POST-EXERCISE MACRONUTRIENT CONSUMPTION & RESISTANCE TRAINING

THE EFFECT OF DIFFERING POST-EXERCISE MACRONUTRIENT CONSUMPTION ON RESISTANCE TRAINING-INDUCED ADAPTATIONS IN NOVICES

By

Joseph Hartman, B.Sc. Kin.

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Author: Joseph Hartman, B.Sc. Kin. (Laurentian University)

Supervisor: Dr. Stuart Phillips

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ABSTRACT

Resistance exercise training results in skeletal muscle hypertrophy due to accumulated periods of protein accretion, the magnitude of which may be influenced by post-exercise feeding. One study suggests that hypertrophy is maximized by immediate provision of protein (26); how the type of protein ingested in this period affects protein accretion is not known. **PURPOSE:** To investigate the effect of consuming different post-exercise beverages - milk (MLK), an isonitrogenous and isoenergetic soy drink (PEC), or an isoenergetic control (CON) - on body mass, fat-bone free mass (FBFM), muscle fibre area, and strength during a 12-week progressive whole body resistance training program, in untrained men. **METHODS:** 34 men were randomized to post-exercise consumption of MLK (n=12; fat free), PEC (n=11; soy) or CON (n=11; maltodextrin) using single-blinded allocation, with characteristics- 22.5 ± 0.6 yr, 25.6 ± 0.7 kg/m^2. Participants trained 5 d/wk using a whole body split resistance training program and consumed 500 ml of their assigned drink immediately and 1h post-exercise following every training bout. **RESULTS:** Total body mass increased following training ($P<0.01$) but there were no differences between groups (MLK = 3.2 ± 0.8 kg, PEC = 2.9± 1.4 kg, CON = 2.0 ± 0.8 kg). Similarly, FBFM also increased post-training ($P<0.01$), but was not different between groups (MLK = 3.3 ± 0.6 kg, PEC = 2.7 ± 0.6kg, CON = 2.2 ± 0.6 kg). Muscle type II fibre area increased post-training ($P<0.05$) but was not different between groups (MLK = 1004 ± 249 μm^2, PEC = 650 ± 192 μm^2, CON = 565 ± 120 μm^2). Training resulted in increases in 1RM strength for 13 different exercises ($P<0.01$)

with no difference between groups (range = 29-101%). **CONCLUSION:** Immediate post-exercise provision of either milk (MLK), or an isonitrogenous / isoenergetic soy drink (PEC), as well as an isoenergetic carbohydrate beverage (CON), resulted in similar increases in body mass, FBFM, type II muscle fibre area, and strength. This study demonstrates that intact dietary proteins from milk and soy are effective for promoting skeletal muscle hypertrophy. Moreover, when given equivalent energy immediately post-exercise, proteins consumed as part of a normal diet, outside of the immediate post-exercise period are sufficient for hypertrophic and strength gains. Finally, post-exercise consumption of protein is no more effective than carbohydrate for promoting skeletal muscle hypertrophy when adequate protein is consumed within a regular diet.

Contribution

This study was a joint effort with the contribution of several individuals in order to ensure its overall success. As well, portions of the data collected for this study were used by other students as a Master's thesis, as well as an undergraduate thesis. My personal contribution to the study, and data analysis are as follows. I acted as the study coordinator and for 3 months prior to the beginning of the study I organized the study and recruited all 36 subjects on my own. I performed all the DEXA scans on my own and assisted in all muscle biopsy procedures. I was present for all blood sampling procedures and took over half of the samples personally, and processed all of the samples for further analysis. I oversaw all 1RM tests throughout the study and performed many tests personally. As the coordinator I oversaw a group of 12 trainers who performed the majority of the training.

Following the data collection period I analyzed all the DEXA and 1RM data personally. I also performed the glucose and lactate sample analysis. Diet record data was entered by myself and two other individuals working for the study. Muscle fibre slicing in preparation for ATPase staining was performed jointly by myself and Jason Tang, and I performed all ATPase staining of the samples. Analysis of the muscle fiber area was performed primarily by myself, and Jason Tang analyzed some samples. Insulin analysis was done in coordination with Dan Moore who also had samples to analyze from his own independent study, we were both present for all procedures, and analyzed our own samples personally. I personally feel that although I had lots of help with this project, the amount of work that I performed throughout this study was greater than many Master's projects done independently.

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GLOSSARY OF ACRONYMS

1RM – 1 repetition maximum

AA – amino acid

BCAA – branched chain amino acid

BMC – bone mineral content

CON – isoenergetic control

CHO – carbohydrate

DXA – dual energy x-ray absorptiometry

EAA – essential amino acids

EAC – essential amino acid carbohydrate

eIF2B – eukaryotic initiation factor 2B

FBFM – fat and bone free mass

FFM – fat free mass

FSR – fractional synthesis rate

MAA – mixed amino acids

MHC – myosin heavy chain

MLK – fat free milk

NPB – net protein balance

N – nitrogen

PB – protein breakdown

PEC – isonitrogenous / isoenergetic control

PS – protein synthesis

RIA – radioimmunoassay

TBM – total body mass

1.1 INTRODUCTION

Skeletal muscle constitutes the largest mass of protein in the human body. It is well known that resistance exercise, when performed regularly and at sufficient intensity, increases skeletal muscle protein mass. Many acute studies have focussed on the combined effects of resistance exercise and amino acid supplementation on muscle protein anabolism. Resistance exercise alone provides a potent stimulus for protein synthesis and, over time, muscle hypertrophy (49, 59). Recent studies have found that consumption of amino acids post-exercise stimulates net muscle protein synthesis, primarily due to an increase in muscle protein synthesis rather than a decrease in breakdown (67, 84). Also, the provision of glucose alone during the post-exercise period, while it promotes a more positive protein balance, does not result in a net positive protein balance (12). Thus, protein is an important component to include in a post-exercise meal in order to promote muscle protein accretion.

Protein source is an important determinant of postprandial protein metabolism in response to feeding (3, 21, 22). So called "slow" and "fast" proteins demonstrate differing protein kinetics (3, 21). Slow proteins such as casein have a greater postprandial retention rate compared to a fast protein such as whey (3, 21). Milk (at 20% whey and 80% casein) and soy proteins have been classified as slow and fast protein sources respectively (14, 28), and their ability to stimulate muscle protein synthesis in different tissue compartments varies. Soy protein promotes greater protein synthesis efficiency (defined as the fraction of intracellular amino acids flux of appearance that is

incorporated into protein (28)) in the splanchnic bed, whereas milk promotes greater protein synthesis efficiency within the peripheral tissues, most likely muscle (28). This implies that milk might be superior for promotion of skeletal muscle protein accretion than soy when consumed post-exercise, since milk proteins would provide a greater stimulus for muscle protein synthesis.

This review will focus on *in vivo* studies performed on human subjects. The influence of amino acid consumption will be examined in detail, along with possible mechanisms underlying the responses observed following resistance exercise. The influence of protein composition and its source on muscle protein accretion will be discussed, along with adaptations to resistance training.

1.2 RESISTANCE EXERCISE AND PROTEIN TURNOVER

1.2.1 PROTEIN BREAKDOWN FOLLOWING RESISTANCE EXERCISE

Resistance exercise has a profound effect on protein metabolism both acutely and chronically. Controversy exists as to whether protein breakdown is increased during exercise; however, evidence clearly demonstrates that skeletal muscle proteolysis is elevated post-exercise in the fasted state (7, 59). The response of muscle protein breakdown following exercise has not been extensively studied and differing results have been found. Some studies have reported increases in rates of muscle protein breakdown post-exercise (24, 25, 62), whereas others have reported no change (63, 64) or decreased (55, 66) protein breakdown in response to exercise. These studies have all examined

protein breakdown based on the rate of excretion of 3-methylhistidine in urine, an amino acid that is post-transcriptionally modified and exists only in actin and myosin and is excreted after protein breakdown. Some of the variability in the literature as to the response of muscle protein breakdown to resistance exercise are likely due to the use of 3-methylhistidine as a marker of skeletal muscle breakdown.

Measurements of protein breakdown based on tracer dilution techniques are likely to be more robust than 3-methylhistidine excretion due to the fact that 3-methilhistidine is simply a marker of protein breakdown whereas tracer dilution techniques are a more direct measure. Biolo and colleagues (4, 6, 9) have utilized a three compartment model using values of labelling and concentrations of amino acids in arterial and venous blood, as well as the labelling of tissue-free tracee to quantify muscle protein breakdown after a bout of resistance exercise (4, 6, 9). They observed that muscle protein breakdown was increased following heavy leg resistance exercise, but to a smaller extent than muscle protein synthesis, resulting in a more positive, and yet negative, net balance. A second approach to examine post-exercise proteolysis used a precursor product method that determined the decay in enrichment of labelled phenylalanine in the venous blood and the muscle intracellular free amino acid pool (102). When this methodology was applied following intense knee extension exercise, it was observed that the rate of muscle protein breakdown was increased by ~50% in untrained human subjects (59, 60). Hence based on the results of tracer studies there is general agreement that protein breakdown is increased following resistance exercise.

1.2.2 PROTEIN SYNTHESIS FOLLOWING RESISTANCE EXERCISE

An acute bout of resistance exercise has a dramatic stimulating effect on the rate of muscle protein synthesis (49, 59). Conversely, there are some reports of a lack of response in muscle protein synthesis following resistance exercise (70, 86). However, the subjects examined in these latter studies (70, 86) were highly trained, and their response might be expected as the result of a biological adaptation to a stressor such as exercise (see below). From the results of various studies (7, 8, 9, 49, 59) it can be stated with good confidence, that when the stimulus is sufficient, that intense resistive contractile activity stimulates muscle protein synthesis. The exercise-induced increase in muscle protein synthesis in the fasted state results in a less negative net protein balance following resistance exercise, but the overall net balance remains negative (59). It is not until the ingestion of amino acids post-exercise that net protein balance becomes positive (8). This relationship will be discussed further.

1.2.2.1 TIME COURSE OF PROTEIN SYNTHESIS

Resistance exercise can stimulate muscle protein synthesis for up to 48 h following an acute bout (49, 59). MacDougall and colleagues (49) measured protein synthesis in humans for 36 hours following a single bout of resistance exercise and found that muscle protein synthetic rates were elevated by 50% 4 h post-exercise, and by 109% at 24 h post-exercise. At the 36 h time point protein synthetic rates had returned to baseline. Phillips and colleagues (59) observed that a bout of heavy resistance exercise

increased mixed muscle fractional synthesis rates by 112% at 3-h, 65% at 24 h, and 34% at 48 h post-exercise. Phillips et al (59) hypothesized that the discrepancy between the two studies at the 48 h time point may be due to the training status of the subjects, since the time course of muscle fractional synthesis rates after exercise may be different in trained vs. untrained subjects. This hypothesis is reinforced by findings that in trained subjects there was an attenuated rate of protein synthesis following a bout of resistance exercise when compared to the untrained state (61), which in theory would result in a blunted protein synthetic response in the trained state.

1.2.3 PROTEIN BALANCE FOLLOWING RESISTANCE TRAINING

Resistance exercise training is well known to induce hypertrophy of the trained muscles (40, 47). The effects of resistance exercise training on human muscle protein

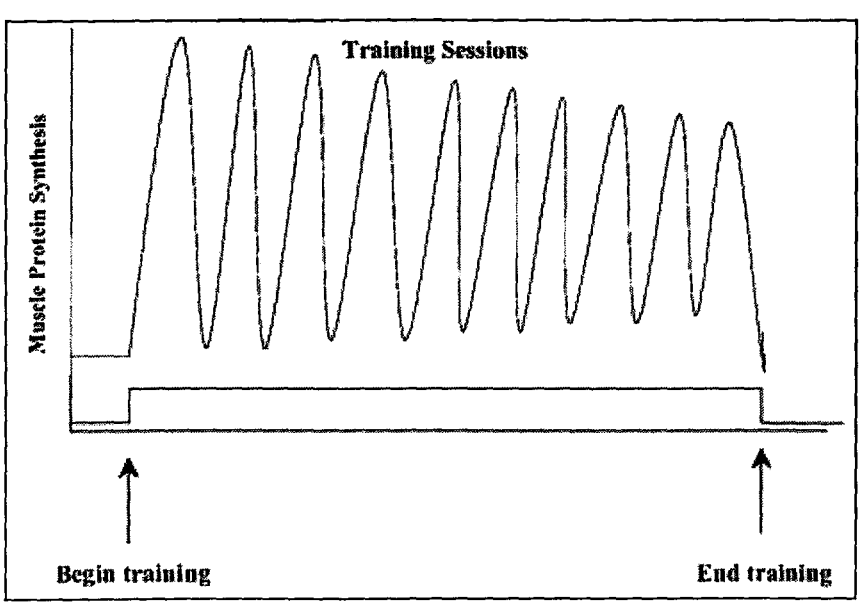

Figure 1. Hypothetical response of muscle protein synthesis to repeated workouts during a resistance exercise training program. Rennie and Tipton (69).

synthesis and breakdown have not been extensively studied. Intuitively, muscle hypertrophy resulting from resistance exercise is caused by an increase in muscle protein synthesis that exceeds protein breakdown in the resting recovering muscle. However, an important question is the extent to which the basal rate of resting protein turnover is elevated as a result of training. In a recent review Rennie and Tipton (69) hypothesized that assuming muscle protein synthesis responds to a repeated stimulus of a constant size (e.g. applied in a square wave pattern) as do other physiological systems (e.g. maximal oxygen consumption capacity and aerobic power), then it is likely that a series of training stimuli will result in a series of responses each of which is progressively reduced (see Figure 1). Thus, theoretically, an increased stimulus should be necessary post-training to initiate a response similar to that observed before training.

The results of a cross-sectional analysis by Phillips and colleagues (60) indicate that resistance trained subjects display an attenuated response to an acute bout of resistance exercise compared with sedentary, untrained controls. The response of muscle protein turnover was compared between these two groups, both at rest and after intense resistance exercise. Protein turnover was greater in the exercised leg than in the control leg. Most importantly, the increase in both muscle protein synthesis and breakdown in the trained subjects was decreased by ~50% compared with that in the untrained subjects. There were no significant differences between trained and sedentary groups in the resting values of protein synthesis and breakdown, although trained athletes displayed slightly higher fractional synthetic rates at rest (60).

A more recent study by Phillips and colleagues (61) examined resistance training induced adaptations in skeletal muscle protein turnover in the fed state. Conclusive evidence for the attenuation of the acute response of muscle protein turnover in response to a single bout of resistance exercise was observed. In this longitudinal study young, untrained men completed 8 weeks of resistance training. It was found that resistance training attenuated the acute exercise-induced rise in fractional synthesis rate by ~20% (61). The results from this study clearly demonstrate that resistance training leads to an attenuation of the acute response of muscle protein turnover in response to a single bout of resistance exercise, which reinforces the hypothesis of Rennie and Tipton (69). Further evidence of a down regulatory adaptation to resistance exercise has been observed in rats. Farrell and colleagues (27) reported that post-exercise muscle protein synthesis was reduced in rats that had been resistance trained for 8 weeks compared with untrained rats.

Most studies indicate that training-induced muscle growth is a result of an accumulated set of anabolic responses to each individual exercise training session, rather than a change in basal net protein balance. However, there is some evidence that presents conflicting results. It has been reported that resistance exercise training, for as little as 2 weeks, increases resting muscle protein synthesis in both young and elderly humans (96, 98). The findings in young subjects may have been confounded by the fact that the initial pre-training measures were made at rest, and the post-training measures were made within 24 h after the last bout of resistance exercise (96). As mentioned previously protein synthesis rates can be elevated for up to 48 h following a bout of resistance

exercise (59). Therefore, it would be difficult to differentiate the response of muscle to the chronic effect of training from the response to the last acute exercise bout. In other words, any chronic training-induced increases in basal muscle protein synthesis are likely simply an artefact from the last workout bout, and not due to the chronic effect of training. In the elderly, it may simply be that training may have normalized an age or inactivity-related reduction in the basal rate of protein turnover. However, results from Phillips and colleagues (61) contradict these findings. They observed that following training, at rest 72-h after the last training bout, muscle protein fractional synthesis and breakdown rates were greater in the trained state. The increase in protein synthesis was greater than the increase in breakdown thus creating an elevated net protein synthesis rate at rest. This adds conclusive evidence demonstrating an increased basal rate of protein turnover following 8 weeks of resistance training in young males. Obviously, more research is needed in order to solidify these findings, but evidence does suggest that basal protein turnover is upregulated following resistance training.

1.3 AMINO ACID SUPPLEMENTATION

1.3.1 AMINO ACID SUPPLEMENTATION AT REST

It is now generally accepted that amino acid availability is an independent regulator of muscle protein turnover (1). It has been observed that increased amino acid availability stimulates muscle protein synthesis (1, 8). Biolo and colleagues (8) observed that hyperaminoacidemia resulting from intravenous infusion of amino acids, increased

protein synthesis at rest. A recent study examined the latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids and found that muscle protein synthesis responds rapidly to increased availability of amino acids but then returns to basal concentrations, despite continued amino acid availability (2). Bohé and colleagues (2) found that a square-wave increase in the availability of plasma amino acids took between 30 min and 1-h to have any measurable effect on muscle protein synthesis. From the 1h mark muscle protein synthesis was markedly stimulated by ~2.8 fold for a period of 1.5 h before falling to a value not significantly different from the basal value for the subsequent 4 h (2).

The results from Bohé et al (2) suggest that as previously observed, overfeeding protein does not increase the size of lean body mass, and excess amino acids are simply oxidized (55, 64, 81) or their carbon skeletons are stored as fat. As well, the time course of protein synthesis observed during the continuous amino acid infusion seems to suggest that amino acids would be more efficiently utilized for maintaining lean body mass when given in divided doses (such as meal feedings) rather than with a continuous infusion application (2).

1.3.2 AMINO ACID SUPPLEMENTATION AND RESISTANCE EXERCISE

As previously discussed, during recovery from resistance exercise in the fasted state, muscle amino acid transport and protein turnover are accelerated, resulting in a less negative net protein balance (49, 59). Although protein balance is less negative in the fasted state, it does not shift to positive values. Research into the combined effects of

resistance exercise and hyperaminoacidemia have revealed that the infusion of amino acids following resistance exercise, increased muscle protein synthesis more than at rest, and the normal exercise-induced increase in muscle protein breakdown was prevented, resulting in net protein accretion (8). Thus, provision of amino acids following resistance exercise has an anabolic effect on muscle. This area has not been extensively studied, therefore key studies in the area of post-exercise amino acid supplementation are examined in greater detail in the following section.

