DIETARY SUPPLEMENTATION & RESISTANCE TRAINING PROGRAMS DESIGNED TO PROMOTE INCREASES IN MUSCLE MASS

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Declaration

I, Paul John Cribb, declare that the PhD thesis entitled *Dietary Supplementation & Resistance Training Programs Designed to Promote Increases in Muscle Mass* is no more than 100,000 words in length, exclusive of tables, figures, appendices, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

July 28th 2006

Table of Contents *page*

Abstract

Lifestyle strategies that focus on building/preserving skeletal muscle mass will enhance the health of a wide sector of the population and possibly, diminish the severity of many ageing-related illnesses. The focus of this dissertation was to examine the effects of strategic intervention with dietary supplements and exercise designed specifically to promote an increase in muscle mass (hypertrophy). Three separate trials were completed using healthy adult males (aged 18-36 years). Each trial utilized a randomized, double-blinded design that involved 10-11 weeks of structured RE training and matched groups that supplemented their diets with whey protein (WP), creatine monohydrate (CrM) and/or carbohydrate (CHO) (separately and in various combinations as well as at strategic times of the day). Assessments included body composition (lean mass, fat mass and body fat %), maximum (absolute) strength in three weight lifting exercises, and vastus lateralis muscle biopsies for determination of muscle fibre types (I, IIa and IIx), cross-sectional area (CSA), energy metabolite and glycogen concentrations as well as contractile protein content. The results of study-1 (chapter-3), demonstrated that despite the consumption of a high protein intake by all groups and no differences between the groups before the study, supplementation with CrM and/or WP resulted in greater ($P < 0.05$) improvements in strength (in three assessments) compared to supplementation with an equivalent dose of CHO. These improvements correlated strongly ($r \geq 0.7$; $P < 0.01$) with the differences ($P < 0.05$) in skeletal muscle morphology that were detected among the groups. The results from study-2 (chapter-4) demonstrated that a CrM-containing WP-CHO supplement provided a significantly greater improvement in body composition (increase in lean mass and decrease in body fat %; $P < 0.05$), greater gains in strength and muscle hypertrophy (type-IIa and IIx muscle fibre CSA and contractile protein content; $P < 0.05$) compared to a group given the same supplement without CrM. In study-3 (chapter 5) the effects of supplement-timing (i.e., the strategic consumption of a WP-CrM supplement immediately before and after exercise) was compared to the consumption of the same supplement at times outside of the pre- and post-workout period. Supplement-timing resulted in a better improvement in body composition ($P < 0.05$), greater gains in strength (in 2 out of the 3 assessments) ($P < 0.05$) and muscle hypertrophy ($P < 0.05$). Very few studies involving exercise training and supplementation have reported favourable changes in body composition alongside alterations at the cellular (fibre type specific hypertrophy) and subcellular (contractile protein content) levels. The research provides data on non-pharmaceutical, cost-effective strategies that could be easily implemented by a wide sector of the population to build/maintain muscle mass throughout the lifespan, and therefore, reduce the severity of many ageing-related illnesses as well as the economic burden on the health care system.

Preface

The research within this dissertation has undergone peer-review and has been presented at the following academic gatherings:

- Experimental Biology Meeting, 2003
  (featured presentation by the American Physiological Society)
- The Australian Association for Exercise and Sports Science's Annual Meeting, 2004
  (Awarded Young Investigator of the Year).
- The American College of Sports Medicine's Annual Meeting, 2002, 2003, 2005
  (selected for oral presentation each year)
- The Australian Academy of Technological Sciences and Engineering's Annual Meeting 2004 (awarded an Early Career Research Fellowship).
- The Victorian State Government's Sport and Recreation Industry Awards 2004
  (first prize in Applied Research in Sports Science).
- Fresh Science 2006.
  A national award sponsored by the Federal and Victorian Governments.

published in the following journals

- International Journal of Sports Nutrition and Exercise Metabolism, 2006
- Medicine and Science in Sports and Execise, 2006
- The Journal of Strength and Conditioning Research, 2007

and monographs

- The United States Dairy Export Council, 2004, 2005, 2006
- Dairy Management Inc. (USA), 2004, 2006

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To Mick, thank you for showing me how a scientist *should* think. To Alan, whom I not only consider a mentor but also a friend, thank you for all those extra hours spent eloquently refuting seemingly endless trivial requests from belligerent manuscript reviewers. Also, for making the time to review these chapters in your holidays, (*I'd apologise to Helen but I know it wouldn't do any good!*)

There is always a stack of people that should be acknowledged by a post-grad when they finally complete a doctoral dissertation but I particularly want to thank my colleagues at the EMU lab, Emma Rybalka, Matt Cooke and Chris Stathis, thank you for your friendship and some very funny times (*great weekend winery tours*) but more importantly, for sharing your laboratory expertise. In particular, I'd like to acknowledge Dr Andrew William's expertise and help in conquering the histochemical and metabolite assays presented in these studies. Emma, Matt, Chris and Andy; each of you are talented, assiduous scientists and owe it to yourselves to fulfil your potential. Thank you also to Dr. Andrew Garnham, the cycling-physician, for pedalling across town in all kinds of weather to perform the biopsies that were essential in this research.

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(like the Master Card commercial says… *priceless!*) AST provided completely unconditional support throughout my studies, for which I will always be grateful.

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Finally, thank you to my reviewers for your precious time. Each of you has already had a profound influence in my development; the standard of your research is what I aspire to in the years ahead. Additionally, for my American reviewers, all spelling is in Australian/English format.

I think that's everyone, so……. game on!

List of Figures

page

List of Tables

page

Abbreviations

AA, amino acids
ACTH, adrenocorticotropin
ACSM, American College of Sports Medicine
ADP, adenosine diphosphate
AHA, American Heart Association
ANT, adenine nucleotide translocase
AMP, adenosine monophosphate
AMPK, AMP-dependent protein kinase
ATP, adenosine triphosphate
BMR, basal metabolic rate
BCAA, branch chain amino acids
CHO, carbohydrate
Cr, creatine
CrM, creatine monohydrate
CK, creatine kinase
CPK, creatine-phosphokinase
CSA, cross-sectional area
Cys, cysteine
DEXA, dual energy X-ray absorptiometry
DNA, deoxyribonucleic acid
EAA, essential amino acids
eIF-2, eukaryotic translation initiation factor2
eIF4E, eukaryotic translation initiation factor 4E
eIF4G, eukaryotic translation initiation factor 4G
4E-BP1,initiation factor 4E-binding protein 1
EMG, electromyographic
ERK1/2, ex-cellular signal-regulated kinase 1/2
FAK, focal adhesion kinase
FLRG, follistatin-like related gene
GH, growth hormone
GDF-8, growth differentiation factor 8
Glu, glutamate
Gln, glutamine
GSK3, glycogen synthase kinase 3
GSH, glutathione
HPA, hypothalamic-pituitary-adrenal axis
IGF-1, insulin-like growth factor 1
IGFBP, insulin-like growth factor binding proteins
IL-1, 1β, 1ra, 6,8,10; interleukin cytokines
LBM, lean body mass
MAPK, mitogen-activated protein kinase(s)
mATPase, myosin ATPase
MEF-2, myocyte enhancer factor 2
MHC, myosin heavy chain
MGF, mechano-growth factor
MPS, muscle protein synthesis
MPB, muscle protein breakdown
MRF, myogenic regulatory factors
mRNA, messenger ribonucleic acid
mTOR, mammalian target of rapamycin
MyoD, myoblast determination factor
NFAT, nuclear factor of activated T-cells
NPB, net protein balance
NSCA, National Strength & Conditioning Association
P_i , phosphate
PCr, phosphocreatine
p38, p38 stress-activated protein kinase
p70 S6k, p70 S6 (ribosomal protein) kinase
PI3K, phosphatidylinositol 30-kinase

PKB/Akt, protein kinase B

PRO, protein

PRO-CHO, protein-carbohydrate

RDA, Recommended daily allowance

RE, (conventional) resistance exercise

RM, repetition maximum

SR, sarcoplasmic reticulum

SRE1, serum response element 1

SRF, serum response factor

TGF-β, transforming growth factor-β

TNF-α, tumour necrosis factor-alpha

WP, whey protein

Chapter 1

Introduction

1.1 Populations that would benefit from this research

Athletic performance and vanity aside, there are many important reasons for wanting to know more about how to build muscle; not the least being the underestimated role of muscle mass in healthy ageing. Sarcopenia is the unexplainable, age-related loss of muscle mass which has a negative impact on strength, power, functional ability and daily living (Evans, 1997). This phenomenon is wide spread among apparently healthy older adults; recent estimates indicate that approximately 45% of people 65 years or older in the United States exhibit sarcopenia (Janssen et al., 2004) and 20% are functionally disabled (Manton, 2001). An accumulating amount of evidence suggests that sarcopenia underlies many other undesirable conditions that are associated with ageing, such as osteoporosis, diabetes, unwanted weight gain, an increased susceptibility to illness, falls and related injuries (Scheibel, 1985; Dutta, 1997; Evans, 1997; Doherty, 2003;). The direct healthcare costs attributable to sarcopenia each year in the United States could be as high as $18.5 billion (Janssen et al., 2004).

There is little doubt that sarcopenia is a multifaceted phenomenon and its mechanisms are yet to be clearly identified (Marcel, 2003; Roubenoff, 2003). However, the reduction in functionality can be attributed to reduced motor unit activation (Jakobi & Rice 2002), muscle mass (Frontera et al., 1991; Lynch et al., 1999) and quality (Frontera et al., 2000a; Klein et al., 2001). The quality of skeletal muscle (from here on known as muscle), can be defined as the efficiency to perform its various functions per unit of mass (Balagopal et al., 1997a). While there appears to be a relationship between a quantitative loss of muscle and diminished functional capacity (Frontera et al., 1988; 1990; 2000b), cross-sectional data on older adults also suggests that the rate of decline in muscle strength accelerates with age (Hughes et al., 2001). Of even greater concern is the longitudinal data (spanning 10-12 years) that shows the decline in leg muscle strength can be 60% greater than estimates from a cross-sectional analysis in the same population (Hughes et al., 2001). Although functional capacity depends on both the quality and quantity of muscle mass, a major problem is that the minimal amount required to maintain health and independent living with advancing age is unknown. However, the rate of muscle mass decline with age is thought to be fairly consistent; approximately 1–2% per year past the age of 50 years (Sehl et al., 2001), but a reduction of 30% or more is thought to limit normal function (Bortz, 2002). Some researchers suggest that sarcopenia may be reversible (at least to a certain extent) (Roubenoff, 2003). Others

believe that tomorrows older adults should be concerned with building a greater "starting reserve capacity" of lean body mass (LBM)[a] today to ensure they avoid the unknown threshold that precedes physical frailty and compromised health (Marcel, 2003). In the year 2000, there was an estimated 600 million people on the planet aged 60 and over. This figure will rise to 1.2 billion by 2025, and 2 billion by 2050 (W.H.O. 2003). Therefore, the prevention, early detection and treatment of sarcopenia is needed to alleviate the load of a rapidly ageing population.

Ageing is also associated with changes in body composition that may carry severe metabolic consequences. While muscle mass declines dramatically between the age of 50 and 75 years by approximately 25%, this is accompanied by a substantial increase in body fat. The results of cross-sectional research (Baumgartner et al., 1995; Van Loan, 1996; Gallagher et al., 1997; Forbes, 1999) suggest that the average adult can expect to gain approximately 1 pound (0.45kg) of fat every year between ages 30 to 60, and lose about a half pound of muscle over that same time span; a change that is equivalent to a 15 pound (6.8kg) loss of muscle and a 30 pound (13.6kg) gain in fat (Forbes, 1999). These age-related changes in body composition have metabolic repercussions. Muscle tissue has a large working range of ATP turnover rates and tremendous potential to consume energy. Due to its mass, muscle is a highly important thermogenic tissue and a prime determinant of basal metabolic rate (BMR) (which for most of us is the largest single contributor to daily energy expenditure) (Elia et al., 2003). Therefore, muscle tissue is not only important for maintenance of a healthy weight, by virtue of its mass and mitochondrial content it is also the largest site of lipid oxidation (Perez-Martin et al., 2001; Heilbronn et al., 2004). This means that muscle not only plays an integral role in fat metabolism but also the maintenance of lipoprotein and triglyceride homeostasis (Thyfault et al., 2004). Muscle is also the primary site of glucose disposal in the post-prandial state (Perez-Martin et al., 2001). Exercised muscle promotes healthy glucose metabolism (Henriksen, 2002). Therefore, maintenance of this metabolically active tissue (with elevated mitochondrial potential) would also reduce the risk for the development of type-II diabetes (Perez-Martin et al., 2001).

To confirm these assumptions, cross-sectional data shows that older men and women generally have a decreased ability to mobilize and oxidize fat, as well as possess a slower BMR in comparison to their younger counterparts (Calles-Escandon et al., 1995; Nargy et al., 1996; Levadoux et al., 2001). Additionally, this age-related decline in BMR and fat metabolism is suggested to be related more to a reduction in LBM than ageing *per se* (Calles-Escandon et al., 1995; Nargy et al., 1996; Levadoux et al., 2001). In fact, the preservation of muscle mass

[a] *As muscle mass constitutes approximately 60% of all lean body mass, these terms are often used interchangeably, particularly with regard to alterations in body composition.*

throughout ageing may reduce the decline in BMR and possibly reduce the degree of body fat accumulation that is characteristically observed in older adults (Evans 1997; Marcel 2003). Unlike aerobic fitness capacity (Broeder et al., 1992), LBM is an important determinant of BMR (Calles-Escandon et al., 1995; Nargy et al., 1996; Levadoux et al., 2001). For this reason, strategies that preserve LBM are thought to be the cornerstone of any successful attempt at weight loss (Broeder et al., 1992; Poehlman et al., 1998). The prevalence of overweight adults in the United States increased from 47% in 1976-1980 to 65% in 1999-2002 (DHHS, 2004). (These percentages are thought to be similar in Australia although no accurate data has been obtained in recent years). Therefore, it is clear that lifestyle strategies that focus on maintaining muscle mass will enhance health and may prevent or reduce the severity of many ageing-related illnesses, as well as reduce a significant economic burden on the health care system (Janssen et al., 2004).

Besides its locomotive and metabolic implications, muscle tissue is the body's largest reservoir of bound and unbound proteins (amino acids) and constitutes 50-75% of all proteins in the human body (Waterlow, 1995). While quantitative estimates suggest that about 1-2% of muscle is synthesized and broken down on a daily basis, the mass of this tissue means that it accounts for up to 50% of whole body protein turnover (Rennie & Tipton 2000). This impact (on whole body protein turnover) is a clear reflection of the essential role that muscle tissue plays in the regulation of whole body amino acid metabolism. Muscle is the main reservoir and synthesis site of mino acids (AA) that are constantly exported to meet an array of physiological demands. One example is glutamine, the essential fuel that powers many aspects of immune function and cell turnover (Curi et al., 2005; Rutten et al., 2005). Therefore, the preservation of muscle mass is critically important to populations living with conditions such as HIV, various forms of cancer and intestinal conditions such as Crohn's disease and Colitis. Although clinically unrelated, all of these conditions promote cachexia; muscle wasting that manifests from a systemic inflammatory response (Hack et al., 1997; Kotler, 2000). This chronic immune response causes a dramatic increase in whole body protein turnover that leads to excessive breakdown of muscle tissue in an attempt to provide the chemical energy that is required. A decline in muscle mass signals a progression in the illness but also underlines mortality (Hack et al., 1997; Kotler, 2000). Despite significant advances in the medication strategies used to combat these illnesses, cachexia is still a major problem that has not been resolved (Hack et al., 1997; Kotler, 2000).

Whether it's to out perform the competition or maximize personal potential, athletes (recreational and professional) are competitive by nature. This drive to succeed and a growing awareness that nutritional choices can influence exercise performance has fuelled an explosion in the interest of nutritional ergogenic aids; dietary compounds that may enhance muscle strength,

size and athletic performance (Bradley et al., 2001). Recent decades have also seen a growing awareness of exercise as part of a healthy lifestyle (DHHS, 2004; CSEPHC, 1998). The sports nutrition industry has expanded beyond its non-commercial roots into a $2 billion a year growth market. With revenue sales of dietary supplements projected to reach in excess of $4.5 billion in the United States alone by 2007 (Tallon, 2003), this defines an enormous expectation for potential benefit. However, very few dietary supplements that are marketed as ergogenic aids have science-based evidence of their effectiveness. Despite the public's interest and the previously discussed clinical implications, there is a paucity of research-based information on cost-effective, nutritional strategies that may help build and maintain muscle mass.

1.2 Factors that regulate the size of human skeletal muscle mass

The mechanisms that regulate the maintenance of human muscle mass are complex and influenced by numerous factors such as physical activity, nutrition, hormones, disease and age (Rennie et al., 2004). Once fully differentiated, the number of muscle cells stays constant (within a few percent) so that, with a few exceptions, a gain or loss of muscle tissue can only occur via an increase (hypertrophy) or a decrease (atrophy) in size of the existing muscle cells (Hargreaves & Cameron-Smith 2002). The quality and quantity of muscle protein is essentially maintained through a constant remodelling process (protein turnover) that involves continuous synthesis and breakdown (Rasmussen & Phillips 2003). Protein turnover is a complex, regulated process but is essentially controlled by the initiation of protein synthesis and proteolytic pathways. There are a number of regulators that affect protein turnover either by stimulation or inhibition of protein synthesis and protein degradation (Liu et al., 2006) (figure 1.1). For example, insulin and IGF-1 prevent contractile protein degradation (IGF-1 inhibits degradation via the ubiquitin pathway). Cortisol and myostatin are known inhibitors of protein synthesis; cortisol, cytokines and ubiquitin proteins activate protein degradation. Conversely, insulin, amino acids, mechanical loading and the anabolic hormones are considered stimulators of protein synthesis. The focus of this chapter is to discuss protein turnover and its regulators in relation to the factors that may increase the size of human muscle mass. Specifically, those factors are resistance exercise (RE) and macronutrient intake. However, when considering all aspects that may influence the size of human muscle mass, one must keep in mind that the regulatory network that controls this process may not reside exclusively within muscle.

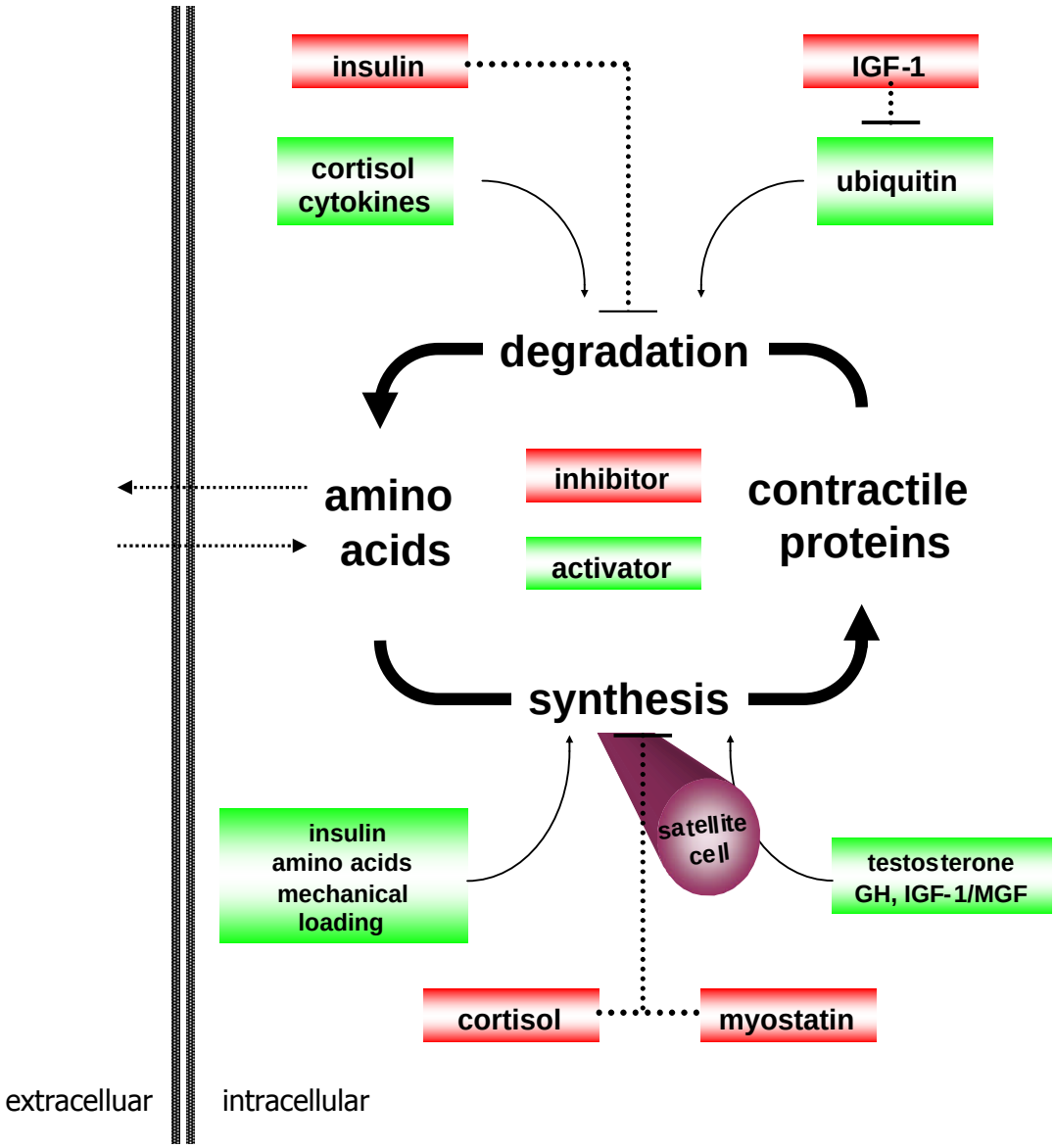


Figure 1.1 Regulators that affect muscle protein turnover.
A number of regulators affect protein turnover either by stimulation or inhibition of protein synthesis and protein degradation. For example, (shown in red) insulin and IGF-1 prevent contractile protein degradation whereas cortisol and myostatin inhibit protein synthesis. Cortisol, cytokines and ubiquitin proteins (shown in green) activate protein degradation. Conversely, insulin, amino acids, mechanical loading and the anabolic hormones (in green) activate protein synthesis.

Some rather convincing evidence suggests that the regulation of whole body protein metabolism (and the size of muscle mass) involves a regulatory circuit between muscle, blood (plasma) and liver AA metabolism (Hack et al., 1997). As mentioned previously, cachectic conditions fail to conserve muscle proteins and convert abnormally large amounts of AA into glucose, and release large amounts of nitrogen as urea. The conversion of AA into glucose is linked to the rate at which ammonium ions in the liver are converted into either urea or glutamine (Gln). In contrast to Gln biosynthesis, urea production requires hydrogen carbonate anions (HCO_3^-) and thereby generates protons (figure 1.2). Carbamoylphosphate is the rate-limiting step for urea biosynthesis, and is limited by the availability of HCO_3^- anions, which are scavenged by protons. A series of studies involving humans living with and without cachectic conditions strongly suggest that the hepatic catabolism of cyst(e)ine (both Cys and its disulphide twin, cystine) is a key regulator of whole body protein metabolism (Kinscherf et al., 1996; Hack et al., 1996; 1997; 1998; Holm et al., 1997). The hepatic catabolism of cyst(e)ine into sulphate (SO_4^{2-}) and protons (H^+) down-regulates urea production in favour of Gln biosynthesis, thereby retaining nitrogen in the muscle AA reservoir (figure 1.3). The necessary hepatic catabolism of cyst(e)ine is an essential proton-generating process that inhibits carbamoylphosphate synthesis and thus, shifts whole body protein metabolism toward the preservation of the muscle AA pool and maintains the size of muscle mass (Hack et al., 1997). This pathway is essentially textbook biochemistry. Nevertheless, a substantial amount of data suggests that the availability of cyst(e)ine in plasma determines the threshold at which AA are converted into other forms of chemical energy that ultimately influences body composition (Kinscherf et al., 1996; Hack et al., 1996; 1997; 1998; Holm et al., 1997).

The term “controlled catabolism” is used to explain this regulatory circuit (figure 1.3) (Hack et al., 1997). The controlled release of AA by muscle in the post-absorptive state is compensated by AA uptake and protein synthesis in the post-prandial state (Holm et al., 1997). The most important function of this regulatory circuit is to ensure that any time urea production is too high and plasma AA are accordingly too low, controlled muscle catabolism is triggered. This leads to export of cyst(e)ine, an increase in plasma cyst(e)ine and a down regulation of the hepatic urea production. However, in cachectic conditions this process is thought to be disturbed. The etiologically unrelated illnesses mentioned previously are characterized by high glycolytic activity (increased lactic acid production) in muscle, increased hepatic urea production and abnormally high post-absorptive venous plasma glutamate (Glu) levels combined with a low plasma Gln/Cys ratio. Often, these characteristics are observed before wasting becomes evident (Droge et al., 1996; Lundsgaard et al., 1996; Dworzak et al., 1998; Hack et al., 1997; 1998). However, it is also interesting that similar biochemical symptoms (and subsequent alterations in body composition) have been observed in healthy adults.

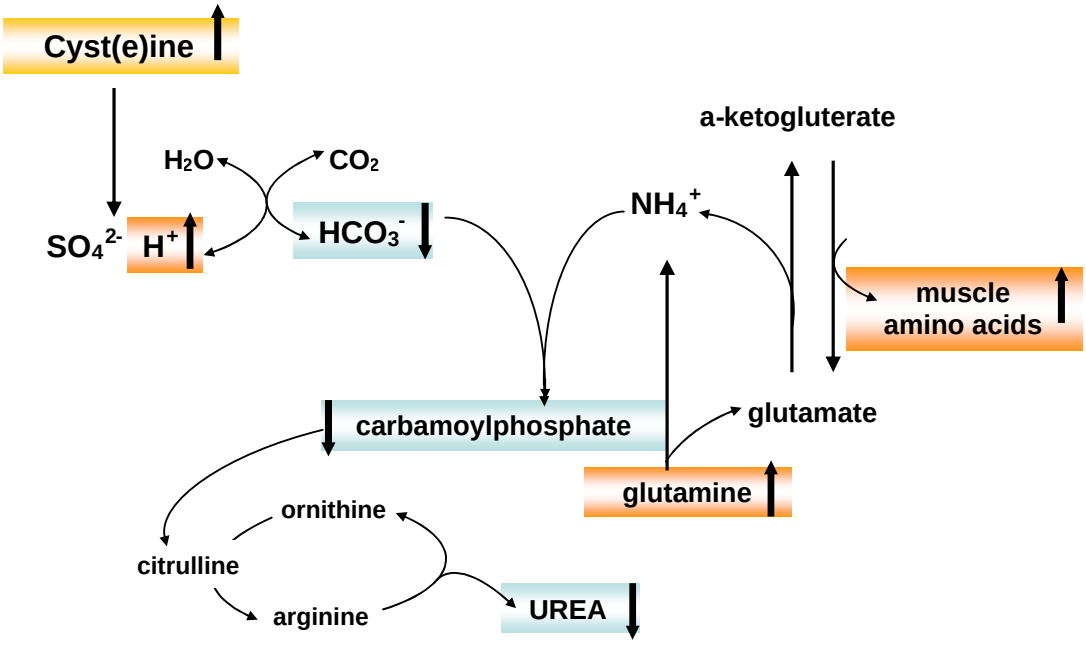


Figure 1.2 Urea biosynthesis and the influence of the amino acid cysteine.
Urea production requires hydrogen carbonate anions (HCO_3^-) and thereby generates protons. Carbamoylphosphate is the rate-limiting step for urea biosynthesis, and is limited by the availability of HCO_3^- anions, which are scavenged by protons. The hepatic catabolism of cyst(e)ine is an essential proton-generating process that inhibits carbamoylphosphate synthesis and down-regulates urea production (Hack et al., 1997).

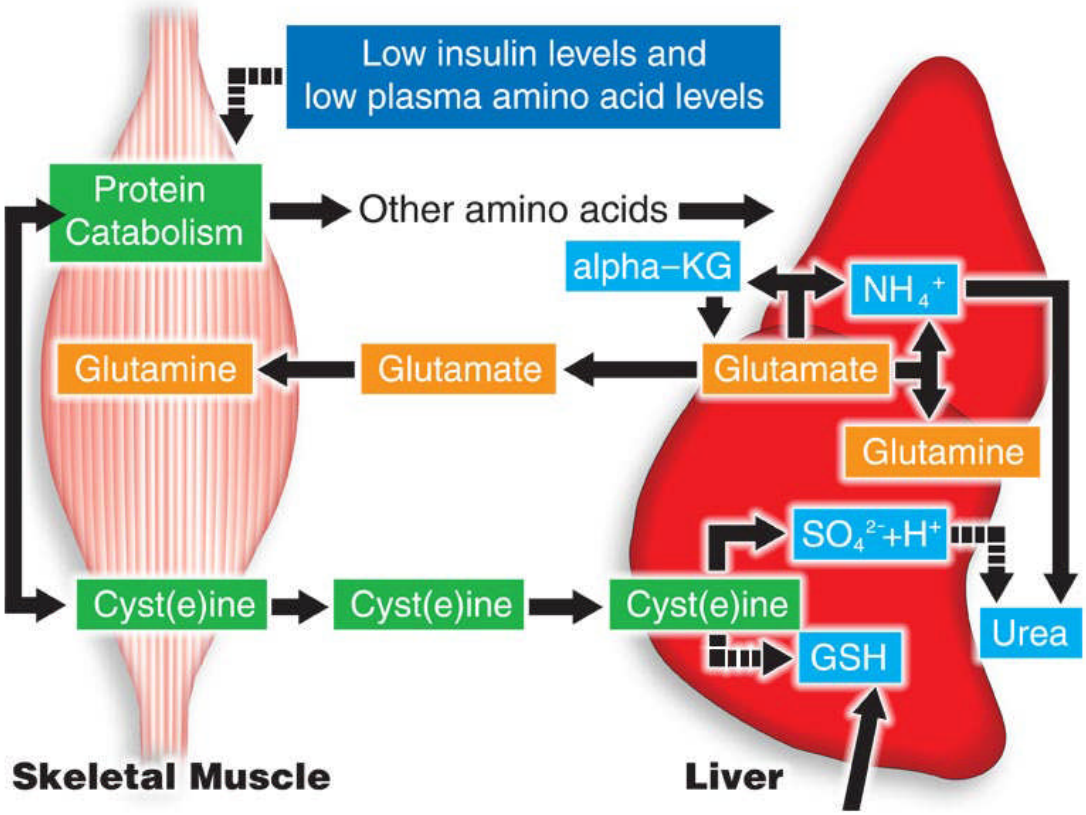


Figure 1.3 Regulatory circuit of whole body protein metabolism (featuring cysteine). The most important function of this regulatory circuit is to ensure that any time urea production is too high and plasma AA are accordingly too low, controlled muscle catabolism is triggered. This leads to the export of cyst(e)ine that is catabolized within the liver into sulphate and protons; a process that promotes glutathione (GSH) synthesis, down regulation of urea production and shifts whole body nitrogen economy towards the preservation of the muscle AA pool. Adapted from research by Hack et al. (1997).

