nu13020661. PMID: 33670701; PMCID: PMC7926405.
54. Pennings B, Boirie Y, Senden JM, Gijsen AP, Kuipers H, van Loon LJ. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. Am J Clin Nutr. 2011 May;93(5):997-1005. doi: 10.3945/ajcn.110.008102. Epub 2011 Mar 2. PMID: 21367943.
55. Takahara, T., Amemiya, Y., Sugiyama, R. *et al.* Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes. *J Biomed Sci* 27, 87 (2020). https://doi.org/10.1186/s12929-020-00679-2
56. Orrù S, Imperlini E, Nigro E, Alfieri A, Cevenini A, Polito R, Daniele A, Buono P, Mancini A. Role of Functional Beverages on Sport Performance and Recovery. Nutrients. 2018 Oct 10;10(10):1470. doi: 10.3390/nu10101470. PMID: 30308976; PMCID: PMC6213308.
57. Malla N, Nørgaard JV, Lærke HN, Heckmann LL, Roos N. Some Insect Species Are Good-Quality Protein Sources for Children and Adults: Digestible Indispensable Amino Acid Score (DIAAS) Determined in Growing Pigs. J Nutr. 2022 Apr 1;152(4):1042-1051. doi: 10.1093/jn/nxac019. Epub 2023 Feb 18. PMID: 35102372.
58. van der Heijden I, West S, Monteyne AJ, Finnigan TJA, Abdelrahman DR, Murton AJ, Stephens FB, Wall BT. Algae Ingestion Increases Resting and Exercised Myofibrillar Protein Synthesis Rates to a Similar Extent as Mycoprotein in Young Adults. J Nutr. 2023 Dec;153(12):3406-3417. doi: 10.1016/j.tjnut.2023.08.035. Epub 2023 Sep 15. PMID: 37716611; PMCID: PMC10739781.
59. Monteyne AJ, Coelho MOC, Porter C, Abdelrahman DR, Jameson TSO, Jackman SR, Blackwell JR, Finnigan TJA, Stephens FB, Dirks ML, Wall BT. Mycoprotein ingestion stimulates protein synthesis rates to a greater extent than milk protein in rested and exercised skeletal muscle of healthy young men: a randomized controlled trial. Am J Clin Nutr. 2020 Aug 1;112(2):318-333. doi: 10.1093/ajcn/nqaa092. PMID: 32438401.
60. Cardoso Alves S, Díaz-Ruiz E, Lisboa B, Sharma M, Mussatto SI, Thakur VK, Kalaskar DM, Gupta VK, Chandel AK. Microbial meat: A sustainable vegan protein

source produced from agri-waste to feed the world. Food Res Int. 2023 Apr;166:112596. doi: 10.1016/j.foodres.2023.112596. Epub 2023 Feb 16. PMID: 36914347.
61. Wilkinson SB, Tarnopolsky MA, Macdonald MJ, Macdonald JR, Armstrong D, Phillips SM. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. Am J Clin Nutr. 2007 Apr;85(4):1031-40. doi: 10.1093/ajcn/85.4.1031. Erratum in: Am J Clin Nutr. 2013 Aug;98(2):512. PMID: 17413102.
62. Kurek MA, Onopiuk A, Pogorzelska-Nowicka E, Szpicer A, Zalewska M, Półtorak A. Novel Protein Sources for Applications in Meat-Alternative Products-Insight and Challenges. Foods. 2022 Mar 25;11(7):957. doi: 10.3390/foods11070957. PMID: 35407043; PMCID: PMC8997880.
63. Monteyne AJ, Coelho MOC, Murton AJ, Abdelrahman DR, Blackwell JR, Koscien CP, Knapp KM, Fulford J, Finnigan TJA, Dirks ML, Stephens FB, Wall BT. Vegan and Omnivorous High Protein Diets Support Comparable Daily Myofibrillar Protein Synthesis Rates and Skeletal Muscle Hypertrophy in Young Adults. J Nutr. 2023 Jun;153(6):1680-1695. doi: 10.1016/j.tjnut.2023.02.023. Epub 2023 Feb 22. PMID: 36822394; PMCID: PMC10308267.
64. Lanng SK, Oxfeldt M, Pedersen SS, Johansen FT, Risikesan J, Lejel T, Bertram HC, Hansen M. Influence of protein source (cricket, pea, whey) on amino acid bioavailability and activation of the mTORC1 signaling pathway after resistance exercise in healthy young males. Eur J Nutr. 2023 Apr;62(3):1295-1308. doi: 10.1007/s00394-022-03071-y. Epub 2022 Dec 19. PMID: 36536114.
65. Vangsoe MT, Joergensen MS, Heckmann LL, Hansen M. Effects of Insect Protein Supplementation during Resistance Training on Changes in Muscle Mass and Strength in Young Men. Nutrients. 2018 Mar 10;10(3):335. doi: 10.3390/nu10030335. PMID: 29534456; PMCID: PMC5872753.
66. Bernárdez-Vázquez R, Raya-González J, Castillo D, Beato M. Resistance Training Variables for Optimization of Muscle Hypertrophy: An Umbrella Review. Front Sports Act Living. 2022 Jul 4;4:949021. doi: 10.3389/fspor.2022.949021. PMID: 35873210; PMCID: PMC9302196.
67. Phillips SM. Short-term training: when do repeated bouts of resistance exercise become training? Can J Appl Physiol. 2000 Jun;25(3):185-93. doi: 10.1139/h00-014. PMID: 10932036.
68. Devries MC, McGlory C, Bolster DR, Kamil A, Rahn M, Harkness L, Baker SK, Phillips SM. Leucine, Not Total Protein, Content of a Supplement Is the Primary Determinant of Muscle Protein Anabolic Responses in Healthy Older Women. J Nutr. 2018 Jul 1;148(7):1088-1095. doi: 10.1093/jn/nxy091. Erratum in: J Nutr. 2019 Mar 1;149(3):542. doi: 10.1093/jn/nxy220. PMID: 29901760.
69. Burke, R., Piñero, A., Mohan, A.E. *et al.* Exercise Selection Differentially Influences Lower Body Regional Muscle Development. *J. of SCI. IN SPORT AND EXERCISE* (2024). https://doi.org/10.1007/s42978-024-00299-4
70. Wakahara T, Miyamoto N, Sugisaki N, Murata K, Kanehisa H, Kawakami Y, Fukunaga T, Yanai T. Association between regional differences in muscle activation in one session of resistance exercise and in muscle hypertrophy after resistance training. Eur J Appl Physiol. 2012 Apr;112(4):1569-76. doi: 10.1007/s00421-011-2121-y. Epub 2011 Aug 21. PMID: 21858666.

71. Fukutani, A., Kurihara, T. Comparison of the muscle fascicle length between resistance-trained and untrained individuals: cross-sectional observation. *SpringerPlus* 4, 341 (2015). https://doi.org/10.1186/s40064-015-1133-1
72. Aagaard P, Andersen JL, Dyhre-Poulsen P, Leffers AM, Wagner A, Magnusson SP, Halkjaer-Kristensen J, Simonsen EB. A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. J Physiol. 2001 Jul 15;534(Pt. 2):613-23. doi: 10.1111/j.1469-7793.2001.t01-1-00613.x. PMID: 11454977; PMCID: PMC2278719.