Intravenous infusion of amino acids is not a practical means of delivering amino acids to humans. Based on the results of Biolo and colleagues (8) Tipton and coworkers (85) examined the effect of orally administered amino acids post-exercise on net protein balance. It was observed that net protein balance was significantly higher during

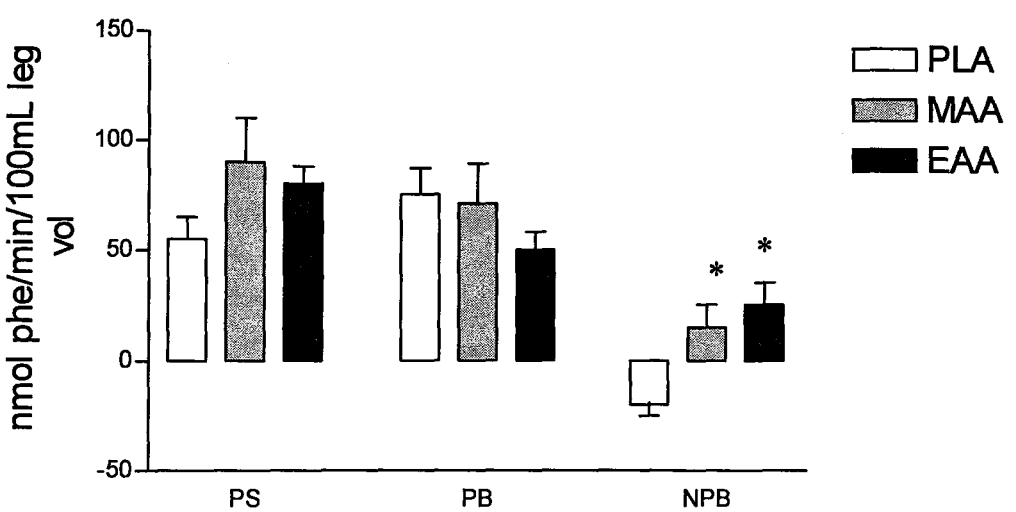

Figure 2. Muscle protein synthesis (PS), protein breakdown (PB), and net muscle protein balance (NPB) after exercise during consumption of solutions of placebo (PLA), 40 g of mixed amino acids (MAA), and 40 g of essential amino acids (EAA). *significantly different from PLA. Tipton et al. (85)

ingestion of both essential amino acids and essential plus non-essential amino acids conditions compared to a placebo following a bout of resistance exercise (see Figure 2). Of importance, whereas net protein balance was negative during the placebo condition, it changed to positive following the ingestion of the solutions containing amino acids. Thus, the combination of amino acid ingestion following resistance exercise has a net anabolic effect greater than that of amino acid ingestion alone. Muscle net balance following ingestion of mixed amino acids (MAA) and essential amino acids (EAA) drinks were similar to the values previously reported by this group following infusion of amino acids (8). Tipton and colleagues (85) concluded that following resistance exercise an oral amino acid supplement was just as effective as amino acid infusion for stimulating muscle protein anabolism.

As mentioned previously, the combination of exogenous amino acids consumed orally (85) or infused intravenously (8), and resistance exercise are synergistic and combine as a potent stimulator of muscle protein synthesis. These conclusions are made based on the assumption that the stimulation of net muscle protein synthesis is additive to the balance that would occur during a normal day in the absence of exercise and amino acid ingestion. The increase in muscle protein synthesis in response to oral ingestion of amino acids following exercise is large, but it is also transient (67, 85, 87). There exists uncertainty as to the impact of these transient responses on chronic changes in muscle metabolism and muscle mass.

Tipton and colleagues (84) measured the acute anabolic muscle response to resistance exercise and ingestion of essential amino acids, and extended their

investigation over a 24 h period, at rest and following resistance exercise. It was observed that the muscle protein balance increased, primarily due to an acute stimulation of muscle protein synthesis by exercise and ingestion of EAA, and that the response was additive to the basal response over a full 24 h period (84). Thus, a bout of resistance exercise combined with consumption of amino acids results in stimulation of protein synthesis above normal day-to-day concentrations. Although this was observed over a 24 h period it might not be representative of the chronic changes observed with resistance training and amino acid ingestion. It is likely that the acute effects of amino acid

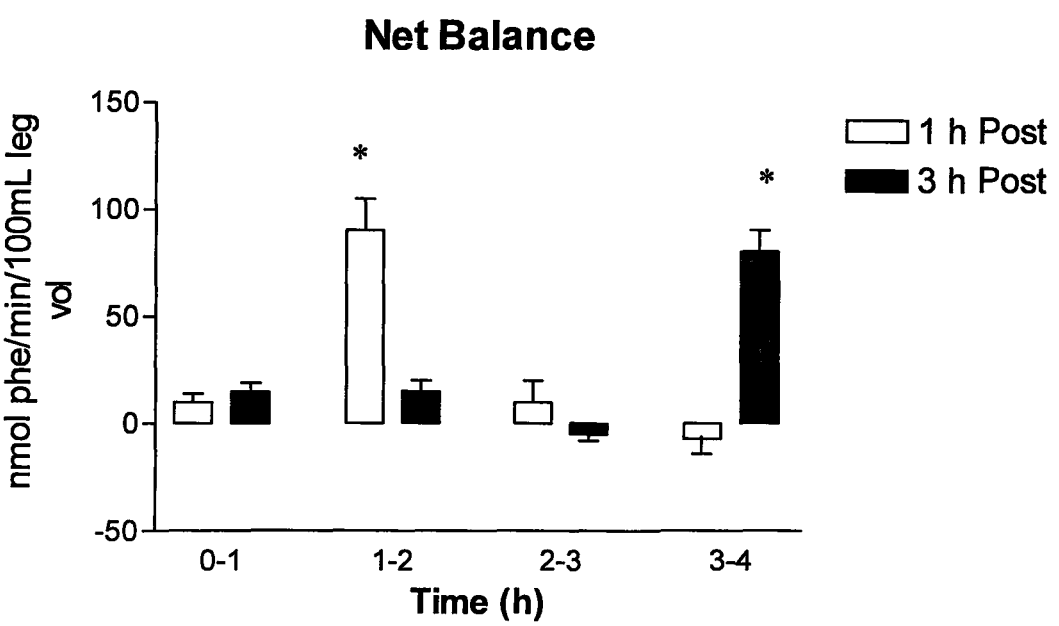

Figure 3: Phenylalanine net balance across leg after resistance exercise. EAA drink was ingested 1h (1-h post) and 3h (3-h post) postexercise. *Significantly different from placebo and predrink values, P<0.05. Rasmussen et al. (67)

ingestion and resistance exercise are attenuated with chronic training and supplementation, as is observed following resistance training (61), and will result in a

blunted response to the stimulus. Future studies should examine the chronic changes in protein turnover due to exercise and amino acid supplementation.

1.3.3 TIMING OF AMINO ACID SUPPLEMENTATION FOLLOWING RESISTANCE EXERCISE

The work of Tipton and coworkers (84) revealed that any meal containing sufficient amino acids that is consumed within 24 h of resistance exercise results in a greater net muscle protein accretion than simply ingesting the amino acids alone. This leads to the question as to whether there are specific times following a bout of resistance exercise that may result in greater increases in net protein balance subsequent to the ingestion of amino acids. It was observed by Rasmussen and colleagues (67) that the timing of a carbohydrate/amino acid supplement did not have a pronounced impact on muscle protein net balance following resistance exercise. Subjects randomly consumed a treatment drink (6 g essential amino acids, 35 g sucrose) or a flavoured placebo drink 1h or 3-h following a bout of resistance exercise on two separate occasions. Phenylalanine was infused and a three-compartment model for determination of leg muscle protein kinetics was used. They concluded that essential amino acids and carbohydrates stimulate muscle protein anabolism by increasing muscle protein synthesis to an equivalent extent when ingested at either 1-h or 3-h after resistance exercise (67) (see Figure 3). Thus, as long as amino acids are consumed within 3-h of resistance exercise the magnitude of net positive protein synthesis is not affected.

Tipton and colleagues (87) later evaluated whether consumption of an oral essential amino acid carbohydrate supplement (EAC) before exercise results in a greater anabolic response than supplementation after resistance exercise. It was observed that during exercise, net protein balance switched from negative to positive following the consumption of EAC solution pre-exercise, and remained significantly higher than the post-exercise consumption group for 1-h post-exercise. They concluded that the response of net muscle protein synthesis to consumption of an EAC solution immediately before resistance exercise is greater than when the solution is consumed subsequent to exercise, primarily because of an increase in muscle protein synthesis as a result of increased delivery of amino acids (87). These findings are based upon an apparent feeding-induced increase in blood flow in the pre-exercise supplementation group. It must be pointed out that the blood flow values of the pre-exercise supplementation group are significantly higher than the post-exercise supplementation group, both during exercise, and 1-h post-exercise. The higher blood flow values observed in the pre-exercise supplementation group translate into greater delivery and uptake of amino acids. Although amino acid delivery is dependant on blood flow (6), the high blood flow values observed in the pre-exercise supplementation group are inordinately high compared with the post-exercise supplementation group, which indicates that the higher positive balance in the pre-exercise supplemented group is due almost entirely to a blood flow effect.

Evidence indicates that significant increases in net protein balance do occur when amino acids are consumed 1-h and 3-h post-exercise (67), yet it is uncertain whether these acute and transient increases will translate into chronic muscle hypertrophy. In the

elderly, evidence suggests that early intake of an oral protein supplement after resistance exercise is important for the development of hypertrophy in skeletal muscle in response to resistance training (26). In this study, 13 men trained for 12 weeks (three times per week) receiving oral protein (10g) in liquid form immediately after (P0), or 2-h after (P2) each training session. Significant muscle hypertrophy did occur in the P0 group, whereas no significant increases in muscle hypertrophy were present in the P2 group. It is unusual that in the P2 group there was no observation of hypertrophy following the 12 week training program since previous studies investigating the effects of resistance training in the elderly, where no specific dietary restrictions were imposed, found significant muscle hypertrophy in response to training (16, 29). The authors suggest that since dietary restrictions ended 2-h post-exercise the possibility remains that if the P2 group ingested a meal shortly following the supplement then the max effective dose of protein was exceeded and could have blunted the hypertrophic response (26). In support of the findings of Esmark and colleagues (26) a resistance training study involving rats found that the timing of a mixed meal ingestion after each training session influenced net protein synthesis over a 10 week training period. The group that was fed immediately post-exercise increased hind-limb muscle mass more than the group fed 4 h later, although the 4 h group did demonstrate hypertrophy (79).

Collectively, evidence suggests that post-exercise supplementation with amino acids is an important stimulus for muscle protein synthesis and allows for a positive net protein balance. There appears to be a minimum 3-h window in which amino acid supplementation stimulates protein synthesis following an acute bout of resistance

exercise in young healthy humans. Timing of the post-exercise supplement does seem to be important in the elderly, although there is no evidence to corroborate the findings of Esmarck et al (26), in younger men and women or confirmation from another study in the elderly.

1.4 MECHANISMS

1.4.1 INFLUENCE OF INSULIN

Most studies examining the influence of insulin and its interaction with exercise and amino acids have been performed using rodent models; few have examined the relationship in humans. One study by Biolo and colleagues (9) examined the effect of a local insulin infusion post-exercise, on the rates of protein synthesis and breakdown. It was found that insulin infusion post-exercise did not affect the rate of protein synthesis, but significantly decreased the rate of protein degradation, thus creating a more positive protein net balance (9). Thus, since insulin creates an optimal environment for net protein gain to occur, this information appears to indicate that carbohydrates should be included in a post-exercise feeding in order to increase insulin concentrations and enhance skeletal muscle net protein balance. A study by Roy and colleagues (70) also found that carbohydrate supplementation post-exercise elevated insulin concentrations, and allowed for a decrease in myofibrillar protein breakdown, resulting in a more positive whole body protein balance.

There appears to be a minimum concentration of insulin required for a full response of translation initiation and protein synthesis to occur. A study by Fluckey et al. (30) had rats either exercise or remain sedentary. The rats were subsequently subjected to bilateral hind-limb perfusion in which one leg was perfused with medium containing insulin and the other with medium devoid of the hormone. The perfusion medium additionally contained physiological concentrations of amino acids. It was observed that in the presence of insulin, protein synthesis was stimulated in the perfused exercised muscle preparations compared with those from sedentary rats. In contrast, the exercise-induced stimulation of protein synthesis was not observed when insulin was eliminated from the perfusion medium (30). These findings concur with previous findings and indicate that insulin, in combination with prior contractions, induces a stimulation of protein synthesis and without the presence of this hormone there is a lack of response altogether.

In rodents, it has been found that eukaryotic initiation factor 2B (eIF2B) is responsible for the initiation of protein synthesis following resistance exercise (39, 47). Subsequent research has observed an interaction between insulin, eIF2B and resistance exercise. It appears that a minimal concentration of plasma insulin is required to generate a stimulation of muscle protein synthesis and eIF2B activity in response to resistance exercise (27, 39, 41, 42).

1.4.2 AVAILABILITY OF AMINO ACIDS

Evidence from human studies strongly suggests that amino acid availability post-exercise allows net protein synthesis to occur in skeletal muscle (8, 67, 84, 85, 86, 87). Recent evidence in swine has found that reduced amino acid availability inhibits muscle protein synthesis and decreases activity of eIF2B (44). Kobayashi and colleagues (44) concluded that a decline in plasma amino acid concentrations below the normal basal value signals an inhibition of muscle protein synthesis, and the corresponding changes in eIF2B activity suggest a possible role for this peptide chain initiation factor in mediating the response (44). This finding is intriguing since it seems that both insulin concentrations (9, 30) and the availability of amino acids (44) can influence the activity of eIF2B and modulate muscle protein synthesis. Future studies in this area are needed in order to verify the interaction.

1.4.3 INFLUENCE OF PROTEIN SOURCE

Proteins elicit different postprandial responses depending on their source and composition, which could result in varying concentrations of incorporation into the different protein pools within the body. So called "fast" and "slow" proteins have been shown to differently modulate postprandial protein accretion (3). Whey and casein, the protein fractions found in milk, have been recognized as fast and slow proteins respectively (3, 21). Whey protein induces a dramatic but short-lived increase in plasma

amino acids while casein induces a more prolonged plateau of moderate hyperaminoacidemia, likely due to a slowed rate of gastric emptying and slower digestion and assimilation (3, 21). The underlying responses of protein turnover induced by these two protein fractions are quite different. Whole body protein breakdown was inhibited by 34% after casein ingestion but not after whey, while postprandial protein synthesis was stimulated by 68% with a whey protein meal and to a lesser extent (31%) by a casein meal. In addition, whole body leucine oxidation was lower following consumption of casein than with whey protein. The end result was that net leucine balance over the 7 h period following a casein or whey meal was more positive with casein than with whey (3, 21). From the results of these studies it can be concluded that the speed of amino acid absorption after protein ingestion has a major impact on the postprandial metabolic response to a single protein meal. The slowly absorbed casein promotes postprandial protein deposition by an inhibition of protein breakdown without excessive increases in amino acid concentration; by contrast, a fast dietary protein stimulates protein synthesis but also oxidation. The observed decrease in proteolysis following consumption of casein can likely be attributed to the duration of the postprandial hyperaminoacidemia. Amino acids themselves have been shown to inhibit proteolysis (33) and the hyperaminoacidemia is much more prolonged with slow than with fast proteins (3, 21). The observed decrease in protein breakdown is unlikely due to insulin since the extent of insulinemia was either not different (3) or higher following the fast proteins (21). The two major constituent proteins in milk are whey and casein, but they are found in quite different proportions, 80% casein and 20% whey protein indicating that milk on

aggregate would act like a slow protein rather than a fast protein due to its larger casein protein fraction (14).

The quality of milk and soy proteins have been assessed both independently and compared directly. Several studies have found that isolated soy protein is of high nutritional quality, comparable to that of animal protein sources (38, 73, 99), and the level of amino acids in plant protein, including soy protein isolates is much higher (per unit of protein) than that required by adults (100). Soy and milk have been directly compared using the N balance technique. These comparisons have highlighted the fact that well processed isolated soy proteins are indistinguishable from milk as a protein source for maintenance of short-term N balance in adult human nutrition (38, 73). On the basis of this evidence it can be assumed that the quality of isolated soy protein is high and if differences do exist between soy and milk proteins in their ability to build skeletal muscle, it is not due to differences in the quality of the protein. In an early review examining soy protein, Young hypothesized that the differences between milk and soy milk in building skeletal muscle is due to the difference in their pattern of digestion (100).

Recently published studies have found that milk and soy proteins display varying posprandial kinetics, milk acting like a slow protein and soy as a fast protein. The differences in postprandial nitrogen metabolism of intact dietary proteins is different following ingestion of milk and soy, mainly because of a greater deamination of soy amino acids than of milk amino acids (13, 31, 46, 50), and of their differing capacity to support protein synthesis (23). In a recent study by Bos et al (14) that directly compared the postprandial kinetics of dietary amino acids from soy and milk in humans, it was

found that soy derived amino acids were digested more rapidly and were directed toward both deamination pathways and liver protein synthesis more than milk derived amino acids. They hypothesized that differences between soy and milk protein may have arisen from the combined influence of a relatively unbalanced amino acid composition in the soy protein and its lower digestibility, resulting in a less favorable pattern of amino acids reaching the periphery (14). A study by Fouillet et al. (28) utilized compartmental modeling techniques to simulate the kinetics of dietary N movement following the ingestion of sucrose and soy or sucrose and milk proteins. They found that protein synthesis efficiencies in the splanchnic bed were significantly affected by the protein source in the meal, and reached 23 and 30% 8 h after milk proteins and soy proteins ingestion respectively. Conversely protein synthesis efficiencies in the peripheral tissues reached 32 and 26% 8 h after milk and soy protein ingestion respectively (28). This would indicate that milk protein promotes greater accretion of peripheral protein, possibly skeletal muscle, than soy protein. The lower whole body retention of dietary N observed experimentally with soy compared with milk protein was associated with the following: 1) a more rapid intestinal transit and absorption of dietary N from soy protein; 2) its increased rate of transfer of amino N to urea, concurrent with its similar sequestration in the splanchnic bed; and 3) subsequent reduction in uptake by the peripheral area (28). It is known that milk proteins used in the Fouillet study (28) contained higher concentrations (~120%) of branched chain amino acids (BCAA) than soy proteins, and these amino acids that are transferred largely to extrasplanchnic tissues (5, 92). Certain BCAA, in particular leucine are also stimulators of muscle protein anobolism (39, 94),

and a higher proportion of BCAA could explain the higher peripheral protein synthesis efficiencies reported following milk protein ingestion versus soy protein (28).

The synergistic effect of resistance exercise and post-exercise protein feeding on overall net protein balance has been well documented (8, 26, 51, 67, 85). A study by Wilkinson et al. (92) examined the effect of milk versus soy drink ingestion following heavy resistance exercise. They observed greater protein synthetic rates and net amino acid uptake in skeletal muscle following milk ingestion compared to soy (92), suggesting greater overall accretion of skeletal muscle in individuals consuming milk post-exercise than those consuming soy. It does appear that milk proteins or protein of an animal origin, promote skeletal muscle accretion of a larger magnitude than that of soy proteins or protein of a vegetable origin.

1.4.4 CARBOHYDRATE INGESTION

Insulin and exercise are well known regulators of protein metabolism (6, 93). At rest, insulin has a stimulatory effect on muscle protein synthesis, which is observed only when amino acid availability is maintained or kept high (6, 9). However, while insulin inhibits proteolysis it has no effect on muscle myofibrilar protein breakdown (6, 9). Interestingly, following resistance exercise the effect of insulin is reversed and no additional increase in muscle protein synthesis is observed (9, 12, 70), while the exercise-induced increase in muscle protein breakdown is significantly attenuated by insulin (9, 12, 43, 51). There is some debate over the effect of insulin on protein synthesis rates

since evaluation of the effect of insulin on muscle is complicated by the fact that systemic insulin infusion causes a pronounced hypoaminoacidemia. This decrease in amino acid concentrations tends to counteract any direct action of insulin to stimulate muscle protein synthesis (95). A practical method of elevating post-exercise insulin concentrations is by consumption of a carbohydrate containing meal. This method has been proven to significantly elevate glucose and insulin concentrations sufficiently to result in a decrease in myofibrilar protein degradation (11, 51, 70).

Carbohydrate and protein are synergistic in their ability of promote protein accretion (31, 51, 67). At rest the addition of sucrose to a protein meal halved the early (0-2 h) deamination peak of dietary nitrogen and reduced endogenous protein oxidation over the first 4 h; both were reduced by 18-24 % over the 8 h period after the meal compared to a protein meal (50). Similarly, Gaudichon and colleagues (31) found that carbohydrates reduced amino acid catabolism during the postprandial phase, both directly and through their insulin releasing effect. Mariotti et al (50) found that there was a marked delay of gastric emptying with a protein sucrose meal compared to a protein meal, which caused the soy protein to act more like a slow protein when ingested with carbohydrate allowing for a more moderate hyperaminoacidemia and promoting protein accretion. An underlying mechanism for this effect may be related to p70 S6 kinase. A recent study found that insulin and amino acids activate p70 S6 kinase through different pathways, and leucine stimulates protein synthesis through a nutrient signaling mechanism that is independent of insulin (34). Following resistance exercise the combined effect on net muscle protein synthesis of carbohydrate and amino acids given

together is roughly equivalent to the sum of the independent effects of either given alone (51). During the 3-h period after ingestion of carbohydrate plus amino acids, net muscle protein synthesis was significantly greater than following carbohydrate ingestion alone (51). Although carbohydrate ingestion does have an influence on overall net protein balance the amplitude of its effect is small in comparison to amino acids alone (12). The main benefit of carbohydrate ingestion following exercise is when it is taken in combination with protein (51).