In several trials that collectively involved 75 moderately well-trained men (between 20-60 years of age), correlations were detected between changes in body composition (both LBM and fat mass) and post-absorptive plasma AA Gln, Glu and Cys (Kinscherf et al., 1996). In these trials, the participants undertook 4-8 weeks of high-intensity (anaerobic-based) exercise training while plasma AA were monitored. The participants with a high plasma Gln/Cys ratio maintained or increased LBM and decreased fat mass during the program. Conversely, a low Gln/Cys ratio, accompanied by a significant increase in Glu (25 to about 40 mM), was indicative of a reduction in LBM and changes in T lymphocyte numbers (Kinscherf et al., 1996). Additionally, the participants with the lowest Gln/Cys ratio at the start of the exercise programs lost the most LBM and the reductions in LBM was accompanied by body fat accumulation. In one of these trials, a group supplemented with a cyst(e)ine precursor, N-acetyl-cysteine (NAC) (400mg/day) during the exercise program increased plasma cysteine levels and demonstrated a beneficial change in body composition (i.e., an increase in LBM and or a decrease in fat mass) (Kinscherf et al., 1996). Aside from these longitudinal investigations, cross-sectional data on AA exchange rates in healthy adults aged from 28 to 70 years also reveal a number of conspicuous relationships between post-absorptive plasma Gln, Cys and body composition.

Firstly, this cross-sectional data demonstrates that plasma Cys levels exhibit by far the strongest age-dependant change of any AA (Kinscherf et al., 1996; Holm et al., 1997; Hack et al., 1997; 1998). Secondly, older adults (60 years and over) demonstrate lower Gln exchange rates and Gln/Cys ratios than their younger counterparts (Hack et al., 1997; 1998). Thirdly, a highly significant ($P < 0.001$) correlation between a low Gln/Cys ratio and increased body fat was observed across all age groups (Hack et al., 1997). A highly significant correlation between decreased LBM and plasma Cys/thiol ratio was also observed in older adults (Hack et al., 1998). Collectively, these results underline the importance of Cys in the regulation of LBM (Hack et al., 1996). However, this data also points toward an age-related decrease in the efficiency of cyst(e)ine catabolism to regulate hepatic urea production (Hack et al., 1997). That is, the liver of an older person with a given plasma Cys level appears to convert less AA to Gln than a young, healthy individual. Therefore, muscle stores would be relied upon increasingly with advancing age to meet the metabolic demands for Gln. Some have speculated that the end result of this malfunction is a steady but aggressive catabolism of muscle tissue throughout the lifespan (Hack et al., 1997; 1998). Based on the findings of these investigations, a regulatory relationship between muscle, plasma, liver cyst(e)ine concentrations and whole body protein metabolism is evident. In particular, the concentration of cyst(e)ine appears to determine the threshold at which other AA are converted into other forms of chemical energy that ultimately influences body composition.

One mechanism that may disturb this regulatory circuit and induce excessive muscle loss is the high level of circulating cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) which are a feature of cachectic conditions such as cancer and HIV (Strassmann et al., 1992; Kotler, 2000) but are also characteristic of older adults (Visser et al., 2002; Krabbe et al., 2004; Toth et al., 2005). These cytokines increase the requirements for L-gamma-glutamyl-L-cysteinylglycine (glutathione); the centrepiece of all cellular antioxidant defences. Glutathione (GSH) regulates re-dox status, cytokine production and many aspects of metabolism such as DNA and protein synthesis, cell proliferation and apoptosis (Townsend et al., 2003; Wu 2004). Chronic inflammation increases γ-glutamyl-cysteine synthetase activity in the liver. As Cys is the rate limiting AA in GSH synthesis (Wu 2004), conditions that promote a chronic inflammatory response are thought to create an unfavourable competition for a limited hepatic cyst(e)ine reservoir — an underlying mechanism of oxidative stress and a cachectic environment that promotes muscle wasting (Hack et al., 1997; Bounous & Molson 2003; Townsend et al., 2003).

However, no matter how tempting it maybe to speculate, the probability is that no single mechanism may be solely responsible for changing the size of human muscle mass. Therefore, clinical interventions that focus on increasing or maintaining muscle mass may need to be multifaceted. The application of stable isotope tracer technology, advances in immunohistochemical techniques, more accurate body composition assessment and powerful microarray methods are all contributing to a better understanding of the aspects that ultimately control the size of human muscle mass. Advancements in these techniques have also provided greater insights into the physiological responses from two major factors that serve to positively affect protein turnover and increase the size of human muscle mass; RE and macronutrient intake.

1.3 Resistance exercise: Adaptations and influences that may affect muscle hypertrophy

RE is regarded as fundamental to the development and maintenance of muscle mass in adults (Feigenbaum & Pollock 1999). Conventional RE typically involves the controlled movement of weighted devices such as barbells, dumbbells and machines (with fulcrums and loaded weight stacks) where muscles undergo concentric (shortening), isometric (static) and eccentric (lengthening) actions against a constant external load — the magnitude of which is limited by the individual's concentric strength. Conventional RE training typically involves the use of heavy loads lifted in sets of 1 to 12 maximum-effort repetitions (Feigenbaum & Pollock 1999; Kraemer et al., 2002). This form of exercise has been studied quite intensively over the last 25 years as an intervention to offset age-related changes in body composition, strength and functional ability

(Barry & Carson 2004; Hunter et al., 2004) as it is a most potent activator of the cellular and molecular mechanisms that promote muscle anabolism (Rasmussen & Phillips 2003). The functional adaptations that occur with conventional RE (from here on referred to as simply, RE) are typically interpreted within the context of two components; a central component that accounts for training-induced changes in motor unit recruitment (Enoka, 1998), and a peripheral component that describes changes occurring at the level of the muscle tissue (Jones et al., 1989). Both aspects will be discussed in the following section.

The value of RE training for increasing functional strength and muscle mass became a topic of increasing interest within the scientific community once DeLorme and Watkins (1948) demonstrated the importance of progressive loading for the rehabilitation of injured World War II military personnel. A major reason for this interest is the remarkable plasticity of muscle tissue (Booth & Baldwin 1996). Muscle responds specifically to current functional demands; fibres can undergo extensive remodelling within their contractile apparatus to meet new functional requirements (Booth et al., 1998). Improvements in strength and/or muscle mass are the result of this remodelling process which involves transformations at the cellular and molecular levels (Staron et al., 1990; Booth & Thomason 1991). For example, it has been proposed that fibre type classifications, based on myosin ATPase (mATPase) histochemistry, represent a continuum that span the functional demands of the muscular system. During contractile activity muscle fibres are recruited from type-I (slow twitch) $\rightarrow$ Ic $\rightarrow$ IIc $\rightarrow$ IIac $\rightarrow$ IIa $\rightarrow$ IIab $\rightarrow$ IIb (fast twitch) (Staron & Johnson 1993). RE typically involves large contractile efforts but slow shortening velocity (Staron et al., 1984). Therefore, initial adaptations to heavier contractile loads occur via the expression of slower isoforms of contractile proteins (Williams & Neufer 1996; Dunn & Michel 1997). Of the contractile proteins within muscle, the myosin heavy chains (MHCs) are the largest subunits. The MHCs make up the majority of the myosin filament that forms the cross bridge according to the sliding filament theory and is the site of mATPase activity. The MHC isoform expressed within a muscle fibre reflect the contractile properties of the histochemically-delineated fibre type (Staron & Johnson 1993). Nine different MHC isoforms have been identified in mammalian muscle; four of which are expressed in adult rodent muscle (Booth and Baldwin 1996) (MHC type-I, IIa, IIx, and IIb). Three, (type-I, IIa, and IIx) are expressed in most human adult muscles[b] (Adams et al., 1993). The differing isoforms with their unique mATPase activity reflect the fibre type and correlate with speed of contraction; MHC I being the slowest and MHC IIx the fastest and most powerful (Bottinelli et al., 1994; Fry et al., 1994; Williamson et al., 2001).

[b] *A gene for a type-IIb MHC has not been identified in humans. Fibres that have been classified as type-IIb express the type-IIx isoform rather than type-IIb. Therefore, human muscle fibres are often classified as type-I, IIa and IIx (Smerdu et al., 1994; Ennion et al., 1995).*

The unique adaptability of muscle tissue appears to reside in the ability of the fibres to transcribe different isoforms of MHC protein (Staron & Johnson 1993; Wright et al., 1997). It is these alterations in the phenotypic expression of the MHCs that provide a mechanism of adaptation to stresses placed upon the muscle, such as increased and decreased usage. The polymorphism of the MHCs play a major role in the adaptability and contractile efforts necessary for various types of exercise (Wright et al., 1997). Furthermore, MHC isoform expression does appear to change along with the fibre type transitions that are a consequence of chronic RE (Fry et al., 1994; Staron, et al., 1994; Williamson et al., 2001). Fibres containing predominantly MHC IIx are not well suited for consistent activity; the available literature suggests that these fibres readily convert to type-IIa during heavy contractile loading (Fry et al., 1994; Staron et al., 1994; Williamson et al., 2001). As a result, muscle fibre recruitment profiles related to training intensity (i.e., the load used) and speed of contraction, play an important role in dictating down-regulation in the expression of the type-IIx gene (Willoughby & Nelson 2002). While MHC isoform (and subsequent fibre type) transitions tend to go in the direction from type-IIx to type-IIa, there is little or no change to type-I regardless of the modality of exercise (Staron et al., 1984; Staron & Johnson 1993). Changes in the relative abundance or rate of translation of the mRNAs encoding the different MHC isoforms must precede major changes in the protein isoform composition by several weeks. This is due to the slow turnover of MHC in human muscle (Balagopal et al., 1997). This delay between exercise-induced changes in MHC mRNA isoform expression and alterations in protein isoform distribution is the basis for mismatches between mRNA and protein expression in individual fibres during RE training (Andersen et al., 1997). Thus, MHC protein isoform concentrations may not be representative of gene expression early in a training program (Staron & Johnson 1993; Fry et al., 1994; Willoughby & Nelson 2002). It is clear that functional changes occur within muscle in response to RE training and these peripheral adaptations involve extensive remodelling of qualitative or intrinsic contractile properties such as MHC isoform composition (Duchateau et al., 1984; Jones & Rutherford 1987; Alway et al. 1989) and alterations in fibre type distribution (Staron et al., 1984). However, due to its apparent locomotive and metabolic benefits, muscle hypertrophy is the peripheral adaptation that has received an increasing amount of attention in recent years (Rennie et al., 2004; Volek, 2004; Phillips et al., 2005).

Hypertrophy can be described as an increase in the cross-sectional area (CSA) of the muscle itself (determined by magnetic resonance imaging) or the individual fibres (Staron et al., 2000). Muscle fibre hypertrophy is commonly determined by needle biopsy and ATPase staining (Hather et al., 1991; Green et al., 1999; Ahtiainen et al., 2003). RE-induced muscle hypertrophy is essentially the result of a net increase in muscle proteins (Phillips, 2000). It is presumed that contractile (myofibrillar) protein volume increases in direct proportion with exercise-induced fibre

hypertrophy (MacDougall et al., 1977; Always et al., 1989; Shoepe et al., 2003). It is also presumed that sarcoplasmic or non-contractile proteins (such as alpha-actinin, myomesins, desmin, dystrophin, nebulin, titin, and vinculin) increase in proportion with the increased synthesis of myofibrillar protein (Alway et al., 1989; Phillips, 2000). However, one recent study has shown that RE training "refines" the acute stimulus so that it is preferentially directed towards contractile protein synthesis more so than non-contractile proteins (Kim et al., 2005). The increase in myofibrillar protein that is associated with hypertrophy includes an increase in the number of myosin and actin filaments inside each sarcomere (Booth & Thomason 1991) as well as the addition of new sarcomeres in a parallel force-producing arrangement (Roman et al., 1993; Staron et al., 1994; McCall et al., 1996). As the contractile proteins make up at least 80% of the fibre space (Phillips, 2000), minimal increases in contractile proteins may contribute significantly to muscle fibre hypertrophy (Wilborn & Willoughby 2004). Aside from athletic populations, significant muscle hypertrophy during RE training has been documented in male and female adults of all age groups (Frontera et al., 1988; Staron et al., 1991; Donnelly, 1993; Häkkinen et al., 2000; Bamman et al., 2003), including the frail elderly (90+ years old) (Fiatarone et al., 1990; 1994). However, the exact mechanical prerequisites that are responsible for this adaptation remain elusive.

Under maximal activation, each type of muscle contraction (concentric, isometric and eccentric) is capable of stimulating hypertrophy (Adams et al., 2004). However, conventional RE involves voluntary contraction of muscles as they undergo concentric, isometric and eccentric actions against a constant external load, the magnitude of which is limited by the individual's concentric strength. Eccentric strength is generally 20-50% greater than concentric strength (Bamman et al., 1997). This means that eccentric loading is always submaximal during conventional RE. Nonetheless, high-overload RE is shown to cause significant myofibrillar disruption (including Z-band disruption) (Paul et al., 1989), even in individuals that lift weights regularly (Staron et al., 1992). Furthermore, submaximal eccentric loading (approximately 80% of maximum concentric strength) is shown to provide more severe, prolonged myofibrillar disruption, soreness, and force deficit than concentric-only lifting with the same load (Gibala et al., 1995), a finding that has also been confirmed in RE-trained individuals (Gibala et al., 2000). From the research available, eccentric loading appears to be essential to obtaining an optimal hypertrophy response from RE (Hortobagyi et al., 1990; Hather et al., 1991; Higbie et al., 1996; Hortobagyi et al., 1996). Also, the mechanical damage that results from RE seems to be an important stimulus for remodelling and hypertrophy (MacDougall 1986; Gibala et al., 2000). However, whether the damage obtained from RE is causally related to the growth response has not been clarified; nowhere in the literature has it been shown that muscle damage is an essential component of hypertrophy (Kraemer et al., 2002).

Although hypertrophy has been documented in a variety of populations that have undertaken RE training, the magnitude of this response may vary considerably between individuals in both men and women (Hubal et al., 2005). One study that utilized a large cohort (over 500 participants) report that some participants demonstrated increases in muscle size over 10 cm^2 combined with 100% improvements in strength while others undertaking the exact same program showed little or no change in strength or hypertrophy (Hubal et al., 2005). The hypertrophy response can extend to each of the major fibre types (Green et al., 1999). However, the magnitude of the response is typically much greater in the type-IIa and IIx subgroups (Staron et al., 1990; Hather et al., 1991; Kraemer et al., 1995; McCall et al., 1996). Therefore, inherent fibre type proportions can influence an individual's potential for hypertrophy. However, hypertrophy is a multifaceted phenomenon that appears to be influenced by a multitude of factors such as age; gender; endocrine profiles; nutrition (that particularly affects anabolic processes such as energy production and protein turnover); strength development; training status; and possible genetic differences as well as the type of RE program performed (Kraemer et al., 1999; Roth et al., 2001; Newton et al., 2002; Beunen & Thomis 2004). Unfortunately, very few studies have directly assessed the impact that these variables may have on the hypertrophy response to RE training. Some of these aspects are discussed briefly in following paragraphs of this section, others such as protein turnover (1.4), the molecular events (1.5), nutrition (1.6 & 1.7) and RE programming (1.10) are the focus of separate sections within this chapter.

Ageing

It is clear that ageing changes muscle physiology. Advancing age appears to reduce maximum voluntary muscle strength (and power), protein and enzyme synthesis rates, sarcoplasmic reticulum (SR) Ca^{2+} uptake and the expression of myo-specific genes (Yarasheski 2003; Nair 2005). However, what is truly fascinating is that RE training can reverse or at least improve each of these aspects (Yarasheski et al., 1993; Hasten et al., 2000; Jubrias et al., 2001; Newton et al., 2002; Hunter et al., 2004). In line with these findings, other studies have shown that ageing *per se* does not diminish the capacity for muscle fibre hypertrophy (Frontera et al., 1988; Hikida et al., 2000; Hagerman et al., 2000) or the ability to gain muscle mass (Frontera et al 1988; Hunter et al 2004; Binder et al., 2005). A major contributor to the age-related decline in muscle mass is thought to be the loss of alpha-motor neuron input that occurs with ageing (Brown, 1972). Yet the research available on this topic suggests that older adults respond with strength and power improvements in a similar manner to young adults (Häkkinen et al 2001; Newton et al., 2002). Also, ageing does not appear to change absolute and relative BMR alterations in response to RE (Lemmer et al., 2001). As discussed in the previous section, an age-related decline in muscle mass

is associated with chronic, low-grade inflammation that is characterized by increased levels of TNF-α and other pro-inflammatory cytokines (such as IL-6), and markers of inflammation (such as C-reactive protein) (Visser et al., 2002; Krabbe et al., 2004). However, RE training is shown to reduce muscle TNF-α protein levels in older adults (Greiwe et al., 2001a). This training-induced reduction in muscle TNF-α protein was associated with an increase in muscle protein synthesis. Additionally, Bautmans et al. (2005) report that 6 weeks of RE training resulted in favourable changes in heat-shock proteins (such Hsp70) in monocytes and lymphocytes in older adults, and these changes were associated with strength gains and modulation of circulating cytokines. The heat-shock proteins protect cellular integrity during stressful situations such as oxidative stress and infection. The Hsp70 in particular is thought to play a role in RE-induced muscle hypertrophy (Kilgore et al., 1998). However, its production is negatively correlated to an age-related increase in circulating IL-6 and TNF-α (Njemini et al., 2002). Therefore, RE training appears to be an effective stimuli to preserve muscle mass in older adults (Hunter et al., 2004), and this may partly be due to the ability of RE to modulate the production of cytokines and heat-shock proteins (Greiwe et al., 2001a; Bautmans et al., 2005). However, when hypertrophy-related responses in younger and older adults (60 years and over) have been compared directly, some clear differences have been observed.

Some (Balagopal et al 1997; Toth et al., 2005), but not all (Volpi et al., 2001), studies report that older adults possess basal muscle protein synthesis (MPS) rates that are 19-40% lower than younger adults. Also, older adults appear to possess a diminished capacity to synthesize new muscle in response to anabolic stimuli when compared directly to their younger counterparts. For example, in response to a single bout of RE (of the same relative intensity) the stimulation of MPS is shorter-lived in older adults (Sheffield-Moore et al., 2005). This response is also accompanied by a greater release of muscle AA and a more vigorous acute-phase response of plasma proteins (particularly albumin), suggesting a differential hepatic and muscle response to RE between young and older adults (Sheffield-Moore et al., 2005). When compared directly to younger adults, older adults also demonstrate a diminished anabolic sensitivity to the consumption of protein via decreased intramuscular expression, and activation (phosphorylation), of the signalling proteins that initiate muscle protein synthesis (Cuthbertson et al., 2005). Additionally, these anabolic deficits were associated with marked increases in NFκB, an inflammation-associated transcription factor (once again providing a link between ageing-related inflammatory responses and a decline in muscle mass). These acute-response investigations are supported by the few longitudinal RE training studies that have directly compared chronic adaptations between young and older adults. For instance, hypertrophy, strength and LBM gains in older adults are smaller in magnitude when compared directly to younger adults (Häkkinen et al 1998b; Lemmer et al., 2001; Dionne et al.,

2004). The most recent example is that by Dionne et al. (2004) who assessed the impact of a 6-month RE program on LBM, resting energy expenditure (REE) and insulin sensitivity (glucose disposal) in 19 younger (18-35yrs) and 12 older (55-70yrs) non-obese caucasian women. In the younger women, the RE program resulted in a gain in body mass (due to an increase in LBM), increased REE and glucose disposal. Conversely, the older women showed a reduction in fat mass and a lesser capacity to gain LBM with no improvement in insulin sensitivity or REE. Thus, younger women appear to possess a greater capacity for metabolic changes in response to RE compared to the older women (Dionne et al., 2004). Aside from a diminished response to anabolic stimuli, other age-related aspects that may influence the hypertrophy response include; a higher concentration of circulating cytokines that retard protein synthesis rates (Visser et al., 2002; Toth et al., 2005), lower concentrations of anabolic hormones in circulation (Kraemer & Ratamess, 2005; Toth et al., 2005), and differences in muscle gene expression (Welle et al., 2003). Although an age-related diminished capacity for hypertrophy is evident, no studies have attempted to assess at what stage in life these chemical and physical alterations take place. Also, it is not known what the main instigators are. For example, could it be hormonal, physical activity or quality of nutrition? Do these factors have a combined effect on gene regulation? If so, what are the genes that are modulated? Recent work has confirmed that a lifelong exercise program offers real protection against the increasing levels of oxidative stress that damage cellular structures and cause the ageing of tissue (Rosa et al., 2005). However, it is not known whether a life-long commitment to RE training may provide a preventative effect against an age-related decline in muscle mass. These are important questions that need to be answered before wide scale prescriptions can be made to an ageing population.

Gender

With regard to sex-specific differences, earlier studies suggested little difference between men and women in their capacity to build muscle mass (Cureton et al., 1988; Staron et al., 1994; O'Hagen et al., 1995). However, a recent study (Hubel et al., 2005) that utilized a large cohort (342 women, 243 men) reported some clear gender-specific differences in strength and hypertrophy development. This longitudinal training study assessed the hypertrophic response to 12 weeks of RE in the upper arm muscles of untrained men and women. Results revealed a 2% difference between men (20%) and women (18%) in hypertrophy that was considered highly significant ($P < 0.001$). Previous studies that examined this topic showed sex-specific differences of 6-7% but due to the smaller (n), these differences were deemed not statistically significant (Cureton et al., 1988; O'Hagen et al., 1995). Therefore, sex-specific differences in hypertrophy may have gone undetected in previous studies due to a lack of statistical power. Hubal et al. (2005) also reported

that the women in their study outpaced the men considerably in relative gains in strength. Therefore, although males probably experience greater absolute increases in hypertrophy, strength and muscle mass (Lemmer et al., 2001), women may experience equivalent or greater changes in strength (Hubel et al., 2005). However, this may also be due to a female's lower initial starting strength level (Hubel et al., 2005). From this study it's also clear that the large variations in hypertrophy that are evident among individuals do not appear to be sex-specific, at least not in healthy young adults (Hubel et al., 2005). No long-term studies (greater than 12 weeks) have examined sex-specific hypertrophy responses to RE. However, cross-sectional data on male and female bodybuilders (Alway et al., 1992) combined with the results of longer term (6 month) training studies on females suggest that the hypertrophy response in females, in the long term, is generally lesser in magnitude than what has been characteristically observed in males (Kraemer et al., 2000; Nindl et al., 2001b). This difference in capacity for hypertrophy could be due to apparent differences in anabolic hormonal concentrations (Kraemer et al 1991; Häkkinen et al., 2000b). For example, circulating testosterone concentrations are generally 10 times higher in males than in females (Wright, 1980).

Endocrine responses

Anabolic hormonal responses are integral in the regulation of tissue growth and energy substrate metabolism and therefore, are thought to play an important role in the hypertrophy response to RE (Kraemer & Ratamess 2005). Plasma concentrations of circulating anabolic hormones such as growth hormone (GH), testosterone, and IGF-1 diminish with age and this has been associated with the age-related decline in muscle mass (Kraemer et al., 1999). However, the contribution of these age-associated hormonal alterations to the size of muscle mass is unclear (Shroeder et al., 2005). While GH is shown to stimulate protein synthesis in humans (Fryburg et al., 1992), cross-sectional (Gallagher et al., 1997; Melton et al., 2000) and longitudinal studies (Morley et al., 1997; Harman et al., 2001) have shown that (serum total and free) testosterone concentrations in particular are, important regulators of net myofibrillar protein balance. Muscle fibre hypertrophy is also shown to be proportional to increases in circulating testosterone concentrations, even in the absence of RE (Sinha-Hikim et al., 2002). The action of growth factors (such as IGF-1) are thought to be secondary to androgen activity (Bamman et al., 2001). Therefore, the acute elevations in circulating testosterone (bound and unbound) that are consistently observed after RE (Kraemer et al., 1990; 1995; 1999; Staron et al., 1994; Häkkinen et al., 1995; McCall et al., 1999; Raastad et al., 2000; Ahtiainen et al., 2005a) appear to be an important component in the development of muscle hypertrophy. However, the role these acute perturbations play in the remodelling/adaptation process is yet to be fully elucidated (Kraemer & Ratamess 2005). What is

clear is that the acute increase in circulating anabolic hormones such as GH, testosterone and IGF-1 that are observed in response to RE are not gender specific — although responses are typically higher in men than women (Kraemer et al., 1991; Häkkinen & Pakarinen 1995; Linnamo et al., 2005). It is also interesting to note that the magnitude of these acute increases are independent of the individual's absolute level of strength (Kraemer et al., 1998a). Some training variables such as, the amount of load used, volume (Kraemer et al., 1990; 1991; Gotshalk et al., 1997; Similos et al., 2003), and exercise selection (Kraemer et al., 1990; Volek et al., 1997a; Hansen et al., 2001), have also been shown to influence the magnitude of the acute hormonal response to RE. What these differences may contribute to chronic adaptations is uncertain as physiological outcomes were not documented in these studies.

The data on RE's ability to influence hormonal responses over the long term, and how this may contribute to changes in muscle mass, is equivocal. For example, while RE training is thought not to alter resting GH or testosterone concentrations (Häkkinen et al., 1987; 2000b; Hickson et al., 1994; Ahtiainen et al., 2003; Kraemer & Ratamess 2005), some studies report an increase followed by a reduction in circulating testosterone in response to different training phases (Raastard et al., 2000; Ahtiainen et al., 2003). Other research suggests that training may improve the acute anabolic hormonal response to an RE workout. That is, higher levels of GH (Rubin et al., 2005) and testosterone and/or lower cortisol concentrations (Kraemer et al., 1995; 1999b; Ahtiainen et al., 2005b) have been observed in trained as opposed to untrained individuals after a workout. Conversely, others report no differences in acute training responses between trained and untrained participants (Ahtiainen et al., 2003). However, it is clear that the hypertrophic response is specific to the loaded muscle(s) (Kraemer & Ratamess 2005). Therefore, activation by a systemic hormone would require load-mediated modulation of the hormone's efficacy in the exercised muscle. The load-mediated modulation of receptor expression and/or binding affinity in muscle has been demonstrated in rodents (Deschenes et al., 1994) and humans during RE training (Bamman et al., 2001; Willoughby & Taylor 2004). This may explain localization of the growth response with elevated blood anabolic hormones. In healthy humans, at least one study has shown that sequential bouts of heavy RE increase blood testosterone concentrations and muscle androgen receptor expression that correspond to subsequent increases in myofibrillar protein (Willoughby & Taylor 2004).

Energy production

Aside from a localized modulation by anabolic hormones, RE is shown to promote other peripheral adaptations that may contribute to, or be a product of, muscle hypertrophy. An earlier examination (McDougall et al., 1977) of biochemical changes within muscle revealed that 5

months of RE increased resting concentrations of ATP (18%) in previously sedentary individuals. An increase in resting ATP from exercise training is thought to be a function of increased mitochondrial number and size. However, some (Hather et al., 1991; Wang et al., 1993), but not all studies (Alway et al., 1988; Chilibeck et al., 1999) have shown that RE-induced hypertrophy is accompanied by a proportional increase in mitochondrial proteins. Unlike endurance training, RE training does not appear to improve the capillary-to-fibre ratio, with similar capillary densities in bodybuilders and sedentary subjects documented (Tesch 1984). Conversely, an increase in fibre CSA in response to an RE training program does not appear to compromise fibre capillarization or oxidative potential in healthy adults (Tesch et al., 1990; Green et al., 1999). The study by MacDougall et al. (1977) also demonstrated that RE may promote an increase in resting concentrations of creatine (Cr) (39%), phosphocreatine (PCr) (22%) and muscle glycogen levels (66%) in previously untrained participants. However, more recent work suggests that chronic RE training probably does not induce further increases in the phosphagen (ATP-ADP and PCr-Cr) (Rawson & Volek 2003), or muscle glycogen (Haff, 2003) reservoirs. Nevertheless, muscle contraction and athletic performance during maximal effort, short term activity (such as RE) is dependant on the maximum rate of ATP regeneration via the phosphagen and glycolysis/glycogenolysis systems.

In this regard, the ATP-ADP system is considered a "co-factor" (albeit an essential one) necessary for biological function within key compartments of the cell that require energy. However, it is now generally accepted that the availability of PCr is most critical to the continuation of muscle force production and performance during high intensity exercise (Balsom et al., 1994; Greenhaff 1997). The PCr-Cr system (encompassing its site-specific CK isoenzymes) plays a pivotal, multifaceted role in muscle energy metabolism. The PCr-Cr system integrates all the local pools (or compartments) of adenine nucleotides; the transfer of energy from mitochondrial compartments to that in myofibrils and cellular membranes as well as the feed back signal transmission from sites of energy utilization to sites of energy production. The main roles of the PCr-Cr system are illustrated in figure 1.4. The first main function of the PCr-Cr system is that of a temporal energy buffer for ATP regeneration achieved via anaerobic degradation of PCr to Cr and rephosphorylation of ADP. This energy buffering function is most prominent in the fast-twitch/glycolytic fibres; these fibres contain the largest pool size of PCr (Tesch et al., 1989). The energy (ATP) required for high intensity exercise is met by the simultaneous breakdown of PCr and anaerobic glycolysis of which the PCr-Cr system provides up to one-third of the total energy required (Greenhaff et al., 1994). The second major function of the PCr-Cr system is that of a spatial energy buffer (or transport system) and involves aerobic metabolism. In this capacity, the

PCr-Cr system serves as an intracellular energy carrier connecting sites of energy production (mitochondria) with sites of energy utilization (Na^+/K^+ pump, myofibrils and the SR) (figure 1.4).