Although post-exercise provision of an amino acid containing meal is essential for accretion of skeletal muscle, controversy exists with respect to the importance of immediate provision of this meal. Some evidence suggests that in the elderly the immediate provision of amino acids is crucial (26), whereas acutely, in young males it does not seem to be essential (67). The presence of increased concentrations of insulin has been identified as being a fundamental component in promoting skeletal muscle accretion. The provision of carbohydrate alone following resistance exercise increases insulin levels and allows for a more positive skeletal muscle net protein balance post-exercise, mainly by reducing protein breakdown levels. Moreover, a post-exercise meal containing both amino acids and carbohydrate is more effective at promoting skeletal muscle net positive balance than either consumed alone. Milk and soy proteins elicit different postprandial kinetics, and milk proteins are more effective at stimulating protein synthesis in the peripheral area than soy proteins. Following resistance exercise milk proteins are more effective than soy proteins for increasing protein synthetic rates and net

amino acid uptake in skeletal muscle. The differences between milk and soy are mainly due to differences in digestion kinetics and not in their amino acid composition.

1.5 STATEMENT OF PURPOSE

The provision of a protein containing meal post-exercise is crucial for the accretion of skeletal muscle (7, 8, 84, 85, 87, 92). How the source of this protein can affect the degree of hypertrophy while resistance training is yet undetermined. At rest it has been demonstrated that milk proteins are more effective at promoting protein synthesis in the peripheral areas than soy proteins (28). Following resistance exercise milk proteins are more effective at stimulating skeletal muscle protein synthesis and net amino acid uptake (92). The aim of the present study was to test the hypothesis that the post-exercise consumption of milk will be more effective than an isoenergetic and isonitrogenous control drink from soy (PEC), and both the milk and soy will be more effective than an isoenergetic control (CON) at promoting resistance training adaptations observed in increases in the following variables:

1. Fat and bone free mass (FBFM)
2. Muscle fibre area
3. 1 RM Strength

Chapter II

The effect of differing post-exercise macronutrient consumption on resistance training-induced adaptations in novices

2.1 INTRODUCTION

Resistance exercise training is a potent stimulus for skeletal muscle hypertrophy (19, 45, 72, 82). Acutely, resistance exercise stimulates skeletal muscle protein synthesis rates and allows for a more positive protein net balance (49, 59). Although less negative, net protein balance does not become positive until after the consumption of a post-exercise protein containing meal (7, 8, 84, 85, 87, 92). Hence, protein is an important macronutrient to include in a post-exercise meal. Moreover, while the provision of carbohydrate alone in the post-exercise period promotes a more positive protein balance, a net positive protein balance is not attained (12, 51).

Postprandial protein metabolism in response to feeding is affected by the protein source (3, 21, 22). Aptly named "fast" and "slow" proteins demonstrate differing protein kinetics (3, 21). Fast proteins such as whey have a lower postprandial retention rate compared to a slow protein such as casein (3, 21). Milk (20% whey, 80% casein protein fractions) and soy proteins have been classified as slow and fast protein sources respectively (14, 28), and the muscle protein synthetic response in different tissue compartments varies following ingestion of each source. Milk proteins promote a greater protein synthesis efficiency (defined as the fraction of intracellular amino acids flux of appearance that is incorporated into protein (28)) within the peripheral tissues, most likely muscle, whereas soy proteins promote greater protein synthesis efficiency in the splanchnic bed (28). These data imply that milk may be superior for promotion of skeletal muscle protein accretion than soy when consumed post-exercise, because milk

proteins would provide a greater stimulus for muscle protein synthesis. The purpose of the present study was to examine the influence of varying post-exercise macronutrient consumption (milk, soy beverage and maltodextrin) on resistance training adaptations in young novice weightlifters. Our hypothesis was that individuals consuming milk immediately and 1-h post-exercise would observe larger gains in strength, lean muscle mass, and muscle fibre area than a group consuming a soy beverage, and both the milk and soy beverage groups would observe larger gains than a group consuming a maltodextrin only beverage.

2.2 METHODS

36 young healthy men (22.5 ± 0.6 y, 82.1 ± 2.2 kg, 1.8 ± 0.01 m, 25.6 ± 0.7 kg/m^2) were recruited to participate in the study. Volunteers completed a routine medical screening questionnaire, and based on their responses all were deemed healthy. Written informed consent was obtained from all volunteers. The study was approved by the Hamilton Health Sciences Research Ethics Board. None of the participants were engaged in regular physical activity (<2 d/wk) at the start of the study and had not done so for the previous 6 months.

2.2.1 EXPERIMENTAL PROTOCOL

Prior to the study and during the 6^{th} and 12^{th} weeks of training participants were required to complete 3 d weighted diet records (analyzed using Nutritionist V, First.Data

Bank, San Bruno, Ca) to determine macronutrient consumption. Subjects were randomly assigned to consume either milk (MLK) (n=12; fat free milk), an isonitrogenous isoenergetic control (PEC) (n=12; soy beverage) or an isoenergetic control (CON) (n=12; maltodextrin beverage) drink consumption groups using single blinded allocation. All drinks had similar strawberry flavor and were visually opaque and of similar colour. Participants consumed one 500 mL drink immediately post-exercise under direct supervision of an investigator, and a second 500 mL drink 1-hr post-exercise. Participants were instructed to be at least 2-h post-prandial before training and were instructed to refrain from consumption of foodstuffs for 2-h post-exercise, with the exception of water. Compliance with the immediate post-exercise drink was 100% because of direct investigator supervision. The 1-h post-exercise drink consumption compliance was reported to be 100 % by the subjects.

Following the pre-training testing procedures two subjects dropped out of the study for personal reasons, one from the PEC and one from the CON groups. Thus a total of 11 subjects remained in both the PEC and CON groups, who completed all aspects of testing/training. All subjects completed 12 weeks of a whole body split routine resistance training program, 5 d/wk and pre, mid and post-testing as described below. Compliance with the training program was 98 ± 0.4 %.

2.2.2 PRE, MID AND POST TESTING

One repetition maximal strength (1RM) testing was conducted over a 3 d period (days 1-3) for each of 13 exercises pre, 4 wk, 8 wk and post training. The legs were tested on the first day (leg press, leg curl, leg extensions, standing calf raises), followed by pushing exercises (seated military press, bench press, vertical bench press, chest fly, and seated machine triceps extensions), and, finally, pulling exercises (latissimus pull-down, seated wide-grip row, seated narrow low row, and seated biceps curl). Subjects initially performed a warm-up set of 8-10 repetitions at approximately 50 % of their estimated 1RM. The first set was the first attempt at the predicted 1RM. A successful lift was judged as being through the full range of motion of the exercise and was performed with proper technique as assessed by an investigator. There was a 2-min rest period between each successive attempt of a new 1RM. If a subject could not lift the initial 1RM, the weight was reduced accordingly and a 2-min rest period was provided before the next 1RM attempt. 1 RM tests on the guided motion machines (all except leg press) subjects started from the initial position of the machine (either at full extension or flexion depending on the exercise) and progressed through the range of motion unaided. The leg press testing consisted of subjects initially having legs at full extension, then lowered the weight until knee angle was 90° of flexion and subjects then lifted the weight into full extension.

Percutaneous muscle biopsies were taken from the vastus lateralis muscle under local anaesthetic (2 % Lidocaine) using a 5 mm Bergstrom needle modified for manual

suction. Samples were immediately dissected of all fat and connective tissue, oriented and mounted in optimal cutting temperature (OCT) embedding medium that was prechilled in isopentane-cooled in liquid nitrogen, snap frozen, and stored at -80°C until subsequent analysis. The remainder of each biopsy was frozen directly in liquid nitrogen.

Body composition (total body mass (TBM)), fat-free mass (FFM), fat and bone-free mass (FBFM) and body fat mass and percentage were assessed using dual energy x-ray absorptiometry (DXA: Model QDR-1000/W, Hologic Inc., Waltham, MA). Participants were scanned during the same time of day pre and post. Whole body scans were performed from head to toe in the single beam mode and bone mineral content (BMC), fat, and lean mass were calculated using the version 5.56 software. All participants were scanned with their hands suppinated at their sides and feet between 20-30 cm apart. All scans were performed by the same investigator and positions were recorded in order to replicate them during post training. During each test the standard bar was placed to the right of the subject and a phantom spine was scanned each day for day-to-day reliability. In a reproducibility experiment using this machine it was found that the coefficients of variation (CV) were 1.6, 1.4, and 1.8 %, for whole-body BMC, lean mass, and fat mass, respectively (19).

All participants were also required to have a one-time response to a leg workout and supplement consumption evaluated during the 7th week of training. Participants arrived in the laboratory at least 2-h post-feeding as instructed throughout the training program and had a plastic catheter (20 Ga) inserted into their anticubital vein. Blood samples were obtained at rest, immediately post-exercise, and every 30 min up to 2-h

post-exercise. Samples were collected in tubes containing heparin for plasma analysis, glucose was analyzed immediately (Yellow Springs Instruments, Yellow Springs, OH, USA), the remainder was centrifuged at 5000 rpm for 5 min at 4 °C. Plasma was transferred and frozen (-20°C) until further analysis (see below).

2.2.3 EXERCISE TRAINING

Following all pre-training testing, participants began a full body split resistance training program 5 d/wk. Sessions were split into one of three training groups (see Table 1), the muscle group that was trained only once the prior week began the following week. All training sessions were monitored by an investigator and a ratio of no more than 2 participants to 1 investigator was maintained throughout the study to ensure proper technique and compliance with exercise intensity.

Table 1. Exercise groupings

	Day 1 (Legs)	Day 2 (Pushing)	Day 3 (Pulling)
Exercises	Leg press, Leg extension, Leg curl, Seated calf raises	Military press, Bench press, Seated bench press, Chest Flys, Seated tricep extension,	Lateral pull down, Wide row, Narrow row, Back flys, Seated bicep curls

The program utilized only guided motion and cable machines (Badger Magnum, Milwaukee, WI) to ensure safety and to reduce the learning required for performance of the exercises. Training was initiated at approximately 80 % of pre 1RM for each exercise, a goal of 2 sets of 10-12 repetitions and was set for weeks 1 and 2.

Table 2. Exercise training protocol

Week 1-2	2 Sets	10-12 reps
Week 3-5	3 Sets	10-12 reps
Week 6-7	3 Sets, with 3rd set to failure	8-10 reps
Week 8-10	3 Sets, with 3rd set to failure	6-8 reps
Week 11-12	3 Sets, with 3rd set to failure	5-6 reps

In subsequent exercise sessions intensity was adjusted so that 3 sets of 6-12 repetitions were performed, with set 3 being performed to failure (see Table 2). The load was continuously adjusted according to subject performance in order to remain within the desired repetition range. Training logs were kept in order to record training intensity and volume of each session. Subjects were instructed not to engage in any new additional training but were encouraged to maintain previous activity levels. Twenty four participants had 100 % compliance and 10 had 90 % or above for an overall compliance rate of 98 ± 0.4 %.

2.2.4 HISTOCHEMICAL ANALYSIS

The OCT embedded biopsy samples were serially sectioned (10 μm thick) on a cryostat microtome (Model HM500OM, Micron International, Walldorf, Germany) at a sample and cabinet temperature of -20°C. Samples were stained for myosin ATPase activity after preincubation at a pH of 4.6 (15) (50 mM potassium acetate, 17.5 mM calcium chloride) for a duration of 7 min with pre and post training samples assayed simultaneously. Slides were then rinsed in distilled water and incubated in 3mM ATP using an alkaline solution (75mM glycine, 40.5mM calcium chloride, 75mM NaCl,

67.5mM NaOH, adjusted to pH 9.4) for 45min at 37°C and agitated at regular intervals in a temperature controlled incubator shaker (G24 Environmental Incubator Shaker, New Brunswick Scientific Co., New Brunswick, NJ). Slides were then rinsed in distilled water and incubated in 1% $CaCl_2$ for 3min at room temperature. Slides were once again rinsed in distilled water and incubated in 2% $CoCl_2$ for 3min at room temperature. Slides were then rinsed in distilled water and incubated in 1% ammonium sulphide for 30 seconds at room temperature. Slides were then rinsed a final time in distilled water and dehydrated by submersing for 2min in the following ethanol concentrations (70, 80, 90, 95 and 100%). Samples were then cleaned twice using xylene. Slides were blotted dry using kimwipes and coverslips were mounted using Permount (Fisher SP15) and allowed to dry overnight.

A total of 100-500 fibres were available for analysis from each sample. Fibre analyses were performed using image analysis software (Image Pro Plus, Media Cybernetics, Silver Springs, MD) interfaced with a microscope (Olympus BX60, Melville, NY) and a digital camera (SPOT Diagnostic Instruments, Inc., Sterling Heights, MI). Custom macro programs within the software were used to calculate individual fibre areas and raw data was exported to an Excel Spreadsheet (Microsoft), percent fibre area and percent fibre type were calculated within Excel. Three to four fibre types were identified per sample (I, IIa, IIx, and IIax) from the staining pattern by setting cut-off limits resulting in the creation of optical density 'bins' according to the darkest (type I), lightest (type IIa) and intermediate (type IIx) fibres. Sample images were converted to 8-bit, 256 grayscale images, which linearly scale each pixel and assign values from between

0 (black) to 255 (white). By setting lower and upper threshold values optical density bins were created that were: 0-95 for dark areas, 100-175 for intermediate areas, 180-255 for light areas. Using these cutoffs the three fibre types were more objectively classified.

2.2.5 INSULIN AND GLUCOSE ANALYSIS

Insulin concentrations were analyzed by radioimmunoassay (RIA) (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Briefly, frozen serum samples were thawed vortexed and centrifuged to clear lipemic samples. 200 μL of sample and 1 mL of ^{125}I insulin were added to 12x75 mm polypropylene insulin-specific antibody coated tubes and gently vortexed. Samples were analyzed in duplicate for post, 30 min post, 60 min post and 120 min post. Tubes were incubated for 24 hrs at room temperature (~21°C) and then decanted thoroughly. Radioactivity was analyzed using a gamma counter (5000 Minaxi Auto Gamma, Packard, Downers Grove, IL) for a duration of 1 min. Glucose was analyzed immediately following sample collection on whole blood (2300 STAT Plus-D, Yellow Springs Instrument, CO, USA)

2.2.6 STATISTICAL ANALYSIS

All anthropometric and histochemical data were analyzed using analysis of variance (ANOVA) with a 3 by 2 design (between factor = CON/MLK/PEC; within factor = PRE/POST training), and analysis of covariance (ANCOVA) was used on type II fibre area with pre fibre area used as a covariate. Glucose data were analyzed using

ANOVA with a 3 by 6 design (between factor = CON/MLK/PEC; within factor = PRE/POST; 30/60/90/120 min post-exercise). Insulin data were analyzed using ANOVA with a 3 by 4 design (between factor = CON/MLK/PEC; within factor = POST/30/60/120). Tukey *post hoc* analysis was employed to make pair-wise comparisons following identification of significant interactions. Correlation matrices, using Pearson's product correlations, were used to analyze the relationship between energy intake and lean mass gains, protein intake and lean mass gains, and lean mass gains and strength gains. Statistical significance was considered to be at level $P \leq 0.05$. Statistical analysis was performed using a computerized statistical package (Statistica 5.1, Statsoft, Tulsa, OH).

2.3 RESULTS

2.3.1 ANTHROPOMETRY

Thirty four subjects, 22.5 ± 0.6 y, 82.1 ± 2.2 kg, 1.8 ± 0.01 m, 25.6 ± 0.7 kg/m^2 completed training. There were no differences between groups before training MLK; 23 ± 1 y, 24.7 ± 1.0 kg/m^2, PEC; 22 ± 0.7 y, 27.8 ± 1.1 kg/m^2, CON; 22.7 ± 1.4 y, 24.5 ± 1.4 kg/m^2. There was an increase in total mass post training in all groups ($P<0.05$). All three groups also increased FBFM post-training ($P<0.05$) with a weak trend observed; MLK had the greatest gains in FBFM (5.5%), PEC observed slightly lower gains (4.0%), and CON had the smallest gains (3.6%) following training (time by group interaction, $P=0.47$). A decrease in body fat percentage was observed following training ($P<0.05$) in all groups, whereas total fat mass was unchanged over the training period (Table 3, Figure 4).

2.3.2 DIETARY ANALYSIS

There were no differences in habitual energy intake or the percentage of macronutrient ingestion at baseline. Protein ingestion increased during the study above habitual intakes for all groups in both absolute ingestion and relative to body mass ($P<0.05$). The relative ingestion of protein also increased throughout the study in all groups ($P<0.05$). No differences were observed for CHO and fat ingestion throughout the study (Table 4).

Table 3. Body composition determined by DXA

	CON			MLK			PEC		
	PRE	**POST**	**% Δ**	**PRE**	**POST**	**% Δ**	**PRE**	**POST**	**% Δ**
Total Mass (kg)	77.5±2.2	79.5±4.6*	2.6±0.8	77.9±3.2	81.1±2.7*	4.4±1.2	86.8±4.2	89.7±4.7*	3.2±1.5
Body Fat %	16.7±1.9	16.1±1.7*	-1.9±3.1	15.8±1.1	14.9±1.0*	-4.4±2.1	19.8±1.5	19.3±1.5*	-2.6±3.2
Fat Mass (kg)	13.5±2.1	13.3±1.8	-0.8±4.2	12.7±1.3	12.3±1.1	-2.2±3.2	17.5±1.8	17.7±2.0	1.0±4.7
Fat Bone Free Mass (kg)	61.2±2.5	63.4±2.7*	3.6±0.9	62.5±1.9	65.7±1.6*	5.5±1.0	66.2±2.8	68.9±3.1*	4.0±0.8

Results are mean ±SE

*Significant main effect for Time ($P<0.05$)

Table 4. Diet analysis before training and at 6 and 12 weeks of training

	CON			MLK			PEC		
	PRE	MID	POST	PRE	MID	POST	PRE	MID	POST
Energy Intake (Mj/day)	12.4±1.2	14.4±1.5	14.6±1.7	12.1±0.8	12.3±0.9	13.3±0.8	12.2±0.6	12.5±1.1	12.3±1.5
Protein Intake (g/kg/day)	1.3±0.1	1.7±0.2*	1.8±0.3*	1.4±0.1	1.5±0.1*	1.6±0.1*	1.2±0.1	1.6±0.2*	1.4±0.2*
Protein intake (g/day)	100±6	134±15*	139±18*	114±10	123±9*	134±9*	101±9	137±15*	128±13*
CHO intake (g/day)	416±45	460±36	448±52	376±34	343±37	395±29	364±40	339±31	360±47
Fat intake (g/day)	97±10	115±14	126±18	100±7	98±9	103±8	107±12	107±10	99±12
Protein, % total caloric intake	14±1	16±1*	16±1*	16±1	17±1*	17±1*	14±1	19±1*	18±1*
CHO, % total caloric intake	55±3	56±4	52±3	51±2	47±4	51±2	50±3	46±2	49±2
Fat, % total caloric intake	30±2	30±2	32±2	32±2	30±2	30±1	33±2	32±1	31±1

Results are mean ± SE

*Significant main effect for time ($P<0.05$)

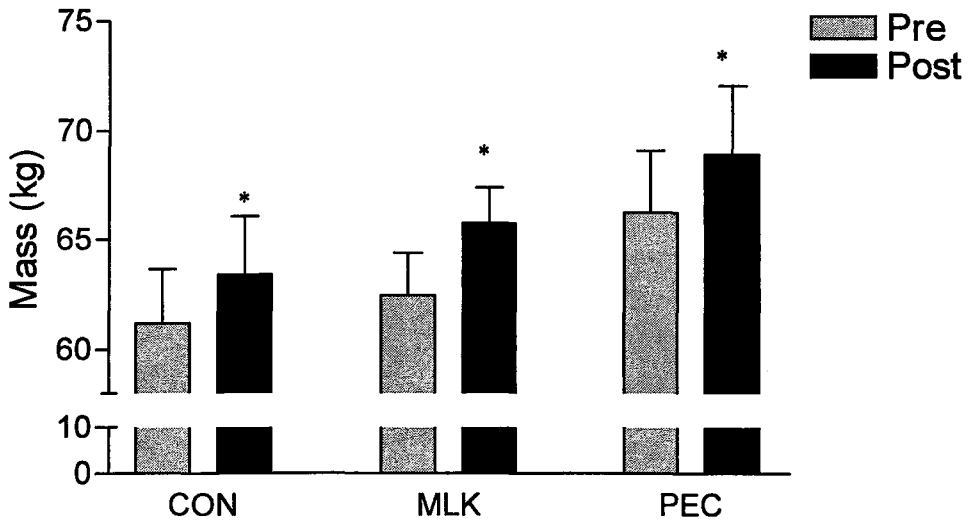

Figure 4. FBFM pre and post training (DXA). * Significant main effect for Time ($P<0.05$), time by group interaction ($P=0.47$).

2.3.3 HISTOCHEMISTRY

CON had a significantly larger type II fibre area than MLK and PEC before training ($P<0.05$). Following training there were large increases in type II fibre area MLK (22.2%), PEC (12.2%), and CON (9.5%) ($P<0.05$) (see Figure 6, Table 5) but not for type I in all groups (see Figure 5, Table 5). No differences were observed for % fibre area before training. Significant increases occurred for Type IIa % fibre area, while a significant decrease in Type IIx % fibre area was present ($P<0.05$) (see Table 6).

Table 5. Fibre area pre and post training

	CON			MLK			PEC		
	PRE	**POST**	**%Δ**	**PRE**	**POST**	**%Δ**	**PRE**	**POST**	**%Δ**
Type I Area (μm^2)	5424±267	5611±286	3.6±2.5	4177±161	4504±206	8.4±5.3	4620±360	4889±357	6.8±4.1
Type II Area (μm^2)	6110±345¶	6674±355*	9.5±1.9	5046±248	6050±213*	22.2±7.2	5366±142	6016±227*	12.2±3.7

Results are Mean ±SE

¶ Main effect for group (P<0.05) (ANCOVA performed to account for these differences)

* Main effect for time (P<0.05)

Table 6. % Fibre Area pre and post training

	CON		MLK		PEC		Average	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
Type I Area (%)	41±4	42±2	32±3	33±4	35±2	37±3	36±2	37±2
Type IIa Area (%)	35±4	44±3*	44±3	50±3*	42±4	47±3*	40±2	47±2*
Type IIb Area (%)	24±2	16±3*	24±3	16±2*	23±3	16±2*	24±1	16±1*

Results are Mean ±SE

*Significant main effect for time (P<0.05)

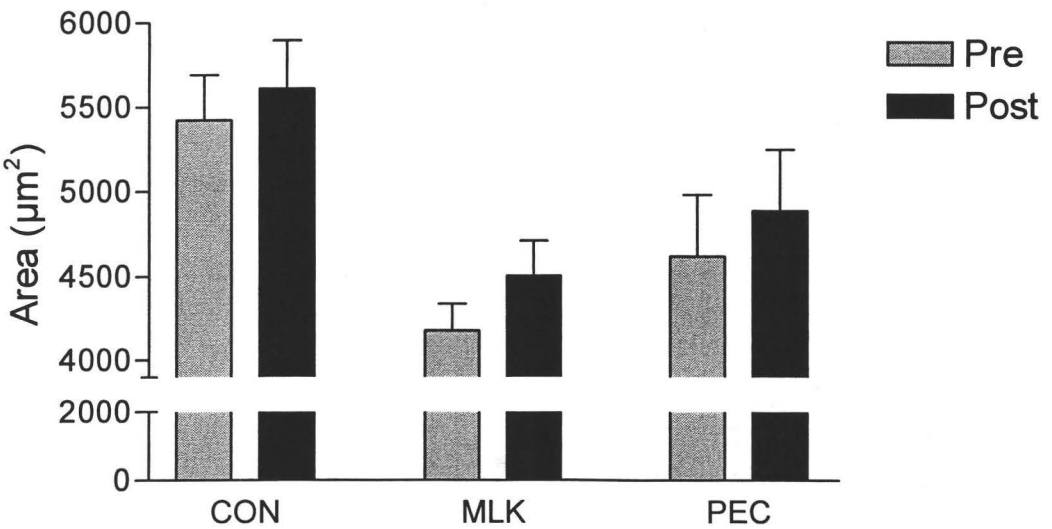

Figure 5. Type I fibre area pre and post training

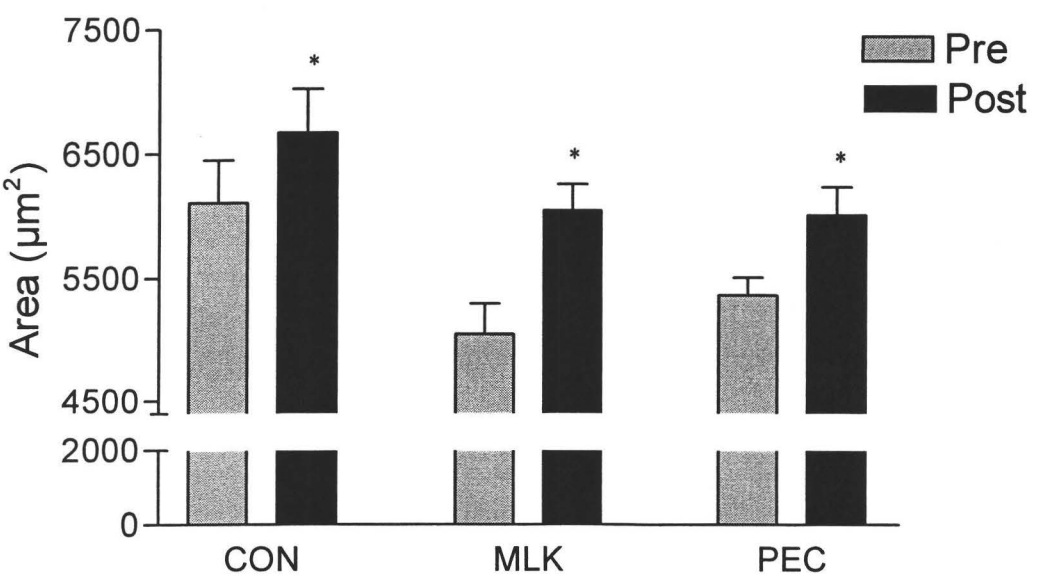

Figure 6. Type II fibre area pre and post training. * Main effect for Time ($P<0.05$), group by time interaction ($P=0.18$)

2.3.4 STRENGTH MEASUREMENTS

No differences were present in 1RM strength before training. All strength measurements increased significantly post training ($P<0.05$) and no group differences were present (see Table 7, 8 and 9).

Table 7. 1RM (pushing exercises) strength pre and post training

	CON			MLK			PEC		
	PRE	POST	%Δ	PRE	POST	%Δ	PRE	POST	%Δ
Shoulder Press (kg)	53±6.3	74±8.6*	38.4	69±3.9	90±3.7*	29.2	64±4.7	90±7.1*	39.8
Bench Press (kg)	63±5.9	104±7.4*	65.0	75±4.6	111±4.4*	48.8	70±5.5	107±7.1*	52.3
Vertical Bench Press (kg)	74±6.7	129±10.0*	72.6	86±4.5	134±7.0*	55.3	85±7.9	131±11.3*	53.3
Triceps Press Down (kg)	40±2.5	58±2.8*	46.4	48±3.5	65±2.9*	33.3	40±3.4	65±3.3*	61.2
Chest Flys (kg)	76±7.2	120±9.0*	56.6	87±5.2	141±11.5*	61.6	87±5.7	135±9.9*	55.3

Results are Mean ±SE

*Significant main effect for time ($P<0.05$)

Table 8. 1RM (pulling exercises) strength pre and post training

	CON			MLK			PEC		
	PRE	POST	%Δ	PRE	POST	%Δ	PRE	POST	%Δ
Lateral Pull Down (kg)	62±3.2	82±4.4*	32.2	69±3.0	89±3.8*	29.5	65±4.5	85±4.5*	31.8
Wide Row (kg)	59±4.4	83±4.8*	40.0	67±2.8	94±3.4*	40.5	65±6.5	94±5.6*	44.8
Narrow Row (kg)	62±4.7	85±4.5*	37.5	68±2.8	96±3.4*	40.7	68±6.0	94±5.2*	38.9
Biceps Curl (kg)	38±4.5	70±5.6*	81.4	50±3.3	76±4.4*	51.7	52±4.3	74±3.7*	42.5
Rear Flys (kg)	54±3.0	81±4.5*	49.1	64±3.7	93±5.1*	45.4	66±5.7	93±5.8*	42.0

Results are Mean ±SE
*Significant main effect for time (P<0.05)

Table 9. 1RM (leg exercises) strength pre and post training

	CON			MLK			PEC		
	PRE	POST	%Δ	PRE	POST	%Δ	PRE	POST	%Δ
Leg Press (kg)	208±83.3	342±47.0*	63.9	183±15.9	368±23.9*	100.4	237±22.8	477±48.7*	101.4
Leg Curl (kg)	70±3.9	106±6.2*	50.8	70±6.1	108±5.9*	53.8	83±5.1	117±7.5*	41.3
Leg Extension (kg)	103±7.0	151±16.6*	45.8	106±4.9	154±5.8*	45.5	123±5.9	186±9.2*	50.7

Results are Mean ±SE
*Significant main effect for time (P<0.05)

2.3.5 GLUCOSE AND INSULIN RESPONSE TO EXERCISE AND SUPPLEMENT CONSUMPTION

Glucose concentrations were significantly higher at rest in the MLK group ($P<0.05$), but this difference was no longer present post-exercise. A significant time by group interaction was present ($P<0.01$) and Tukey Post-hoc analysis revealed the following: (i) CON glucose concentrations were significantly higher at 30-min post-exercise than MLK and PEC ($P<0.05$); (ii) Glucose concentrations were elevated above post-exercise concentrations at the 30-min time point in CON group only ($P<0.05$); (iii) Glucose concentrations remained constant throughout the sampling period for both the MLK and PEC groups (see Figure 7).

Insulin concentrations between groups were not different post-exercise. A significant time by group interaction was present ($P<0.01$) and Tukey Post-hoc analysis revealed the following: (i) CON insulin concentrations were significantly higher at 30, 60 and 120-min post-exercise than MLK ($P<0.05$) and at the 60 and 120-min post-exercise for the PEC group ($P<0.05$); (ii) Insulin concentrations were significantly elevated at the 30-min time point above post-exercise concentrations in all groups ($P<0.05$); (3) conversely at 60 and 120-min insulin concentrations were significantly elevated above post-exercise concentrations in CON only ($P<0.05$) (see figure 8).

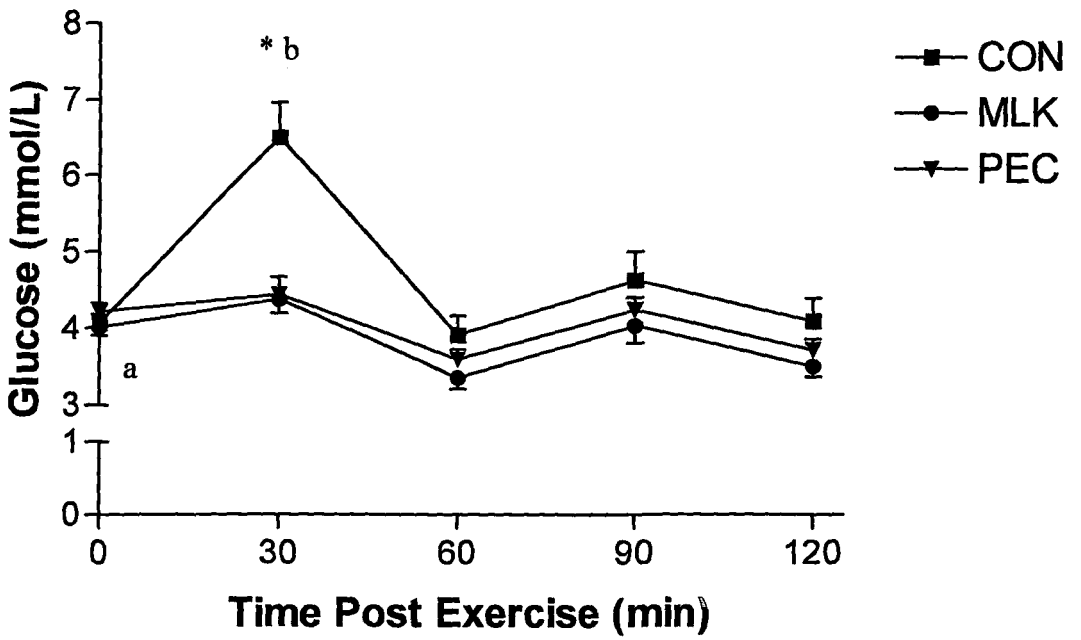

Figure 7. Blood glucose response to leg exercise and supplement consumption. Results are Mean ±SE, b Significantly different from a ($P<0.05$),*Significantly higher than MLK and PEC at same time point ($P<0.05$)

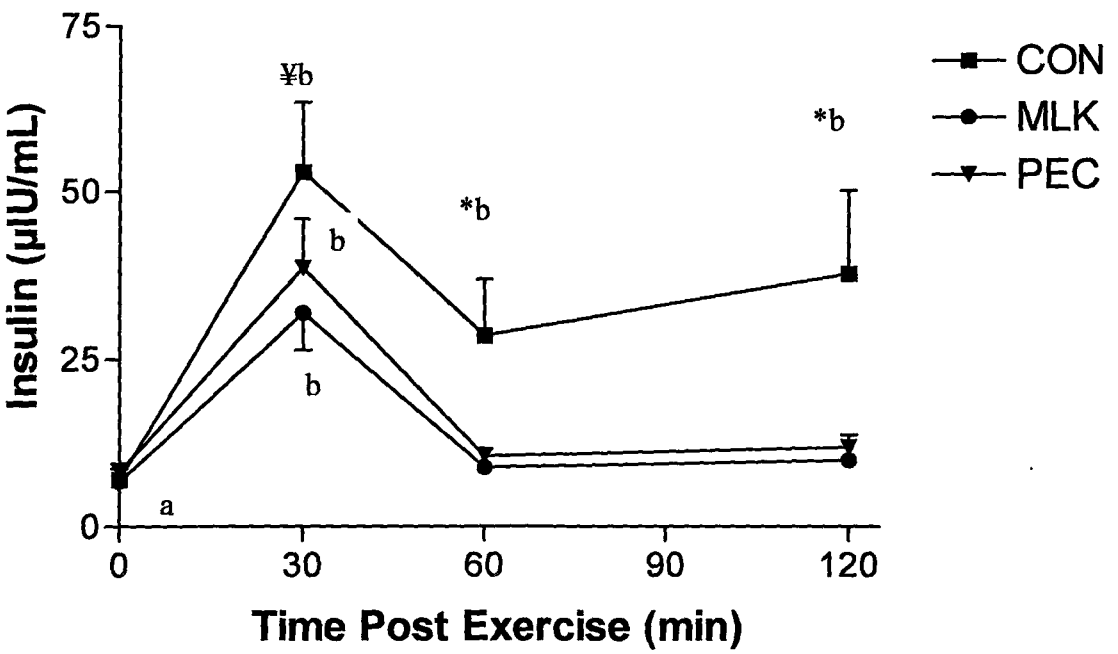

Figure 8. Blood insulin response to leg exercise and supplement consumption. Results are Mean ±SE, b Significantly different from a (P<0.05), * Significantly higher than MLK and PEC at same time point (P<0.05) ¥Significantly higher that MLK at same time point (P<0.05)

2.3.6 CORRELATIONAL ANALYSES

Lean mass gain did not correlate with either absolute or relative protein intake, or absolute caloric intake (P>0.05). Lean mass gain was significantly correlated with leg press strength gains (P<0.05) but not with bench press strength gains (P>0.05).

2.4 DISCUSSION

The major novel findings of the present study were that intact dietary proteins are an effective stimulus for skeletal muscle accretion and promote hypertrophy of large magnitude, when consumed immediately post-exercise, and carbohydrate consumption alone is adequate to support hypertrophy when sufficient protein is consumed as part of a balanced diet. Fat and bone-free mass increased over the 12 week training program in all groups and the magnitudes of the increases in FBFM observed in this study were large; the MLK group gained 3.2 ± 0.6 kg, PEC 2.7 ± 0.6 kg, and CON 2.2 ± 0.6 kg. To our knowledge, this is the first study to utilize intact dietary proteins as a post-exercise supplement in conjunction with a chronic resistance training program. In comparison to the lean mass gains observed in studies of similar, or slightly shorter duration using amino acids or hydrolyzed, isolated protein fractions, our lean mass gains were similar (20, 72). This implies that intact dietary proteins are as effective as amino acids or isolated protein fractions at promoting skeletal muscle hypertrophy. Data from Wilkinson et al. (92) support this contention: these investigators observed that intact milk and soy proteins were an effective stimulus for increased muscle protein synthesis following an acute bout of resistance exercise, and that the magnitude of the increase in synthesis rates were similar to those of previous work that provided amino acids post-exercise, when discrepancies in blood flow were accounted for (11, 84, 85, 87). Therefore, intact dietary proteins are an effective stimulus for muscle protein synthesis that promote skeletal muscle accretion to the same degree as amino acids or isolated protein fractions.

The amount of FBFM gained by the MLK group was 3.2 ± 0.6 kg (5.5%), by the PEC group 2.7 ± 0.6kg (4.0%), and by the CON group 2.2 ± 0.6 kg (3.6%) and there was no difference in gains between the groups (P=0.47). However, there was a notable trend in type II muscle fibre area (P=0.18), which reflected a similar pattern of change between the groups (MLK = 1004 ± 249 μm^2 (22%), PEC = 650 ± 192 μm^2 (12%), CON = 565 ± 120 μm^2 (9.5%)). It is difficult to argue, based on the P value for the FBFM interaction (P=0.47), that we have made a type 2 statistical error. The data from the type II fibre area revealed a stronger trend (P=0.18 for a group by time interaction) that indicated a greater hypertrophic gain in the MLK group. A post-hoc power analysis revealed that with alpha set at 0.05 and β at 0.7 we could detect a significant difference in type II fibre area between MLK and CON with 4 more subjects per group and between MLK and PEC with 7 more subjects per group. Unfortunately, the acute post-exercise increase in protein consumption in the PEC and MLK group versus the CON group may have been obscured by the overall increase in daily protein intake in all groups. In a recently published study that compared the effect of a protein containing supplement to a carbohydrate only supplement on lean mass gains over a ten week training period, although not significant, greater lean mass gains were seen in the protein-only versus the carbohydrate-only group (P=0.077) (20). An acute study by Miller et al also provides support to our assertion; they observed that a drink containing both amino acids and carbohydrate significantly elevated net muscle protein synthesis rates following a resistance exercise bout, whereas a drink containing only carbohydrate did not significantly elevate net protein synthesis rates (51). Over a twelve week period these acute differences could translate into larger

accretion of skeletal muscle in a group consuming a mixed carbohydrate protein drink compared to a group consuming solely carbohydrate. A study by Fouillet et al. (28) comparing milk and soy proteins directly also lends support to our theory. They observed that protein synthesis efficiencies were significantly greater in the peripheral area following milk protein ingestion compared to soy proteins. This suggests that longitudinally within our study, the MLK group would accrue more skeletal muscle than the PEC group.