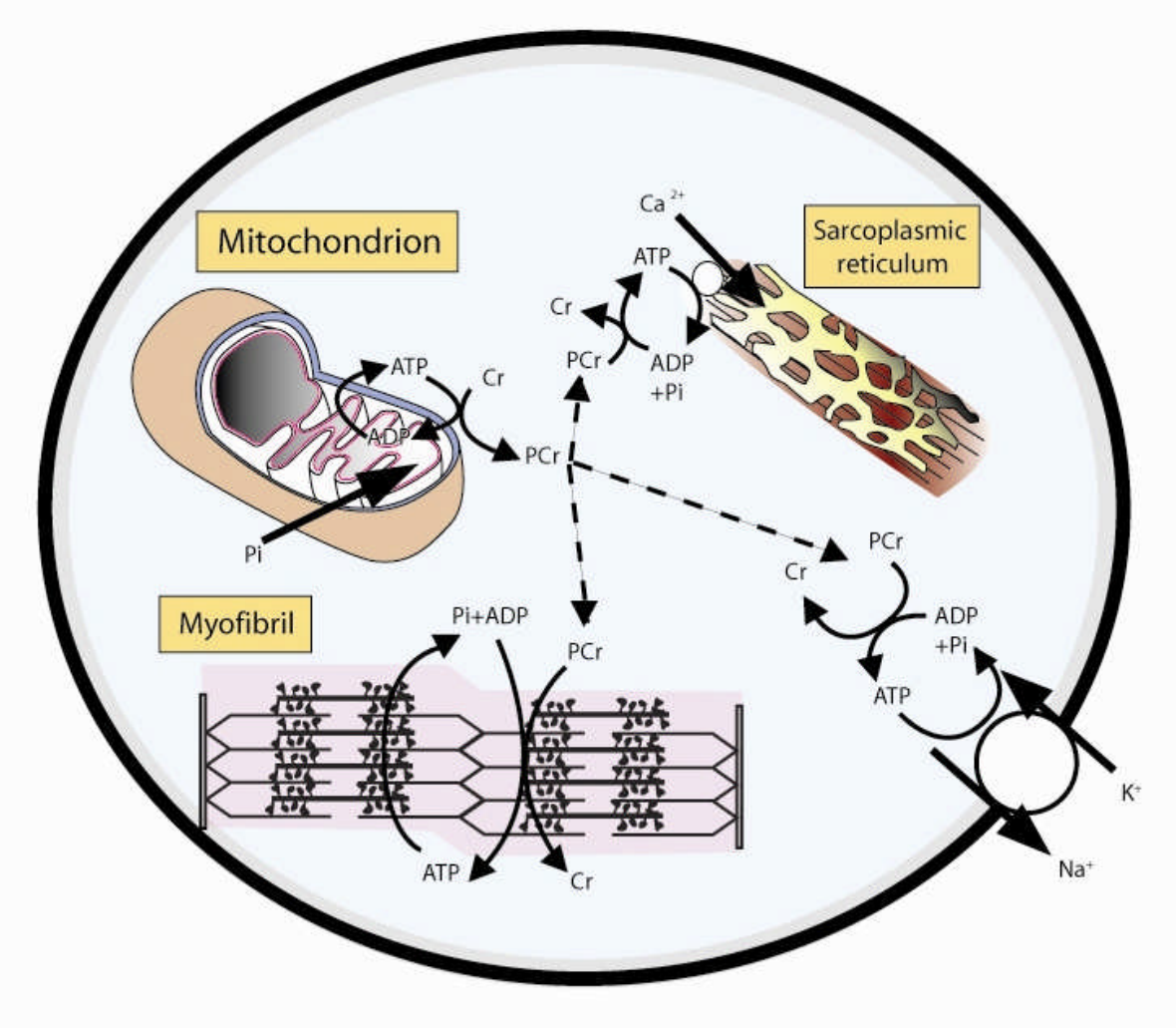


Figure 1.4 The main functions of the Cr-PCr system in a muscle fibre.
The first is that of a temporal energy buffer for the regeneration of ATP via anaerobic degradation of PCr to Cr and rephosphorylation of ADP. The second major function of this system is that of a spatial energy buffer or transport system that serves as an intracellular energy carrier connecting sites of energy production (mitochondrion) with sites of energy utilization, such as Na^+/K^+ pump, myofibrils and the SR.

This process has been coined the Cr-P_i shuttle (Bessman & Geiger 1981) to describe the specificity of this system. Meaning, Cr literally shuttles energy from the mitochondrion to highly specific sites and then returns to regenerate energy exactly the equivalent to its consumption at those sites (Bessman & Geiger 1981). These reactions can only occur via compartment-specific CK isoenzyme located at each of the energy producing or utilizing sites that transduce the PCr to ATP (Wallimann et al., 1992). The mechanism of action of all the CK isoenzymes involves functional coupling with adenine nucleotide translocase (ANT) in the mitochondria and with ATPases in myofibrils and cellular membranes based on the metabolic channeling of adenine nucleotides (Vendelin et al., 2004). This mitochondrial-based energy transport system may be more prominent in the slow-twitch fibres as these fibres characteristically contain a smaller PCr pool and a relatively higher mitochondrial CK isoenzyme compared to the fast-twitch fibres. Conversely, the percentage of cytosolic CK is smaller in the slow-twitch fibres compared to the more glycolytic fibres (Wallimann et al., 1992). Another function of the PCr-Cr system is the prevention of a rise in ADP that would have an inhibitory effect on a variety of ATP-dependant processes, such as cross-bridge cycling. A rise in ADP production would also activate the kinase reactions that ultimately result in the destruction muscle adenine nucleotides (Greenhaff, 1997). Therefore, the removal of ADP via the CK reaction-induced rephosphorylation serves to reduce the loss of adenine nucleotides while maintaining a high intracellular ATP/ADP ratio at the sites of high energy requirements (Hultman & Greenhaff 1991).

The CK reaction during the resynthesis of ATP takes up protons (Wallimann et al., 1992) and therefore, another function of this PCr-Cr system is the maintenance of pH in exercising muscle. In a reversible reaction (catalysed by the site specific CK), Cr and ATP form PCr and ADP (figure 1.5). The formation of the polar PCr "locks" Cr within the muscle and maintains the retention of Cr because the charge prevents partitioning through biological membranes (Greenhaff, 1997). When pH declines (i.e., during exercise when lactic acid accumulates), the reaction will favour the generation of ATP. Conversely, during recovery periods (i.e., periods of rest between exercise sets), where ATP is being generated aerobically, the reaction will proceed toward the right and increase PCr levels. The notion that maintenance of PCr availability is crucial to continued force production and performance during high intensity exercise is further supported by research that demonstrates the rate of PCr utilization is extremely high during the first seconds of intense contraction— high anaerobic ATP regeneration rates result in a 60-80% fall in PCr (Bogdanis et al., 1995). Not only is the depletion of muscle PCr associated with fatigue (Hultman & Greenhaff 1991), the resynthesis of PCr and the restoration of peak performance are shown to proceed in direct proportion to one another, despite low muscle pH during recovery (Bogdanis et al., 1995).

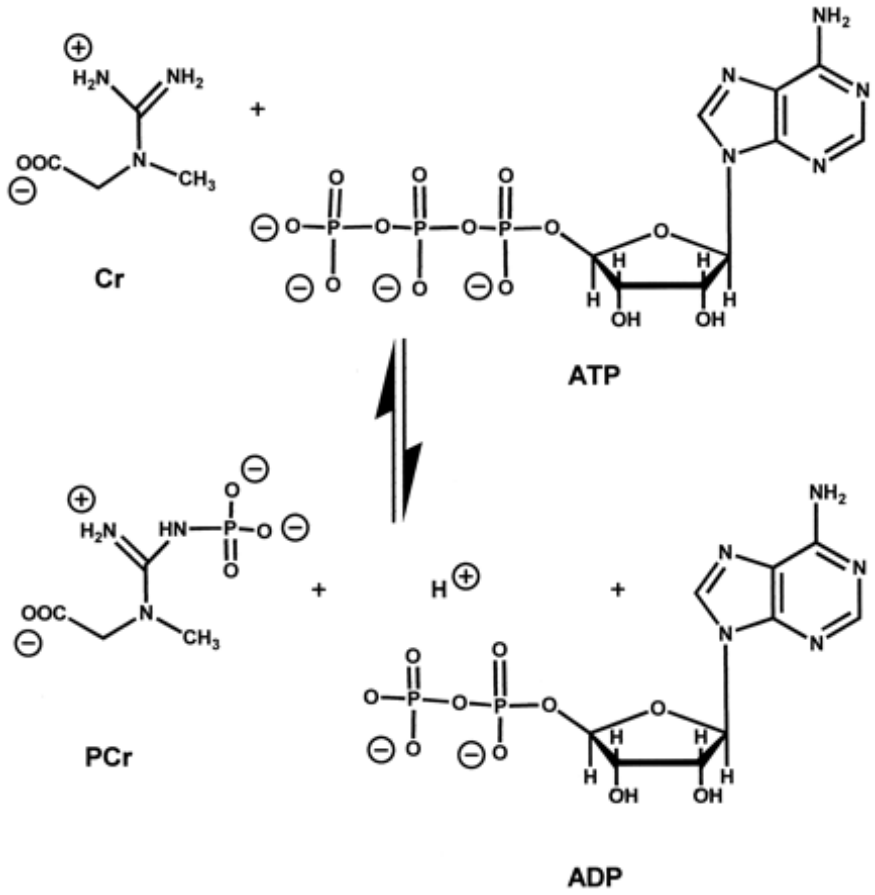


Figure 1.5 The reversible phosphorylation of Cr by ATP to form PCr and ADP

In fact, PCr availability correlates with performance during short term power production (Bogdanis et al., 1995). Therefore, energy supply appears to be more critical for generating power than the direct effect of protons on contractile mechanisms. While the Cr-P_i shuttle clearly plays an integral role in the efficient delivery of energy for muscle contraction, this system may also serve another important anabolic function and that is the supply of energy for the synthesis of muscle proteins during hypertrophy (Bessman & Savabi 1988).

Once Ingwall et al. (1974; 1976) demonstrated that increasing Cr availability selectively stimulated the rate of synthesis of contractile proteins (actin and myosin) in culture, Bessman et al. (1980) proposed that the Cr-P_i shuttle may play a role in contractile-specific protein synthesis via an increase in PCr "traffic" within the intervening space between the mitochondrion and the contractile apparatus during muscle contraction. These researchers postulated that a protein synthesizing microsome lay adjacent to, or may even be a part of creatine-phosphokinase (CPK) site at the myofibril where it would receive some of the ATP liberated when this CK isoenzyme transfers ATP to the cross-bridge binding site (Bessman et al., 1980). Therefore, this protein synthesizing complex would benefit from an increase in Cr-P_i shuttle activity that would occur during contractile activity. The researchers demonstrated evidence of this protein synthesizing component via two experiments. Firstly, protein synthesis was measured in muscle and in hepatocytes (*in vitro*) as affected by fluorodinitrobenzene (FDNB). Increasing concentrations of FDNB inhibited muscle protein synthesis in parallel with the inhibition of CPK activity but had no effect on protein synthesis in hepatocytes (which have little CPK activity) (Carpenter et al., 1983). A second set of experiments by this group were even more specific; they demonstrated that PCr was a better energy donor for protein synthesis by microsomes than the ATP-ADP system (Savabi et al., 1988). The general concept put forward by Bessman's group is that net protein synthesis is affected only by the increase of energy supplied to the contractile apparatus (Bessman & Savabi 1988). This is supported by studies that suggest increasing Cr availability increases myosin synthesis and/or differentiation of myogenic satellite cells (Ingwall et al. 1974; 1976; Young & Denome 1984; Vierck et al., 2003). Therefore, while PCr availability appears to be all-important to maintenance of muscle force production and performance during high intensity exercise, it may also play a role in the synthesis of muscle protein, and therefore, hypertrophy during RE. A revelation in exercise physiology in recent decades has been the confirmation that resting concentrations of PCr and Cr can be increased via dietary supplementation (Balsom et al., 1994). This strategy is shown consistently to provide an ergogenic benefit, particularly during repeated bouts of high intensity muscle contraction (Terjung et al., 2000). Supplementation to specifically increase PCr availability and augment muscle hypertrophy during RE is an important aspect of this dissertation and is covered in greater detail later in this chapter (section 1.9).

Along with the phosphagen system, glycolysis and glycogenolysis are considered to be important energy contributors during RE (Lambert & Flynn 2002). In fact, during one set of 12 maximum-effort repetitions in the arm curl exercise, just over 82% of ATP demands were estimated to be met by glycogenolysis (MacDougall et al., 1999). A single bout of high-intensity RE characteristically results in a significant reduction in muscle glycogen of 30-40% (Robergs et al., 1991; Tesch et al., 1998; MacDougall et al., 1999; Haff et al., 2000). As little as 3 (maximum-effort) sets of 12 repetitions in the barbell curl exercise can reduce glycogen stores in the biceps by 25% (MacDougall et al., 1999). This RE-induced reduction in muscle glycogen is particularly evident in type-II muscle fibres (Tesch et al., 1998). While these fibres contain a higher concentration of glycogen than the type-I fibres (approx. 26%), the type-II fibres also exhibit a much higher rate of glycogenolysis (64%) during intense activity (Greenhaff et al., 1991). A preferential depletion of glycogen in type-II fibres has also been demonstrated after other forms of high-intensity, short term exercise (Green, 1978; Vollestad et al., 1992). The type-II fibres are responsible for maximum force production, and low glycogen levels in these fibres have been associated with compromised performance during RE (Lambert et al., 1991; Haff et al., 1999; Leveritt & Abernethy 1999). Muscle damage is reported to increase the requirement for carbohydrate (CHO) for optimal glycogen synthesis (Costill et al., 1990), and the muscle damage that occurs from RE may reduce the capacity to store glycogen (O'Reily et al., 1987). Alternatively, high muscle glycogen stores appear to offer an ergogenic benefit during high intensity exercise (Balsom et al., 1999). Increasing CHO ability before (Lambert et al., 1991), during (Haff et al., 2001), and after RE (Haff et al., 1999) results in improved work capacity. Therefore, the implementation of a CHO supplementation regime during RE training may prevent decreases in performance and stimulate an increase in muscle glycogen resynthesis as well as improve the potential for greater physiological adaptations. However, dietary strategies that promote the maintenance or increase of muscle glycogen during RE is a topic that has received little investigation (Haff, 2003). Clearly, muscle glycogen is an important fuel source during RE, the amount of glycogen stored within muscle has the potential to influence force production, work capacity and therefore chronic adaptations (i.e. hypertrophy and strength). For these reasons, strategies that promote the maintenance or increase in muscle glycogen during RE training warrant investigation.

The relationship between strength and hypertrophy

Maximal voluntary strength is typically measured by repetition maximum (RM) or isometric/dynamic torque (Kraemer et al., 1995; Aagaard et al., 2002). Muscle hypertrophy and strength development are closely related. For example, force production is usually proportional to

muscle fibre CSA (Kraemer, 2000). An increase in muscle fibre CSA is thought to underline most of the improvements in force production and strength that are achieved during RE training (Shope et al., 2003). However, hypertrophy also contributes to improved force production by altering muscle architecture such as, an increase in the pennation angle and fascicle length (increase in the number of sarcomeres in series) of pennate muscles (Kawakami et al., 1993; 1995; Kearns et al., 2000; Kumagai et al., 2000; Aagaard et al., 2001). These alterations in muscle architecture appear to play an important role in expression of functional strength (Brechue & Abe 2002). Hypertrophy increases the pennation angle of pennate muscles that results in an increase in force production (Kearns et al., 1998). Although it is the changing pennation angle that allows for greater sarcomere packing per CSA, the benefit of greater fascicle length in these muscles is the maintenance of an increase in force per CSA; greater fascicle length limits the change in pennation angle associated with muscle hypertrophy to improve force production per CSA (Kearns et al., 1998, 2000; Kumagai et al., 2000). To underline the influence of fascicle length in functional strength, a study of experienced weight-lifters demonstrated that greater fascicle lengths in the triceps and vastus lateralis was closely related to greater 1RM strength in the barbell squat, deadlift and bench press (r values ranged from 0.63 to 0.54; $P < 0.01$) (Brechue & Abe 2002). While an increase in fascicle length is an alteration in muscle architecture that is considered an important contributor to improved force production and functional strength (Abe et al., 1999; Kearns et al., 2000; Kumagai et al., 2000), the magnitude of this adaptation is positively associated with increases in LBM (Brechue & Abe 2002). Therefore, it is clear that muscle morphology provides important contributions to the development of strength during RE training. However, neural adaptations are also thought to play a prominent role in strength development, both in the initial stages of a training program (Morianti & DeVries 1979; Carson & Reik 2001; Carroll et al., 2002) and in the longer term (Sale, 1992; Häkkinen et al., 1988; 1998b).

The capacity to generate force is essentially dependent on motor unit activation (Sale, 1992). The motor unit serves as the functional unit of the neuromuscular system. In most mammalian skeletal muscles, motor units are comprised of a single motor neuron and the multiple muscle fibres that it innervates (McComas, 1996). Motor unit populations differ between muscles; in general, small muscles such as the external rectus of the eye, lumbricals, and interossei muscles have few muscle fibres per motor unit (100 or less), whereas larger muscles such as the medial gastrocnemius can have up to nearly 2,000 muscle fibres per motor unit (Lee et al., 1975). To initiate movement, motor units are recruited according to their size; from small to large (Henneman 1965; Sale, 1992). Maximal force production requires the recruitment of all motor units. This includes the high threshold motor units that must be recruited at a high-enough firing rate to produce maximal force (Sale, 1992). Untrained individuals appear not to be able to voluntarily

recruit the highest-threshold motor units (Moranti, 1992). Training often results in greater force production, in the presence or absence of hypertrophy (Morianti & DeVries 1979; Abernethy et al., 1994; Phillips, 2000). Therefore, a large part of the strength improvements observed in response to RE is thought to be a result of an improved ability to recruit all motor units that initiate and control movement (Rutherford & Jones 1986; Akima et al., 1999; Leong et al., 1999). RE training is shown to enhance neural drive (i.e., recruitment and rate of firing) (Aagaard et al., 2002), improve recruitment order efficiency (Sale, 1992) and increase the synchronization of the motor units (Milner-Brown et al., 1973; Felici et al., 2001). This aspect is an important component of force development as greater synchronization ensures that a greater number of motor units are firing at any one time. Strength improvements from RE training can also be linked to an improvement in the deactivation of antagonist muscles along with the improved activation of agonist muscles (Häkkinen et al., 1998a) as well as decreased Golgi tendon organ inhibition (Sale, 1992). Training can also alter the manner in which muscles are recruited by the central nervous system (Carroll et al., 2001). This is associated with a change in the input–output properties of the corticospinal pathway, such that a greater degree of muscle activation is generated by the same amount of cortical input (Carroll et al., 2002). A reduction in the cortical input is necessary to elicit a given level of force that may serve to benefit the production of coordinated movements by reducing the level of central drive and thus minimizing the potential for functional interference within the motor cortex (Carson et al., 1996; 1998). Therefore, it is apparent that both hypertrophy and neural factors influence the expression of strength. However, the magnitude to which each of these aspects contributes to strength development during a training program is not clear.

Training status

Untrained individuals or "novices" (those with no RE training experience or who have not trained for several years) generally respond readily with significant improvements in strength and hypertrophy to a wide variety of RE protocols (Gettman et al., 1978; Morganti et al., 1995; Campos et al., 2002; Goto et al., 2005). Strength gains develop rapidly in untrained participants; they are clearly evident within the first 4-8 weeks of training (Hickson et al., 1994; Staron et al., 1994; O'Bryant et al., 1998) and neural adaptations are thought to play a prominent role in this early stage. It is presumed that muscle hypertrophy follows improvements in strength as hypertrophy becomes evident 6 to 8 weeks into an RE program (Phillips, 2000). However, the contribution of neural and morphological adaptations to strength development can be influenced by the complexity of the exercise (Chilibeck et al, 1998). For example, one study has shown that over a 20 week training period, hypertrophy of the upper extremity muscles occurred during the first 10 weeks whereas the lower extremity and trunk muscles did not hypertrophy until the last 10 weeks

(Chilibeck et al, 1998). The authors of this research believed that for simple motor tasks such as the biceps curl exercise, early gains in strength may occur concurrently with muscle hypertrophy. On the other hand, more complex movements associated with the trunk (e.g., bench press) and legs (e.g., leg press) require a longer period for neural adaptation, and thus delay muscle hypertrophy. However, the reasons for this are unclear.

"Trained" or "experienced" individuals have been defined as those with approximately 6 months of consistent RE training (Kraemer et al., 2002). The term "advanced" or "highly trained" refers to those individuals with years of consistent training experience who have also attained significant strength and/or muscle hypertrophy (Häkkinen et al., 1988). Trained individuals are generally considered to have much slower rates of improvement than untrained individuals (Giorgi et al., 1998; Häkkinen et al., 1998b; Schiotz et al., 1998), but very little is known about neurological and hypertrophy contributions in the development of strength in these participants. Several time courses in the literature propose that generally, neural adaptations and changes in protein quality (i.e., myosin isoforms) provide most, if not all the early strength improvements observed in the initial stages of a training program (Moritani, 1992; Sale, 1992; Wathen et al., 2000). The contribution of neural factors is thought to diminish as training continues beyond 6 to 8 weeks (Sale, 1992; Moritani 1992; Wathen et al., 2000). Histochemically determined fibre CSA studies show that hypertrophy becomes evident after 6 weeks of training but this response is also suggested to plateau after 12 to 16 weeks of training. Therefore, according to the most of the timelines proposed in the literature (Sale, 1992; Wathen et al., 2000; Fleck & Kraemer 1997), little or no significant improvements in strength or hypertrophy occur after 16 weeks of training! In fact, the use of anabolic drugs has been proposed as the only way a trained individual may experience significant hypertrophy (Sale, 1992). Nevertheless, further (significant) hypertrophy has been reported with continued training (after the initial 6 weeks) in both men and women (Frontera et al., 1990; Staron 1990; Kraemer et al., 1995; Green et al., 1999). In a year-long RE training program, older men and women were shown to increase muscle strength within the first 8 weeks but muscle fibre hypertrophy did not occur until after 30 weeks of training; well after most of the strength gains had occurred (Pyka et al., 1994). There is also evidence that suggests the hypertrophic response can continue with chronic training. For instance, subjects who have participated in resistance training programs spanning several years (Alway et al., 1988; Klitgaard et al., 1990; Kadi et al., 1999) typically have larger muscle fibres than previously sedentary individuals who have trained for only 3–6 months (MacDougall et al., 1980; Kraemer et al 1985; Alway et al., 1989; McCall et al., 1996). Additionally, significant hypertrophy and strength improvements have been documented in longitudinal studies involving trained (Häkkinen et al., 1985; Staron et al., 1991; McCall et al., 1996; Volek et al., 1999; Cribb et al., 2006) and even highly-trained

individuals (Häkkinen et al., 1988). However, it also appears as though these athletes are also able to experience significant neurological adaptations that result in strength improvements over the longer term (one to two years) (Häkkinen et al., 1988). Therefore, although the adaptations maybe less in magnitude, clearly RE-trained individuals still possess the capacity for neurological and morphological adaptations that result in significant improvements in strength and hypertrophy.

While training is presumed to diminish the capacity for significant hypertrophy (Sale, 1992; Kraemer et al., 2002), an alternate consideration is that the methodologies used to estimate hypertrophy have not been sensitive enough to detect small but significant increases in protein accretion. For example, more recent AA (isotope) kinetic studies clearly show that a net gain in muscle protein is possible after a single workout (if AA are supplemented at this time) (Tipton et al., 1999; 2001; 2003). By definition, this is hypertrophy.

The idea that the hypertrophy response to RE training may be improved under certain conditions is a consideration that has only received attention by the scientific community in recent years (Volek, 2004). An aspect that warrants consideration when discussing chronic adaptations to prolonged RE training is that many earlier longitudinal studies did not control some important variables that are now known to influence strength and hypertrophy development. For instance, it is well-established that an improvement in strength will increase the potential for hypertrophy; strength gains usually lead to increases in muscle CSA (Atha, 1981; Saltin, 1983; Tesch, 1992). However, the level of supervision of participants during RE training studies has been identified as a limiting factor in strength development (Mazzetti et al., 2000). A personal training approach to RE supervision (i.e., one-to-one or one-to-two instruction during each workout) is shown to ensure better control of workout intensity and greater strength improvements during training (Mazzetti et al., 2000). Yet, very few training studies document this level of supervision in their research protocols. It is also clear that the type, timing and quantity of macronutrient intake can potentially influence strength and hypertrophy development (as discussed in section 1.5 of this chapter). However, many of the longitudinal RE training studies that form our current body of knowledge and shape our current perceptions do not mention any attempt to control or monitor dietary intake (Häkkinen et al., 1985; 1987; 1991; 1995; 1996; 1998; 2001; Staron et al., 1990; 1991; 1994; Hather et al., 1991; Adams et al., 1993; Ploutz et al., 1994; Hortobagyi et al., 1996; Ostrowski et al., 1997; Chestnut & Docherty 1999; Takashi et al., 2000; Abe et al., 2000; Campos et al., 2002). Therefore, it is quite possible that much of the research that has assessed strength and hypertrophy may have overlooked some important variables that can influence the chronic adaptations to RE training. To support this view, recent studies that have examined the effects of strategies designed to optimize the hypertrophy response have revealed a much greater capacity for improvements than

previously assumed. For example, although chronic training is presumed to diminish the hypertrophic response, training studies that have utilized a personal-training approach to supervision of trained participants and dietary intervention have reported average increases in muscle fibre CSA of 35% (for all fibre types) (Volek et al., 1999), and gains in LBM of up to 6% (Cribb et al., 2006). These responses were previously thought only to be possible via the use of anabolic steroids (Sale, 1992). Obviously, there are limitations to the extent of any physiological adaptation that can be achieved from physical training. However, it is equally apparent that we know very little about the aspects that may improve the hypertrophy response from RE. Therefore, the typical expectations (and limitations) that are currently associated with muscle hypertrophy may be some what premature.

1.4 Resistance exercise, protein turnover and muscle hypertrophy

Any adaptive change in muscle mass such as hypertrophy must involve alterations in protein turnover. That is, the difference between rates of MPS and muscle protein breakdown (MPB) determines net protein balance (NPB) (Rassmussen & Phillips 2003). A single bout of RE results in the acute stimulation of MPS (50-100% above basal values), that peaks within 3-24 hours and remains elevated at a diminishing rate for up to 48 hours post-exercise (Chesley et al., 1992; Biolo et al., 1995; Phillips et al., 1997; Yarasheski et al., 2001). However, studies that have assessed the rate of MPB and MPS simultaneously after a single bout of RE in both the fed (Phillips et al., 2002), and fasted state (Biolo et al., 1995; Phillips et al., 1997), demonstrate that both processes are stimulated equally so that NPB remains negative. A positive NPB is not obtained until exogenous AA are provided (Biolo et al., 1997). The muscle degradation that occurs in response to RE is thought to be an important component of the remodelling response (Laurent & Millward 1980). However, it is the stimulation of MPS that appears to be the facilitating process that underlines net protein accretion and hypertrophy (Rennie et al., 2004; Cuthbertson et al., 2005). Early research established that in response to loading, an increase in muscle mass correlated with the magnitude of stimulation of MPS (Goldberg, 1968). Since then, other studies have confirmed that the rate of MPS is the critical regulatory event that leads to load-induced hypertrophy (Wong & Booth 1990; Phillips et al., 1997; Baar & Esser 1999). While these studies examined the impact of RE on mixed MPS, more recent work has confirmed that strenuous exercise can stimulate an increase in myofibrillar, sarcoplasmic and connective tissue protein synthesis rates (Louis et al., 2003; Miller et al., 2005; Mittendorfer et al., 2005). Chronic training also appears to influence this response. For example, untrained participants demonstrate large increases in both contractile and non-contractile MPS (Louis et al., 2003; Mittendorfer et al., 2005). However, after an 8 week training program, this acute response is modulated so that the stimuli

becomes preferential toward an increase in contractile synthesis more so than non-contractile proteins (Kim et al., 2005). This recent finding confirms previous studies that reported the stimulation of mixed MPS in response to RE was most likely to be an increase in MHC protein synthesis (Hasten et al., 2000; Balagopal et al., 2001). However, while these studies provide important insights into the acute physiological responses to RE, very little work has examined the chronic effects (longer than 8 weeks) of training on muscle protein turnover.

One cross-sectional study has demonstrated a comprehensible training effect on protein turnover (Phillips et al., 1999). That is, in response to a single workout, RE-trained participants demonstrated smaller increases in MPS rates and little or no change in rates of MPB, unlike untrained participants who showed large increases in both rates of MPS and MPB (while working at the same relative exercise intensity) (Phillips et al., 1999). Data obtained from a subsequent longitudinal study by the same researchers confirmed that the magnitude of stimulation of mixed MPS in response to the same absolute load is diminished after 8 weeks of training (Phillips et al., 2002). However, the training program still provided significant increases in protein turnover rates (both MPS and MPB) at rest and in the hours after RE (Phillips et al., 2002). These increases were also noted in the previously mentioned cross-sectional study but did not reach statistical significance (Phillips et al., 1999). Therefore, a RE training program is capable of evoking a substantial increase in resting and post-exercise protein turnover. However, recent work by Kim et al. (2005), suggests that this increase may be modulated by training. Kim et al. (2005), employed a single leg RE training protocol for 8 weeks (the collateral leg served as the control) and results showed that the training program attenuated the acute large increase in mixed MPS. However, the stimulatory response in contractile protein synthesis rates was of similar magnitude in both the trained and untrained leg muscles after the program (Kim et al., 2005). Another interesting finding was that training resulted in an increase in resting mixed MPS rates, which is indicative of an extensive remodelling response (Rassmussen & Phillips 2003) but resting myofibrillar protein synthesis rates remained unchanged. While these results may appear to be contradictory, it may be that training attenuates the large acute response in mixed MPS observed in untrained participants (Phillips et al., 1997; 1999; 2002) but not the stimulatory effect on myofibrillar protein synthesis rates (Kim et al., 2005). Once again it is important to remember that this 8 week study utilized (previously) untrained participants. There is no longitudinal data on the effects of RE on muscle protein turnover in RE-trained individuals. Therefore, we do not know if these responses may change with prolonged training or if different RE programs are capable of altering these responses and adaptations.

The potent stimulatory effect of RE on protein turnover is indicative of an extensive muscle protein remodelling process. An important part of this remodelling process is not only the repair of extensive ultrastructural damage but also degradation and removal of damaged proteins before new proteins can be incorporated into the contractile machinery. This extensive degradation process that must occur requires activation of proteolytic pathways (DeMartino & Ordway 1998; Ordway et al., 2000). Moreover, as damage appears to be reduced after subsequent bouts of muscle contraction (Ebbling & Clarkson 1989; Clarkson et al., 1992; Willoughby et al., 2003), this suggests an adaptive response within the actual pathways of protein degradation. Intriguingly, this "protective response" has been documented after just one subsequent bout of RE performed almost 6 weeks after a previous bout (Stupka et al., 2001). The biochemical characteristics of this adaptation include an attenuated release of CK, changes in the inflammatory response and ubiquitin-conjugated protein content (Stupka et al., 2001) as well as reduced expression of ubiquitin, E2 protein, and 20S proteasome mRNAs (Willoughby et al., 2003). While extracellular (inflammatory activation such as lysosomes) and intracellular (ubiquitin) proteolytic pathways exhibit adaptive responses, the mechanism(s) of proteolysis in response to chronic loading activity such as RE, are not well understood (Reid, 2005).