Strength increases over the 12 week training study were identical between groups. The only interaction present was between the pre and 4 week strength measurements for biceps curls, the CON group gained significantly greater strength. These strength gains can be attributed mainly to neuromuscular adaptations since it has been demonstrated that neural factors account for most of the strength improvements observed in early phases of resistance training (53, 76, 77), and were not the result of larger hypertrophy resulting from the supplement that this group was consuming. Clearly the strength gains observed in this study were of large magnitude and highly significant, ranging from 29% to 101%, and were among the highest reported for a study of this duration (20, 26) and were similar between groups. It is unlikely that a type 2 statistical error could explain these results because strength gains were greater in CON for 4 exercises, MLK for 2, PEC for 4, and there were equivalent increases in the MLK and PEC groups for 1 exercise, and in all groups for 2 exercises. Thus, it appears that the differing post-exercise supplements had no effect on strength gain over the 12 week training period.

Dietary analysis revealed that all groups increased protein ingestion during the training program. This was expected for both the MLK and PEC groups since they were receiving protein in their post-exercise drinks, however the CON group also increased protein consumption even though their post-exercise drinks contained no protein. To our knowledge, this is the first documented case in which protein consumption increased to such a degree during a resistance training program in a group whose supplement contained no additional protein (72, 82). One study has found that rats enhance protein selection in response to a protein deprivation period which could explain the response observed within the present study (10). One could also hypothesize that the human body is homeostatic and chooses an optimal protein intake. No other differences were present in macronutrient consumption as well as total energy intake. Protein consumption during the training program was high, between 1.4 and 1.8 g/kg/d for all groups which is within the range recommended by ACSM/ADA/DC position stand (56). This amount of protein would by all estimates be adequate to support protein requirements during a resistance training program (45, 80, 81), and between group differences would not influence the degree of hypertrophy in any group in this study. Participants were required to refrain from consumption of any food or beverage for a 2-h period prior to each resistance exercise session and only consumed their supplement during the 2-h post-exercise period, and these stringent guidelines could have affected participants eating habits. In a study of similar design no differences were observed in dietary intakes pre to post training even though participants were receiving a protein supplement equivalent to 0.13 ± 0.01 g protein/kg body wt (26). In the present study training times were scheduled so that they

would not interfere with regular eating habits but if one assumes that training sessions took approximately 1-h, there was a 5 h time frame where participants consumed only 340 kcals in the form of the supplements that were provided. This could have accounted for the lack of increase in overall energy consumption.

Glucose and insulin responses to a resistance exercise bout and supplement consumption were significantly higher in the CON group. This was expected considering that the CON group received solely carbohydrate post-exercise. Both the MLK and the PEC groups displayed significant elevations in blood insulin concentrations at the 30 min post-exercise time point, and at no other time point. This rise in insulin is important for supporting the increase in protein synthesis when amino acids are provided, and also decreasing protein breakdown following resistance exercise (9, 30). The combination of both these effects would create an environment favoring the accretion of skeletal muscle. That each group showed significant increases in insulin all drinks would have created a favourable environment for muscle hypertrophy; however, a rise in muscle protein synthesis is not observed when exogenous amino acids are not provided post-exercise (9, 12, 70). Hence the lack of amino acids in the CON drink would have blunted the protein synthetic response post-exercise when compared to the MLK and PEC groups due to the lack of exogenous amino acids needed to induce the rise in protein synthesis (9), which would also decrease intracellular amino acid concentrations. Statistically, the CON group gained the same amount of FBFM as the MLK and PEC groups. Acute studies have shown that the provision of amino acids post-exercise are essential in shifting protein balance (i.e., synthesis minus breakdown) from negative to positive and promoting the

accretion of skeletal muscle (20, 67, 84, 85, 87). Roy and colleagues (70) observed that post-exercise consumption of a glucose drink significantly decreased protein breakdown (assessed by 3-methylhistidine excretion) and urinary urea nitrogen excretion, and did not significantly increase muscle protein FSR, resulting in a more positive protein balance due, the authors hypothesized, to the elevated concentrations of insulin they observed. Borsheim and colleagues (12) also observed an improved net muscle protein balance following carbohydrate consumption and this improvement was due primarily to a progressive decrease in muscle protein breakdown. They also remarked that the effect of carbohydrate ingestion was minor and delayed compared with the previously reported effect of ingestion of amino acids (12). It is evident, however, that the effect of carbohydrate ingestion on synthesis and breakdown rates may be a delayed effect, when compared to amino acids. The phenylalanine net balance observed in the previous study was only significantly improved at the 3-h post-exercise time point, and unfortunately, analysis did not extend beyond this point (12). It is possible that the true effect was missed and occurred beyond the 3-h point. Similarly, a study by Miller et al. (51) observed a similar effect where net phenylalanine uptake continuously rose over the 3-h post-exercise time point in a group who consumed only carbohydrate post-exercise. Consequently, the true effect of carbohydrate ingestion post-exercise may take over 3-h to reach its full magnitude. In the present study it is likely that individuals would have consumed a protein-containing meal at the 2-h time point post-exercise when diet restrictions were lifted. The CON group still had elevated insulin concentrations at the 2-h time point creating an optimal environment for protein accretion, as amino acids had

now been consumed. Data from Phillips and colleagues has established that protein synthesis rates are elevated for up to 48 h following an acute bout of resistance exercise (59); one would assume that any protein consumed over this period would allow for a more positive balance and increase skeletal muscle accretion. This could also influence the gains observed in both the MLK and PEC groups as they were consuming a regular mixed diet throughout the training program that contained protein from various sources.

The gains in FBFM and type II fibre area observed in the CON group also highlight the fact that protein consumption during the 2-h post-exercise period is not essential for hypertrophy and strength gains to occur in young individuals. A landmark study by Esmarck et al (26) found that in elderly subjects, a lack of protein consumption during the 2-h post-exercise period blunted strength gains and eliminated hypertrophy. Methodological differences were present between the Esmarck study and the present one, such as during the post-exercise period the control group (P2) received a drink containing no calories, whereas the CON group in the present study received carbohydrate. Although carbohydrate supplementation post-exercise does improve net balance post-exercise, it does not allow for a positive muscle protein balance, even at the 3-h time point post-exercise (12), so it is unlikely that the carbohydrate given during the post-exercise period can account for the large gains in muscle mass in the present study. Although an acute bout of resistance exercise elevates protein synthesis rates similarly in the young and the elderly (35, 97) and muscle protein anabolism can be stimulated by oral amino acids in the elderly as well as in the young (88, 89), it is likely that the duration, and possibly the magnitude of the elevation in net muscle protein balance is shorter in the

elderly compared to younger individuals. There is no direct evidence for this theory, although indirect evidence for this is observed in the larger gains found within the present study compared to previous studies of similar duration in the elderly (17, 26) as well as in a study by Welle et al. (91) directly comparing the hypertrophic response of young and elderly subjects. The greater hypertrophy in young individuals is likely the result of a more sustained elevation of protein synthesis rates compared to older subjects. This could account for the differences observed between the present study, and the Esmarck study (26).

Peripheral and splanchnic metabolism of dietary nitrogen is different for soy and milk proteins (28); milk proteins stimulate uptake of amino acids to a larger degree following resistance exercise than soy proteins (92). Within the present study a sample size of 11 (PEC) and 12 (MLK) was not large enough to translate the acute differences in protein synthesis between milk (MLK) and soy (PEC) (92) into significant differences in chronic training induced FBFM and fiber area gains. There does not appear to be any difference in the amino acid composition of soy and milk. Analysis of the composition of both the soy and milk protein used within the present study revealed that amino acid composition of the two proteins were remarkably similar (see Appendix 10). Furthermore several nitrogen balance studies have found that the overall quality of isolated soy proteins is equivalent to that of milk in adults since both can support similar N balance (73, 101). Therefore, intuitively one would expect that the gains in skeletal muscle would be quite similar between soy and milk. It would appear that the major difference between milk and soy proteins in stimulating protein synthesis is their

digestion rate (100). Several studies have highlighted these differences and have found that soy acts like a "fast" protein and milk a "slow" protein (14, 23, 28). Soy is associated with a faster transfer of dietary N into urea and a higher level of incorporation into the serum protein pool (14).

Alternatively, Mariotti et al. (50) have found that the addition of carbohydrate (sucrose) to soy protein allows it to act more like a slow protein, resulting in a marked delay of gastric emptying versus a soy protein only meal. The effect of carbohydrate could have had a large influence within the present study considering that the soy drink contained carbohydrate to maintain energy equivalency, and could have allowed the soy to act more like a slow protein, possibly negating the differences in digestion between soy and milk. Prior research from our lab established a greater uptake of amino acids following milk ingestion compared to soy ingestion with the same drink composition as the present study (92). The largest differences, although not statistically significant, were at the 2 and 3-h time points post-exercise following consumption of a single drink (92). Within the present study a second drink was consumed 1-h post-exercise, a factor that can also affect post-prandial kinetics of fast proteins. Repeated whey protein ingestion caused a shift in post-prandial kinetics of this protein from fast to slow (21) and displayed a better leucine balance over a 7 h period compared to a single dose. Within the present study the repeated ingestion of the soy protein drink could also have caused it to act more like a slow protein than a fast protein. It should also be mentioned that the two hour post-exercise time frame may have been too short a duration for differences between soy and milk proteins to be present. Most studies examining the differences between slow and

fast proteins examine postprandial kinetics for up to 7 h (14, 21, 23, 50). Since it is likely that participants in the present study consumed a meal immediately following the diet restriction period post-exercise, postprandial kinetics of the soy and milk proteins could have been affected.

Correlational analyses were performed to assess the relationship between dietary intakes and gains in FBFM. Absolute and relative protein intakes were not correlated with FBFM gains ($P>0.05$) lending evidence to the theory that once minimum protein requirements are met, further protein consumption does not increase lean mass (72). A third correlation was performed between FBFM gains and absolute caloric intake and again no significant interaction was present ($P>0.05$). A recently published article by Rozenik and colleagues (72) had hypothesized that once protein requirements are met, overall energy consumption is the largest determinant of lean mass gain while resistance training. It would appear from the results of the present study that this is not the case. Leg press strength gains were significantly correlated with FBFM gains ($P<0.05$), whereas bench press strength gains were not. It is surprising that one strength measure would be correlated with FBFM gains and another would not, the reason for this is unclear.

2.5 CONCLUSION

Strength and lean mass gains observed within this study were of large magnitude, and illustrate the fact that designer supplements such as amino acids or isolated protein fractions are not needed to induce large gains in muscle mass while resistance training. In reality intact dietary proteins are more than sufficient to maximize skeletal muscle accretion while resistance training. Our results also indicate that simply consuming carbohydrate post-exercise is a sufficient stimulus to induce hypertrophy when adequate protein is being consumed as part of a balanced diet. It also appears that immediate consumption of protein during the 2-h post-exercise period is not essential in younger subjects illustrating that differences exist between young and elderly populations with respect to protein consumption during the 2-h post-exercise period. Our results also question the application of the many acute studies to actual longitudinal results. Acute studies have shown that muscle protein net balance does not become positive when only carbohydrate is ingested post-exercise; they have stated that the effect of carbohydrate ingestion post-exercise on muscle net balance is "minor" compared to that of amino acids (12). These large differences between carbohydrate and amino acids observed in acute studies do not translate into significant differences in hypertrophy. Past research has illustrated the importance of protein consumption post-exercise (8, 11, 84, 85, 87) as well as differences between milk and soy proteins (14, 28) which did not convert into significant differences in hypertrophy over a 12 week resistance training program between the various groups within the present study. This calls into question the

generalizability of some acute studies into long term effects as it appears that the large differences observed in acute protocols are largely diluted longitudinally. Future training studies basing their sample size calculations on results from acute studies should use trepidation when doing so, and increase sample sizes in order to account for these shortcomings. Differences between soy and milk protein sources, if present, are minute and these results illustrate the fact that as long as adequate protein is ingested within the diet, that additional supplements are not needed.

Future studies examining differences between soy and milk should attempt to use soy and milk proteins only, without carbohydrate present so that the true differences between the proteins can be examined without interference from carbohydrate. Stricter diet guidelines should also be implemented during the post-exercise period to allow the post-exercise drinks to have their full effect without interference from additional food sources. In addition the results from the present study illustrate the inherent differences between acute and longitudinal studies. Researchers must attempt to replicate some of the results observed in acute studies with longer term experiments in order to confirm the results they have obtained acutely.

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APPENDIX 1

DIET RECORD DATA, and ANOVA TABLES

Diet Record Raw Data: Pre Training

	Subject	Age	Wt (kg)	avg kJ	avg kcal	kcal/kg	PRO	PRO/kg	CHO	CHO/kg	FAT	FAT/kg
Group 1	S1	18	51.8	10316.2	2468	47.64	88	1.70	349	6.74	85	1.64
	S2	19	100.0	16795.2	4018	40.18	96	0.96	687	6.87	106	1.06
	S3	22	84.6	18237.3	4363	51.57	114	1.35	550	6.50	151	1.78
	S4	22	77.7	11695.6	2798	36.01	83	1.07	389	5.01	103	1.33
	S5	20	82.1	8836.5	2114	25.75	90	1.10	312	3.80	57	0.69
	S6	32	81.0	13024.9	3116	38.47	102	1.26	451	5.57	106	1.31
	S7	31	75.6	13133.6	3142	41.56	129	1.71	459	6.07	102	1.35
	S9	21	75.5	7160.3	1713	22.69	84	1.11	144	1.91	88	1.17
	S10	21	99.5	10688.3	2557	25.70	106	1.07	431	4.33	45	0.45
	S11	22	75.1	18040.9	4316	57.47	135	1.80	544	7.24	147	1.96
	S12	22	67.2	8472.9	2027	30.16	83	1.24	255	3.79	78	1.16
Group 2	S13	32	68.0	9831.4	2352	34.59	76	1.12	319	4.69	92	1.35
	S14	20	72.8	14567.3	3485	47.87	136	1.87	552	7.58	90	1.24
	S15	20	71.5	8999.5	2153	30.11	85	1.19	254	3.55	94	1.31
	S16	22	89.5	14813.9	3544	39.60	190	2.12	407	4.55	134	1.50
	S17	23	83.5	13292.4	3180	38.08	108	1.29	393	4.71	95	1.14
	S18	22	96.6	13325.8	3188	33.00	126	1.30	381	3.94	123	1.27
	S19	22	82.5	5889.6	1409	17.08	69	0.84	155	1.88	58	0.70
	S20	19	64.0	15185.9	3633	56.77	135	2.11	547	8.55	110	1.72
	S21	21	70.0	12209.8	2921	41.73	117	1.67	320	4.57	132	1.89
	S22	23	92.8	12995.6	3109	33.50	104	1.12	371	4.00	112	1.21
	S23	21	82.0	10692.4	2558	31.20	93	1.13	313	3.82	79	0.96
	S24	32	90.5	13643.5	3264	36.07	139	1.54	505	5.58	83	0.92
Group 3	S25	19	70.0	12636.1	3023	43.19	117	1.67	414	5.91	107	1.53
	S26	19	99.0	12226.5	2925	29.55	105	1.06	315	3.18	142	1.43
	S27	20	82.6	15453.5	3697	44.76	86	1.04	507	6.14	139	1.68
	S28	22	102.6	10600.5	2536	24.72	83	0.81	190	1.85	72	0.70
	S29	21	72.1	10805.3	2585	35.85	73	1.01	422	5.85	74	1.03
	S30	23	99.7	11135.5	2664	26.72	131	1.31	344	3.45	87	0.87
	S32	23	83.6	13020.7	3115	37.26	101	1.21	407	4.87	105	1.26
	S33											
	S34	24	100.6	6855.2	1640	16.30	58	0.58	176	1.75	80	0.80
	S35	22	72.6	8803.1	2106	29.01	108	1.49	266	3.66	72	0.99
	S36	21	97.5	20130.9	4816	49.39	155	1.59	600	6.15	197	2.02

Diet Record Raw Data: 6 wk

	Subject	Age	Wt (kg)	avg kJ	avg kcal	kcal/kg	PRO	PRO/kg	CHO	CHO/kg	FAT	FAT/kg
Group 1	S1	18	51.0	13070.9	3127	61.31	107	2.10	454	8.90	100	1.96
	S2	19	101.5	17217.4	4119	40.58	128	1.26	718	7.07	98	0.97
	S3	22	84.7	17188.2	4112	48.55	155	1.83	489	5.77	129	1.52
	S4	22	76.4	9066.4	2169	28.39	70	0.92	392	5.13	42	0.55
	S5	20	82.0	11469.9	2744	33.46	141	1.72	320	3.90	94	1.15
	S6	32	81.5	15064.7	3604	44.22	143	1.75	451	5.53	150	1.84
	S7	31	74.3	11357.1	2717	36.57	130	1.75	501	6.74	131	1.76
	S9	21	79.8	8598.3	2057	25.78	83	1.04	296	3.71	57	0.71
	S10	21	100.0	11549.3	2763	27.63	102	1.02	365	3.65	102	1.02
	S11	22	77.0	25610.9	6127	79.57	249	3.23	560	7.27	216	2.81
	S12	22	69.0	18605.2	4451	64.51	167	2.42	519	7.52	149	2.16
Group 2	S13	32	71.8	10032.0	2400	33.43	86	1.20	296	4.12	100	1.39
	S14	20	73.1	12243.2	2929	40.07	131	1.79	388	5.31	87	1.19
	S15	20	72.5	8364.2	2001	27.60	93	1.28	230	3.17	81	1.12
	S16	22	91.0	12163.8	2910	31.98	113	1.24	420	4.62	69	0.76
	S17	23	87.5	14349.9	3433	39.23	97	1.11	398	4.55	83	0.95
	S18	22	97.3	15428.4	3691	37.93	172	1.77	435	4.47	130	1.34
	S19	22	86.3	12802.1	3063	35.49	128	1.48	363	4.20	96	1.11
	S20	19	68.3	12280.8	2938	43.02	135	1.98	41	0.60	60	0.88
	S21	21	74.1	8723.7	2087	28.16	117	1.58	344	4.64	103	1.39
	S22	23	92.5	8360.0	2000	21.62	84	0.91	272	2.94	68	0.74
	S23	21	81.5	15269.5	3653	44.82	153	1.88	382	4.69	146	1.79
	S24	32	92.0	17861.1	4273	46.45	178	1.93	557	6.05	156	1.70
Group 3	S25	19	71.5	14450.3	3457	48.35	234	3.27	421	5.89	98	1.37
	S26	19	100.0	12824.2	3068	30.68	139	1.39	336	3.36	117	1.17
	S27	20	81.0	13610.1	3256	40.20	146	1.80	440	5.43	98	1.21
	S28	22	101.7	15311.3	3663	36.02	130	1.28	308	3.03	115	1.13
	S29	21	73.9	9317.2	2229	30.16	91	1.23	299	4.05	78	1.06
	S30	23	104.6	11867.0	2839	27.14	165	1.58	312	2.98	108	1.03
	S32	23	87.9	15386.6	3681	41.88	158	1.80	410	4.66	147	1.67
	S33	25	78.9	10353.9	2477	31.39	107	1.36	298	3.78	98	1.24
Omitted	S34	24	102.4	4226.0	1011	9.87	46	0.45	130	1.27	38	0.37
	S35	22	72.6	13405.3	3207	44.17	186	2.56	269	3.71	150	2.07
	S36	21	104.1	16929.0	4050	38.90	114	1.10	508	4.88	139	1.34