For instance, the signals generated during muscle contraction that modulate these pathways (and pathway-related gene transcription) are not clear. In particular, it is uncertain what the molecular modifications that stimulate ubiquitin conjugation to muscle proteins are, or the rate-limiting step(s) in targeting and degradation of substrate proteins (Reid, 2005). In comparison, the mechanisms that underline MPS (the transcription and translation of mRNA into protein) have been elucidated to a much higher degree than proteolytic responses. This is mainly because the protein-synthetic machinery forms a cohesive metabolic unit with the ribosome and the endoplasmic reticulum. For instance, regulatory changes in the machinery of protein synthesis can be linked relatively clearly with an increase in the synthesis of protein or phosphorylation of a signalling complex, whereas in the case of proteolytic pathways, a change in the activity of key components of the system may occur with no, or apparently opposite, changes in the extent of net protein balance (Attaix et al., 2001). However, it is clear that the autophagic-lysosomal pathway is responsible for the bulk of proteolysis (Kadowaki & Kanazawa 2003), and the ubiquitin-proteasome pathway plays a significant role in the fine control of the degradation of specific proteins (Lecker et al., 1999). The ubiquitin-proteasome pathway probably also has a prominent role in the contraction-induced remodelling of muscle (Ordway et al., 2000). Additionally, the autophagy system appears to be physiologically controlled by plasma AA. Recently, the amount of leucine within muscle, but also other AA such as glutamine, tyrosine, phenylalanine, proline, methionine, and histidine in the liver, has been identified in the regulation of this process

(Kadowaki & Kanazawa 2003). However, despite a concerted effort over the last 30 years to examine this key aspect of muscle protein metabolism (Waterlow 1995; Carson et al., 1997; Baar et al., 1999; Kadowaki & Kanazawa 2003; Rennie et al., 2004), in general, this appears to be all that is known about the proteolytic mechanisms that underline the adapatational responses to RE.

1. 5 The molecular events associated with hypertrophy

Significant advances in our understanding of the processes responsible for muscle hypertrophy are mainly due to a greater understanding of the mechanisms that underline MPS. Once MPS was recognized as a critical regulatory event for load-induced changes in muscle mass, this response became a major focus that has lead to an increased knowledge base in the logistics that control the size of human muscle mass. Therefore, this section will focus exclusively on the molecular processes and pathways that regulate MPS in response to RE and nutrient consumption. The paragraph below provides a very simplistic overview of the molecular processes involved and this is followed by a brief discussion of the main events.

RE induces mechanical stress (tensile, compressive and shear) and transient changes in sarcoplasmic calcium concentrations, energy substrate levels, the re-dox state as well as increases the availability of hormones and cytokines (No.1 figure 1.6). Sufficient changes to any (or all) of these variables are thought to activate a network of signal transduction pathways that transfer the mechanical stimuli into specific chemical signalling within the cell. Once activated, these pathways activate transcription factors that alter the expression of muscle genes within the nucleus (No.2 figure 1.6). Active, nuclear transcription together with receptor binding of muscle growth-factors, androgens and glucocorticoids change the expression of the major muscle growth regulators such as IGF-1/MGF, myostatin and other muscle genes (No.3 figure 1.6). Whereas IGF, insulin and RE all appear to (partially) recruit the same pathway which activates protein synthesis via increased translational initiation (No.4 figure 1.6), AA intersect and stimulate MPS via mTOR; a central protein in the signal transduction pathway of MPS (No.5 figure 1.6). While AA activate mTOR, an increased energy demand sensed by AMPK inhibits the phosphorylation of mTOR and the stimulation of MPS (No.6 figure 1.6). Growth factors (IGF/MGF) and myostatin appear to play a key regulatory role in the proliferation and fusion of satellite cells to the muscle fibre (No.8 figure 1.6); a process that is considered integral to muscle hypertrophy.

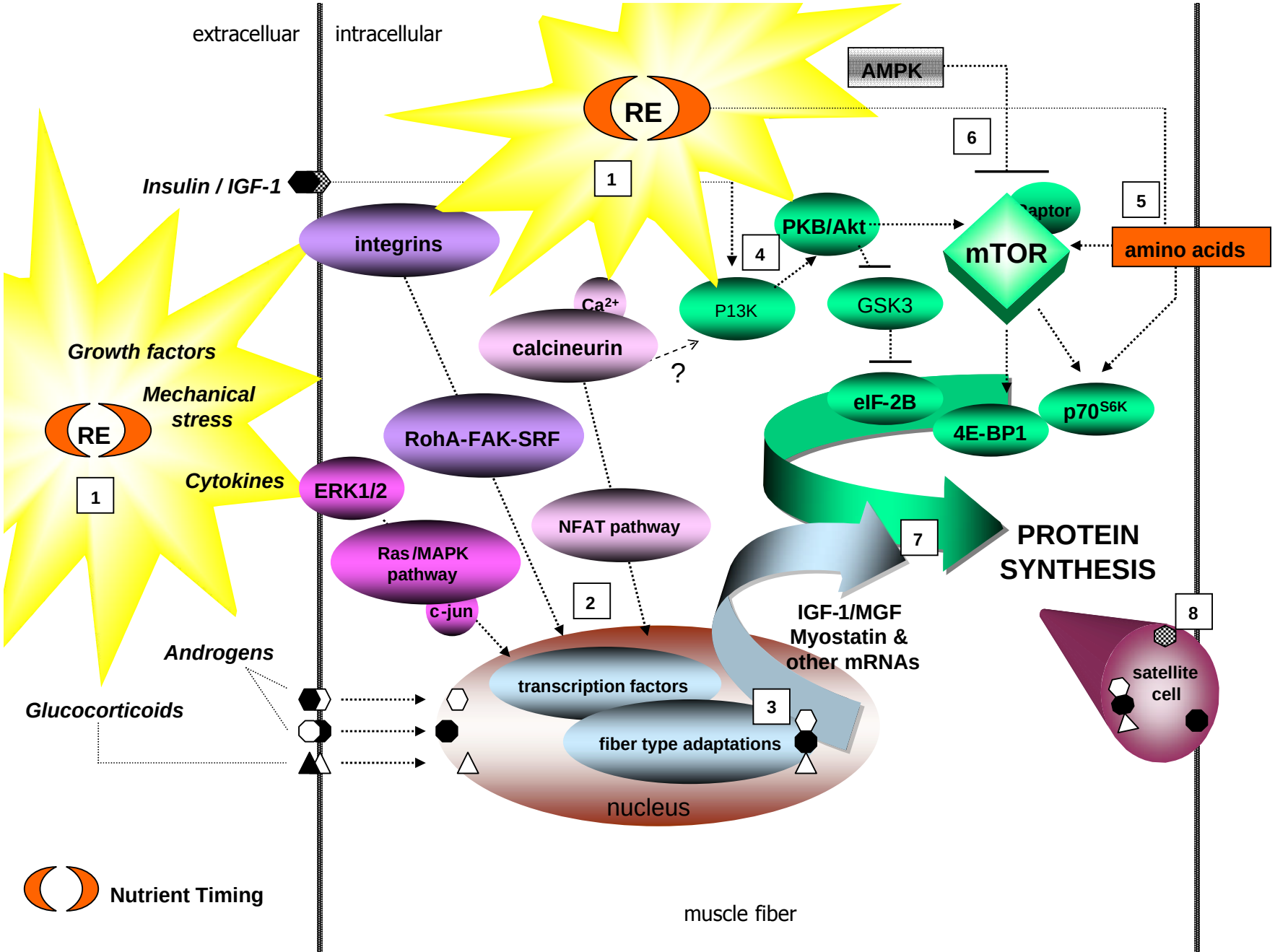


Figure 1.6 Signalling pathways that lead to muscle protein synthesis

The stimulation of protein synthesis within muscle might be mediated by pre-translational (alteration in the abundance of mRNA), translational (alteration in protein synthesis per unit of mRNA) or post-translational (transformation of the protein such as phosphorylation) events (Booth et al.,1998). However, the rate limiting step of MPS appears to be translation initiation (Mathews et al., 1996; Sonenberg 1996); a complex, multiple step process in which the mature mRNA transcript exits the nucleus and is coupled to the ribosomal machinery (No.7 figure 1.6). Translation initiation is thought to be regulated by three key proteins that are in turn controlled by post-translational modification (Merrick, 1992). These three proteins are, eukaryotic initiation factor 2 (eIF-2B) eukaryotic initiation factor 4E binding protein 1 (4E-BP1), and 70-kDa S6 protein kinase (p70-S6k) (Mathews et al., 1996). While eIF-2B is thought to regulate general protein synthesis, 4E-BP1 and p70-S6k control tissue growth-related protein synthesis (Mendez et al., 1997). In particular, p70-S6k has been confirmed as a major regulatory kinase in the activation of MPS in response to RE (Karlsson et al., 2004).

In research that has now proved to be seminal, Baar and Esser (1999) established that phosphorylation of p70-S6k was not only increased following mechanical loading (designed to mimic high intensity RE), the increase in p70-S6k activity correlated with increases in muscle mass. Next it was shown that translation (the actual process of protein synthesis using mRNA as the template and the signal transduction pathways that regulate it), is selectively activated by contractions designed to mimic RE (Nader & Esser 2001). Upstream activators of p70-S6k, such as protein kinase B (PKB) and mammalian target of rapamycin (mTOR) were then identified as crucial for muscle hypertrophy (Bodine et al., 2001; Rommel et al., 2001). For example, specific inhibition of mTOR with rapamycin results in a 95% blockade of hypertrophy (Bodine et al., 2001). Signalling to PKB (also known as Akt) via PI3 kinase is probably involved in this pathway (Rommel et al., 2001). Evidence obtained from *in vivo* and *in vitro* work implicate the phosphorylation of mTOR with the subsequent activation of not only p70-S6k but also 4E-BP1 which allows the association of the ribosomal scaffolding proteins eIF4E and eIF4G. In rodent models, this signalling cascade (i.e., PI3/PKB-mTOR-p70-S6k/4E-BP1) has been linked strongly to the stimulation of MPS in response to mechanical loading designed to mimic RE (Bodine et al., 2001; Pallafacchina et al., 2002; Bolster et al., 2003; Atherton, 2005). In humans, phosphorylation of 4E-BP1 and p70-S6k are shown to be stimulated in parallel with both myofibrillar and sarcoplasmic MPS rates after intense isometric exercise (Rennie et al., 2001). A persistent, long-lasting rise in p70-S6k phosphorylation, with smaller transient rises in PKB/Akt phosphorylation have been observed in humans after more conventional high-overload RE (Cuthbertson et al., 2002; personal communication with MJ Rennie). Therefore, the PI3/PKB-mTOR-p70-S6k/4E-BP1 cascade is a likely pathway that activates MPS in humans in response to RE (No.4 figure 1.6).

Whereas the PI3/PKB-mTOR-p70-S6k/4E-BP1 pathway is associated with muscle growth, the calcineurin/NFAT pathway and to a lesser extent the Ras/MAPK pathway, is thought to control muscle fibre type distribution (No.2 figure 4) (Serrano et al., 2001). Calcineurin is a cytoplasmic calcium-regulated phosphatase that is thought to be activated in overloaded muscles via the chronic increases in intracellular calcium that occur under these conditions (Panchenko et al., 1974). Overload-induced fibre hypertrophy and fibre type transformations are shown to be prevented *in vivo* by administration of calcineurin-specific inhibitors (Dunn et al., 1999). Therefore, calcineurin appears to be crucial in signalling the adaptive responses to RE (Dunn et al., 2000). Once activated, calcineurin is thought to signal downstream genes involved in regulating muscle fibre size via dephosphorylation of its substrate transcription factors such as NFAT (nuclear factor of activated T cells) (Dunn et al., 2000). Additionally, various NFAT isoforms can activate genes which have been implicated in fibre transitions and hypertrophy (Olson & Williams, 2000). Another likely candidate that appears to link mechano-chemical transduction to gene expression and, ultimately, muscle growth is the integrin-meditated RhoA-FAK-SRF pathway (Carson & Wei 2000) (No 2. figure 1.6). The intergrins are membrane-associated proteins that act as primary sensors for relaying an array of physical or mechanical signals from the surrounding environment into the interior of the cell. Serum response factor (SRF) is a transcription factor substrate of FAK that binds to the α-actin gene via the serum response element (SRE1) (Lee et al., 1992). SRE1 is a hypertrophy regulatory element that is thought to activate specific contractile protein genes to produce more mRNA in response to overload conditions (Carson et al., 1995). This pathway provides a transcriptional link between membrane, the genome and subsequent expression of muscle protein (Carson & Wei 2000). Muscle hypertrophy may also require activation of the mitogen-activated protein kinase (MAPK)-signalling cascade. The MAPK pathway (also associated with the extracellular signal-regulated kinase-ERK1/2) (No.2 figure 1.6) is thought to be important to exercise-induced muscle morphology as it activates several myogenic transcription factors (Widegren et al., 1998). Interestingly, a single bout of RE has recently been shown to activate some of the MAPK associated signalling proteins (such as p38) in young but not older adults (Williamson et al., 2003; Karlsson et al., 2004; Creer et al., 2005).

The stimulation of protein synthesis within skeletal muscle by the consumption of a mixed macronutrient meal is due primarily to the essential amino acids (EAA) (Rennie et al., 2006). Of the EAA, the branch chain amino acids (BCAA) are the most potent at stimulating protein synthesis via upregulation of the initiation of mRNA translation (Kimball & Jefferson 2006). The consumption of EAA-containing meals results in the phosphorylation of p70-S6k and the eukaryotic initiation factor proteins eIF4E and eIF4G (Cuthbertson et al., 2005; Rennie et al., 2006); a mechanism that not only promotes global translation of mRNA but also contributes to

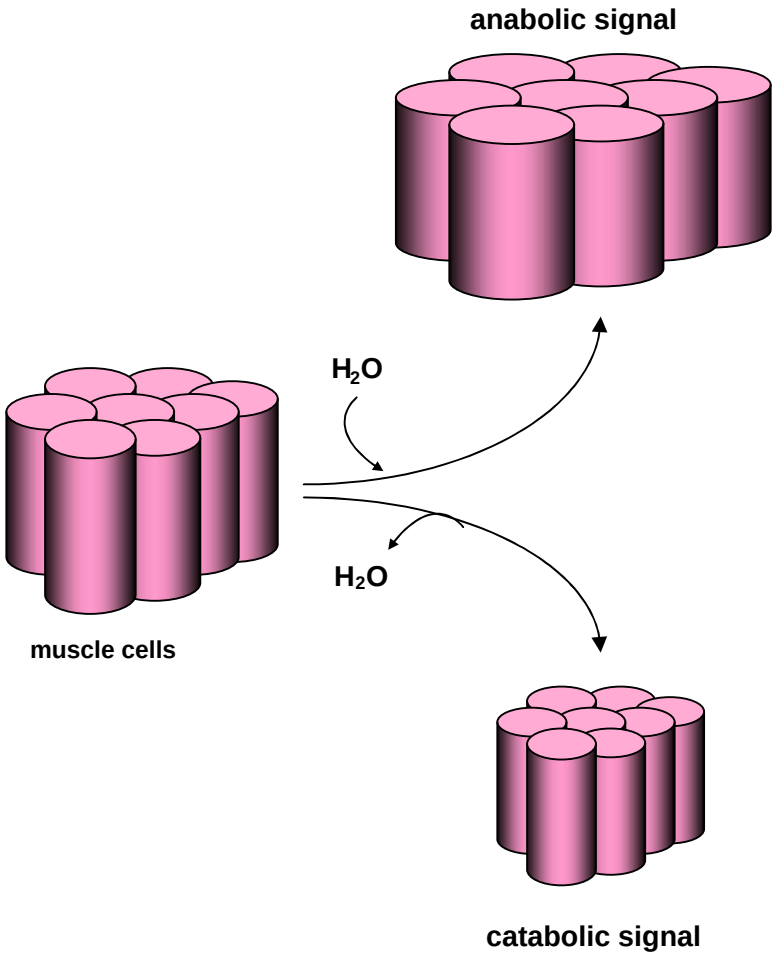
processes that mediate discrimination in the selection of mRNA for translation (Kimball & Jefferson 2006) (No.5 figure 1.6). Insulin stimulates glucose uptake, glycolysis, and glycogen synthesis within muscle via the activation of PI3-PKB(Akt)-GSK-3 signalling pathway (Kimball et al., 2002). While RE also seems to (partially) recruit the same signalling pathway as insulin (No 4. figure 1.6), AA can stimulate MPS without a large amount of insulin (Cuthbertson et al., 2005). In fact, the amount of insulin required for full activation of MPS appears to be quite low (Bennett et al., 1989; Bohe et al., 2003). Unlike RE or insulin, the presence of AA do not appear to stimulate MPS via activation of PI3 and PKB(Akt) (Greiwe et al., 2001b; Liu et al., 2002). It is also clear that the activation of MPS by AA does not involve gene expression (at least in the short term) (Svanberg et al., 2000). However, recent studies in humans (Cuthbertson et al., 2005) have confirmed *in vitro* (Christie et al., 2002) and *in vivo* (Hara et al., 1998; Anthony et al., 2000) work that demonstrates AA stimulate MPS directly via activation of the Raptor-mTOR complex (and its regulatory proteins S6K1 and 4E-BP1) (No.5 figure 1.6).

The large (289 kDa) raptor-mTOR complex is expressed more in muscle than other tissues (Kim et al., 2002). It is nutrient sensitive and contains multiple binding sites (Proud, 2002). The major role of mTOR is to integrate various signals of the energetic status within the cell with environmental stimuli to control cell growth (Deldicque et al., 2005a). The mTOR complex appears to be a focal intersection point in the MPS signalling cascade, that is, both RE and AA activate mTOR (Deldicque et al., 2005a). In rodent muscle, phosphorylation of mTOR is shown to be preferentially localized in the type-IIa (tibialis anterior) fibres (Parkington et al., 2003). This may be one explanation for the greater hypertrophy that is consistently observed in these fibres in response to RE training (Kraemer et al., 1995; McCall et al., 1996; Volek et al., 1999). Whereas AA and RE can activate mTOR, increased energy demand sensed by AMPK inhibits mTOR (Bolster et al., 2002). This may partly explain the high stimulation of MPS that occurs after and not during exercise (Bylund-Fellenius et al., 1984). However, MPS (and hypertrophy) can also be stimulated independently of mTOR (Bodine et al., 2001).

Enhanced activity of eIF-2B is shown to occur after RE (Farrell et al., 1999) or administration of AA (Kimball et al., 1998). The eIF-2B complex is the only one of the three regulators of translation initiation (the rate limiting step of MPS) that is not under direct control of mTOR (Deldicque et al., 2005a). In this pathway (No.4 figure 1.6), PKB phosphorylates and in this instance, inactivates the protein kinase GSK-3 which in turn phosphorylates eIF-2B. Phosphorylation of eIF-2B results in the inhibition of its guanine nucleotide exchange activity, which stimulates global protein synthesis (Bolster et al., 2002). Thus, activation of PKB indirectly results in enhanced eIF-2B activity through inhibition of GSK-3. Some AA such as leucine can

also activate MPS independently of mTOR (and phosphorylation of 4E-BP1 and p70-S6k) (Anthony et al., 2000; 2002). Therefore, it appears that both RE and AA can stimulate MPS via both mTOR-dependant and independent pathways. Intriguingly, the various AA may differ in how they activate MPS (Meijer & Dubbelhuis 2004). For example, the BCAA are thought to be important signalling molecules in translation initiation (Kimball & Jefferson 2006) where as other AA such as Gln may stimulate MPS via an indirect mechanism such as an increase in cell volume (figure 1.7).

Figure 1.7 Regulatory effects of cell volume



Cell swelling increases
Protein synthesis
Glycogen synthesis
Lactate uptake
Pentose phosphate shunt
Amino acid uptake
Ketoisocaproate oxidation
Acetyl-CoA carboxylase
MAP kinase activity
Glutathione (GSH) efflux
Actin polymerization
Microtubule stability
mRNA levels of c-jun and inhibits protein degradation

Cell swelling decreases
Glycogenolysis
Glucose-6-phosphatase activity
Carnitine palmitoylt'ase I activity
Urea synthesis from $NH4^+$
Viral replication

Modulators of cell volume
Insulin/glucogon/IGF-1
Amino acids, particularly Gln
Hydroperoxides (oxidative stress)
Na^+/H^+ and Cl^-/HCO_3^- exchange
K^+ channel blockers
Conjugated bile acids
Energy substrates such as creatine
cAMP

Adapted from research by Lang et al., 1998.

Very small alterations in cell volume (hydration) act as separate and potent regulators of cellular function (Haussinger et al., 1993; 1995). For example, an increase in muscle cell volume acts as an independent, anabolic signal that stimulates a number of anabolic processes such as an increase in AA transport and protein synthesis (Stoll et al., 1992), inhibition of protein degradation (Haussinger et al., 1990; Vom Dahl et al., 1996), and stimulation of glycogen synthesis (Peak et al.,

1992) (figure 1.7). Conversely, a reduction in cell volume evokes catabolic and anti-proliferative responses (Lang et al., 1998). Cell volume is dynamic and changes within minutes under the influence of certain substrates, hormones, AA, glucose and oxidative stress (Lang et al., 1998). In humans, a close relationship between the cellular hydration state in muscle and nitrogen balance has been shown in a variety of cachectic conditions irrespective of the underlying disease (Haussinger et al., 1993). Therefore, cellular hydration's impact on protein turnover may provide a better understanding of the catabolic environment that leads to the excessive muscle degradation that is apparent in various disease states. While the signalling mechanisms that link changes in cell volume to anabolic and catabolic events is unclear, the pathway is thought to resemble that activated by growth factors. That is, phosphorylation of the MAP kinases and c-jun (Agius et al., 1994). The activation of this signalling cascade may also (partly) explain the influence of cell hydration on gene expression (Lang et al., 1998). However, there also appears to be a relationship between proteolytic activity and cellular hydration, regardless of whether the latter is modified by hormones, AA, bile acids, K^+ channel blockers (such as Ba^{2+}) or anisotonic exposure (Lang et al., 1998). Therefore, hydration changes may be the common mechanism that underlines proteolysis control by these heterogeneous effectors. Gln is considered a most potent activator of increasing cell volume (Haussinger et al., 1993). Gln triggers an increase in cell volume via an increase in intra-cellular osmolarity following Na^+ dependant transport across the cell membrane (Low et al., 1996). *In vitro* and *in vivo* studies have demonstrated that the concentration of Gln within various cells (including muscle) regulate cell volume, protein synthesis rates (Haussinger et al., 1995; Low et al., 1996) and nitrogen balance (Haussinger et al., 1993). Recent *in vitro* work has demonstrated the importance of Gln-induced cell swelling in the phosphorylation of mTOR and S6 kinase (Fumarola et al., 2005). In this study, decreasing cell volume (via depravation of Gln) prevented the activation of mTOR and S6 kinase, independent of energy status. Conversely, both complexes were activated by increased AA availability (this was particularly prominent with leucine). The most important finding from this work was that the activation of mTOR and S6 kinase required the presence of Gln and the accompanying increase/maintenance of cell volume that Gln provides (Fumarola et al., 2005).

So far the discussion within this section has identified the signal-transduction pathways that lead to the activation of MPS via RE, insulin or the presence of AA. However, the combination of RE and AA is shown to stimulate MPS beyond the rate that can be achieved by either one alone. The magnitude of this effect appears to be synergistic rather than simply additive (Biolo et al., 1997; Tipton et al., 1999). Recent work suggests this may be due to enhanced phosphorylation of at least one of the key signalling proteins that controls translation initiation (Karlson et al., 2004). When an oral dose of BCAA (total of 100mg/kg) was provided to healthy participants before,

during, and after RE, a greater site-specific phosphorylation of the p70-S6k protein in muscle was observed (Karlson et al., 2004). The bout of exercise alone led to a marked increase in ERK1/2 (p38) MAPK phosphorylation, but this was completely suppressed upon recovery and unaltered by the BCAA. Furthermore, phosphorylation of the ribosomal protein S6 was increased in the recovery period only during the BCAA administration. This study provides further evidence that AA and RE-induced signalling of MPS may converge at one point; the mTOR complex (No 4 and 5. figure 1.6). However, a recent study has shown that muscle glycogen availability can also influence the activation of MPS during RE (Creer et al., 2005). In this study, two groups of athletes (cyclists) performed a single bout of RE. One group performed RE with (diet-induced) low glycogen stores and failed to activate PKB/Akt unlike the group that performed RE with high muscle glycogen levels. However, in this study, the phosphorylation of ERK1/2 (and a ribosomal S6 kinase) was unaffected by glycogen availability (Creer et al., 2005). Therefore, the presence of AA and CHO during RE may provide the best chance of maximal activation of the signalling pathways that stimulate MPS.

Muscle is a tissue in which gene expression is regulated to a large extent by the load-sensitive signalling complexes and pathways discussed in the previous paragraphs of this section. Recovery and adaptation to exercise in general, is associated with transcriptional activation of specific genes involved with muscle growth, vascularization and metabolism (Hargraves & Cameron-Smith 2002). In healthy humans, significant elevations in various immune, metabolic and myo-specific mRNA have been identified in response to a single bout of RE (Chen et al., 2003; Zambon et al., 2003). While this response could occur within minutes of muscle activation (Puntschart et al., 1998), gene expression is predominantly thought to take place in the recovery period; anywhere from 3-12 hours post exercise (Pilegaard et al., 2000) but can be detected up to 48 hours after RE (Hespel et al., 2001). The research to date indicates that large scale changes in transcription regulation play a key role in the metabolic shifts and muscle remodelling responses to exercise training (Pilegaard et al., 2000; Richardson et al., 2000; Chen et al., 2002). While recent work has shown that prior training history can modify the acute gene responses in skeletal muscle to subsequent exercise (Coffey et al., 2005), it is not understood how such transcriptional changes are regulated during training. A large proportion of the changes in protein turnover that are observed in response to RE are probably posttranscriptional, due mostly to increased translational efficiency (Welle et al., 1999). However, using gene chip microarrays, Chen et al. (2002) revealed that RE may lead to selective changes in gene expression through both transcriptional and translational mechanisms.

The work by Chen et al. (2002) showed that 65% of the mRNAs whose abundance was altered by RE, were detected in both the total and polysomal analyses; thus indicating transcriptional mechanisms. However, another 25 mRNAs whose expression in the total mRNA analysis was not altered, but their abundance in the polysomal (i.e., active) fraction changed with exercise; indicating regulation through alterations in translation. Using a small group of young, healthy males, the same research group (Chen et al., 2003) then revealed a high similarity (~50%) in the expression responses observed between human undergoing eccentric contractions and the data obtained from the previous analyses on rats (Chen et al., 2002). Some changes that were specific to humans included greater inflammatory responses and vascular remodelling as well as the confirmation of c-fos as an important transcription factor in the response to exercise-induced damage. However, it is worth noting that the RE protocol used in this research (sitting and rising from a chair 300 times) was more like a test of muscular endurance rather than a bout of high overload RE. Therefore, while this data is novel, the exercise protocol used may limit its application in deciphering the overall molecular program of hypertrophy in response to conventional RE training.

Adult skeletal muscle fibres are unique in that they are multinucleated cells. Each myonucleus controls the production of mRNA and protein synthesis over a finite volume of cytoplasm, a concept known as the DNA unit or myonuclear domain (Cheek, 1985; Hall & Ralston 1989). Essentially, a skeletal muscle fibre consists of a mosaic of overlapping nuclear domains (Hall & Ralston 1989). Satellite cells are undifferentiated myogenic cells that lie under the basal lamina of the skeletal muscle fibre. Activation of these satellite cells results in their incorporation into the muscle fibres as new myonuclei (Moss, 1971). If satellite cell proliferation is inhibited, muscle fibre growth or recovery from atrophy is inhibited (Rosenblatt et al., 1994). The exercise-induced muscle damage-recovery process seems to have a similar cellular mechanism to postnatal growth in that satellite cells become activated and fuse with the damaged muscle fibres (Goldring et al., 2002). The process of myoblast fusion is thought to be integral to hypertrophy as it maintains myonuclear domain size and thus the capacity for muscle protein synthesis. That is, hypertrophy of a muscle fibre is thought to be dependant upon the insertion of new nuclei to maintain a constant ratio of nuclei to cytoplasmic volume (McCall et al., 1998). However, few studies have investigated the possible modulation of satellite cells and myonuclear number to accommodate RE-induced hypertrophy in normal human muscles. The results of a recent study (Kadi et al., 2004) involving healthy adults confirmed previous work (Kadi & Thornell 2000; Roth et al., 2001) that RE training does enhance the size of the satellite cell pool. However, the hypertrophy of individual muscle fibres observed by Kadi et al. (2004) was not accompanied by an enhancement of the myonuclear number. In this study, 30 days of training was sufficient to induce an increase in the

satellite cell pool and an additional 60 days of training further enhanced satellite cell frequency (Kadi et al., 2004). Interestingly, the increase in satellite cell number following the RE program was maintained for a long time after the cessation of training (up to 90 days) (Kadi et al., 2004). These results support the hypothesis that in normal skeletal muscles, the transcriptional activity of individual myonuclei may not be maximal (Newlands et al., 1998). That is, the transcriptional capacity of existing myonuclei could sustain increased protein synthesis following RE training, the size of each myonuclear domain simply increases and controls a greater amount of cytoplasm, but once the transcriptional activity of existing myonuclei reaches a certain level, additional myonuclei provided by satellite cell proliferation and fusion to the fibre become required to support a greater cytoplasmic volume (Allen et al., 1995; Hikida et al., 1998; Kadi & Thornell 2000). In general, it appears that an increase in myonuclear number only occurs when mammalian (including human) muscle fibres hypertrophy by more than 26% (Cabric & James, 1983; Allen et al., 1995; Hikida et al., 1998; Roy et al., 1999; Kadi & Thornell 2000). The existing myonuclei are able to increase their rate of protein synthesis and support a moderate expansion of the cytoplasmic area in smaller hypertrophy responses (Giddings & Gonyea 1992; Kadi et al., 2004).

From the extensive literature on myogenic differentiation, it is clear that growth factors play crucial roles in the formation of muscle (Florini et al., 1991). One growth factor in particular, insulin-like growth factor-1 (IGF-1) appears to be involved with the expression of a complete spectrum of muscle-specific proteins (Florini et al., 1991; 1992). IGF-1 stimulates muscle protein synthesis (Jurasinsk et al., 1995), and myoblast proliferation and differentiation *in vitro* (Florini 1996). IGF-1 is also capable of promoting muscle growth by activating the regulators of translational initiation via the PI3K-PKB-mTOR pathway (Song et al., 2005). On the basis of their mRNA transcripts, three isoforms of IGF-1 have been identified in human muscle, they are; IGF-IEa, IGF-IEb, IGF-IEc (Yang & Goldspink 2002; Hameed et al., 2003a). One of these, IGF-IEa is expressed both in working and nonworking muscle (McKoy et al., 1999), and is very similar to the isoform produced by the liver and probably has a similar function (Hameed et al., 2003a). The isoform IGF-IEc in humans has been dubbed the mechano-growth factor (MGF); not to be confused with IGF-IEb in rats that has also been called MGF (Hameed et al., 2003b). MGF is the mechano-sensitive splice variant of IGF-1 that is expressed specifically and rapidly in response to tissue damage (Haddad & Adams 2002; Hill & Goldspink 2003). MGF is thought to play an important role in muscle hypertrophy firstly by direct stimulation of myofibrillar protein synthesis but also activation of satellite cell proliferation and differentiation, followed by fusion of differentiated myoblasts to hypertrophying fibres (Adams, 1998).

One of the special functions of the MGF isoform (and its unique carboxyl peptide sequence) is to activate the muscle satellite (stem) cells for division (Yang & Goldspink 2002; Hill & Goldspink 2003). Whereas the IGF-IEa peptide is localized throughout the cytoplasm, the MGF peptide is localized in the nucleus and is expressed only in response to tissue damage (as it is thought to prevent apoptosis). MGF has a short half life and does not appear to survive in the extracellular compartment for any appreciable length of time (Yang & Goldspink 2002; Hill & Goldspink 2003). For these reasons, MGF is thought to have a unique role in replenishing the stem cell pool for repair and growth throughout life (Goldspink, 2005). The positioning of the satellite cells (under the basal lamina) for proximal activation by MGF also adds to the weight of evidence that suggests a key role of this IGF-splice variant in the muscle regenerative/hypertrophy response to RE. However, only two studies have examined the expression of the IGF-1 splice variants in human muscle in response to loading. The data from these studies suggest that in response to RE, MGF is increased for a short time, soon after exercise, and this response was detected in young adults, but not older adults (Hameed et al., 2003a). In general, the expression of all IGF-I variants (a,b and c) appears to be down-regulated during the initial stages of recovery from RE (Psilander et al., 2003). Unfortunately, a more detailed time course of IGF-I mRNA expression after RE is not known. However, the small amount of research that has been completed on this topic has shown that IGF-1 mRNA content measured during recovery from exercise is highly variable among subjects; some exhibit a marked increase while other show no change (Hameed et al., 2003; Psilander et al., 2003a). These large variations in IGF-1 expression might help to explain the wide variation in hypertrophy responses that have been observed in a large group of people undertaking the exact same RE program (Hubel et al., 2005).

The efficacy of muscle IGF-I is dependent not only on its expression but also on its availability, which is regulated by a family of six IGF binding proteins (Kraemer & Ratamess 2005). For example, in muscle, IGFBP-4 has a high affinity for IGF-I and thus inhibits its myogenic effects, whereas IGFBP-5 may facilitate (Florini et al., 1996) or inhibit (James et al., 1996) IGF-I-stimulated differentiation under certain conditions. Additionally, IGFBP-1 has been shown to inhibit IGF-I-stimulated protein synthesis (Frost & Lang 1999). In humans, RE is shown to produce alterations in these binding proteins such as a decreased expression of IGFBP-4 (Bamman et al., 2001), that may alter the availability of active IGF-1 to tissues. These alterations caused by RE have been detected in circulation over a 13 hour period (Nindl et al., 2001). Therefore, the impact that RE has on the IGF-I system may not be completely related to the total amount of IGF-I that is in circulation but rather the manner in which IGF-I is partitioned among its family of binding proteins. However, another possible mechanism for increasing muscle IGF-I availability is via androgen activation (Bamman et al., 2001). The results of one study suggest that

IGF-I action in muscle is secondary to androgen activity (Bamman et al., 2001). Rodent and human studies confirm that RE increases androgen receptor expression and number along with IGF-1 mRNA (Deschenes et al., 1994; Bamman et al., 2001). Therefore, although the mechanism(s) are not clear, muscle androgen and IGF-I activities appear to be related. To summarize the role of IGF-1 (and its related splice variants) in the hypertrophy process; it is most likely to be multifaceted involving localized activation via the short-lived MGF and the longer-lasting (~12 hours) alterations in circulating IGF-1 binding proteins, as well as alterations in androgen receptor expression. However, the integration of each of these aspects within the overall picture of muscle accretion during RE training has not been elucidated. In particular, the residual effect that RE appears to have on serum IGF-1 binding proteins (that appears to last up to 12 hours) may provide a beneficial "carryover" effect when consecutive days of RE are performed. This may be one advantage to training a different muscle group each day; a strategy that is characteristic of bodybuilders.

Whereas growth factors are essential to activating satellite cell proliferation, the progression of satellite cells into myoblasts requires the regulation of muscle specific proteins belonging to the basic-helix-loop-helix family of transcription factors collectively called myogenic regulatory factors (MRFs) (Murre et al., 1989). Members include MyoD, myogenin, myf-5, MRF-4 and MEF-2 which collectively function as dominant activators of muscle differentiation (Perry & Rudnick 2000). Animal studies show overloaded muscle undergoing hypertrophy contains increased mRNA levels of the MRFs (Carson & Booth 1998; Lowe et al., 1998; Mozdziak et al., 1998). More recently, myogenin, MyoD, and MRF4 mRNA levels were shown to be transiently elevated (from 100-400%) in human muscle in response to RE for up to 24 hours (Psilander et al., 2003). After dimerization with a ubiquitous E protein, the MRFs bind to an E-box domain and activate downstream muscle genes, such as MLC, troponin I, and desmin (Lin et al., 1991; Wentworth et al., 1991; Li & Capetanaki 1993) which are building blocks of larger protein complexes that synthesize functional and contractile elements. Therefore, the MRFs may contribute to the hypertrophy response by regulating the transcription of more complex structures. In line with such a possibility, a correlation between changes in muscle mass and MRF expression have been observed in three studies (Hespel et al., 2001; Alway et al., 2002a; 2002b). One example is the study by Hespel et al. (2001) that showed the MRF4 protein content in human muscle correlated (r = 0.73; $P < 0.05$) with the concomitant change in muscle fibre CSA after 10 weeks of RE. In addition, several studies have shown that muscle injury induces satellite cell activation along with MRF expression (Rantanen et al., 1995; Marsh et al., 1997; Launay et al., 2001). The MRFs appear to play a role in myoblast proliferation and differentiation (Murre et al., 1989). Myogenin has been suggested to regulate the expression of a protein that is required for satellite cell fusion (Lowe &

Alway 1999). As satellite cell proliferation, differentiation, and fusion with muscle fibres are necessary for continued hypertrophy, the MRFs might be involved in regulating muscle growth over the long term. However, the MRFs are also shown to be up-regulated during atrophy (Voytik et al., 1993; Alway et al., 2001). One explanation for the observed up-regulation of the MRFs during both hypertrophy and atrophy could be that these myo-specific transcription factors regulate other processes that coincide with changes in muscle mass such as the phenotypic expression of the MHCs and fibre type. MyoD and myogenin have been implicated in regulating muscle fibre type (Hughes et al., 1993; Voytik et al., 1993). In rodent muscle, myogenin has been found in slow-twitch fibres and MyoD in fast-twitch fibres (Hughes et al., 1993; Voytik et al., 1993). In humans, one study has shown that type-IIx MHC expression is associated with MyoD mRNA, whereas type-I and IIa MHC mRNA (the slower twitch isoforms) were associated with myogenin mRNA expression, 6 hours after a single bout of high overload RE (Willoughby & Nelson 2002). This research confirmed previous work by Mozdziak et al. (1998) that MyoD and myogenin play a role in MHC isoform gene expression in response to overload.

When attempting to describe gene transcription regulation in the complex picture of muscle hypertrophy, it is also important to consider that the process may depend on the suppression of some genes and increased transcription activity of others. A predominant action of a RE training program in the overall hypertrophic response may be the selective inhibition of transcription or degradation of specific mRNA prior to selective expression of other genes (Cameron-Smith, 2002). One example of this selective inhibition may be the cytokine myostatin, also known as growth differentiating factor-8 (GDF-8). A member of the transforming growth factor-β (TGF-β) superfamily, myostatin appears to negatively regulate muscle growth (McPherron et al., 1997). In adult muscle, myostatin expression is thought to decrease muscle size probably by way of a glucocorticoid receptor meditated mechanism (Ma et al., 2001) to inhibit satellite cell activation and induce proteolysis (Ma et al., 2003). However, myostatin may also inhibit myoblast differentiation by down-regulating MRF expression (Langley et al., 2002). Myostatin mRNA expression is increased in immobilized (inactive) muscle (Carlson et al., 1999), whereas loading is shown to reduce the expression of this gene (Wehling et al., 2000). In humans, one study reported that 9 weeks of concentric-only RE resulted in a decrease in myostatin mRNA (Walker et al., 2004). Based on this data, one would assume that RE would be an effective activity that down-regulates myostatin expression to promote hypertrophy. However, the role of RE in nullifying myostatin's negative effects on muscle hypertrophy may not be so straight forward. Another study that involved healthy males undertaking 12 weeks of high overload RE training, reported an increase in glucocorticoid receptor and myostatin mRNA expression despite significant hypertrophy (Willoughby, 2004). In this study, serum follistatin-like related gene (FLRG) was

shown to increase (127%) with a concomitant down-regulation of the activin IIb receptor. These findings suggest that RE training may increase myostatin mRNA expression (and serum myostatin levels) in the presence of hypertrophy, and the increase in myostatin expression is nullified by a concomitant increase in its inhibitory-binding protein and decrease in its cell receptor (Willoughby, 2004). Therefore, while some studies suggest that for hypertrophy to occur, myostatin expression must be down-regulated, other work suggests that hypertrophy occurs in the presence of an increase in myostatin via a training-induced mechanism that inhibits this cytokine's negative effect on muscle growth.

Clearly, many questions remain unanswered with regard to the molecular events that ultimately result in muscle hypertrophy. This discussion is concluded by highlighting some of the controversial or unexplained phenomenon to which clarification would be particularly valuable for improving the efficacy of RE prescription. Firstly, the initial events within muscle contraction that activate PI3K/PKB and therefore MPS, are unknown. The IGF-1 response would be too slow and the influence of insulin is thought to be minimal (Kimball et al., 2002). While calcium release (Ji et al., 2002) and/or integrin signalling (MacKenna et al., 1998) are the likely candidates, so far there is no evidence that links either of these to the PKB/m-TOR signalling cascade. Secondly, if one keeps in mind that chronic RE is supposed to diminish the acute MPS response, then the impact of training on the signalling pathway(s) that lead to MPS need to investigated. If the molecular signalling responses that lead to MPS are down-regulated by training, then attempts to identify possible sites of amplification/up-regulation within these pathways should be considered. Finally, exactly how the EAA trigger the phosphorylation of mTOR and the MPS cascade remains unclear. This is particularly important to clarify as recent work by Cuthbertson et al. (2005) has revealed a diminished anabolic response to protein consumption in older humans via reduced activation of the initiating protein complexes (such as mTOR). In light of this information, it now appears crucial to find non-pharmaceutical ways to amplify the molecular signalling process that activate MPS. The data produced by Karlsson et al. (2004) with regard to the synergistic effect of BCAA and RE on MPS, via the greater phosphorylation of at least one of the initiatory proteins, underscores the important role that strategic nutritional intervention may play in enhancing the hypertrophy response to RE.

1.6 Acute responses to nutrient intake & resistance exercise associated with hypertrophy

Early studies that utilized isotope labelled AA and tracer limb exchange methods established that the large increase in whole-body synthesis observed after the consumption of a meal is due mainly to the changes in protein synthesis rates in muscle. In fact, in response to feeding, muscle mass contributes more than half of the total increase in whole body protein synthesis (Rennie et al., 1982). Other studies that have used similar methods (Svanberg et al., 1996; 1997; 1999; 2000; Volpi et al., 1999; 2003; Rasmussen et al., 2002; Rennie et al., 2002) have established the following; 1) the consumption of mixed macronutrient meals alter rates of MPS and promotes a positive NPB; 2) MPS increases approximately 30-100% in response to a meal and the major contributing factor to this response is AA; 3) the effect of insulin on MPS is dependant on the availability of AA; 4) the net accretion of muscle protein that is observed after a meal is due mostly to the large increase in MPS (inhibition of MPB contributes but to a lesser extent) and 5) the anabolic response to feeding is rapid but transient. That is, during the post-prandial phase (1-4 hours after the meal) MPS is elevated while MPB is reduced. MPB increases in the post-absorptive state (approx 5 hours after the meal) and MPS rates decline. Therefore, accretion of protein only occurs in the fed state. More recently, it has also been confirmed that meal consumption stimulates both myofibrillar and sarcoplasmic protein synthesis (3-fold compared to basal values) (Mittendorfer et al., 2005). While there may be some small differences between muscle groups (such as triceps versus soleus and vastus lateralis), in general, the stimulation of MPS by AA in humans appears not to be influenced by anatomical location or fibre-type (Mittendorfer et al., 2005).

The development of muscle hypertrophy would involve repeated disruption/damage to certain muscle fibres that later must undergo a recovery phase such as a coordinated qualitative and quantitative change in muscle proteins. The physiological processes responsible for these events would be influenced by the availability of hormones, cytokines and growth factors which are in turn, influenced by the presence (or absence) of the macronutrients. For example, it is clear that the intake of CHO and protein (PRO) at a time close to RE (i.e., the hours just before and/or after a workout) alters the acute hormonal and protein turnover response patterns to create an environment that probably helps to optimize conditions for recovery (Kraemer et al., 1998b; Volek, 2004). The consumption of proteins (or EAA) and RE have a synergistic effect on MPS that results in a positive net balance (Biolo et al., 1997; Tipton et al., 1999). The strategic intake of CHO (glucose) before or after RE does not appear to alter the MPS response (Roy et al., 1997; Borshiem et al.,

2004) but may reduce myofibrillar breakdown CHO (Roy et al., 1997). The combination of CHO (35g) with EAA (6g) after RE is shown to provide a synergistic effect on MPS and NPB that is much greater than either macronutrient alone (Miller et al., 2003). In fact, when this combination is consumed 1 or 3 hours after a single bout of RE, MPS rates were shown to increase up to 400% above pre-exercise values; the highest value ever recorded (Rasmussen et al., 2000). The same small dose of EAA and CHO is also shown to promote a similar anabolic effect within muscle when administered just before RE (Tipton et al., 2001). While these investigations utilized AA solutions, other studies have confirmed that whole proteins, such as whey and casein, evoke an anabolic response that is similar in magnitude to free form AA (Levenhagen et al., 2001; Tipton et al., 2004; Wilborn et al., 2005). Therefore, it is clear that nutrient-timing (i.e., the consumption of PRO and CHO before and/or after RE) not only augments the anabolic response, most importantly, it shifts net protein balance to a positive state (albeit for a transient time). This result can be (at least partly) attributed to changes in the acute hormonal response pattern.

Few studies have examined the effects of macronutrient intake on RE-induced hormonal response patterns (Chandler et al., 1994; Roy et al., 1997; Kraemer et al., 1998b; Tarpenning et al., 2001). This is some what bemusing and disappointing given the large number of studies that have assessed endocrine responses to RE, and the important role that hormones play in the regulation of gene expression and protein metabolism. The following paragraphs of this section provide a brief discussion on the acute endocrine (and associated immune) responses to RE and pre-post macronutrient intake in relation to muscle hypertrophy.

Compared to a low-caloric (non-insulin stimulating) placebo, a high calorie PRO-CHO supplement (total; 7.9 kcal/kg, 1.3g CHO/kg, and 0.7g protein/kg per day) consumed 2 hours before and just after RE is shown to provide higher blood insulin levels in the hour after exercise (Kraemer et al., 1998b). A macronutrient-induced stimulation of insulin is expected to improve the anabolic response by increasing the uptake of nutrients (AA and glucose) while decreasing MPB (Wolfe & Volpi 2001). Thus, the stage is set for the initial recovery phase. However, while insulin is clearly associated with improving the anabolic response to RE, the impact of nutrient-timing on other hormones such as GH, testosterone, cortisol and growth factors such as IGF-1 is less clear.

An increase in protein synthesis, lipolysis, and glucose conservation are all hallmark responses to increased availability of GH (Thissen et al., 1994; Rennie 2003). The nutrient-timing study by Kraemer et al. (1998b) demonstrated that PRO-CHO supplementation enhanced acute serum GH responses for 30 minutes after exercise compared to the non-caloric placebo. This response was observed on the first day but not the following two days of training (Kraemer et al.,

1998b). The reason for this is not clear. Chandler et al. (1994) also reported an increase in serum GH in response to a similar dose of PRO-CHO immediately and 120 minutes after RE. In contrast, Williams et al. (2002) reported no significant effect of PRO-CHO on the GH response to RE. The regulation of hepatic IGF-1 is characteristic of GH (Thissen et al., 1994). Kraemer et al. (1998b) also reported that nutrient-timing with the PRO-CHO supplement elevated serum IGF-1 levels for 30 minutes after RE in 2 out of 3 training days. After a 6 month training program, an increase in (resting) plasma IGF-1 has been observed in response to the daily consumption of a PRO-CHO supplement (42g PRO, 24g CHO) as opposed to CHO (70g) alone (Ballard et al., 2005). Furthermore, Willoughby et al. (2005) recently reported that 10 weeks of heavy RE combined with protein supplementation before and after each workout was effective at increasing serum IGF-1 and muscle MGF mRNA expression. Although nutrient-timing during RE may increase serum IGF-1 concentrations, the anabolic action of this growth factor on tissue is thought to reside in alterations within its binding proteins (Friedlander et al., 2001). Separately, meal consumption (Lee et al., 1997) and RE (Nindl et al., 2001) appear to influence the regulation of the IGF-1 binding proteins. However, no studies have examined the impact of nutrient intake and RE on the IGF-1 binding proteins and muscle anabolism.

Testosterone is known to promote anabolism by augmenting the synthesis of muscle protein, and the consumption of PRO-CHO before and after RE appears to have an effect on circulating levels of this hormone. The nutrient-timing study by Kraemer et al. (1998b) also assessed acute testosterone responses. The researchers reported an acute increase in circulating testosterone followed by a sharp decrease (to levels that were significantly lower than baseline) when the PRO-CHO supplement was administered before and after RE. This response was consistently observed on each of the three training days assessed. Studies by Chandler et al. (1994) and Bloomer et al. (2000) have also shown a similar response. Collectively, these studies indicate that although RE evokes an increase in circulating testosterone, nutrient intake close to RE results in a decrease in circulating testosterone concentrations in the hours post-exercise; a finding that is directly opposite to RE studies that did not involve nutrient intake. This rapid decrease in blood testosterone levels in response to PRO-CHO consumption close to RE may be due to increased metabolic clearance of this hormone such as increased uptake by muscle. At least one study supports this assumption. Chandler et al. (1994) showed that a decline in circulating testosterone in response to nutrient-timing after RE was not linked to a decrease in lutenizing hormone production. Nutrient-timing provides a dramatic increase in MPS in the hours after a workout (Tipton et al., 1999; 2003). Therefore, the drop in circulating testosterone could be due to increased uptake by muscle to facilitate this process. To further support this contention, Volek (2004) reported that a

meal-induced decrease in post-workout circulating testosterone corresponded with an increase in muscle androgen receptor content.

The adreno-cortical steroids such as cortisol (or glucocorticoids) serve to induce catabolic processes within muscle that are thought to be essential to the remodelling response (Nieman et al., 2004). However, the data on the response pattern of this hormone to RE and macronutrient consumption is conflicting. Cortisol is regulated by pituitary adrenocorticotropin (ACTH), which in turn, is under the influence of hypothalamic corticotrophin-releasing hormone (Smith, 2000). An important action of the glucocorticoids is local and systemic modulation of inflammatory mechanisms related to cytokine-meditated cortisol secretion that occurs via the hypothalamic-pituitary-adrenal (HPA) axis (Braun & von Duvillard 2004). The HPA axis is sensitive to a variety of different stressors including exercise and feeding. Exercise causes numerous changes in immunity that reflects physiological stress and immunosuppression, whereas macronutrient consumption has the potential to alter this response (Nieman et al., 2004). For example, CHO supplementation before, during and/or after exercise is proposed to sustain glucose availability and reduce substrate stress which results in the attenuated stimulation of the HPA axis and lower cortisol production (Braun & von Duvillard 2004). In turn, this is thought to reduce perturbations in immune status that lead to lower cytokine production (Febbraio et al., 2003) which attenuates the inflammatory response (Braun & von Duvillard 2004). However, very few studies have assessed cytokine responses to RE. One recent study by Neiman et al. (2004) reported that the ingestion of a 6% CHO solution did not alter modest increases in plasma IL-6, IL-10, IL-1ra, and IL-8 (and muscle gene expression of these cytokines) in a group of strength-trained participants that lifted weights for 2 hours. Many aspects of protein turnover are controlled by the classic steroid-hormone binding mechanism. Like testosterone, cortisol also binds to a cytoplasmic receptor and activates a receptor complex so that it can enter the nucleus, bind specific response elements on DNA and act directly at the genetic level. In doing so, cortisol (and other hormones) alter the transcription and subsequent translation of specific proteins (Carson, 1997). These processes are thought to be integral to tissue remodelling and adaptation to exercise. This may be one reason why the majority of studies that have assessed cortisol responses to RE show that macronutrient consumption does not alter the response pattern of this hormone (Kraemer et al., 1998b; Bloomer et al., 2000; Koch et al., 2001; Williams et al., 2002). However, one study has shown that supplementation with CHO blunted the cortisol response to RE and this had a positive effect on muscle hypertrophy (Tarpenning et al., 2001).

In the study by Tarpening et al. (2001), CHO supplementation (6% solution) resulted in significantly greater gains in both type-I (19.1%) and type-II (22.5%) muscle fibre CSA than a

placebo after 12 weeks of RE. The difference in the cortisol response accounted for 74% of the variance ($r = 0.8579$, $P = 0.006$) of change in type-I muscle fibre area, and 52.3% of the variance ($r = 0.7231$, $P = 0.04$) of change in type-II muscle fibre area. This study is unique because it is one of few that have linked an acute change in the pathway of adaptation (i.e., decrease in post-exercise cortisol) to a chronic adaptation (i.e., increased muscle size). RE training is shown to reduce the magnitude of the cortisol response to this exercise (Staron et al., 1994; Kraemer et al., 1999) and this change appears to be mediated by a reduction in ACTH responses to the exercise stress (Kraemer et al., 1999). Based on these findings, a reduction in cortisol responses over the longer term have been proposed as one possible mechanism of protein accretion during RE training (Kraemer et al., 1999). Therefore, the effects of CHO supplementation reported by Tarpenning et al., (2001) warrant further investigation.

To summarize the literature regarding the acute responses to macronutrient intake and RE; firstly, it is clear that the strategic consumption of PRO and CHO close to RE (nutrient-timing) augments the acute anabolic response but more importantly, shifts protein balance to a positive state in the hours following exercise. Secondly, nutrient-timing appears to achieve this effect by providing an abundance of material during high-intensity muscle contraction that alters the acute hormonal and protein turnover response patterns. However, the chronic affects that may arise from these acute metabolic perturbations remains largely, unknown. Very little work has linked diet-induced alterations in protein turnover to chronic adaptations such as strength or hypertrophy development. The immune and endocrine systems are likely to be critical components in the pathways that intergrate diet, RE and hypertrophy. Therefore, a greater focus on this topic is warranted if we are to ascertain a better understanding of how to optimize the hypertrophy response to nutrient intake and exercise.

1.7 Chronic responses to nutrient intake & resistance exercise associated with hypertrophy

RE is fundamentally anabolic but is only able to shift NPB to a positive value in the presence of protein-containing meals. RE and meal consumption interact synergistically to provide a NPB that is greater than what can be obtained from feeding alone. The chronic response of this interaction between RE and meal consumption on NPB is thought to be muscle hypertrophy (Phillips et al., 2005). However, to date, no studies have linked the acute metabolic perturbations (described previously), to chronic adaptations such as increases in muscle size and strength. Nonetheless, there is an increasing amount of evidence that supports the theory that nutrient-timing results in greater muscle hypertrophy. For instance, nutrient-timing with protein (15g of EAA

before and after RE) is shown to result in higher net protein accretion over a 24 hour period (Tipton et al., 2003). In terms of longitudinal investigations, a number of RE training studies report significantly greater muscle fibre hypertrophy (Esmarck et al., 2001; Andersen et al., 2005) or statistical trends for greater LBM accretion from nutrient-timing. In a group of older (untrained) adults (74 ± 2 yrs) undertaking RE for 12 weeks, immediate post-workout provision of a mixed-macronutrient supplement resulted in significant muscle fibre hypertrophy (22% increase in CSA of the type-IIa fibres) and a 7% increase in total thigh muscle CSA. In contrast, a matched group that was fed 2 hours after exercise did not demonstrate hypertrophy (Esmarck et al., 2001). In a group of physically active (but not RE-trained) young males Chromiak et al. (2004) examined the effects of post-workout PRO-CHO supplementation on LBM gains during 10 weeks of RE training. The group given a PRO-CHO supplement (25g PRO, 76g CHO) within an hour of finishing each RE session (4 times/week for 10 weeks) demonstrated a 3.4kg increase in LBM compared to a 1.5kg gain seen in a control group given a (92g) dose of CHO (Chromiak et al., 2004). In another study, Rankin et al. (2004) utilized untrained males and a similar supplement protocol to Chromiak et al. (2004), and obtained the same result. Only one study has examined the effects of pre- and post- nutrient consumption on chronic adaptations to RE training (Andersen et al., 2005). In this study, after 14 weeks, the group of young men provided with a protein supplement before and after each workout (4x/week, 25g), demonstrated hypertrophy of the type-I and type-II muscle fibres, whereas no change above baseline was observed in a matched group given an equivalent dose of CHO.

When interpreting the results of these nutrient-timing studies it's important to remember that the investigations restricted the intake of certain macronutrients (namely protein) in the hours surrounding the workout. That is, the participants were not permitted to consume any other nutrients other than the designated supplement up to 3 hours before and after each workout (Esmarck et al., 2001; Chromiak et al., 2004; Rankin et al., 2004; Andersen et al., 2005). Therefore, the results obtained may have less relevance in a real-world setting. Meaning, strength athletes and others that desire muscle mass gains would not usually go without consuming a protein-rich meal for 3 hours after exercise. Nonetheless, based on these findings, it has been suggested that the strategic use of a PRO-CHO supplement just before and after each workout may provide the ideal anabolic situation for muscle hypertrophy (Volek, 2004). However, no research has examined the effects of supplementation with PRO-CHO before and after RE in the presence of a normal eating pattern. Additionally, no studies have examined whether this supplement-timing strategy provides greater benefits in terms of muscle hypertrophy or strength development compared to supplementation at other times during the day.

Although nutrient consumption close to RE promotes muscle anabolism in the short term, a single bout of RE affects (muscle) protein turnover for at least 24 hours, even in trained individuals (Chesley et al., 1992; MacDougall et al., 1995). Therefore, the type, timing and quantity of the energy-yielding macronutrients (CHO, PRO and fat) consumed throughout each day also has the potential to influence net protein accretion and chronic adaptations. For example, meal type, quantity and frequency is shown to influence the potentially substantial gains and losses of body proteins that occur during the diurnal cycle of feeding and fasting (Millward et al., 1995; 1996). Total energy intake also appears to influence the hormonal response patterns associated with hypertrophy (Forbes et al., 1989; Sallinen et al., 2004). For example, a short term increase in daily energy intake above maintenance requirements not only increases whole body protein synthesis, it also elevates resting concentrations of GH and testosterone along with an increase in LBM (Forbes et al., 1989; Fern et al., 1991; Jebb et al., 1996). Serum IGF-1 concentrations have the potential to influence chronic adaptations to RE as they are shown to correlate with the synthesis rates of MHC in both young and older adults (Balagopal et al., 1997b). The restriction of dietary energy and/or protein is shown to have a negative effect on serum IGF-I concentrations whereas an intake above maintenance requirements is shown to increase the circulating concentrations of this growth factor (Thissen 1994). Overall energy needs during any form of exercise training are principally determined by the individual's training load (i.e., the intensity x frequency x duration of daily workouts), body mass and exercise goals (Hawley et al., 1995). As protein synthesis is an ATP-dependant process, it is generally accepted that a slight increase in daily energy (kilojoule) intake above that needed for weight maintenance would augment muscle mass gains during RE training (Lambert et al., 2004). While overall energy intake would appear to be an important aspect with regard to dietary influences on the hypertrophy response, no research has determined what amount is required to enhance muscle mass gains during RE training (Lambert et al., 2004).

Aside from overall energy intake, it is clear that the type and quantity of macronutrients consumed each day has the potential to influence adaptations during RE training. For example, the quantity and composition of dietary fat has been shown to impact resting (Volek et al., 1997a) and exercise-induced circulating testosterone (free and bound) concentrations (Raben et al., 1992; Sallinen et al., 2004). Additionally, certain dietary fats such as conjugated linoleic acid (CLA) and eicosapentaenoic acid (EPA) have shown potential to improve body composition (Gaullier et al., 2004; Bhattacharya et al., 2005) or promote LBM directly (Barber 2001; Whitehouse et al., 2001). Therefore, the effects of quantity and composition of dietary fat intake on muscle and strength development during RE is a topic that deserves further attention. While dietary fat makes an important contribution to overall energy intake, to date, no direct evidence suggests increasing dietary fat intake *per se*, would improve adaptations to RE training (Lambert et al., 2004).

Particularly, not at the expense of the PRO and CHO; the macronutrients that predominantly affect muscle (and whole body) anabolism and catabolism.