Diet Record Raw Data: 12 wk

	Subject	Age	Wt (kg)	avg kJ	avg kcal	kcal/kg	PRO	PRO/kg	CHO	CHO/kg	FAT	FAT/kg
Group 1	S1	18	50.8	12966.4	3102	61.06	134	2.64	390	7.68	116	2.28
	S2	19	101.0	23508.3	5624	55.68	191	1.89	904	8.95	172	1.70
	S3	22	85.0	11908.8	2849	33.52	113	1.33	289	3.40	121	1.42
	S4	22	80.0	13572.5	3247	40.59	101	1.26	518	6.48	93	1.16
	S5	20	80.0	11068.6	2648	33.10	95	1.19	383	4.79	84	1.05
	S6	32	82.0	9116.6	2181	26.60	77	0.94	294	3.59	83	1.01
	S7	31	77.3	10073.8	2410	31.18	87	1.13	342	4.42	78	1.01
	S9	21	83.4	12673.8	3032	36.35	129	1.55	453	5.43	81	0.97
	S10	21	99.9	13735.5	3286	32.89	144	1.44	360	3.60	144	1.44
	S11	22	78.0	26547.2	6351	81.42	288	3.69	531	6.81	279	3.58
	S12	22	68.2	15695.9	3755	55.06	175	2.57	468	6.86	137	2.01
Group 2	S13	32	72.3	9471.9	2266	31.34	95	1.31	306	4.23	75	1.04
	S14	20	71.8	14345.8	3432	47.80	132	1.84	448	6.24	128	1.78
	S15	20	74.2	14496.2	3468	46.74	163	2.20	412	5.55	131	1.77
	S16	22	94.0	12715.6	3042	32.36	130	1.38	411	4.37	100	1.06
	S17	23	87.5	9806.3	2346	26.81	102	1.17	265	3.03	49	0.56
	S18	22	96.1	11921.4	2852	29.68	113	1.18	342	3.56	110	1.14
	S19	22	86.0	10993.4	2630	30.58	116	1.35	305	3.55	78	0.91
	S20	19	69.2	13075.0	3128	45.20	146	2.11	448	6.47	87	1.26
	S21	21	75.8	12159.6	2909	38.38	127	1.68	359	4.74	109	1.44
	S22	23	94.0	11942.3	2857	30.39	120	1.28	405	4.31	92	0.98
	S23	21	83.5	16080.5	3847	46.07	201	2.41	395	4.73	139	1.66
	S24	32	89.5	19115.1	4573	51.09	169	1.89	650	7.26	144	1.61
Group 3	S25	19	71.8	14981.1	3584	49.92	216	3.01	394	5.49	122	1.70
	S26	19	103.3	13062.5	3125	30.25	154	1.49	278	2.69	130	1.26
	S27	20	82.0	9405.0	2250	27.44	79	0.96	305	3.72	69	0.84
	S28	22	102.1	11110.4	2658	26.03	141	1.38	281	2.75	89	0.87
	S29	21	73.3	9660.0	2311	31.53	69	0.94	366	4.99	73	1.00
	S30	23	104.4	9325.6	2231	21.37	114	1.09	253	2.42	87	0.83
	S32	23	91.4	13242.2	3168	34.66	167	1.83	389	4.26	106	1.16
	S33	25	80.8	10633.9	2544	31.50	106	1.31	336	4.16	84	1.04
	S34	24	103.6	8431.1	2017	19.47	106	1.02	269	2.60	61	0.59
	S35	22	73.0	9329.8	2232	30.58	100	1.37	290	3.97	82	1.12
	S36	21	108.5	26530.5	6347	58.50	160	1.47	803	7.40	196	1.81

Diet Record Raw Data Summary

Pre Training

		Age	Wt (kg)	avg kJ	avg kcal	kcal/kg	PRO	PRO/kg	CHO	CHO/kg	FAT	FAT/kg
MEAN		22	82.25	12227.77	2925.30	36.17	106.21	1.32	386	4.79	101.36	1.26
SD		4	12.73	3328.23	796.23	10.26	27.65	0.37	132	1.72	31.53	0.39
SE		1	2.18	570.79	136.55	1.76	4.74	0.06	23	0.29	5.41	0.07

6 Wk

		Age	Wt (kg)	avg kJ	avg kcal	kcal/kg	PRO	PRO/kg	CHO	CHO/kg	FAT	FAT/kg
MEAN		22.53	83.64	13069.35	3126.64	38.21	131.69	1.62	380.04	4.64	106.85	1.31
SD		3.74	12.80	3924.67	938.92	12.82	42.90	0.61	126.79	1.70	37.15	0.50
SE		0.64	2.20	673.08	161.02	2.20	7.36	0.10	21.75	0.29	6.37	0.09

12 Wk

		Age	Wt (kg)	avg kJ	avg kcal	kcal/kg	PRO	PRO/kg	CHO	CHO/kg	FAT	FAT/kg
MEAN		22.53	84.52	13314.78	3185.35	38.39	134.12	1.63	401.24	4.84	109.68	1.33
SD		3.74	13.05	4526.79	1082.96	13.22	45.01	0.63	144.25	1.65	43.87	0.57
SE		0.64	2.24	776.34	185.73	2.27	7.72	0.11	24.74	0.28	7.52	0.10

Average KJ ANOVA for all time points

	SS	Degr. of	MS	F	p-level
Group	3.966195E+07	2	1.983098E+07	0.5654	0.574069
Error	1.052225E+09	30	3.507417E+07		
TIME	2.458279E+07	2	1.229139E+07	1.7692	0.179254
TIME*Group	1.525160E+07	4	3.812901E+06	0.5488	0.700576
Error	4.168571E+08	60	6.947619E+06		

Average PRO ANOVA for all time points

	SS	Degr. of	MS	F	p
Group	4	2	2	0.0006	0.999366
Error	90334	30	3011		
TIME	17321	2	8661	9.1424	0.000342
TIME*Group	3442	4	860	0.9083	0.464981
Error	56838	60	947		

Average PRO/kg ANOVA for all time points

	SS	Degr. of	MS	F	p
Group	0.5117	2	0.2559	0.3898	0.680563
Error	19.6913	30	0.6564		
TIME	2.2669	2	1.1334	7.8953	0.000904
TIME*Group	0.7159	4	0.1790	1.2467	0.301003
Error	8.6135	60	0.1436		

Average CHO ANOVA for all time points

	SS	Degr. Of	MS	F	p
Group	132337	2	66168	1.8155	0.180187
Error	1093390	30	36446		
TIME	7448	2	3724	0.4347	0.649465
TIME*Group	22824	4	5706	0.6661	0.618029
Error	513999	60	8567		

Average CHO/kg ANOVA for all time points

	SS	Degr. Of	MS	F	p
Group	38.790	2	19.395	3.9110	0.030950
Error	148.775	30	4.959		
TIME	0.542	2	0.271	0.1936	0.824495
TIME*Group	6.408	4	1.602	1.1448	0.344288
Error	83.967	60	1.399		

APPENDIX 2

SUBJECT CHARACTERISTICS

Subject Characteristics Pre

	Subject	Age	Weight (kg)	Height (m)	BMI (kg/m2)
Group 1	S1	18	51.8	1.80	15.99
	S2	19	100.0	1.87	28.60
	S3	22	84.6	1.86	24.59
	S4	22	77.7	1.73	25.96
	S5	20	82.1	1.81	25.14
	S6	32	81.0	1.83	24.21
	S7	31	75.6	1.68	26.91
	S9	21	75.5	1.85	22.06
	S10	21	99.5	1.74	33.05
	S11	22	75.1	1.93	20.16
	S12	22	67.2	1.73	22.45
Group 2	S13	32	68.0	1.80	20.99
	S14	20	72.8	1.83	21.86
	S15	20	71.5	1.84	21.12
	S16	22	89.5	1.87	25.59
	S17	23	83.5	1.86	24.14
	S18	22	96.6	1.72	32.65
	S19	22	82.5	1.81	25.18
	S20	19	64.0	1.73	21.38
	S21	21	70.0	1.74	23.25
	S22	23	92.8	1.78	29.29
	S23	21	82.0	1.82	24.76
	S24	32	90.5	1.85	26.44
Group 3	S25	19	70.0	1.87	20.02
	S26	19	99.0	1.82	29.89
	S27	20	82.6	1.73	27.60
	S28	22	102.6	1.83	30.64
	S29	21	72.1	1.78	22.76
	S30	23	99.7	1.84	29.61
	S32	23	83.6	1.80	25.80
	S33	25	78.0	1.74	25.76
	S34	24	100.6	1.77	32.11
	S35	22	72.6	1.55	30.22
	S36	21	97.5	1.78	30.95
N=	34				
Mean		22.5	82.1	1.79	25.62
SD		4	12.6	0.07	4.10
SE		1	2.2	0.01	0.70

Subject Characteristics Post

	Subject	Age	Weight (kg)	Height (m)	BMI (kg/m2)
Group 1	S1	18	50.7	1.80	15.74
	S2	19	100.5	1.87	28.74
	S3	22	86.0	1.86	24.70
	S4	22	79.8	1.73	26.40
	S5	20	80.5	1.81	24.81
	S6	32	81.5	1.83	24.33
	S7	31	76.2	1.68	27.77
	S9	21	83.3	1.85	23.78
	S10	21	99.8	1.74	33.32
	S11	22	79.1	1.93	20.81
	S12	22	68.3	1.73	22.75
Group 2	S13	32	71.9	1.80	22.31
	S14	20	71.8	1.83	21.80
	S15	20	74.4	1.84	21.30
	S16	22	92.5	1.87	26.45
	S17	23	88.5	1.86	25.87
	S18	22	96.3	1.72	32.96
	S19	22	85.8	1.81	26.34
	S20	19	68.9	1.73	22.89
	S21	21	76.7	1.74	24.92
	S22	23	94.9	1.78	29.29
	S23	21	83.9	1.82	25.27
	S24	32	89.8	1.85	26.56
Group 3	S25	19	71.4	1.87	20.59
	S26	19	105.2	1.82	31.46
	S27	20	81.3	1.73	27.40
	S28	22	102.3	1.83	30.94
	S29	21	73.5	1.78	23.04
	S30	23	104.1	1.84	30.83
	S32	23	91.6	1.80	27.65
	S33	25	80.9	1.74	26.42
	S34	24	104.1	1.77	32.56
	S35	22	72.7	1.55	29.97
	S36	21	109.8	1.78	34.25
N=	34				
Mean		22.5	84.6	1.79	26.30
SD		4	13.2	0.07	4.22
SE		1	2.3	0.01	0.72

APPENDIX 3

BODY COMPOSITION RAW DATA DETERMINED BY DXA, AND ANOVA TABLES

DXA scan results Pre training

	Subject	Fat (grams)	Lean + BMC (grams)	BMC (grams)	Lean (grams)	% Fat	Total (grams)
Group 1	S1	5271.90	44025.00	1877.04	42147.96	10.69	49296.90
	S2	27615.70	69851.80	3323.14	66528.66	28.33	97467.50
	S3	9810.40	71754.50	2974.04	68780.46	12.03	81564.90
	S4	15164.40	61447.10	2518.37	58928.73	19.79	76611.50
	S5	18873.50	62060.30	2831.28	59229.02	23.32	80933.80
	S6	10173.70	68840.40	3087.86	65752.54	12.88	79014.10
	S7	14814.00	61245.90	2577.13	58668.77	19.48	76059.90
	S9	7958.50	65435.00	2637.30	62797.70	10.84	73393.50
	S10	23644.50	75431.00	3024.14	72406.86	23.87	99075.50
	S11	7242.80	66108.40	2736.76	63371.64	9.87	73351.20
	S12	8354.60	57038.20	2643.68	54394.52	12.78	65392.80
Group 2	S13	8517.80	57902.70	2610.47	55292.23	12.82	66420.50
	S14	9429.70	60082.10	2355.25	57726.85	13.57	69511.80
	S15	6808.20	63845.40	2611.05	61234.35	9.64	70653.60
	S16	11526.20	75195.90	3231.89	71964.01	13.29	86722.10
	S17	14216.00	69115.30	2928.53	66186.77	17.06	83331.30
	S18	17867.20	80149.10	3376.72	76772.38	18.23	98016.30
	S19	15871.20	64873.30	2956.21	61917.09	19.66	80744.50
	S20	8936.30	54475.70	2643.32	51832.38	14.09	63412.00
	S21	8388.80	59716.20	2716.94	56999.26	12.32	68105.00
	S22	20700.50	69279.90	2750.80	66529.10	23.01	89980.40
	S23	15686.50	64994.80	3208.03	61786.77	19.44	80681.30
	S24	14853.40	74242.30	3259.27	70983.03	16.67	89095.70
Group 3	S25	8849.30	58551.70	2644.28	55907.42	13.13	67401.00
	S26	19798.40	78765.30	3304.20	75461.10	20.09	98563.70
	S27	13485.30	69264.30	3072.67	66191.63	16.30	82749.60
	S28	22271.30	82800.60	3474.85	79325.75	21.20	105071.90
	S29	13959.20	57281.90	2481.35	54800.55	19.59	71241.10
	S30	24310.40	75579.20	3551.34	72027.86	24.34	99889.60
	S32	12881.00	70293.60	3099.37	67194.23	15.49	83174.60
	S33	11082.20	66351.80	2965.36	63386.44	14.31	77434.00
	S34	28629.30	72115.80	3269.49	68846.31	28.42	100745.10
	S35	18902.10	52721.10	2251.42	50469.68	26.39	71623.20
	S36	18431.90	78950.30	3565.58	75384.72	18.93	97382.20
N=	34						
Mean		14539.01	66464.29	2898.80	63565.49	17.41	81003.30
SD		6159.92	8690.14	392.41	8335.89	5.29	13146.91
SE		1056.42	1490.35	67.30	1429.59	0.91	2254.68

DXA scan results Post training

	Subject	Fat (grams)	Lean + BMC (grams)	BMC (grams)	Lean (grams)	% Fat	Total (grams)
Group 1	S1	4567.5	44740.6	1837.3	42903.4	9.3	49308.1
	S2	24168.4	74847.3	3338.9	71508.5	24.4	99015.8
	S3	11967.8	73870.0	3103.4	70766.7	13.9	85837.9
	S4	16327.5	62738.8	2605.9	60132.9	20.7	79066.3
	S5	16497.6	62625.7	2804.6	59821.0	20.9	79123.3
	S6	10866.5	70442.9	3145.0	67298.0	13.4	81309.4
	S7	13989.5	62003.1	2484.7	59518.3	18.4	75992.6
	S9	10223.6	71582.5	2752.6	68829.9	12.5	81806.2
	S10	22633.0	76138.9	3048.3	73090.6	22.9	98771.9
	S11	7007.4	70313.2	2692.0	67621.1	9.1	77320.5
	S12	7996.5	58542.6	2653.1	55889.5	12.0	66539.1
Group 2	S13	8275.6	61039.8	2635.0	58404.9	11.9	69315.5
*	S14	8648.8	59287.4	2206.8	57080.6	12.7	67936.2
	S15	7085.1	66448.7	2686.7	63762	9.6	73533.8
	S16	11748.4	78762	3308.4	75453.6	13	90510.4
	S17	14105.8	72369.3	3001.3	69368	16.3	86475.1
*	S18	14597.6	79171.4	3310.1	75861.3	15.6	93769.0
	S19	16363.1	68382.1	2977.6	65404.5	19.3	84745.2
	S20	8213.3	59492.8	2760.9	56731.9	12.1	67706.1
	S21	10397.7	66871.8	2839.8	64032.0	13.5	77269.4
	S22	20557.7	69891.8	2829.6	67062.2	22.7	90449.4
	S23	13947.3	69259.7	3197.9	66061.8	16.8	83207.0
	S24	13429.8	74694.0	3327.6	71366.4	15.2	88123.8
Group 3	S25	8629.5	61218.3	2707.9	58510.4	12.4	69847.8
	S26	22361.6	81654.6	3212.1	78442.5	21.5	104016.1
	S27	10201.3	70187.6	2957.9	67229.6	12.7	80388.8
	S28	19109.1	82907.6	3449.2	79458.4	18.7	102016.7
	S29	12233.4	58061.6	2459.5	55602.1	17.4	70294.9
	S30	24512.0	78504.0	3597.4	74906.6	23.8	103016.1
	S32	15951.9	75045.5	3219.7	71825.8	17.5	90997.4
	S33	11301.6	68826.9	2969.8	65857.1	14.1	80128.4
	S34	28803.2	75471.6	3266.6	72205.0	27.6	104274.8
	S35	18187.2	53728.3	2283.8	51444.5	25.3	71915.4
	S36	23502.7	86747.7	3801.6	82946.1	21.3	110250.4
N=	34						
Mean		14364.97	68996.18	2925.68	66070.51	16.72	83361.14
SD		6000.46	9047.14	412.59	8666.44	4.98	13491.19
SE		1029.07	1551.57	70.76	1486.28	0.85	2313.72

*DXA chopper cut off error during scans, inaccurate results and were not included in analysis

FBFM ANOVA Pre vs Post.

	SS	Degr. of	MS	F	p
Group	10369	2	5185	1.239	0.303528
Error	129683	31	4183		
TIME	1707	1	1707	13.490	0.000899
TIME*Group	296	2	148	1.170	0.323669
Error	3923	31	127		

Body Fat % ANOVA Pre vs Post

	SS	Degr. of	MS	F	p
Group	216.98	2	108.49	2.2603	0.121249
Error	1487.95	31	48.00		
TIME	7.83	1	7.83	6.1397	0.018871
TIME*Group	0.53	2	0.26	0.2073	0.813869
Error	39.56	31	1.28		

Total mass ANOVA Pre vs. Post

	SS	Degr. of	MS	F	p
Group	1016.0	2	508.0	1.604	0.217258
Error	9815.8	31	316.6		
TIME	105.6	1	105.6	22.156	0.000050
TIME*Group	12.0	2	6.0	1.256	0.298853
Error	147.7	31	4.8		

Fat mass ANOVA Pre vs Post

	SS	Degr. of	MS	F	p
Group	3.335941E+08	2	1.667971E+08	2.5251	0.096394
Error	2.047749E+09	31	6.605642E+07		
TIME	4.653739E+05	1	4.653739E+05	0.2498	0.620745
TIME*Group	1.259139E+06	2	6.295695E+05	0.3379	0.715835
Error	5.775242E+07	31	1.862981E+06		

Lean mass gains one way ANOVA

	SS	Degr. of	MS	F	p
Group	3.7252	2	1.8626	0.45793	0.637085
Error	117.9536	29	4.0674		

APPENDIX 4

FIBRE AREA RAW DATA, AND ANOVA TABLES

Fiber Area Pre

Subject		I	IIA	IIAX	IIX	II (Pooled)
1		4842	5156	4655	3756	4356
2		5020	7242	8089	7182	7148
3		5651	7253		6841	6991
4		5447	7660		6458	6992
5		4421	5614	6077	5461	5555
6		5554	6119		5388	6031
7		5152	6034	5657	5410	5810
9		5442	6799		5938	6413
10		7289	7909	9037	8055	7944
11			6016		5395	5679
12			3214		5348	4288
13		3937	5330	5399	5058	5204
14		5080	5608	5805	4408	4875
15		3939	5912		5027	5558
16		3500	3690	3483	3054	3377
17		4467	4898	4395	4259	4683
18		3467	4560		4435	4510
19		3959	5307		4173	5199
20						
21		4626	6623		5749	6192
22						
23		4375	4938	4867	5091	5002
24		4424	5895	5505	5770	5859
25		3155	5269	5093	4382	5029
26		5610	5625	6113	5561	5564
27						
28		4869	5049		4751	5003
29						
30		6103	5921	5090	5043	5541
32		4355	5811	6298	5838	5791
33		3923	5601		4106	5147
34		3669	5284	4558	4667	4890
35						
36		5275	6152		5562	5961
36		18854.19	21989.54		19879.5	21306.88
	Mean	5243.177	6321.5	5697.744	5803.054	6122.217
	SD	2872.91	3186.515	1388.97	2888.029	3066.738
	SE	492.6999	546.4829	238.2065	495.2929	525.9413