CHO alters muscle (and whole body) anabolic and catabolic responses to RE. As these processes are stimulated on a frequent basis during a RE training program, dietary CHO intake has the potential to influence chronic adaptations. For example, muscle glycogen stores affect work capacity during intense exercise (Balsom et al., 1999). The daily maintenance of glycogen stores appears to be directly related to the CHO in the diet. In particular, the amount of muscle glycogen synthesis in the 24-hour period post-exercise is directly correlated (r = 0.84) to the amount of CHO ingested and the timing of that ingestion (Costill, 1991). More specifically related to RE, inadequate CHO intake may compromise adaptations by impairing glycogen resynthesis and performance in subsequent bouts (Haff et al., 1999). Also, the extent of exercise-induced protein degradation is shown to be inversely related to CHO availability (Lemon & Mullin 1980). Aside from the affect of CHO on protein catabolism, the results of one recent study suggests that performing RE with low muscle glycogen stores may impair the activation of MPS (via regulation of the Akt pathway) (Creer et al., 2005). However, despite the obvious importance of an adequate CHO intake, no research has attempted to define the amount that may improve muscle strength and hypertrophy development during RE training (Lambert et al., 2004).

Whereas dietary fat and CHO requirements during RE training have received little attention by the scientific community, protein requirements have been a topic of great controversy for many years and a clear consensus has still not been reached (Lemon, 2001). In particular, it is unclear whether or not a high protein intake above the recommended dietary allowance (RDA) may enhance muscle and strength development (Lemon, 2001). Investigations involving both strength and endurance athletes indicate that exercise does increase the need for AA/protein; approximately 1.5 to 2 times the RDA does appear to be advantageous for muscle and strength development during RE (Lemon, 1992; Tarnopolski et al., 1992). However, at least one longitudinal study (involving untrained, older adults) demonstrated that a protein intake of twice the RDA did not enhance LBM accretion during 12 weeks of RE (Campbell et al., 1995). Conversely, previous work has shown that the quantity of protein in the diet does influence the potentially substantial gains and losses of body proteins that occur during the diurnal cycle of feeding and fasting (Pacy et al., 1994; Price et al., 1994; Millward et al., 1995; 1996). Studies that have examined the impact of protein quantity (from 0.4- 2.5g protein/day) on leucine kinetics (as an index of whole body protein turnover) have found a greater stimulation of protein synthesis from high vs. low protein-containing meals (Gibson et al., 1996; Forslund et al., 1998). Additionally, some (Fern et al., 1991; Forslund et al., 1998), but not all (Tarnopolsky et al., 1992)

short-term studies that have investigated protein intake during exercise suggest that up to 3 or 4 times the RDA will enhance whole body protein synthesis and/or protein accretion in young, healthy adults. These discrepancies in the literature may be due to the methodologies used in each study. For example, the results obtained from nitrogen balance studies suggest that exercise may enhance nitrogen efficiency and therefore, possibly decrease protein requirements (Butterfield & Galloway 1984). Conversely, studies that have utilized the metabolic tracer technique suggest that dietary protein recommendations (based on the nitrogen status technique) may underestimate resting needs by 40-90% (Young et al., 1994; 1999). Regardless of the discrepancies in the literature, reports show that bodybuilders and other strength athletes habitually supplement their diet with extra protein to achieve intakes that are sometimes up to 4 times the RDA (Kleiner et al., 1994; Marquart et al., 1998).

The idea that a high protein intake is essential to gaining muscle mass during RE appears to be imbedded into the culture of strength athletes (Di Paquale 2000; Lemon 2001) and this is probably due to a number of reasons. Firstly, a variety of factors may influence protein requirements such as, total energy intake (Gibson, 1996; Young & Borgonha 1999), CHO availability (Lemon & Mullin 1980), exercise intensity, type and duration (Lemon et al., 1992; Tarnopolsky et al., 1988), training history (Lemon et al., 1992; Tarnopolsky et al., 1992), age (Campbell et al., 2001) and timing of macronutrient intake (Roy et al., 1997; Tipton et al., 2003). Secondly, a high protein intake (up to 3 times the RDA), is reported to be a safe strategy in healthy humans (Poortmans & Dellalieux 2000) that has favourable effects on body composition (i.e., preservation of LBM and greater reduction of body fat) (Fansworth et al., 2003; Layman, 2004). Additionally, when a higher proportion of overall energy intake is protein (21% or 2.5g/kg/day) a positive (significant) whole-body protein balance has been reported along with a negative fat balance (Forslund et al., 1999). Finally, strength athletes may consume a high protein diet simply because they know that the RDA does not take into account what may be necessary to maximize athletic performance (Lemon, 2001). Some renowned scientists in this area recommend that high protein intakes are not necessary for muscle hypertrophy during RE training (Rennie et al., 2004). Others caution that the underlying biology of maintenance AA needs may be much more complicated than simply the support of protein metabolism itself; there are so many gaps in our knowledge of this subject that until the various functions of AA are better understood at both the mechanistic and quantitative level, current dietary recommendations for both healthy and sick humans should remain at an intellectually unsatisfactory empirical level (Reeds & Bolio 2002).

An individual's habitual protein intake may prove to be one of the more important variables that influence the size of human muscle mass since recent work has confirmed that the

concentration of EAA in the blood (plasma) regulates protein synthesis rates within muscle (Bohe et al., 2003). In particular, it is the extracellular, as opposed to the intracellular, concentration of EAA that controls the rate of protein synthesis within muscle (Bohe et al., 2003). That is, when plasma EAA levels are low, MPS rates decline (Kobayashi et al., 2003). Conversely, MPS rates increase in a linear fashion with increased EAA availability (Bohe et al., 2001). This response occurs up to a point where very high plasma concentrations (>2.5-fold normal) saturate this response (Rennie et al., 2002). Once a saturation point is achieved, MPS falls back to basal levels despite continued AA administration (Bohe et al., 2001). This relationship between plasma EAA and MPS has been established via infusion studies in the non-exercised state. However, acute response studies involving oral doses of EAA (Tipton et al., 1999; 2001; 2003), or whole proteins (Tipton et al., 2004; Paddon-Jones et al., 2005a), show a similar (transient) effect. Aside from modulating MPS rates, the presence of AA also inhibits MPB, although not as powerfully as they promote protein synthesis (Rennie et al., 2002). AA inhibit protein breakdown in other tissues such as the liver, perhaps more powerfully than they do in muscle (Mortimer et al., 1991). A 40g oral dose of AA appears to temporarily increase the size of the free AA pool (Tipton et al., 2003) so that while a saturation point may be reached with regard to the stimulatory effect on MPS, an inhibitory effect on MPB may still be obtained (Rennie et al., 2002). Any inhibitory effect of exogenous AA on protein breakdown would (theoretically) decrease the size of the free pool available for protein synthesis, providing a link between the two arms of the processes of protein turnover via the extracellular pool; this would effectively integrate the action on a whole body basis. The bottom line is that the stimulation of MPS is the facilitating mechanism of hypertrophy and the concentration of EAA in the blood is a known regulator of protein synthesis rates within muscle. Therefore, the type, frequency and quantity of protein consumed during the hours of each day would influence muscle protein accretion and the magnitude of hypertrophy obtained from a RE training program. Manipulation of the diet to create and maintain a high concentration of EAA in the blood stream during RE training that would influence muscle protein accretion and hypertrophy represents a rather exciting area of research that is yet to receive systematic investigation.

The results of several recent investigations support the theory that dietary manipulation of EAA availability may influence chronic changes in muscle mass. For instance, there is data that shows a correlation between acute stimulation of MPS (via protein consumption) and chronic changes in muscle mass (Paddon Jones et al., 2004). In this study, subjects were given a EAA supplement three times a day for 28 days during bed rest. Results indicated that acute stimulation of MPS provided by the supplement on day 1 resulted in a net gain of ~7.5g of muscle over a 24 hour period (Paddon-Jones et al., 2004). When extrapolated over the entire 28 day study, the predicted change in muscle mass corresponded to the actual change in muscle mass (~210g)

measured by DEXA (Paddon-Jones et al., 2004). Additionally, the role of protein supplementation in promoting muscle tissue accretion has recently been highlighted (Paddon Jones et al., 2005a). In this study, supplementation (15g of EAA and 30g of CHO) was shown to produce a greater anabolic effect (increase in net phenylalanine balance) than ingestion of a mixed-macronutrient meal, despite the fact that both interventions contained a similar dose of EAA. Furthermore, the consumption of the supplement did not interfere with the normal anabolic response to the meal consumed 3 hours later. This finding is particularly important; the refractory period when re-stimulation of MPS may occur with repeat meal consumption was previously unknown (Rennie et al., 2004). The results of the investigations by Paddon-Jones et al. (2004; 2005a) suggest that supplementation between regular meals may provide an additive effect on net protein accretion due to a more frequent stimulation of MPS. In combination with RE, repeated stimulation of MPS via the frequent consumption of protein (supplements and meals) would most likely influence the size of muscle mass, but this has not been investigated directly. When discussing the possible chronic effects of repeated meal consumption on muscle protein metabolism, the role of insulin must also be considered. Insulin inhibits muscle proteolysis (Fryburg et al., 1995; Bolio et al., 1999), probably via the ATP-ubiquitin-dependent proteolytic system that is responsible for myofibrillar protein breakdown (Reid, 2005). The consumption of a CHO-PRO-containing meal triggers an insulin response; the extent of which is dependant on the glycemic index but also the glycemic load of the meal (Alfenas & Mattes 2005). Therefore, the consumption of frequent CHO-PRO meals throughout a 24 hour period would promote anabolism not only via an increased stimulation of protein synthesis but also a reduction in protein breakdown (Rennie 2005). While it is logical to speculate that these responses may improve the rate of whole body protein accretion during RE, the effects of repeated meal consumption on muscle or whole body protein turnover during RE training has not been investigated.

Strength and hypertrophy training typically involves multiple workouts throughout the week that utilize different muscle groups each training session (Kraemer et al., 2002). However, the impact of daily workouts on net protein balance and accretion within muscle(s) is a topic that has not been considered. For example, the studies that have assessed the metabolic impact of RE and feeding have only examined the acute response to one or two small meals consumed close to a once-off bout of RE within a particular muscle group (Rasmussen et al., 2000; Tipton et al., 1999; 2001; 2004). The measurements obtained may reflect changes in the muscle assessed but they do not represent changes within other muscle groups that would influence protein metabolism at the whole body level. For instance, it is clear that a single bout of RE induces an increased intramuscular "recycling" of AA from protein breakdown and increased inward transport from the AA pool; muscle protein balance does not become positive until exogenous AA are provided

(Biolo et al., 1997). It is also clear that until exogenous AA are provided, the most readily available source of AA for utilization is from an increase in the rate of muscle protein breakdown (Biolo et al., 1995, Phillips et al., 1997). Therefore, regular workouts (performed throughout the week) would provide a systematic stimulation of this process throughout all muscles within the body. Unless the provision of exogenous AA are provided close to RE on every occasion, consecutive workouts (involving different muscle groups) could result in a "robbing Peter to pay Paul" scenario with regard to AA flux and distribution between muscle groups—a response that may reduce or even eliminate a positive balance obtained from a previous days training in another muscle group. It is apparent that a single bout of RE can stimulate protein turnover for at least 24 hours (MacDougall et al., 1995) and a RE program can affect resting protein turnover rates (Kim et al., 2005). Therefore, the "residual" impact of consecutive days of RE on muscle (and whole body) protein turnover clearly has the potential to affect protein accretion and the hypertrophy response to training. The interaction of repeated meal consumption during consecutive days of RE on muscle protein metabolism (and net accrual) is an important area of research that must receive systematic investigation if we are to ascertain a better understanding of the processes that may improve muscle hypertrophy during RE training.

Due to the lack of data that may link acute metabolic perturbations to chronic adaptations from training and dietary intervention, any prudent discussion of hypertrophy should include the characteristics of the one population group that represents a living "physiological model" of this prized adaptation. That is, bodybuilders; men and women that have spent a large portion of their lives in the pursuit of few other athletic/recreational endeavours except the development of muscle mass. Although an extreme model (and one that is not without apparent flaws), the dietary and training characteristics of this group of athletes may provide a better understanding of what is required to systematically increase muscle mass. To achieve muscle hypertrophy and strength improvements, bodybuilders characteristically perform daily RE workouts with different muscle groups and consume high-protein/energy intakes (Lambert et al., 2004). Daily energy intake is typically divided into a number of small, mixed-macronutrient meals that are consumed frequently (every 2-3 hours) over the course of their day (24 hour period) (Marquart et al., 1998; Leutholtz & Kreider 2001). As mentioned previously, this type of eating pattern would provide some important physiological advantages for promoting hypertrophy. For example, protein synthesis in muscle is a continuous activity that requires a balanced supply of twenty different AA (Rennie et al., 2004). The consumption of frequent, protein-rich meals throughout the day may create and maintain a high level of EAA in the bloodstream that would promote a higher rate of MPS and/or a reduction in MPB, reduce the potentially substantial losses of body protein that occur during the diurnal cycle of normal feeding and fasting, and ensure a higher net gain in muscle protein in response to

training. This eating pattern would also promote steady-state blood glucose and insulin levels more so than the traditional three-meals-a-day that most adults follow (Ryan, 2000); thus, minimizing protein breakdown while promoting the inward transport of nutrients to tissues. Therefore, the dietary strategies that bodybuilders follow would appear to provide a favourable bio-environment that is conducive to muscle protein accretion.

1.8 The potential of whey protein to enhance muscle hypertrophy

Although the high protein intakes that many bodybuilders consume can be met easily by simply increasing energy intake, many strength athletes use protein supplements to achieve high protein intakes (Brill & Keane 1994; Marquart et al., 1998; Leutholtz & Kreider 2001). Aside from quantity, one reason for this may be that different types of protein are known to affect whole body protein anabolism and accretion (Biorie et al., 1997; Bos et al., 2003; Dangin et al., 2003) and therefore, have the potential to affect muscle and strength development during RE training (Lemon et al., 2002). The type of protein consumed may influence protein accretion during RE training due to variable speeds of absorption (Dangin et al., 2001; 2003) differences in AA profiles (Bos et al., 2003; Phillips, 2005), unique hormonal responses (Bratusch-Marrain & Waldäusl 1979; Carli et al., 1992) or positive effects on antioxidant defence (Lands et al., 1999). Whey protein (WP) is the collective term for the soluble protein fractions extracted from dairy milk. WP supplements (80%+ protein concentrates and isolates) generally contain a higher concentration of EAA (45-55g/100g of protein) than other protein sources and therefore score highly on most evaluations of protein quality (Bucci & Unlu 2000; Di Pasquale 2000). In particular, they are the richest known source of BCAA such as leucine (up to 14g/100g protein) (Bucci & Unlu 2000). The BCAA have been shown to alter concentrations of circulating GH (Bratusch-Marrain & Waldäusl 1979), insulin (Ferando et al., 1995) and testosterone (Carli et al., 1992) as well as attenuate protein degradation (Blomstrand et al., 1992; MacLean et al., 1994; Coombes et al., 1995). Leucine in particular, is an established modulator of muscle protein metabolism and has been identified as a key regulator in the translation initiation pathway of muscle protein synthesis (Anthony et al., 2001). Oral BCAA supplementation is shown to augment the phosphorylation of p70-S6k; a major regulatory kinase in the activation of MPS in response to RE (Karlsson et al., 2004).

The acute response to a single dose of WP is a higher (but transient) blood AA peak and stimulation of (whole body) protein synthesis when compared to other high quality protein sources such as casein (the other major bovine milk protein) (Boirie et al., 1997; Dangin et al., 2001; 2003). A most relevant finding is that the consumption of WP in mixed-macronutrient meals provides a higher stimulation of MPS and higher net gain in whole body protein in both young and older

adults in comparison to isonitrogenous casein meals (Dangin et al., 2003). Aside from its high concentration of EAA, WP is a rich rare source of Cyst(e)ine residues. WP supplements generally contain a 3- to 4-fold higher concentration of Cys compared to other protein sources (Bucci & Unlu 2000). As discussed earlier in this chapter (refer to figures 1.2 & 1.3), Cys is thought to play a key role in the regulation of whole body protein metabolism and LBM. An abundant supply of Cys in the blood is necessary for hepatic catabolism of this AA into sulphate and protons; a process that down-regulates urea production, promotes GSH synthesis and shifts whole body nitrogen disposal in favour of preserving the muscle AA pool. In humans, WP supplementation (up to 1g/kg/day) is the only protein source shown to augment this pathway of protein metabolism (Lands et al 1999; Middleton et al., 2004), possibly in a dose-dependant manner (Marriotti et al., 2004). Therefore, due to its high concentration of EAA, Cys and ability to promote higher net protein accretion, the incorporation of WP into the diet may enhance muscle strength and hypertrophy development during RE.

Some studies demonstrate that supplementation with WP does enhance some of the adaptations desired from RE. WP is shown in several human (Lands et al., 1999; Burke et al., 2001; Cribb et al., 2006) and rodent (Bouthegourd et al., 2002; Belobrajdic et al., 2004) trials to improve body composition (i.e., an increase in LBM and/or a decrease in fat mass). During 6 weeks of RE training, WP supplementation (1.2g/kg/day) in RE-trained individuals resulted in an almost 2-fold higher gain (2.1 vs. 1.2kg) in LBM and a better gain in bench press strength compared to a CHO control group (Burke et al., 2001). In a double-blinded study that used two groups of matched, RE-trained young men, our laboratory has previously demonstrated a significantly greater gain in LBM and strength in a group provided with a hydrolysed WP isolate (1.5g/kg/day) compared to a matched group given an equivalent dose of casein (Cribb et al., 2006). Other studies have also reported body composition improvements from WP supplementation. In direct comparison to other quality proteins such as casein, WP is shown to maintain LBM and reduce fat mass via enhanced antioxidant (GSH) status (Lands et al., 1999), more efficient fat oxidation in the hours after exercise (Bouthegourd et al., 2002), improved muscle insulin sensitivity (Belobrajdic et al., 2004) or suppression of hepatic fatty acid synthesis along with increased fat utilization by muscle (Morifuji et al., 2005). Recent years have seen an increase in the number of trials that have involved RE training and dairy protein supplementation. However, while a number of these studies report changes in strength and body composition (Demling & DeSanti 2000; Antonio et al., 2001; Burke et al., 2001; Chromiak et al., 2004; Rankin et al., 2004; Cribb et al., 2006) none have compared these changes alongside hypertrophy responses at the cellular level, such as fibre-specific (i.e., type-I, IIa, IIx) CSA as well as the sub-cellular level, such as contractile

protein content. Additionally, no studies have examined the influence of supplementation with WP exclusively on muscle fibre morphology during RE training.

1.9 The potential of creatine monohydrate to enhance muscle hypertrophy

The rationale for the use of nutritional supplements to enhance exercise capacity is based on the assumption that they will confer an ergogenic effect above and beyond that afforded by regular food alone. The proposed ergogenic effect of many nutritional supplements is based on a presumptive augmentation of a metabolic pathway. However, under the rigor of scientific control, ingestion of the nutrient often fails to translate into a quantifiable change in exercise performance. Intriguingly, this does not appear to be the case for creatine monohydrate (CrM) (n[aminoiminomethyl]-N-methylglycine). Probably due to its vital role in the energy (ATP) production pathway, investigations involving Cr can be found as early as 1914 (Folin & Denis 1914). However, studies completed in the early 1990's demonstrated that a CrM "loading phase" (4 x 5g/day for 5 days) consistently elevated muscle Cr concentrations by approximately 25mmol/kg dry mass (Harris et al., 1992), or 15–40% (Balsom et al., 1994). This sparked a series of investigations that attempted to link CrM supplementation with improved exercise performance (Greenhaff et al., 1993; 1994; Birch et al., 1994; Earnest et al., 1995; Casey et al., 1996; Febbraio et al., 1996; Kreider et al., 1996; 1998; McKenna et al., 1999). Since that time, well over 200 studies have examined the effects of CrM supplementation on exercise performance. A majority of these studies have reported an ergogenic effect, particularly during exercise involving repeated, short bouts of extremely powerful activity (Birch et al., 1994; Greenhaff et al., 1993; 1994; Balsom et al., 1995; Casey et al., 1996; Vandenberghe et al., 1996; Rawson & Volek 2003). However, the precise physiological mechanisms linking increased muscle Cr content to improved functional capacity still remain elusive.

Oral supplementation with CrM enters the circulation where active uptake by tissues such as muscle is facilitated by a Na^+ dependent transporter against a concentration gradient (Guimbal & Kilimann 1993). Supplementation is shown to increase not only muscle Cr and PCr concentrations but also other tissues with low baseline Cr content such as the brain, liver and kidney (Dechent et al., 1999; Leuzzi et al., 2000; Ipsiroglu et al., 2001). As discussed earlier in this chapter (in section 1.3 *Energy production*,) the maintenance of PCr availability within the muscle cell is considered essential to continued force production and performance during high intensity exercise. Muscular fatigue during high intensity exercise has been associated with the inability of this tissue to maintain a high rate of anaerobic ATP production from PCr hydrolysis (Katz et al., 1986; Hitchcock 1989; Bogdanis et al., 1995). Conversely, improvements in muscular performance

during high intensity contractions have been associated with greater ATP resynthesis as a direct consequence of increased PCr availability via CrM supplementation (Harris et al., 1992; Greenhaff et al., 1994; Casey et al 1996; Kurosawa et al., 2003). However, CrM loading has also been shown to delay the onset of neuromuscular fatigue (Stout et al., 2000). This has been demonstrated during an incremental cycle ergometer test, "the physical working capacity at the fatigue threshold" (PWC_{FT}); an assessment which utilizes electromyographic (EMG) fatigue curves to identify the power output that corresponds to the onset of the neuromuscular fatigue (Stout et al., 2000). This benefit from supplementation was attributed to an elevated muscle PCr concentration on the transition from aerobic to anaerobic metabolism during exercise (Stout et al., 2000). However, CrM loading is also shown to shorten muscle relaxation time (defined as the time for muscle torque to decrease from 75 to 25% of maximum) during intermittent maximal isometric contraction (Van Leemputte et al., 1999; Hespel et al., 2002). SR Ca^{2+} reuptake by virtue of Ca^{2+}/ATPase pump activity is the rate-limiting step in relaxation of mammalian muscle cells (Dux, 1983). Thus the effect of CrM on relaxation rate suggests that SR Ca^{2+} reuptake is facilitated in Cr-loaded muscle (Van Leemputte et al., 1999; Hespel et al., 2002). This effect may promote an ergogenic action as the relaxation process accounts for an important fraction of total energy consumption during repeated contractions (Bergstrom et al., 1988).

As discussed previously (refer to figure 1.5), the formation of the polar PCr (from Cr and ATP), secures this high-energy phosphate within the muscle and maintains the retention of Cr because the charge prevents partitioning through biological membranes (Greenhaff, 1997). Therefore, supplementation with CrM is thought to enhance the cellular bioenergetics of the phosphagen system by increasing resting PCr concentration within muscle (Hultman et al., 1996; Greenhaff, 1997). However, according to Bessman & Savabi (1988) supplementation would boost the efficiency of the Cr-P_i shuttle and the transfer of high-energy phosphates between the mitochondria and the sites of major energy utilization (via functional coupling with ANT), such as the myofibrils. Thereby enhancing the availability of energy not only for muscle contraction but also the synthesis of contractile proteins (figure 1.8). Bessman et al. (1980) proposed that a protein synthesizing microsome lay adjacent to, or may even be a part of the CK complex at the site of the myofibril where it would receive some of the ATP liberated when this CK isoenzyme transfers ATP to the cross-bridge binding site (figure 1.8). This protein synthesizing complex would benefit from an increase in Cr-P_i shuttle activity that would occur from CrM supplementation and possibly, enhance the synthesis of contractile proteins during hypertrophy. A number of studies that examined the ergogenic effects of CrM supplementation also report a significant increase in body mass and/or LBM (Harris et al., 1992; Balsom et al., 1993; 1995; Earnest et al., 1995). For these

reasons, it was suspected that CrM may improve the development of strength and LBM accretion during RE training (Vandenberghe et al., 1997).

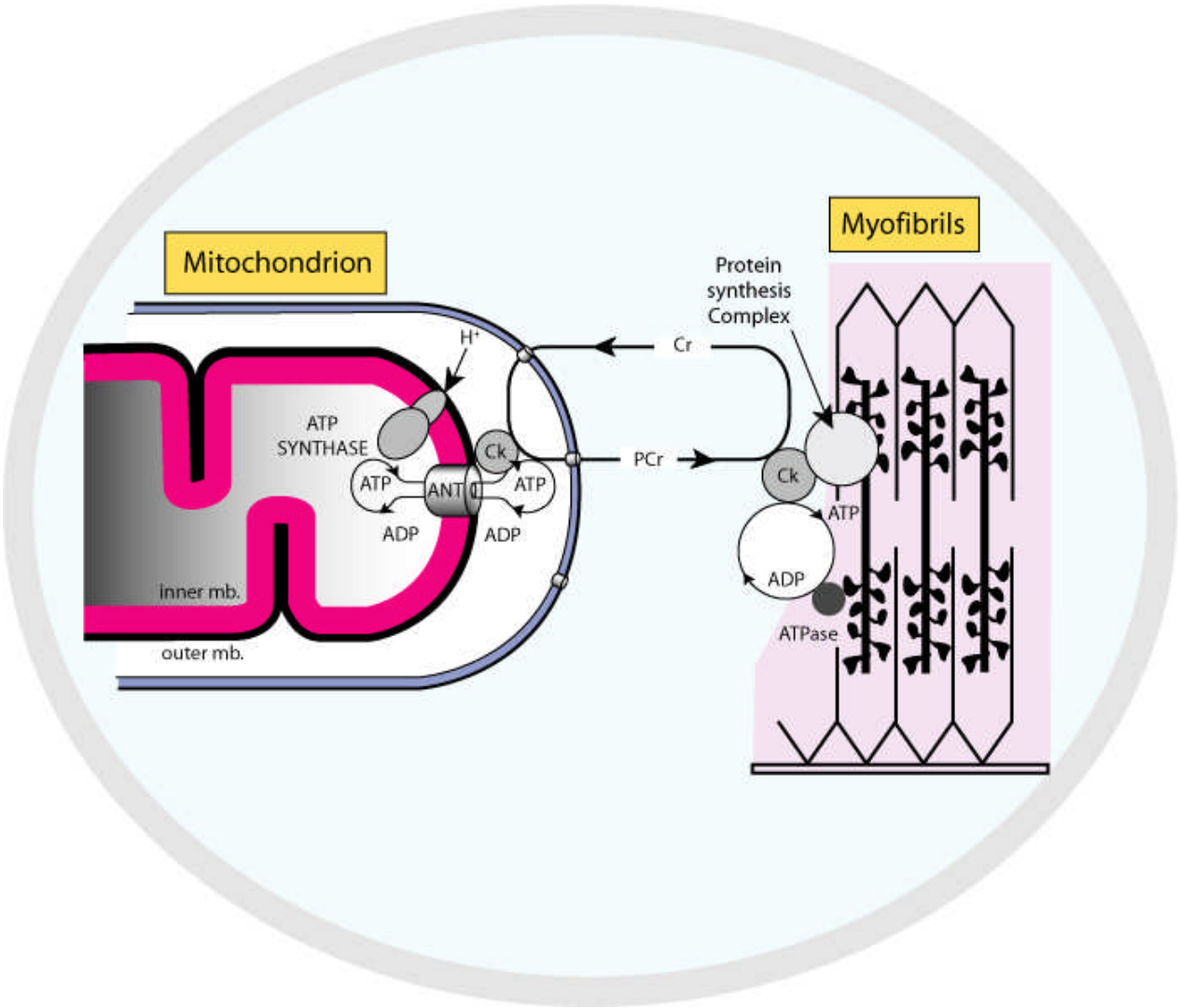


Figure 1.8 The Cr-P_i shuttle and its potential role in contractile-specific protein synthesis. Adapted from work by Saks et al. (1998); Bessman et al., (1980).

Consequently, several studies have confirmed that supplementation with CrM during RE training does improve gains in LBM in both men and women (Earnest et al., 1995; Vandenberghe et al., 1997; Bermon et al., 1998; Kreider et al., 1998; Volek et al., 1999; Becque 2000; Chrusch et al., 2001). Additional evidence suggests that the increase in lean mass and total body water from CrM during RE training is mainly intracellular (an increase in cell volume) suggesting that body cell dry mass has increased (Poortmans & Francaux 1999; Bemben et al., 2001). That is, CrM may promote an increase in cell volume and the subsequent activation of the anabolic mechanisms this phenomenon provides (refer to figure 1.7), resulting in greater accretion of LBM. Surprisingly, only two studies have quantified the extent of specific muscle fibre hypertrophy in response to RE training and CrM supplementation (Volek et al., 1999; Tarnopolsky et al., 2001). Only one managed to demonstrate that supplementation (loading phase followed by 5g/day for 12 weeks) resulted in significantly greater muscle fibre hypertrophy (all fibre types assessed) compared to a matched placebo-treated group.

Aside from a beneficial impact on LBM, a review of 22 studies involving CrM and RE concluded that supplementation does enhance strength and weightlifting performance (Rawson & Volek 2003). That is, when analysed collectively, CrM supplementation during training provides an average 8% better gain in muscle strength (as assessed by 1, 3, or 10RM) than placebo treatment (20 vs 12%)(Rawson & Volek 2003). The average increase in weightlifting performance (maximal repetitions at a given percent of maximal strength) following CrM supplementation is 14% greater than the average increase in weightlifting performance after placebo ingestion (26 vs 12%). The increase in bench press 1RM was as high as 45%, and an improvement in weightlifting performance in this exercise up to 43% (Rawson & Volek 2003). However, despite a substantial amount of data that suggests CrM can improve weightlifting performance, the development of muscle mass and strength, a clear mechanistic explanation for these benefits has remained elusive.

If CrM does enhance skeletal muscle morphology during RE, then one logical explanation would be that supplementation modulates some aspect of protein turnover; either by stimulating MPS or by decreasing MPB (section 1.4). Studies that have directly assessed the effect of CrM on protein turnover have shown that a typical loading phase does not enhance myofibrillar protein synthesis, leg net amino acid balance or decrease leg muscle protein breakdown either at rest (in both the fasted and fed state) (Louis et al., 2003a), or after RE (Louis et al., 2003b). Similarly, Parise et al. (2001) demonstrated that supplementation (loading phase followed by 5g/day for 4 days) did not enhance MPS in men or women but did decrease whole body protein breakdown by approximately 7.5%. Although this data demonstrates a lack of an acute anabolic effect of CrM on muscle protein turnover, it is important to remember that this is not necessarily strong evidence for

a lack of effect from supplementation on the mechanisms of muscle hypertrophy. For example, there is evidence that suggests the magnitude of increase in protein synthesis after exercise may be dependent on the extent of the previous reduction in energy status (a fall in the ATP/ADP ratio) during muscle contraction (Bylund-Fellenius et al., 1984). Other work has shown that CrM supplementation can attenuate the exercise-induced fall in the PCr/Cr ratio and maintain the ATP/ADP ratio during exercise (Kurosawa et al., 2003). Therefore, the CrM loading procedure performed by the participants in the study by Louis et al., (2003) may have blunted the magnitude of acute stimulation MPS normally observed after RE. Additionally, assessments in this study only lasted 4 hours post-exercise. Increasing the availability of PCr (via CrM supplementation) may enhance the efficiency of the Cr-P_i shuttle to deliver energy required for recovery and the synthesis of muscle proteins in the days after RE. However, the relationship between energy status and protein turnover in human muscle is one that has received very little exploration. Further research to resolve these speculations is warranted.

The effect of CrM on muscle morphology during RE may reside in the stimulation of transcriptional changes in muscle gene expression that might occur as a result of increased availability of PCr (and associated ATP/ADP concentration or Ca^{2+} concentration changes during/or after contractile activity). The results of which, in terms of protein accretion, would not be seen for days or weeks after the initial stimuli. To support this notion, Willoughby & Rosene (2001; 2003) demonstrated that supplementation with CrM (6g/day for 12 weeks of RE) resulted in greater (relative) strength, LBM and thigh volume. These benefits were observed alongside increases in the mRNA (type-I, IIa, and IIx), and protein (type-I, IIa and IIx) expression of the MHC isoforms as well as muscle CK mRNA expression, myogenin and MRF-4 mRNA (and protein) expression. It is apparent that Cr-loaded muscle is able to perform at a higher capacity during RE (Rawson & Volek 2003). These transcriptional changes in muscle gene expression that result in greater strength and hypertrophy from CrM during RE may contribute to, or be a product of, CrM's ability to enable muscle to work at a higher capacity. One study by Arciero et al. (2001) provides a classic example of this senario.

Arciero et al. (2001) compared 1RM strength gains in healthy young males after 28 days of CrM supplementation (loading phase followed by 10g/day) with or without RE training. Bench press and leg press 1RM increased 8 and 16% respectively with CrM alone. Compared to the respective 1RM strength gains demonstrated the by group that took CrM during RE training (18% for bench press and 42% for leg press), these results suggest that approximately 40% of the increase in strength over the 4-week period was simply due to the acute effects of CrM on muscle function (Arciero et al., 2001). CrM's ability to enable muscle to work at a higher capacity could

be due to an increased PCr availability for more efficient ATP resynthesis and/or Ca^{2+} handling in muscle, which would allow an athlete to perform more repetitions each set and/or provide more rapid recovery between sets. Clearly, this would enhance the potential for greater muscle strength and hypertrophy during a training program. To provide further evidence of this, Syrotuik et al. (2000) reported that when training volume was kept equal, participants ingesting CrM or placebo experienced similar increases in muscle strength and weightlifting performance following an 8-week resistance training program.

The improvements in strength, LBM and muscle hypertrophy that have been reported from CrM supplementation during RE could be a result of several mechanisms that have been discussed. These include, improved work capacity via a more efficient supply of ATP; an increased expression of the muscle genes and regulatory factors associated with hypertrophy; an increase in satellite cell mitotic activity; or initiation of other anabolic processes which may be secondary to increased cell volume. The evidence available suggests that CrM is a safe supplement to consume during exercise training (Poortmans & Francaux 1999; Groeneveld et al., 2005; Poortmans et al., 2005). This is important as CrM is a widely used supplement among populations that perform RE training (LaBotz & Smith 1999; Jacobson et al., 2001; McGuine, 2002; Sundgot-Borgen et al., 2003; Froiland et al., 2004; Morrison et al., 2004; Kristiansen et al., 2005). However, CrM may also confer a therapeutic benefit (i.e., increased LBM and/or muscle strength) to a wider section of the population such as older adults (Tarnopolsky, 2000), patients with neuromuscular disorders (Tarnopolsky et al., 1999; 2004), McArdle's disease (Vorgerd et al., 2000) and mitochondrial cytopathies (Tarnopolsky et al., 1997). Since 1993, well over 200 studies have examined CrM's ergogenic/therapeutic potential (Rawson & Volek 2003). In comparison, few studies have examined the effects of CrM supplementation on muscle morphology during RE training (Volek et al., 1999; Tarnopolsky et al., 2001; Willoughby & Rosene 2001; Fry et al., 2003). In particular, although a number of studies have assessed changes in strength and body composition in response to supplementation and training (Earnest et al., 1995; Vandenberghe et al., 1997; Bermon et al., 1998; Kreider et al., 1998; Volek et al., 1999; Becque 2000; Chrusch et al., 2001), few have assessed these changes alongside fibre-specific hypertrophy responses (Volek et al., 1999; Tarnopolsky et al., 2001; Fry et al., 2003). Additionally, no studies involving CrM supplementation during RE training have reported changes in strength and body composition alongside adaptations at the cellular level (i.e., fibre-specific hypertrophy) and the sub-cellular level (i.e., contractile protein content). Finally, no studies have examined the effects of combining CrM with WP on muscle fibre characteristics during RE training.

1.10 Resistance exercise program design for muscle hypertrophy

RE is considered a key component in any health program by organizations such as the American College of Sports Medicine (ACSM) (Feigenbaum & Pollock 1999) and the American Heart Association (AHA) (Fletcher et al., 1995; Pollock et al., 2000). RE is also a cost-effective activity that can be easily implemented into the lifestyles of most adults (Roubenoff, 2003). However, despite the well documented benefits, research on program design to optimize hypertrophy is a topic that has received scant attention from the scientific community. RE program prescription for muscle hypertrophy would involve the correct choice of variables, such as frequency, intensity, volume and exercise selection to provide the optimal cellular and molecular responses that promote the desired result. Unfortunately, this information is yet to be clearly identified. Studies that have compared the effects of manipulating program variables have mainly been concerned with strength rather than hypertrophy outcomes (Berger 1962; 1965; Anderson & Kearney 1982; Willoughby, 1993; Stone & Coulter 1994; Tan, 1999). While the development of strength is an important aspect, this chapter has demonstrated that hypertrophy is a multifaceted phenomenon that can be influenced by a myriad of factors. Until we have a better understanding of the various factors that influence hypertrophy (such as age, gender, endocrine profiles, previous training status, nutrition and genetics), program design will remain at best, an educated guess based on protocols and principles that would most likely be effective for the population group concerned. This situation will have to change in the near future. The emerging importance of muscle preservation for healthy ageing and the spiralling healthcare costs related to sarcopenia in a rapidly ageing population will eventually force governments to focus greater attention on supporting research that will determine the most effective exercise programs for hypertrophy. Nevertheless, this section will attempt to provide the reader with a science-based approach to RE program design for optimizing muscle hypertrophy.

A science-based approach to RE prescription can be traced to the post World War II era, when army physician's DeLorme and Watkins designed progressive RE programs for rehabilitation of orthopaedically disabled veterans (DeLome, 1945; DeLome & Watkins 1948). Their studies demonstrated that the use of heavy resistance and a low number of repetitions developed muscular strength, whereas the use of lighter resistance and a higher number of repetitions developed muscular endurance. DeLome and Watkin's research also underscored progression as a fundamental principle in effective RE program design. Progression is defined as the act of moving forward or advancing toward a specific goal. Progression in RE entails structuring the training program for continued improvement in a desired variable over time or until the target goal has been

achieved (Kraemer et al., 2002). Although untrained individuals respond favourably to a wide variety of protocols (Campos et al., 2002; Harris et al., 2004), progression is a fundamental aspect of program design as physiological adaptations take place in a relatively short period of time (Staron et al., 1994). For example, it is clear that training reduces the stimuli of MPS for a given load (Phillips et al., 2002), and less muscle mass is recruited to lift a given load once adaptation has occurred (Ploutz et al., 1994). Therefore, systematically increasing the demands placed upon the muscle(s) is necessary for further improvement (Ploutz et al., 1994). Progressive RE program design involves the proper manipulation of variables to systematically increase the training stress. This can be achieved by increasing the amount of resistance (overload) used, manipulating the number of sets and repetitions completed and altering exercise selection (i.e., progressing to more complex movements). Periodization in RE training refers to planned changes in the training program in a direct attempt to bring about a peak training response (Fleck & Kraemer 1997). In this respect, periodization is closely related to progression. For example, the classic linear strength/power periodization approach follows a general trend of decreasing volume while increasing intensity during a training program so that strength/power is optimized at the end of the program (Fleck, 1999). However, periodization can also involve an undulated manipulation of program variables (Willoughby, 1993). Both approaches are thought to be effective strategies that help to limit natural training plateaus (that point in time where no further improvements takes place) and enable higher levels of adaptation to be achieved (Fleck, 1999).

As identified previously in this chapter, the stimulation of MPS is the facilitating mechanism that underlines hypertrophy (Rennie et al., 2004; Cuthbertson et al., 2005). However, it is also apparent that the degree of overload placed on muscle determines the magnitude of stimulation of MPS (Goldberg, 1968; Baar & Esser 1999; Phillips et al., 2002). Additionally, a close relationship exists between strength and muscle size (Häkkinen et al., 1991; Tesch 1992). Therefore, the classical prescription for muscle hypertrophy would appear to still hold true. That is, size and strength gains are directly proportional to the magnitude of overload placed on muscle (Atha, 1981; Saltin, 1983; Tesch, 1992). An improvement in strength would enable greater overload to be placed on the muscle(s) and therefore, provide further potential for hypertrophy (Tesch, 1992; Kraemer, 1996; 1997). This appears to be the basic recommendation from the leading organizations that prescribe RE, such as the ACSM and the National Strength and Conditioning Association (NSCA). However, despite a very limited amount of data to draw from, both organizations also provide rather specific training recommendations for hypertrophy that are quite distinct from training for strength development. The reason for this is perplexing as a science-based distinction between training for strength and training for hypertrophy is very difficult—the principles of the former are intertwined within the latter. As a result, the following paragraphs will

reveal that the hypertrophy-specific training recommendations made by the leading organizations are based primarily on anecdotal reports and/or highly speculative observations. And, most of these recommendations actually contradict the foundation principles required to induce muscle hypertrophy.

Training load is an important variable as it typically defines the intensity of the exercise (Kraemer et al., 1996). That is, intensity is typically described as the amount of weight used when performing an exercise (Kraemer et al., 1990; Häkkinen et al., 1993; Ahtiainen et al., 2003). This is often defined by the maximum amount of repetitions that can be completed with a given weight (Tan, 1999). For example, the amount of weight that can be lifted once successfully, with correct technique is the participant's 1RM (Stone et al., 2000). Progression in RE is easily implemented by manipulating the training load. This can be achieved by increasing the load on the basis of a load-repetition continuum such as using a percentage of the 1RM (i.e., a higher percentage indicates fewer repetitions can be performed as the weight is heavier, as opposed to using a lower percentage with a lighter load where more repetitions can be completed) (Fleck & Kraemer 1997). Alternatively, progression can be applied by increasing the training load within a prescribed zone (e.g., 8-12 RM) (Tan 1999). With this method, when a specific RM zone is exceeded, a 2-10% increase in load is applied so that the individual continues working within the designated RM zone (Kraemer et al., 2002). Longitudinal studies show that a range of maximal loads (from 1 to 12RM or 100-60% of 1RM) can provide improvements in strength and hypertrophy in the short term (12-16 weeks) (Cureton et al., 1988; Staron et al., 1991; 1994; Kraemer, 1996; Goto et al., 2005). Particularly, untrained individuals respond to a wide variety of loading protocols, even low loads (15-50% of 1RM) (Gettman et al., 1978; Sale, et al., 1990; Moss et al., 1997; Bemben et al., 2000). While recent work has confirmed an "RM continuum" for muscle strength and endurance development (Anderson & Kearney 1982; Stone & Coulter 1994; Tan, 1999), the information on loading protocols to optimize hypertrophy is diverse and somewhat contradictory.

Studies that have assessed muscle hypertrophy responses to different loading protocols have utilized inexperienced (untrained) participants (Taaffe et al., 1996; Moss et al., 1997; Bemben et al., 2000; Goto et al., 2005) and/or short assessment periods (8 to 12 weeks). Consequently, results have been equivocal; a range of maximal loads from 3 to 20RM have been reported to induce hypertrophy (Hisaeda et al., 1996; Ostrowski et al., 1997; Chestnut & Docherty 1999; Campos et al., 2002). It is also pertinent to note that none of these studies mention any attempt to control or monitor dietary intake. This is an important consideration as the type, timing and quality of nutrient intake is thought to influence the hypertrophy response to RE (Lemon et al., 2002). In their accreditation text the NSCA recommends that moderate loads (i.e., 8-12RM) should be used

for hypertrophy-specific training (Conroy & Earle 2000). The reason is uncertain as no references are provided. However, in another chapter of the same text, it is recommended that heavy loads (in the 3-5RM range) are most effective in stimulating growth of all muscle fibres (Kraemer, 2000). The most recent recommendations provided by the ACSM (Kraemer et al., 2002) are that heavier loads (1-6RM) provide optimal strength development whereas the use of moderate loads (8-12RM) will optimize hypertrophy. However, to date, no longitudinal studies have been able to confirm this and the results of two recent 6 month-long training studies refute these training recommendations directly (Kraemer et al., 2004; Ahtiainen et al., 2005b). These studies report no differences in terms of muscle strength and hypertrophy development between the use of heavy (1-6RM) and moderate (8-12RM) training loads. This finding has been confirmed in both trained (Ahtiainen et al., 2005b) and untrained participants (Kraemer et al., 2004). (It is also interesting to note that both of these more recent studies monitored dietary intake!) The development of strength improves the potential for hypertrophy. Therefore, optimizing strength development should be a key feature of program design for hypertrophy. Utilizing a range of maximal training loads (from 12RM to 3RM) in a linear or undulating, periodized fashion would appear to achieve this (Willoughby, 1993; Kraemer 1996; Fleck, 1999).

While it is apparent that muscle hypertrophy can be achieved with a range of maximal loads, a research-based quantifiable prescription for other programming variables such as training volume, rest intervals and training frequency remains elusive. Training volume can simply be defined as the number of sets x repetitions performed x the load used (Tesch, 1992). It does appear that a certain total amount of work is required to produce hypertrophy (Rhea et al., 2002; 2003). For example, multiple-set programs are more effective than single working set programs once an individual progresses past the novice stage (Schlumberger et al., 2001; Rhea et al., 2002; McBride et al., 2003; Kemmler et al., 2004). However, more precise recommendations on training volume for hypertrophy vary tremendously (Tesch, 1992; Conroy & Earle 2000; Kraemer et al., 2002; Rhea et al., 2003). To optimize muscle hypertrophy, organizations such as the NSCA and ACSM recommend the use of high volume (the use of up to 20 working sets for each muscle group), moderate RM loads and short rest intervals (under two minutes) between sets (Baechle et al., 2000; Conroy & Earle 2000). However, none of the literature cited by either organization provides data that directly supports these rather definitive recommendations. The ACSM's rationale for this hypertrophy-specific approach is based on data that shows a greater increase in circulating anabolic hormones (Kraemer et al., 2002). Some (Kraemer et al., 1990; 1991), but definitely not all (Raastad et al., 2000), training studies support this finding. More importantly, at the time of publication no physiological advantages (such as greater strength or hypertrophy development) had been documented from these acute hormonal perturbations. More recently, training studies that have

assessed physiological outcomes from manipulating volume report no physiological advantages from a "hypertrophy-specific" protocol similar to that recommended by the ACSM when compared to a traditional strength training program (Ahtiainen et al., 2005b). The only research-based, quantifiable, prescription for training volume can be found in recent meta-analyses on dose-response relationships for strength development (Rhea et al., 2003; Peterson et al., 2004). These analyses report that experienced individuals obtain the best strength gains by training each muscle group twice a week, with high loads (approximately 80% of the 1RM) and a total of only 4 working (non-warm up) sets per muscle group (Rhea et al., 2003). More competitive (or professional) strength athletes are recommended to use a similar frequency at a slightly higher intensity (85% of the 1RM) and volume, up to 8 workings sets per muscle group (Peterson et al., 2004).

In RE training for sports, exercise selection is based on the movement patterns of the activity. However, if the goal of RE is to induce hypertrophy then the research dictates that exercise selection should be based primarily on overload. This is based on the premise that it is the degree of overload that governs the magnitude of stimulation of MPS and subsequent changes in muscle mass (Goldberg, 1968; Baar & Esser 1999). Therefore, exercise selection for hypertrophy development should be based on the movement patterns that enable the highest amount of overload to be placed on the working muscles. While both single- and multiple-joint exercises are shown to be effective for promoting muscular strength and hypertrophy (Kraemer et al., 1996), multiple-joint (complex) exercises such as squats, deadlifts and presses allow for greater overload and motor neural activation (Escamilla et al., 2001). Therefore, multi-joint exercises would provide a greater scope for strength and hypertrophy development (particularly as the experience of the participant increases) (Haff, 2000). For similar reasons, the use of free-weights (barbells and dumbbells) is thought to be more advantageous for strength and hypertrophy development as opposed to weight-stack machines (Escamilla et al., 1998; 2001). Weight-stack machines require little stabilization of non-moving body parts, provide a fixed plane of movement and limit the synergistic activation of the joints and muscles involved (Haff, 2000). While weighted machines focus the activation to a specific set of prime movers, free-weights require a much higher degree of intra- and intermuscular coordination for controlled movement during exercise execution (Escamilla et al., 1998; 2001; Haff, 2000). Consequently, exercises that incorporate free-weights provide greater motor neural activation than machines for a designated load (Escamilla et al., 1998; 2001).

Some research suggests that the order (sequence) in which exercises are performed will affect strength development (Sfrozo & Touey 1996). To ensure the highest level of overload possible, more complex (i.e. free-weight, multi-joint) exercises should be performed before single-

joint exercises that involve the assistant musculature (Kraemer et al., 2002). Bodybuilders have been known to use a pre-exhaust technique which typically involves performing a single-joint exercise (such as the leg extension) prior to performing a related multi-joint movement (such as the squat or leg press). This training strategy is performed in the belief that it will increase the intensity placed on the working muscle (and therefore stimulate greater hypertrophy). However, the only study to examine this aspect directly has shown that the pre-exhaust technique actually decreases muscle activation and performance during the multi-joint exercise (Augustsson et al., 2003). The pre-exhaust technique reduces the intensity (overload) placed on a muscle, it does not increase it. Therefore, multi-joint, free-weight exercises should be emphasized in programs that aim to optimize muscle strength and hypertrophy, and these exercises should be performed early in the workout, before related single-joint exercises (Kraemer et al., 2002).

A common recommendation for hypertrophy is that muscle(s) should be worked to fatigue by using short rest intervals along with a higher number of sets and/or repetitions (Baechle et al., 2000; Conroy & Earle 2000). This recommendation is based upon some research that suggests metabolite accumulation (H^+, lactate, P_i, Cr, K^+) both inside and outside the cell during high resistance contractions is an important stimulus for hypertrophy (Rooney et al., 1994; Shinohara et al., 1998). While this may be the case, no research has been able to link muscle contraction to fatigue with hypertrophy (Tesch, 1992) and some has refuted this notion directly (Folland et al., 2002). The amount of rest between sets and exercises is shown to affect weightlifting performance (Kraemer, 1997; Richmond & Godard 2004; Willardson & Burkett 2005), strength gains and work capacity during training (Robinson et al., 1995). It is clear that weightlifting performance is compromised by short rest intervals between sets (i.e., 30-60 seconds) and improved by longer rest intervals (two-three minutes), particularly when complex exercises are involved (Richmond & Godard 2004; Willardson & Burkett 2005). Some longitudinal resistance training studies have shown greater strength increases with long versus short rest periods between sets (e.g., 2-3 minutes vs. 30-40 seconds) (Robinson et al., 1995; Pincivero et al., 1997). These findings underline the importance of adequate recovery between sets for optimizing weightlifting performance and maximizing strength development (thus, enhancing the potential for hypertrophy). There is no evidence that demonstrates the use of shorter rest intervals, (as recommended by the NSCA and ACSM for hypertrophy-specific RE training) provides greater advantages, particularly when compared to more traditional strength training protocols that involve longer rest intervals (2-5 minutes). In fact, the research available suggests that the recommendation of shorter rest intervals would be counter-productive to hypertrophy development as it appears to reduce work capacity and/or the amount of overload used (Richmond & Godard 2004; Willardson & Burkett 2005). Based on the data available on this topic, rest intervals of at least two to five minutes between sets

and exercises should be utilized when performing complex exercises with heavy loads. Rest intervals of one to two minutes could probably be used for single joint exercises as they generally involve lighter loads, less muscle mass and therefore, less intensity.

To summarize program design for muscle strength and hypertrophy development, the research suggests that experienced participants will benefit from a periodized program that involves a progressive overload approach with varied maximal repetition ranges (anywhere from 1-12RM). An emphasis on the heavier end of the RM spectrum will provide greater strength improvements and therefore, greater potential for hypertrophy. Multiple-joint, free-weight exercises should form the core of exercise selection and these compound exercises should be performed before the related single-joint exercises. In terms of training volume, the literature on strength development suggests that in trained individuals, best results are achieved when each muscle group is trained twice a week with a total of 4 to 8 working sets per muscle group, each workout.

1.11 Chapter summary

It is clear that lifestyle strategies that focus on building/preserving skeletal muscle mass will enhance the health of a wide sector of the population and diminish the severity of many ageing-related illnesses as well as reduce a significant economic burden on the health care system. RE is a potent stimulator of protein turnover, particularly MPS; which appears to be the facilitating mechanism that underlines an increase in muscle mass (hypertrophy). Therefore, RE is a fundamental component in the development of muscle hypertrophy. However, hypertrophy is a multifaceted phenomenon that appears to be influenced by many factors such as, age, gender, hormones, training status, the type of RE protocol, and genetic factors as well as many nutritional aspects. At present, there is a paucity of data on the impact that these aspects have on the hypertrophy response to RE training. A clear understanding of the processes that lead to hypertrophy is also exacerbated by the multifaceted role of muscle tissue in the regulation of whole body protein metabolism.

The idea that the hypertrophy response to RE training may be enhanced under certain conditions has only been considered by the scientific community in recent years. However, in that short amount of time, a substantial amount of evidence has accumulated that suggests the type, timing and quantity of macronutrient intake does affect muscle (and whole body) protein turnover, and therefore, has the potential to influence chronic adaptations from RE training. One clear example is the acute anabolic effects obtained from the strategic consumption of macronutrients

(namely protein and carbohydrate) close to RE. This nutrient-timing strategy interacts synergistically to stimulate a higher degree of anabolism and provide a net gain in muscle protein. In longitudinal studies, this nutrient-timing strategy is shown to be accumulative and result in greater muscle hypertrophy. However, RE affects protein turnover for at least 24 hours. Therefore, an individual's habitual (daily) protein intake may also prove to be one of the more important variables that influence the size of human muscle mass—particularly since it has recently been confirmed that the concentration of EAA in the blood is a regulator of protein synthesis rates within muscle. Studies that link acute responses to chronic adaptations from macronutrient intake and RE training is an area of research that needs to receive systematic investigation to ascertain a better understanding of what is required to promote muscle hypertrophy in adults. Additionally, the study of conditions that may optimize the hypertrophy response to RE is an exciting area of research that is still very much in its infancy. For example, although chronic training is presumed to diminish the hypertrophic response, studies that have utilized a personal-training approach to supervision with trained participants and dietary intervention with supplements such as WP and CrM, have reported muscle hypertrophy responses that were previously thought only to be possible via the use of anabolic steroids. Further research in this area would not only provide a clearer understanding of the conditions that ensure a better hypertrophy response, it may also help to reduce some of the large gaps in our basic understanding of the mechanisms that lead to this prized adaptation. Clearly, examining the effects of dietary and training strategies that may improve the hypertrophy response to exercise is an area of research that has a number of beneficial implications.

The focus of this dissertation is to examine the effects of dietary intervention with WP and CrM during RE designed to promote hypertrophy in healthy humans. The major aim of this dissertation is to examine the effects of consuming these supplements separately and in various combinations as well as at strategic times of the day, on chronic adaptations (i.e., body composition, muscle strength and fibre-specific hypertrophy). Three separate trials are presented to which a general hypothesis can be applied; supplementation (utilizing the prescribed protocol) will enhance the chronic adaptations that are desired from an RE training program. The specific aims, hypotheses, supplement protocols, methods used and results of each of these trials are presented in the following chapters. It is hoped that information obtained from these studies may contribute to a better understanding of what is required to improve muscle strength and hypertrophy development in healthy adults undertaking RE.

Chapter 2

Methods and Procedures

Each of the studies presented in this thesis utilized a longitudinal design, that is, a RE training/dietary supplementation program with pre- and post- intervention assessments. The same methods and procedures were used in all studies. Details of the experimental design, aims and hypothesis of each study are provided in the appropriate study chapter. The studies will be referred to numerically as follows.

Chapter 3, Study 1. The effects of whey isolate, creatine & resistance training on muscle fibre characteristics, strength and body composition

Chapter 4, Study 2. The addition of creatine to a protein-carbohydrate supplement improves muscle fibre hypertrophy, strength and body composition during resistance training

Chapter 5, Study 3. Supplement-timing improves muscle fibre hypertrophy, strength and body composition during resistance training

2.1 Participants

In all studies, participants were healthy male volunteers aged between 18 and 35 years. The participants were experienced, recreational bodybuilders that had performed RE consistently for at least six months prior to the study. Although the participants' training age ranged from 6 months to 10 years, when statistically analysed, there were no differences in training age between the groups in each trial. All participants were recruited from local gymnasiums and fitness centres via poster advertising and word of mouth. The participants were not paid to partake in the study and were free to withdraw from the research at any time they desired. To qualify, the men (a) had no current or past history of anabolic steroid use; (b) had been training consistently (i.e., 3-5 days per week) for the previous 6 months (c) submitted a detailed description of their current training program; (d) had not ingested any ergogenic supplement for 12-weeks prior to the start of the study; and (e) agreed not to ingest any other nutritional supplements, or non-prescription drugs that may affect muscle growth or the ability to train intensely during the study. All of this information

was obtained via questionnaire. Participants were informed of the potential risks of the investigation before signing an informed consent document approved by the Human Research Ethics Committee of Victoria University and the Department of Human Services, Victoria, Australia and adhered to the principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects.

In the months prior to study 1, several RE workshops were held on weekends in the facility where the RE training was to be completed (Maidstone Fitness Centre, Rosamond Rd, Maidstone, Melbourne Victoria). These workshops were held for screening purposes but also to demonstrate the correct execution/technique of the exercises to be completed in the training program. In these workshops the candidates had to demonstrate the ability to correctly perform each exercise used in the strength tests (as described by Baechle et al., 2000). Aside from these workshops, the participants selected for studies 2 and 3 were given a RE program similar to the one used in the trial (table 2.1) and were overseen while performing this program for 8-12 weeks prior to the start of each trial. The RE training for studies 2 and 3 was held at Lakeside Fitness, Sunshine Rd, Sunshine, Melbourne, Victoria.

A sample size (*n*) of 11 per group has been shown to be sufficient to detect a (DEXA scanned) change in LBM of 2.0% (Chilibeck et al., 1994). Our previous work (Cribb et al., 2006) and others (Volek et al., 1999) that have involved supplementation and supervised RE training have shown increases in LBM of 3 to 6% over 10-12 weeks of training. Other researchers that have undertaken RE training/supplementation studies have determined *a priori* that an *n* of 11 per group is adequate to avoid a type II statistical error (Tarnopolsky et al., 2001). Therefore, studies 2 and 3 commenced each trial with at least 11 participants per group. In study 1, four groups were utilized, therefore an attempt was made to commence this trial with an *n* as close as possible to this number.

2.2 Supplementation

In each of the studies, after baseline testing (see experimental protocols) participants were matched for maximal strength in three weight training exercises (see strength assessments), weighed and then randomly assigned to a dietary supplement in a double-blind fashion. In each study the participants' were instructed to consume their supplement in the prescribed manner for the duration of the study. Each study utilized different supplementation protocols and these are described in the appropriate chapter. The supplements used in the studies presented in chapters 3, 4 and 5 were mixed from the following "stock" supplements.

- A carbohydrate (CHO) supplement that was D-glucose (containing approximately 89g carbohydrate 0; protein 0; fat/100g of supplement.) The rest of the supplement consisted of flavours, colour and electrolytes.
- A protein (PRO) supplement that was whey isolate (containing approximately 86g protein >5g carbohydrate, >1g fat /100g of supplement). The rest of the supplement consisted of flavours, colours and electrolytes.
- Creatine monohydrate; 99.9% CrM (containing approximately 100g CrM/100g of supplement)
- As CrM uptake is dependant on secondary active co-transport with Na^+ both the CHO and PRO supplements contained an equivalent amount of sodium (approx 250mg and 270mg per 100g of supplement for PRO and CHO, respectively).

These supplements were supplied by AST Sports Science, Golden, Colorado USA and contained no other performance enhancing substances. Specifically, they were tested to comply with label claims before leaving the place of manufacture in the USA. The supplements used in each trial were obtained from these stock supplements. That is, each batch was measured, weighed and packaged by the author in identical, unmarked containers and sealed with tamper-proof lids. (A complete nutrient breakdown of all supplements and dosages used in each trial are described in the appendix). Additionally, the whey protein was independently assessed by Naturalac Nutrition LTD (Level 2/18 Normanby Rd Mt Eden, New Zealand) on two separate occasions, and matched labelled ingredients on both occasions. To ensure a double-blinded procedure, Dr. Alan Hayes coded the supplements for distribution to the participants. In each study, the dose was prescribed in grams of supplement per kilogram of body mass per day (g/kg/day). This dose was described to the participant in measured scoops that were provided in the supplement. The participants signed a consent form stipulating that they would follow their habitual daily diet (obtained from dietary records) and take the supplement only as prescribed during the study. Potential changes in body mass were tracked by weighing the individuals each week on a balanced Seca 703 stainless steel digital medical scale (Seca, Perth, WA) at the gym. When the individual showed a substantial increase (i.e. a 2 or more kilo change) in body mass from baseline, they were instructed to increase their supplement serving size in accordance with the increase in body mass. Participants were given an approximate one-week supply of the supplement at the start of each week and asked to return the container before they received the next weeks supply as an act of compliance to the dosing procedure. Additionally, the participants recorded the times that the supplement was consumed in the dietary record books provided to them.