Fibre Area Post

Subject		I	IIA	IIAX	IIX	II (Pooled)
1		4692.528	5544.243	4677.324	3953.704	4673.883
2		6155.062	7922.584		8130.462	7964.16
3		5961.641	7394.555	7276.707	6748.678	7072.025
4		5631.392	7668.377	6095.328	6727.272	7481.758
5		4545.293	5771.071	6591.592	5548.001	5699.556
6		5498.226	7139.903		6877.738	7133.094
7		5185.876	6881.439		6414.86	6695.509
9		5413.067	7470.498		7759.527	7521.205
10		7415.259	7967.109			7967.109
11			6635.816	11274.37	6424.442	6475.913
12			5430.574		3872.905	4734.594
13		4371.394	7044.663		7262.091	7127.38
14		5243.909	5937.485		3904.334	5317.622
15		4319.452	6547.967	4636.919	5040.713	6145.21
16		5334.938	5890.455		6822.006	6120.467
17		4659.849	5897.469	4089.479	4466.815	5534.469
18		3064.486	5167.612		5792.924	5204.395
19		3981.307	5689.533	7136.327	5382.724	5582.689
20						
21		4866.452	7366.594	5919.905	5765.326	7098.885
22						
23		4591.494	6192.386	6502.26	6543.697	6290.422
24		4608.198	6059.38	5993.124	6081.505	6073.58
25		4196.936	6642.647	7403.029	6197.356	6496.386
26		5545.476	5918.269	5401.413	5156.512	5727.38
27						
28		4886.957	5261.208	4928.098	4756.549	5133.929
29						
30		6828.884	7782.029	6866.197	6032.45	6832.844
32		4262.782	6713.53	5595.964	4975.737	5827.997
33		3958.648	6152.753	6870.164	5504.522	5959.991
34		3959.505	5305.646		5496.264	5335.601
35						
36		5475.966	7220.35		6062.594	6813.227
	Mean	4998.556	6538.282	6411.305	5916.593	6334.55
	SD	951.9691	859.8259	1618.762	1055.213	880.4439
	SE	163.2614	147.4589	277.6153	180.9676	150.9949

Type 1 Fibre Area ANOVA Pre vs Post

	SS	Degr. of	MS	F	p
Group	1.377494E+07	2	6.887470E+06	6.031	0.006836
Error	3.083561E+07	27	1.142059E+06		
TIME	3.320723E+05	1	3.320723E+05	2.074	0.161317
TIME*Group	2.450979E+05	2	1.225489E+05	0.765	0.474967
Error	4.322875E+06	27	1.601065E+05		

Type II Fibre Area ANOVA Pre vs. Post

	SS	Degr. of	MS	F	p
"Var1"	9.388842E+06	2	4.694421E+06	3.424	0.047283
Error	3.701767E+07	27	1.371025E+06		
TIME	5.640096E+06	1	5.640096E+06	16.043	0.000436
TIME*"Var1"	1.280662E+06	2	6.403309E+05	1.821	0.181129
Error	9.492250E+06	27	3.515648E+05		

Type II fibre area ANCOVA Pre vs. Post, Pre fibre size as covariate

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	96671	25	167824.9	0.57602	0.569416
2	1	7788961	26	177610.0	43.85430	0.000001
12	2	274659	26	177610.0	1.54642	0.231971

Type II fibre area gains one way ANOVA

	SS	Degr. of	MS	F	p
Intercept	15577922	1	15577922	43.85430	0.000001
"Var1"	1098638	2	549319	1.54642	0.231971
Error	9235718	26	355220		

APPENDIX 5

% FIBRE DISTRIBUTION RAW DATA AND ANOVA TABLES

% Fibre distribution Pre

Subject		I	IIA	IIX
1		38.80	33.35	27.85
2		46.76	29.46	23.77
3		49.99	18.87	31.15
4		35.84	35.77	28.39
5		28.42	50.92	20.66
6		37.26	55.48	7.25
7		64.78	15.60	19.62
9		40.62	33.59	25.79
10		23.13	39.90	36.97
11		56.62	23.07	20.30
12		29.81	46.33	23.86
13		18.22	46.43	35.35
14		21.08	47.42	31.50
15		30.17	39.06	30.77
16		40.67	36.48	22.85
17		24.02	53.39	22.60
18		45.87	33.40	20.74
19		37.01	58.18	4.82
20				
21		47.99	30.26	21.75
22				
23		19.66	48.69	31.65
24		31.71	46.47	21.82
25		34.48	52.71	12.81
26		31.26	31.22	37.52
27				
28		27.23	53.15	19.62
29		39.07	39.10	21.83
30				
32		25.35	55.68	18.98
33		37.38	37.95	24.68
34		40.15	25.50	34.35
35				
36		45.44	38.09	16.47
	Mean	36.06975	40.07756	23.85268
	SD	11.30033	11.62443	8.092878
	SE	1.937991	1.993574	1.387917

% Fibre distribution Post

Subject		I	IIA	IIX
1		44.15	30.58	25.28
2		46.66	39.56	13.78
3		41.81	33.65	24.54
4		44.36	45.17	10.48
5		34.18	50.11	15.71
6		27.67	70.52	1.81
7		35.85	47.17	16.98
9		54.01	38.57	7.42
10		48.21	51.79	
11		44.65	32.74	22.61
12		37.04	39.95	23.01
13		39.02	37.34	23.64
14		38.48	45.13	16.40
15		23.50	56.44	20.06
16		52.81	34.20	12.99
17		26.65	58.00	15.35
18		28.73	66.64	4.64
19		48.35	40.58	11.08
20				
21		43.00	48.50	8.50
22				
23		18.13	58.88	22.99
24		15.81	58.12	26.07
25		38.14	50.90	10.95
26		33.30	48.46	18.24
27				
28		34.97	47.10	17.92
29		43.64	47.97	8.40
30				
32		36.74	39.54	23.72
33		24.95	59.18	15.87
34		37.22	44.88	17.90
35				
36		49.05	35.01	15.94
	Mean	37.39	47.36	15.81
	SD	9.95	9.96	6.43
	SE	1.71	1.71	1.10

Type 1 % Distribution ANOVA Pre vs Post

	SS	Degr. of	MS	F	p
"Var1"	0.083234	2	0.041617	3.2954	0.053019
Error	0.328356	26	0.012629		
TIME	0.003369	1	0.003369	0.4273	0.519038
TIME*"Var1"	0.000694	2	0.000347	0.0440	0.956978
Error	0.204961	26	0.007883		

Type IIA % Distribution ANOVA Pre vs. Post

	SS	Degr. of	MS	F	p
"Var1"	0.117921	2	0.058961	2.7822	0.080369
Error	0.550997	26	0.021192		
TIME	0.045153	1	0.045153	5.4902	0.027048
TIME*"Var1"	0.001925	2	0.000962	0.1170	0.890025
Error	0.213830	26	0.008224		

Type IIX % Distribution ANOVA Pre vs. Post

	SS	Degr. of	MS	F	p
"Var1"	0.013480	2	0.006740	0.4249	0.658498
Error	0.396601	25	0.015864		
R1	0.053147	1	0.053147	9.9661	0.004129
R1*"Var1"	0.001295	2	0.000647	0.1214	0.886213
Error	0.133321	25	0.005333		

APPENDIX 6

BLOOD GLUCOSE CONCENTRATIONS RAW DATA AND ANOVA TABLES

Blood Glucose Concentration Following Exercise bout and Supplement Consumption

Subject #	Pre	Post	30	60	90	120
1	4.49	3.96	5.58	4.44	4.28	4.42
2	4.41	4.42		3.09	5.19	3.27
3	3.81	4	5.93	3.06	4.22	3.74
4	4.19	4.65	7.02	4.61	4.58	3.84
5	4.34	3.88	7.71	5.12	5.8	
6	4.12	3.38	4.74	4.28	2.81	3.71
7	3.77	4.13	6.75	5.13	3.5	3.96
9	3.71	3.61	6.39	3.36	4.17	3.55
10	4.56	4.7	9.84	2.95	7.48	6.64
11	4.24	3.98	5.96	3.11	4.71	3.56
12	4.46	4	4.99	3.89	4.2	4.25
13	3.75	3.25	3.82	2.98	3.03	2.98
14	5.13	3.97	4.48	3.73	3.56	4.02
15	4.18	3.68	3.81	2.84	3.77	2.57
16	6.93	4.84	4.6	3.7	5.51	3.69
17	6.87	3.93	4.42	2.97	3.88	3.07
18	4.37	4.1	5.99	3.67	5.11	3.88
19	3.97	3.96	4.28	2.29	3.94	3.14
20	3.34	4.04	4.1	4.06	3	3.81
21	4.88	4.2	4.4	3.42	3.61	4.08
22	4.44	4.01	4.66	3.49	3.98	3.48
23	4.04	4.06	3.42	3.55	4.1	3.41
24	4.32	4.1	4.55	3.56	4.91	3.96
25	2.91	3.76	3.46	3.24	4.03	3.18
26	4.07	4.07	3.71	3.47	4.82	3.54
27	4.34	4.34	4.34	3.84	4.75	4.33
28	5.75	4.93	3.94	4.11	3.74	4.06
29	4.51	4.64	6	3.9	4.57	4.09
30	3.68	3.76	3.99	3.33	4.1	3.45
32	4.76	4.39	4.96	3.15	4.09	3.73
33	3.84	4.09	4.63	4.32	4.66	4.17
34	5.47	4.02	5.34	3.89	4.78	4.1
35	4.19	3.9	4.23	3.1	3.06	3.28
36	4.71	4.56	4.26	3.19	4.1	2.99
Mean	4.426061	4.101515	5.0225	3.587879	4.295758	3.735313
SD	0.84736	0.385463	1.388322	0.630034	0.915082	0.67963
SE	0.145321	0.066106	0.238095	0.10805	0.156935	0.116556

Glucose ANOVA for all time points

	SS	Degr. of	MS	F	p
group	8.661	2	4.330	2.973	0.066893
Error	42.236	29	1.456		
Time	41.031	5	8.206	21.253	0.000000
Time*group	22.319	10	2.232	5.780	0.000000
Error	55.988	145	0.386		

Tukey Post Hoc

Calculated by hand

Critical Value = SRS * (SQRT (MSERROR / # of OBSERVATIONS Per Mean))

SRS for 5 means and 120 df = 3.98

CV = 3.98 * (SQRT (0.56451 / 11))
CV = 0.9

Blood Glucose Concentrations

	CON	MLK	PEC
Pre	4.19±0.09	4.69±0.33	4.38±0.24
0 (mmol/L)	4.06±0.12	4.01±0.10	4.22±0.11
30 (mmol/L)	6.49±0.47¶*	4.38±0.18	4.44±0.23
60 (mmol/L)	3.91±0.25	3.36±0.14	3.59±0.13
90 (mmol/L)	4.63±0.37	4.03±0.23	4.25±0.16
120 (mmol/L)	4.09±0.30	3.51±0.14	3.72±0.14

Results are Mean ±SEM
*Significantly different from 0 ($P<0.05$),
¶Significantly higher than MLK and PEC at same time point ($P<0.05$)

APPENDIX 7

BLOOD INSULIN CONCENTRATIONS RAW DATA AND ANOVA TABLES

Blood Insulin Concentration Following Exercise and Supplement Consumption

Subject	POST	30	60	120
1	7.41989	81.08067	46.42544	76.36851
2	5.928155	81.88126	11.99006	16.8748
3	4.5482	22.66662	9.292703	8.920859
4	7.193318	73.7416	45.04928	44.3902
5	4.16072	25.03755	12.67528	
6	4.961092	19.8448	19.06828	22.46568
7	4.579464	39.02763	36.05925	19.76047
9	4.831297	23.83654	7.131408	15.47466
10	20.05233	117.4839	89.22261	120.2398
11		44.88347	9.044604	15.20789
12				
13	8.345297	19.18289	10.92889	9.090982
14	5.790235	21.76973	9.52573	10.55532
15	3.053115	21.24178	4.552995	6.775833
16	6.535763	17.10632	5.59524	6.587804
17	7.248184	37.15995	7.731319	14.46104
18	3.619874	35.16587	5.339072	4.539557
19	10.9717	73.15644	14.10068	13.68503
20	5.754802	9.950526		9.847041
21	4.137876	57.23146	6.000555	13.28641
22	12.91802	47.68866	13.22906	12.70557
23	8.064384	24.12768	8.742603	9.847041
24	5.133348	18.60524	12.55079	8.029573
25	8.546286	18.9573	12.11076	9.993664
26	7.059809	26.87772	7.365651	10.75371
27	7.016185	30.54432	7.511244	6.746827
28	15.33575	36.7744	11.36324	13.14718
29				
30	6.913938	41.53286	14.10702	19.50393
32	4.823469	22.80325	15.30025	11.62844
33	5.446974	7.375872	5.819326	4.602952
34	6.460531	50.01159	7.964705	6.549868
35	5.16895	85.55268	15.53551	23.69599
36	15.30414	65.17043	8.56562	12.307
Mean	7.330107	39.2384	14.78246	16.7225
SD	3.914702	25.63988	16.45022	21.00279
SE	0.671366	4.397204	2.821189	3.601948

Insulin ANOVA for all time points

	SS	Degr. of	MS	F	p
group	7631.26	2	3815.63	5.04446	0.012687
Error	23448.39	31	756.40		
TIME	20940.87	3	6980.29	46.71352	0.000000
TIME*group	3073.33	6	512.22	3.42789	0.004212
Error	13896.77	93	149.43		

Tukey Post Hoc

Calculated by hand

Critical Value = SRS * (SQRT (MSERROR / # of OBSERVATIONS Per Mean))

Studentized range statistic (SRS) for 4 means, 80 df: = 3.74

Critical Value = 3.74 (SQRT (301.17 / 11)

CV = 19.5

Insulin Means

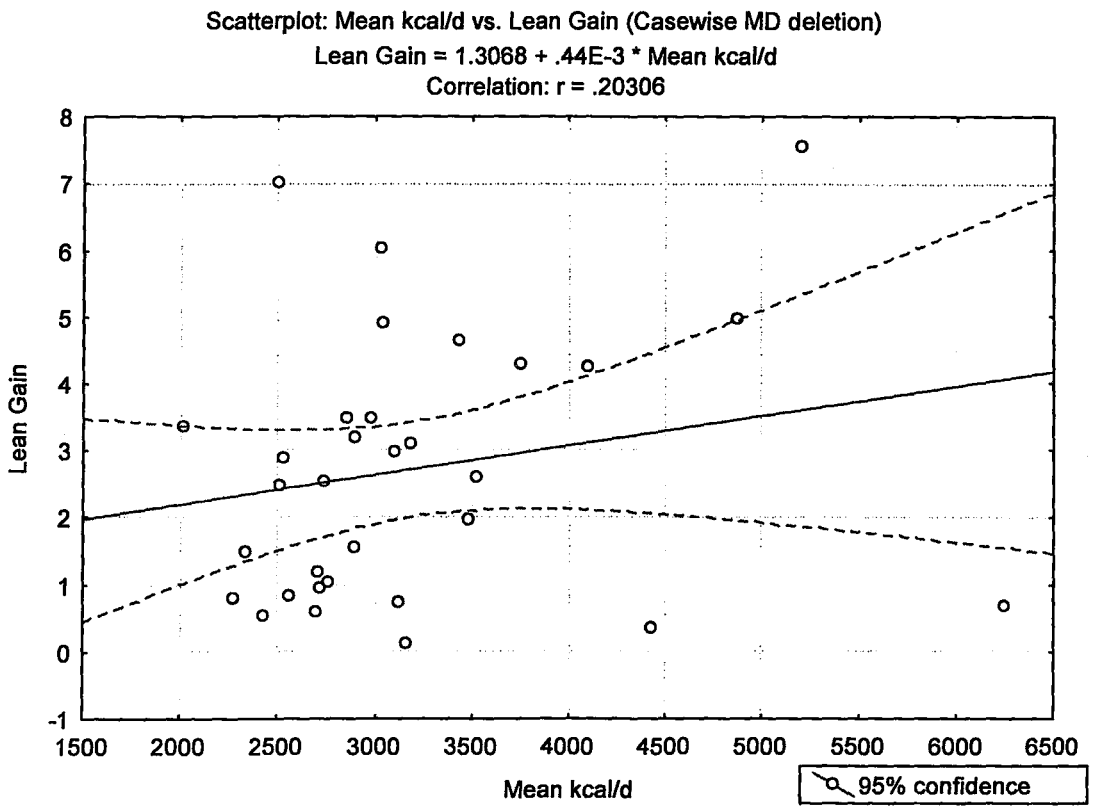
	CON	MLK	PEC
0 (µlU/mL)	7.1	6.8	8.2
30 (µlU/mL)	52.9±*	31.9*	38.6*
60 (µlU/mL)	28.6¶*	8.9	10.6
120 (µlU/mL)	37.7¶*	10.0	11.9

*Significantly different from 0 (P<0.05)
¶Significantly higher than MLK and PEC at same time point (P<0.05)
±Significantly higher that MLK at same time point (P<0.05)

APPENDIX 8

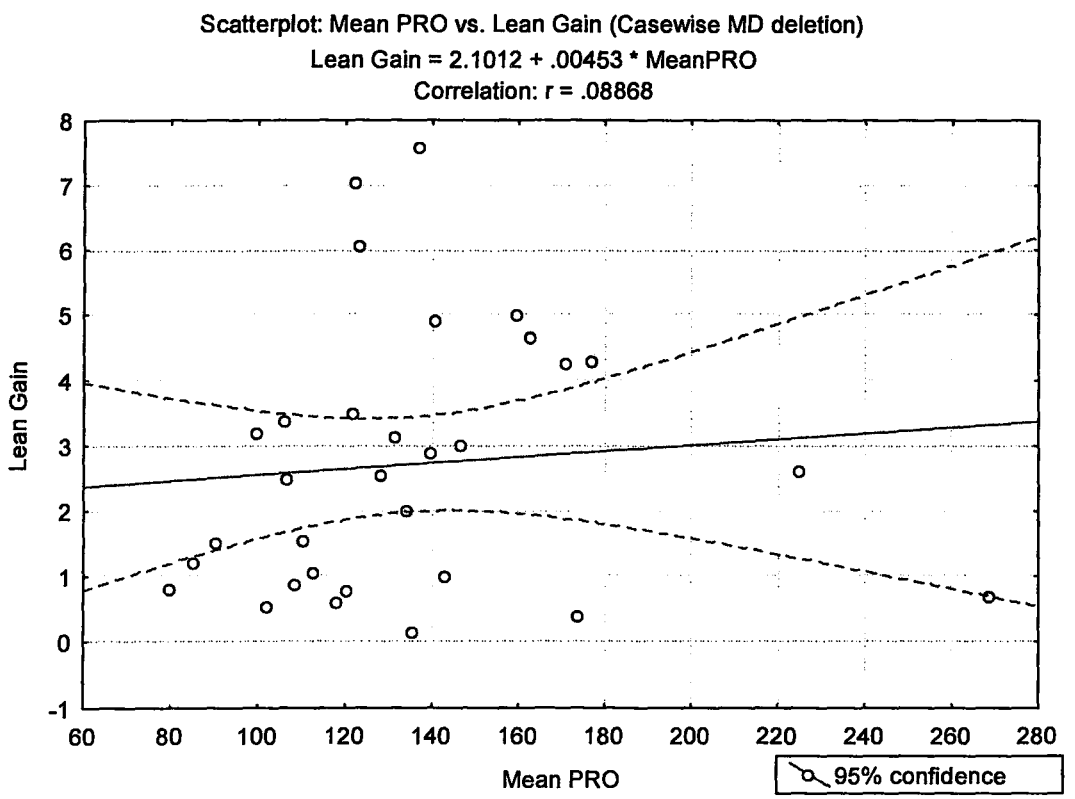
CORRELATIONS

Correlation: Lean Mass Gains and Mean kcal/kg during training



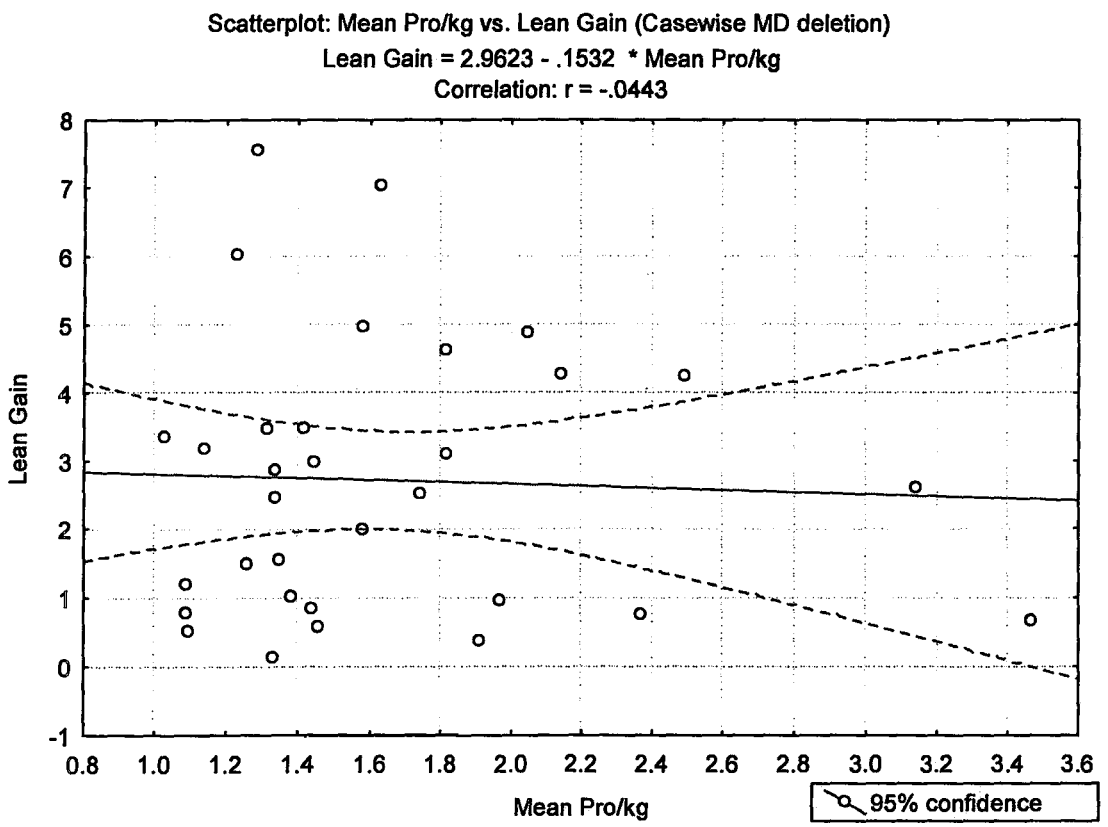
Correlation not significant (P>0.05)

Correlation: Lean Mass Gains and Mean Protein intake during training



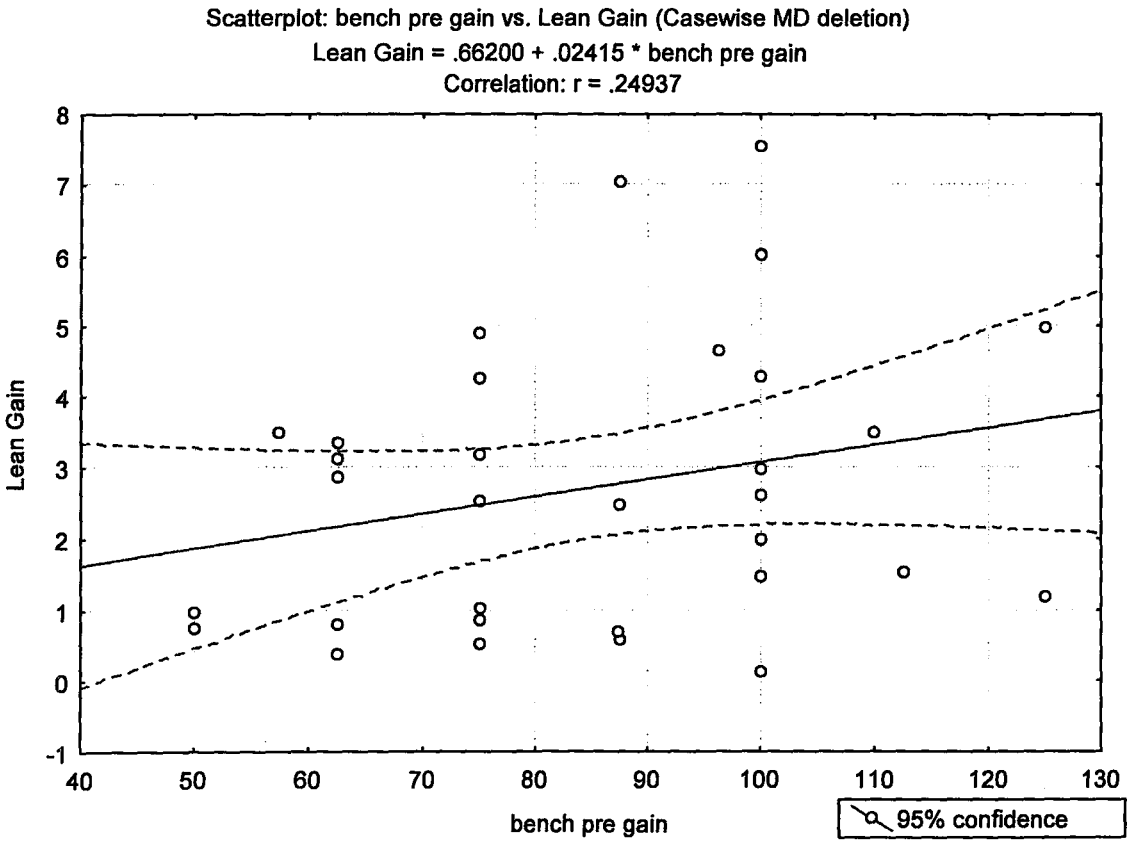
Correlation not significant (P>0.05)

Correlation: Lean Mass Gains and Mean Protein/kg intake during training



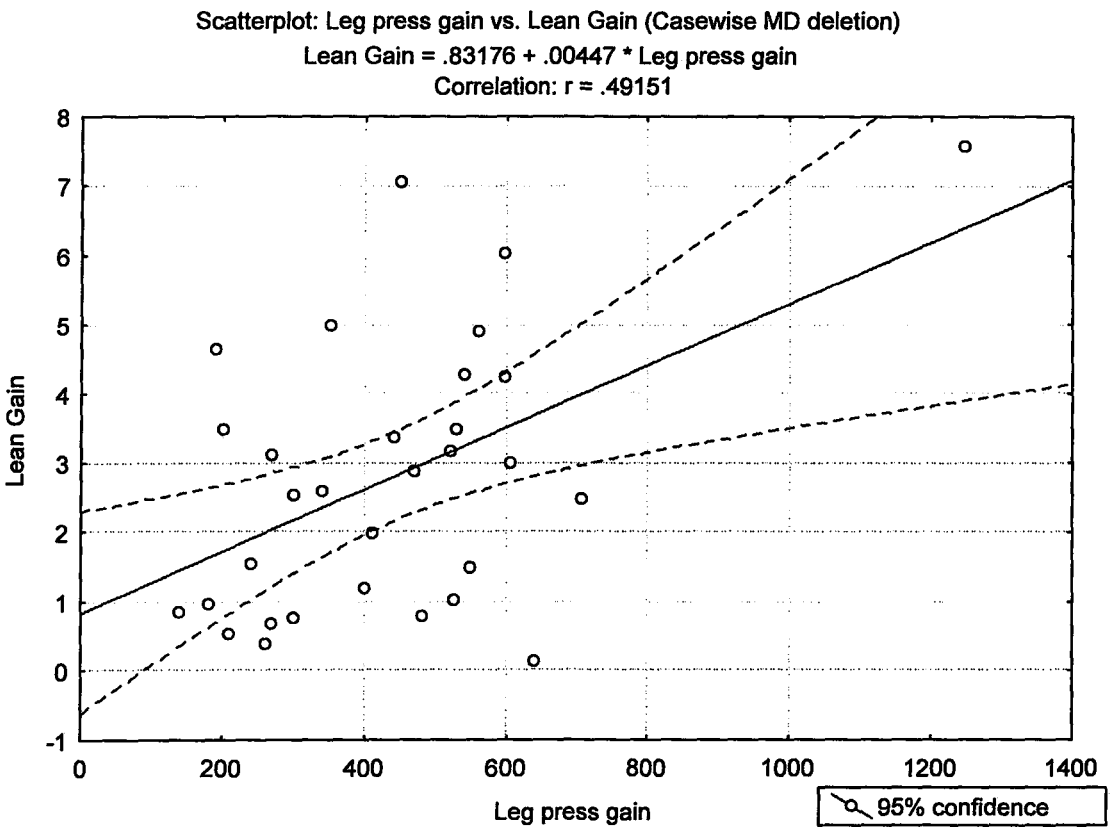
Correlation not significant (P>0.05)

Correlation: Lean Mass Gains and Bench Press Strength Gains



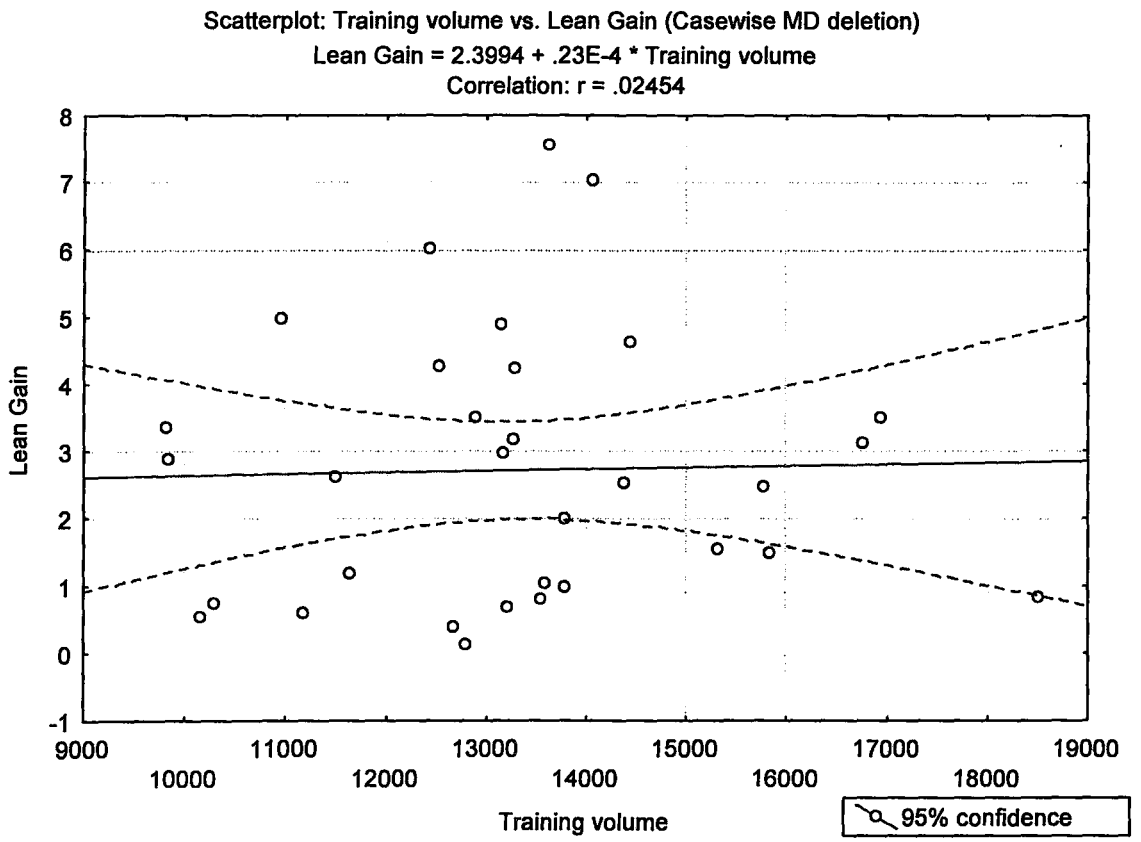
Correlation not significant (P>0.05)

Correlation: Lean Mass Gains and Leg Press Strength Gains



Correlation significant ($P<0.05$)

Correlation: Lean Mass Gains and Training Volume



Correlation not significant (P>0.05)

APPENDIX 9

DRINK COMPOSITIONS

Drink Compostitions

	CON	MLK	PEC
Protein (g)	0	18.2	18.2
Carbohydrate (g)	45	24.5	22.9
Fat (g)	0	1.0	1.6
Total kcal	180	180.1	179

APPENDIX 10

AA COMPOSTION OF MILK AND SOY PROTEINS

AA Composition of Soy and Milk Proteins

	SOY (mg/g PRO)	**%**	**MILK** (mg/g PRO)	**%**
ASP	56	7.2	51	5.6
SER	81	10.5	87	9.6
ASN				
GLU	120	15.5	189	20.8
GLY	29	3.7	18	2.0
GLN				
HIS	22	2.8	24	2.6
TAU				
THR	61	7.9	69	7.6
ARG	51	6.5	29	3.2
ALA	39	5.0	31	3.4
PRO	50	6.5	83	9.1
TYR	19	2.4	27	2.9
CYS				
VAL	31	4.0	55	6.0
MET	14	1.8	23	2.6
ILE	42	5.4	43	4.7
LEU	66	8.5	80	8.8
LYS	51	6.6	60	6.6
PHE	44	5.7	41	4.5
EAA	**329**		**396**	
TOTAL	**773**		**912**	
BCAA	139		178	

APPENDIX 11

ATPase HISTOCHEMISTRY PROTOCOL

ATPASE HISTOCHEMICAL ANALYSIS

Reference: Dubowitz, V. Muscle Biopsy: A practical approach 2ed. London: Bailliere Tindall, 1985.

Adapted by: Snow, R.J. School of Health Sciences, Deakin University, Australia

PART A: CUTTING MUSCLE

1. Store OCT mounted muscle at -80°C.
2. Prior to cutting muscle, place mounted muscle in cryostat for at least 15 min to reach -20°C.
3. Trim the OCT covered portion of the sample at 20μm/cut. Once muscle sample is exposed, reduce thickness of cut to 10μm for ATPase stain.
4. Cut 2-3 samples per slide.
5. Cover slides with paper towel and allow slides to dry overnight at 4°C.
6. Once dried, wrap slides in aluminum foil and store at -80°C until further analysis.

PART B: PREPARATION OF SOLUTIONS

Alkaline Stock Solution, pH 9.4

	Reagent	Manufacturer	Qty
1	Glycine	BioShop Biotechnology Grade - GLN 001	2.8163g
2	$CaCl_2 2H_2O$	BDH 10070/EM Science 10070-34	3.00g
3	NaCl	BioShop Reagent Grade - SOD 002	2.1938g
4	NaOH	BDH Analytical Reagent ACS 816	1.3500g
5	MilliQ H_2O		500mL

1. Dissolve reagents in MilliQ H_20 and bring to volume.
2. Calibrate pH meter prior to adjusting pH to 9.4 with conc. HCl/5M KOH.
3. Store stock solution in fridge (4°C).

Acid Preincubation Stock Solution, pH 4.6

	Reagent	Manufacturer	Qty
1	Potassium Acetate	EM PX 1330-1	2.45g
2	$CaCl_2 2H_2O$	BDH 10070/ EM Science 10070-34	1.30g
3	MilliQ H_2O		500mL

1. Dissolve reagents in MilliQ H_2O and bring to volume.
2. Calibrate pH meter prior to adjusting pH to 4.6 with glacial acetic acid.
3. Store stock solution in fridge (4°C).

5M NaOH (MW: 40.00g/mol) – Dissolve 20.00 g in 100 mL
5M KOH (MW: 56.11g/mol) – Dissolve 28.055 g in 100 mL

Alkaline Preincubation Solution

1. Remove alkaline stock solution from fridge and allow stock solution to reach room temperature.
2. Adjust pH of an appropriate volume (50mL) of alkaline stock solution to 10.50 using 5M NaOH (This should be done with continuous mixing and a pH meter sensitive to 0.001 pH units).

Acid Preincubation Solution

1. Remove acid stock solution from fridge and allow stock solution to get to reach room temperature.
2. Adjust pH of an appropriate volume (50mL) of acid stock solution to 4.30, 4.54, 4.60 with glacial acetic acid (This should be done with continuous mixing and a pH meter sensitive to 0.001 pH units).

ATP Preincubation Solution, pH 9.4 (PREPARE FRESH DAILY)

1. Add 170 mg of ATP (SIGMA A2383) to 100mL volumetric flask and bring up to volume using ALKALINE STOCK SOLUTION.
2. Adjust pH to 9.4.
3. Keep in fridge (4°C) until ready for use.

1% Calcium Chloride Stock Solution

1. Dissolve 10 g of $CaCl_2 \cdot H_2O$ in 1000mL volumetric flask using MilliQ H_2O and bring up to volume.
2. Store at room temperature.

2% Cobalt Chloride

1. Dissolve 5 g of $CoCl_2.6H_2O$ in 250mL volumetric flask using MilliQ H_2O and bring up to volume.
2. Cover in aluminum foil and store at room temperature.

1% Ammonium Sulfide (PREPARE FRESH DAILY)

1. Add 5 mL of 20% ammonium sulfide solution to 100mL volumetric flask.
2. Bring to volume.
3. Store in fume hood until ready for use.

PART C: FIBRE TYPE STAINING PROCEDURE

1. Incubate the sections in acid preincubation solutions adjusted to a pH of 4.30, 4.54 and 4.60; and alkaline preincubation solution adjusted to pH of 10.50 at the following time periods:

pH	Incubation time (min)
10.50	25
4.54	7.5
4.60	7.0
4.30	5.0

2. Transfer slides into plastic staining trough.

3. Rinse slides in distilled water 3 times.

4. Incubate slides in ATP incubation solution for 45 minutes at 37°C, in a temperature-controlled shaker.

5. Rinse slides in distilled water 2 times.

6. Incubate slides in 1% $CaCl_2.2H_20$ (Calcium Chloride) for 3 minutes at room temperature.

7. Rinse slides in distilled water 5 times.

8. Incubate slides in 2% $CoCl_2 \cdot 6H_20$ (Cobalt Chloride) for 3 minutes at room temperature.

9. Rinse slides with distilled water 5 times.

10. Incubate slides in 1% ammonium sulphide for 1 minute at room temperature.

11. Rinse slides in distilled water 5 times.

12. Dehydrate tissue for 2 minutes in each alcohol concentrations (70, 80, 90, 95 and 100% ethanol).

13. Clear sections with xylene. Do this twice in clean xylene @ 2 minutes.

14. Blot off excess xylene using Kimwipes. Mount the coverslips on slides using Permount (Fisher SP15-100). Allow Permount to dry (~1h). Store slides in the dark when not in use.

PART D: CAPTURING IMAGES / IMAGE ANALYSIS

1. Turn on camera and microscope and allow to warm up for 5min.
2. Focus image at 4x magnification.
3. Refocus image at 20x magnification to calculate fibre area (μm^2).
4. Open SPOT Advanced software.
5. Click "Get Image" icon to capture image.
6. Click "Focus" icon to refocus image.
7. Save image as .jpg file.
8. Capture 3-4 images per sample.
9. When finished with microscope and camera:
   a. Remove slide, lower platform, turn off camera, then microscope.
   b. Replace lens and dust covers.
10. Analyze Images using ImagePro Plus to determine fibre area (μm^2).