2.3 Dietary Recordings

Prior to each study, the participants were shown how to record nutrient intake and each participant was asked to submit three written dietary recordings of their daily food/energy intake. Each recording consisted of three days; one weekend day and two mid-week days. Participants were asked to submit one of these recordings before the study, one in the first week and another in the final week of the training/supplementation program. The macronutrient profiles of all recordings were analysed using Nutritionist PRO (First Data Bank, San Bruno, CA, USA). The energy, carbohydrate, and protein intake from these records were statistically evaluated. The participants were weighed on the Seca 703 stainless steel digital medical scale (Seca, Perth, WA) on each occasion. Energy intake was expressed in kJ/kg of body mass per day, protein and carbohydrate were expressed in g/kg of body mass per day. Three-day recalls were selected as longer periods tend to influence under reporting of eating patterns and food intake (Craig et al., 2000; Goris et al., 2001). A similar procedure to that used in these trials has been used to monitor nutrient intake in other studies involving RE and supplementation (Kreider et al., 1998; 2002; Volek et al 1999; Antonio et al., 2001).

2.4 Resistance Training Protocol

In each study, the RE training program began the week immediately after baseline assessments and continued for 10 or 11 weeks. The RE program was essentially the same in each study (table 2.1) and consisted of high-intensity workouts using mostly compound exercises with free-weights. The primary goal of the program was to increase strength and muscle size. The program followed the principles thought to produce effective gains in strength and muscle hypertrophy (discussed in section 1.10 of the previous chapter). Training intensity for the program was initially determined using repetition maximums (RM) from strength tests. A designated RM was applied in each training phase. Once the RM range was reached, the participants were instructed by the trainer to increase the weight used so that the participant continued to work within the prescribed RM range. The progressive overload program was divided into 3 phases; Preparatory (10-8RM), Overload Phase-1 (6RM), and Overload Phase-2 (4RM). Participants were given training sheets to record exercises, sets, repetitions performed and the weight used. These sheets were viewed by the author usually after every workout, or at least on a weekly basis.

All workouts were supervised by the same two qualified instructors; the author (a certified Strength and Conditioning Specialist; NSCA) and a certified personal trainer. All workouts were supervised in a personal training fashion. That is, either a two-to-one or one-to-one personal training. Individual, one-to-one personalized training during RE is shown to provide a greater rate and magnitude of training load increases, which in turn promotes greater strength gains (Mazzetti et al., 2000). The instructors were blinded to the supplement groups at all times during training and strength testing. A roster was also devised so that each participant received an equivalent amount of supervision from each instructor. The following assessments occurred in the week before and after each RE program.

2.5 Strength assessments

Strength assessments consisted of the maximal weight that could be lifted once (1RM) in three weight training exercises such as the barbell bench press, squat and cable pulldown or deadlift. An absolute measure of strength was chosen simply because bodybuilding was the primary aim of this research. That is, the training/supplementation protocols were designed to increase muscle mass, and relative measures of strength tend to favour individuals with less body mass. The 1RM testing protocol and exercise execution guidelines that were followed have been previously documented (Baechle et al., 2000). Briefly, the participant's maximal lift was determined within no more than five single repetition attempts following three progressively heavier warm up sets. Participants were required to successfully lift each weight before attempting a heavier weight. Each exercise was completed before the next attempt and in the same order. Each 1RM strength assessment was performed 5-10 days before and after the training/supplementation program. All strength tests were completed at the same facility that the participants performed the RE training program. Reproducibility for these tests was determined on 2 separate occasions. These resulted in non-significant coefficients of variation (CV) that ranged from 0.5-5%.

2.6 Body Composition

Whole-body composition measurements were determined using a Hologic QDR-4500 dual energy x-ray absorptiometry (DEXA) with the Hologic version V 7, REV F software (Waltham, MA). All scans were performed on the same apparatus, located at Victoria University's Biomechanics Laboratory, by the same licensed DEXA operator. Quality control calibration procedures were performed on a spine and step phantom prior to each testing session according to procedures previously described (Ellis & Shypailo, 1998). Participants were positioned exactly in

the manner as recommended by the manufacturer. The DEXA scans all regions of the body to determine the amount of bone mass, fat mass and lean body mass within each region. Body fat percent is calculated by the software, dividing the amount of measured fat mass by total scanned mass (sum of bone mass, fat mass and lean mass). Most participants were scanned at the same time of the day, that is, in the morning in a fasted state. For longitudinal studies in which small changes in body composition are to be detected, whole body scanning with this instrument has been shown to be accurate and reliable (precision errors 0.8-2.8%) (Prior et al., 1997; Ellis & Shypailo 1998).

Table 2.1 Resistance training program

Monday	Tuesday	Thursday	Friday
General preparatory phase (weeks 1-2) 2 sets, 10 to 8RM			
Barbell squat	Barbell bench press	Barbell deadlift	Standing b'bell press
45^{o}Leg press	Incline b'bell bench press	Pull-ups (wide grip)	Lateral d'bell raise
Stiff-leg deadlift	Dips + weight	One-arm d'bell row	Barbell curl
Calf press	Core stabilization exercise	Seated cable row	Close-grip bench press
Abdominal strength exercise		Abdominal strength exercise	Core stabilization exercise
Overload Phase-1 (weeks 2-6) 2 sets, 6RM			
Barbell squat	Barbell bench press	Barbell deadlift	Standing b'bell press
Barbell stationary lunge	Incline d'bell press	Pull-ups (close grip) + weight	Lateral d'bell raise
Stiff-leg deadlift	Dips + weight	Barbell row	d'bell curl
Calf press	Core stabilization exercise	Abdominal strength exercise	Cable push-down
Abdominal strength exercise			Core stabilization exercise
Overload Phase-2 (weeks 7-10) 2 sets, 4RM			
Barbell squat	Barbell bench press	Barbell deadlift	Standing b'bell press
Barbell lunge	Incline d'bell press	Variety of pull-ups + weight	Lateral d'bell raise
Stiff-leg deadlift	Decline d'bell bench press	Barbell row	Barbell curl
Calf press	Dips + weight	One-arm d'bell row	Close grip bench press
Abdominal strength exercise	Core stabilization exercise	Abdominal strength exercise	Core stabilization exercise

2.7 Muscle sampling, treatment and analyses

All muscle samples were obtained from the vastus lateralis via needle biopsy. After local anaesthesia (1% xylocaine) a small incision of the skin was made (approximately 10 cm to the lateral epicondyle of the right femur), and a muscle sample (50-400mg) was obtained from the vastus lateralis muscle using the percutaneous needle biopsy technique modified for suction (Evans et al., 1982). To minimize potential variations in fibre type distribution, post-training biopsies were obtained from a close proximity to the pre-training biopsy scar at a similar needle depth (approximately 2cms), and pre- and post-training biopsies were performed by the same medical practitioner. One small part of the muscle sample was immediately frozen for metabolite and protein analysis. The remaining tissue was mounted using Tissue-Tek medium after the fibres had been orientated in a longitudinal direction and snap frozen in isopentane pre-cooled in liquid nitrogen and stored at -80^{o}C for histochemical analysis. Biopsies were obtained at Victoria University's Exercise Physiology Laboratory and all muscle analyses were completed at Victoria University's Exercise Metabolism Unit.

The mounted biopsy samples were serially sectioned (12μm thick) on a cryostat microtome at –20^{o}C. Staining for ATPase was used to classify muscle fibre types-I, IIa and IIx based on the methods of Brooke and Kaiser (1970). A preincubation medium of pH 4.54 along with the standard preincubations of 4.3 and 4.6 was included to enable a clear differentiation of the type-II sub-groups (Dubowitz 1985) (figure 2.2). Baseline and endpoint cross-sections were assayed simultaneously. A loaded image of the stained cross-sections was analysed using Analytical Imaging Station (AIS) software™ (Imaging Research Inc. Ontario, Canada) interfaced to a Zuiss BH-2 microscope. This software enables accurate CSA determination and classification of fibre types as staining intensities (hues) can be calibrated and scaled to provide clear threshold values. This minimizes inconsistencies that may occur with operator-determined identification of the type II sub-groups. On most occasions, the hue calibrations allowed for automated detection and calculation of the muscle fibre CSA. Repeated measures yielded a mean deviation of <0.001% for the same fibre. On occasions where hue calibrations did not enable automated detection of CSA, the CSA of the muscle fibre was manually traced with the computer cursor. A range between 50 (Fry et al., 2003) and 400 (Staron et al., 2000) individual muscle fibres have been counted to assess fibre type CSA and proportion during RE training. The studies presented within this dissertation determined fibre type percentage and CSA from sections containing 210 ± 23 (mean ± SE) fibres;

an increase in this number does not appear to reduce the calculated variation to any great extent (Bloomstrand & Ekblom 1982). To assess reproducibility, 37 samples were measured twice on two separate occasions for percentage total fibre area, and mean area of fibres. The intra-assay CV were non-significant, (1.5% and 1.3%, respectively).

For the determination of ATP, PCr, Cr and glycogen, approximately 10 milligrams of muscle tissue was freeze-dried at -20°C for 48 hours, reweighed and then powdered at room temperature. Two milligrams of the powdered muscle was extracted in 0.5M perchloric acid/1mM EDTA and neutralized using 2M $KHCO_3$ on ice as per methods described by Harris et al., 1974). One milligram was used for glycogen determination where the powdered muscle was hydrolysed with 2mM HCL (at 100°C for 2 hours) then neutralized with 0.667Mm NaOH. All extractions were then stored at -80°C prior to analysis. The samples were analysed in triplicate for ATP, PCr, Cr and glycogen using fluorometric analysis (Turner Digital Filter Fluorometer Model 112) according to methods described by Harris et al. (1974). All concentrations are expressed per kilogram of dry weight (kg/dw). The values obtained are in accordance with those published previously from our laboratory (Febbraio et al., 1994; 1995; McAinch et al., 2004). Intra-assay coefficients of variation were determined for each triplicate for 67 samples and resulted in coefficients of 2.85%, 4.45, 3.86%, 5.05% for ATP, PCr, Cr and glycogen respectively.

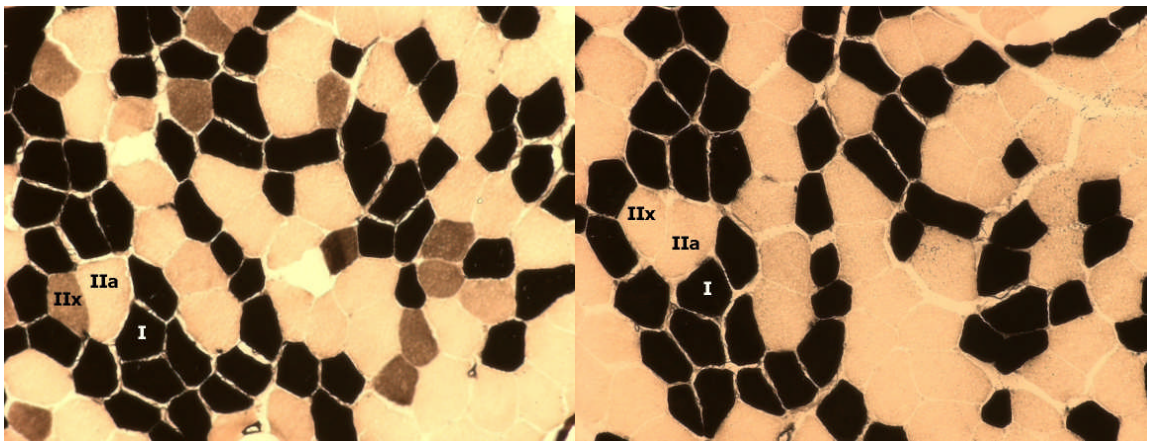
Approximately 5 milligrams of muscle was used to determine contractile protein content. The tissue was weighed and placed in a buffer solution which contained (in mM), KCl 50, KH_2PO4 10, $MgCl_2.6H_2O$ 2, EDTA 0.5, DTT 2 in a 1:50 wet weight: volume ratio, and homogenized using a glass tissue grinder. The muscle sample, buffer, and tissue grinder were kept on ice at all times. Crude homogenates (200µL) of individual muscle samples were set aside for determination of total protein concentration. The remainder of the homogenate was centrifuged at 5ºC for 10 min at 1000G (Heraeus Sepatech Biofuge, USA). The supernatant, containing cytosolic proteins, was discarded, and the pellet containing contractile proteins, was resuspended in the ice-cold buffer solution. Protein concentrations were performed in triplicate using a Bradford Protein Assay (Bio-Rad Protein Assay, Bio-Rad Laboratories, Hercules, CA, USA) with BSA standards and spectrophotometric detection at 595nm (Bradford, 1976). The values obtained were in accordance with others that have used this procedure to measure changes in muscle contractile protein content (Beitzel et al., 2004). To assess reliability, 52 samples were run twice on two separate occasions and intra-assay CV for the two runs resulted in non-significant CV of 3.7% and 2.9% for total and contractile protein, respectively.

To be included in the final data, the participant was required to complete both pre- and post- assessments for a dependant variable, provide all dietary recordings and attend at least 75% of the supervised training sessions. In some instances, the participant chose not to complete the post-training biopsy. If the participant had met all other requirements, their data (other than muscle sample characteristics) was included. Hence, in some cases the *n* of participants varied and this information is provided in the methods section in each study's chapter.

Statistics

The data from each trial was analysed with two-way factorial analysis of variance (group × time) with repeated measures on time (pre- and post-training) (SPSS v 12, Chicago, Illinois). When an interaction was identified, group differences were determined by Tukeys post hoc analysis. Simple regression was used to determine significant relationships among the deltas for selected variables. A *P* value < 0.05 was considered statistically significant. A *P* value < 0.09 was designated a trend.

Figure 2.1 Histochemical determination of muscle fibre types I, IIa and IIx with myosin ATPase staining, pH 4.54 (left) and 4.3 (right).



Chapter 3

The effects of whey isolate, creatine & resistance training on muscle fibre characteristics, strength & body composition

3.1 Introduction

As discussed previously in chapter 1 (section 1.8), certain types of protein affect whole body protein anabolism and accretion and therefore, have the potential to affect muscle and strength development during RE training. WP supplements that contain a high concentration of EAA and cyst(e)ine residues promote higher net protein accretion than other proteins such as casein (Dangin et al., 2003). For these reasons, it has been suggested that supplementation with WP would enhance muscle hypertrophy during RE (Ha & Zemel 2003). While some studies have shown that WP supplementation during RE training results in greater strength and/or LBM gains compared to groups given an equivalent dose of carbohydrate (Burke et al., 2001) or protein (Cribb et al., 2006), none have assessed skeletal muscle adaptations. In chapter 1 (section 1.9) it was also identified that chronic use of CrM is a popular strategy among those that exercise with weights to increase muscle strength, size, and LBM. CrM may also confer these benefits to a wider section of the population, such as older adults and patients with various neuromuscular disorders. The beneficial effects of oral CrM supplementation are thought to be dependant on the extent of Cr accumulation within muscle (Greenhaff, 1997). While repeat oral doses are shown to elevate muscle Cr concentrations this response can be highly variable between subjects (Harris et al., 1992; Greenhaff et al., 1993; Green et al., 1996). For this reason, dietary strategies such as combining CrM with CHO (Green et al., 1996a; 1996b) or protein (Steenge et al., 2000) have been used to enhance Cr uptake.

Many RE training studies that report changes in body composition from dietary intervention have not compared these changes alongside adaptations at the cellular level (i.e., fibre-specific; type-I, IIa, IIx hypertrophy) (Kreider et al., 1996; 1998; Burke et al., 2001; Rozenek et al., 2002;

Brose et al., 2003; Chromiak et al., 2004; Rankin et al., 2004). Those that have reported fibre-specific increases in CSA have not confirmed this hypertrophy response with changes at the sub-cellular level (i.e., contractile protein content) (Volek et al., 1999; Esmarck et al., 2001; Andersen et al., 2005; Phillips, 2005). For example, the combination of CrM with CHO has been shown to promote greater strength and LBM gains during RE than CHO alone (Kreider et al., 1998). CrM combined with WP has also been shown to augment muscle strength and LBM gains during RE when compared to CHO or WP supplementation (Burke et al., 2001). However, no studies have examined the effects of CrM and WP supplementation during RE on strength and body composition alongside muscle characteristics such as fibre-specific hypertrophy and contractile protein accrual. Therefore, the aim of this study was to examine the effects of combining CrM with CHO and with WP in comparison to WP and CHO alone, on strength, body composition, fibre-specific (i.e., type-I, IIa, IIx) hypertrophy, contractile protein and energy metabolite (ATP, PCr, Cr and glycogen) concentrations. The first hypothesis was that supplementation with the Cr-containing supplements would provide greater benefits than CHO or WP alone. Due to the benefits previously reported with WP, a secondary hypothesis was that the combination of CrM and WP would provide greater benefits than the combination of CrM and CHO.

3.2 Methods and Procedures

Thirty-three (33) recreational male bodybuilders met the requirements (outlined in chapter 2) to commence this study that involved pre-post assessments and supplementation (4 groups) during 11 weeks of RE training. After baseline assessments, the men were matched for maximal strength (1RM) in three weight lifting exercises (see strength assessments, Chapter 2) and then randomly assigned to one of four supplement groups in a double-blind fashion; whey protein isolate (WP), CrM and whey protein isolate (CrWP), CrM and carbohydrate (CrCHO), and carbohydrate–only (CHO).

Participants were instructed to consume 1.5 grams of the supplement per kilogram of body mass per day (1.5g/kg/day) while maintaining their habitual daily diet. The chosen supplement dose was based on previously reported intakes of this population (Marquart et al., 1998; Leutholtz & Kreider 2000). The supplements consumed by each group were similar in energy content. For example, an 80kg participant in the WP group consumed 120g/day of a supplement that contained approximately 103g protein, <6g carbohydrate, <1.2g fat and 1864 kJ (447 kcal), whereas an 80kg participant in the CHO group consumed the same dose of a supplement that contained 106g carbohydrate, 0 protein or fat and 1770 kJ (424 kcal). The Cr-containing supplements (CrCHO and CrWP) contained a 1 week loading phase with CrM (0.3g/kg/day) that was followed by a

maintenance phase (0.1g/kg/day) for the duration of the study (weeks 2-11). This CrM protocol has been shown previously to augment muscle strength and hypertrophy during RE training (Volek et al., 1999). In the first week, an 80kg participant in the CrCHO group consumed 120g/day of a loading phase supplement that contained 85g carbohydrate, 24g CrM/ and 1420 kJ (340 kcal), and then a maintenance phase supplement (weeks 2-11), that provided 98.9g carbohydrate, 8.4g CrM and 1651 kJ (396 Kcal). A participant of the same weight in the CrWP group consumed a loading phase supplement that contained 83g protein, <4.8g carbohydrate, <1g fat, 24g CrM and 1500 kJ (359 Kcal) followed by a maintenance phase supplement (weeks 2-11), that contained 96g protein, <5.5g carbohydrate, <1g fat, 8.4g CrM and 1729 kJ (415 kcal). (Nutrient composition of all supplements is provided in Appendix).

The participants were asked to consume their supplement dose in three equal servings throughout the day. That is, the participants were asked to consume one serving mid-morning, one serving as soon as they finished each workout in the afternoon (or similar time on non-training days), and one serving in the evening before sleep. Participants were given approximately a one-week supply of the supplement at the start of each week and asked to return the container before they received the next weeks supply as an act of compliance to the dosing procedure. In addition to having to return the container, the participants were asked to document the time of day they took the supplement in nutrition diaries that were provided. Nutritional intake was monitored via written dietary recalls as described in Chapter 2.

Pre- and post-training assessments were completed in the week before and after the 11 week RE training program. Assessments included; 1RM strength in three exercises (barbell bench press, squat and cable pulldown); body composition and muscle biopsies for histochemical determination of muscle fibre type, CSA, contractile protein content and metabolite concentrations (as described in Chapter 2).

Four (4) participants did not attend the required number of supervised training sessions or provide all dietary records, therefore their data was not included. Additionally, some participants chose not to return for final biopsies (n=3). This reduced the number of the groups to 7=CHO, 5=WP, 8=CrCHO and 6=CrWP (the starting characteristics of these individual's are presented in table 3.1). Statistical evaluation of the data was accomplished by factorial ANOVA; group x time with repeated measures on time. Post-hoc between-group differences were identified by Tukeys analysis. Simple regression was used to determine significant relationships among the deltas for selected variables. A P value < 0.05 was considered statistically significant.

Table 3.1 Baseline characteristics (Values are means ± SD)

Characteristics	CHO	WP	CrCHO	CrWP
Age (yrs)	24 ± 7	24 ± 5	25 ± 6	25 ± 4
Training age (yrs)	6 ± 3	5 ± 2	6 ± 3	4 ± 2
Height (cm)	177 ± 5	181 ± 8	177 ± 6	190 ± 7
Body mass (kg)	76 ± 12	70 ± 11	84 ± 14	84 ± 12
Fat mass (kg)	13 ± 7	11 ± 4	17 ± 7	16 ± 6
CSA type-I (μm^2)	3662 ± 273	3423 ± 88	3656 ± 593	3699 ± 774
CSA type-IIa (μm^2)	4674 ± 803	4529 ± 223	4673 ± 661	4458 ± 919
CSA type-IIx (μm^2)	4253 ± 656	4220 ± 223	4354 ± 972	4057 ± 604
1RM Bench (kg)	99 ± 16	98 ± 13	104 ± 22	106 ± 26
1RM Squat (kg)	125 ± 25	118 ± 26	118 ± 18	123 ± 37
1RM Pulldown (kg)	90 ± 12	86 ± 11	89 ± 18	88 ± 13

3.3 Results

Baseline characteristics (table 3.1) There were no significant differences between the groups prior to the training/supplementation program.

Dietary Analyses The average of three day written dietary recalls for energy (kJ/kg/day), carbohydrate and protein (g/kg/day) are presented in table 3.2. Weeks 1 and 11 data does not include supplementation. No differences were identified between the groups or across time with regard to energy, protein and carbohydrate intake.

Table 3.2 Dietary analyses (values are mean ± SD)

Variable	CHO	WP	CrCHO	CrWP
Energy intake kJ/kg/day				
before	154.1 ± 30.1	174.2 ± 20.1	175.8 ± 25.5	170.8 ± 15.1
week 1	152.8 ± 22.2	169.6 ± 14.6	156.2 ± 15.9	167.1 ± 12.1
week 11	152.4 ± 24.7	163.7 ± 13.8	160.8 ± 17.2	167.1 ± 13.4
Carbohydrate (g/kg/day)				
before	2.9 ± 0.6	4.0 ± 0.6	4.4 ± 1.2	3.8 ± 1.4
week 1	2.8 ± 0.6	3.7 ± 0.4	3.7 ± 1.0	3.9 ± 1.4
week 11	2.7 ± 0.4	4.0 ± 1.2	3.7 ± 0.6	4.7 ± 1.9
Protein (g/kg/day)				
Before	1.6 ± 0.3	1.6 ± 0.2	1.5 ± 0.3	2.1 ± 1.0
week 1	1.7± 0.2	1.7 ± 0.2	1.5 ± 0.3	1.9 ± 0.8
week 11	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.3	1.7 ± 0.7

Body composition DEXA determined body composition is presented in table 3.3. All groups demonstrated an increase ($P < 0.05$) in body mass after the program but no group or group x time interaction were detected. There were no significant interactions detected for fat mass or body fat percentage between the groups or across time. However, a group x time interaction ($P < 0.05$) was observed for lean mass (LBM). While each of the groups demonstrated an increase ($P < 0.05$) in LBM after the program, (CrCHO +3.7kg); (CrWP +3.4); (WP +2.3kg); (CHO +0.7); only the CrCHO group's increase in LBM was significantly greater (post hoc $P < 0.05$) than the CHO group.

Table 3.3 Body mass and composition (mean ± SE)

Variable	CHO	WP	CrCHO	CrWP
Body mass (kg)				
PRE	75.6 ± 4.7	69.7 ± 5.0	84.2 ± 4.9	83.9 ± 4.8
POST[#]	77.0 ± 4.8	72.3 ± 4.3	88.2 ± 5.0	87.9 ± 5.0
Lean mass (kg)				
PRE	62.3 ± 2.8	59.0 ± 3.2	67.0 ± 2.6	67.9 ± 2.6
POST[#]	63.0 ± 2.7	61.3 ± 3.0	71.3 ± 3.0*	71.3 ± 2.8
Fat mass (kg)				
PRE	13.2 ± 2.8	10.6 ± 1.9	16.6 ± 2.6	15.9 ± 2.5
POST	14.0 ± 2.9	11.0 ± 1.6	17.0 ± 2.1	16.6 ± 2.6
Fat %				
PRE	16.9 ± 2.4	14.9 ± 1.7	19.1 ± 1.9	18.5 ± 1.9
POST	17.6 ± 2.5	15.0 ± 1.3	18.8 ± 1.3	18.5 ± 1.9

[#] Training effect all groups; *greater increase than CHO group ($P < 0.05$)

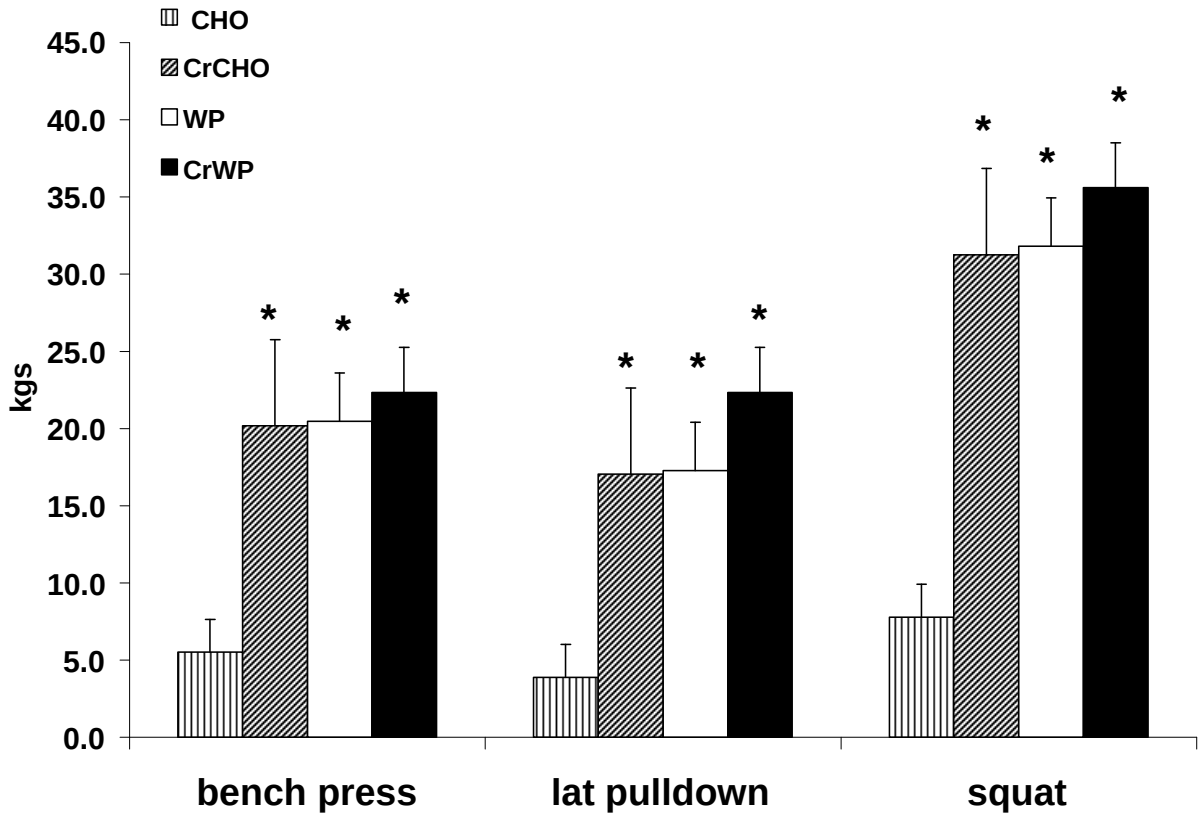
Strength 1RM strength data for three exercises (barbell squat, bench press and cable pulldown) is presented in table 3.4. While all groups demonstrated an improvement ($P < 0.05$) in strength in each exercise after the program, a group x time interaction ($P < 0.01$) was observed for each exercise. The CrCHO, CrWP and WP groups demonstrated a greater increase in strength in each exercise compared to the CHO group (post hoc $P < 0.05$) (figure 3.1) however, no differences were detected between the CrCHO, CrWP and WP groups.

Table 3.4 1RM Strength (mean ± SE)

Variable	CHO	WP	CrCHO	CrWP
Squat (kg)				
PRE	124.5 ± 9.5	118.2 ± 11.8	118.0 ± 6.4	123.0 ± 15.0
POST[#]	132.3 ± 8.8	150.1 ± 12.0*	149.3 ± 9.6*	158.6 ± 14.3*
Bench press (kg)				
PRE	98.6 ± 6.0	97.7 ± 5.6	104.4 ± 7.9	105.6 ± 10.6
POST[#]	104.1 ± 6.1	118.2 ± 7.0*	124.5 ± 7.4*	128.0 ± 10.5*
Pulldown (kg)				
PRE	90.3 ± 4.4	86.4 ± 4.8	89.2 ± 6.4	87.9 ± 5.3
POST[#]	94.2 ± 3.3	101.8 ± 3.4*	106.3 ± 5.9*	110.2 ± 4.5*

[#] Training effect all groups; *greater increase than CHO group ($P < 0.05$)

Figure 3.1 Changes in strength (1RM)



*Greater increase compared to the CHO group ($P < 0.05$) (table 3.4)

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Muscle characteristics There were no changes between the groups or across time with regard to fibre type proportions (table 3.5). Table 3.6 presents muscle fibre CSA and proportion of contractile protein before and after the training program. All groups demonstrated significant hypertrophy in each fibre type after the program. However, a group x time interaction ($P < 0.05$) was detected in CSA for each of the fibre types. The CrCHO and CrWP groups demonstrated a greater increase in CSA across all fibre types (type-I, IIa and IIx) compared to the CHO group (post hoc $P < 0.05$). The CrCHO and CrWP groups also demonstrated a greater increase in CSA in the type-I fibres when compared to the WP group (post hoc $P < 0.05$) (figure 3.2). A trend for a greater hypertrophy of the type-IIa and IIx fibres ($P = 0.077$ and $P = 0.078$, respectively) was also observed in the WP group compared to the CHO group. A group x time interaction ($P < 0.05$) for contractile (myofibrillar) protein was also detected. The CrCHO, CrWP and WP groups each showed a greater increase in contractile protein content compared to the CHO group after the program (post hoc $P <0.05$) (figure 3.3). Additionally, the CrCHO and CrWP groups demonstrated a trend ($P = 0.07$ and $P = 0.08$, respectively) for a greater increase in contractile protein content compared to the WP group.

Table 3.5 Muscle fibre type (%) (mean ± SE)

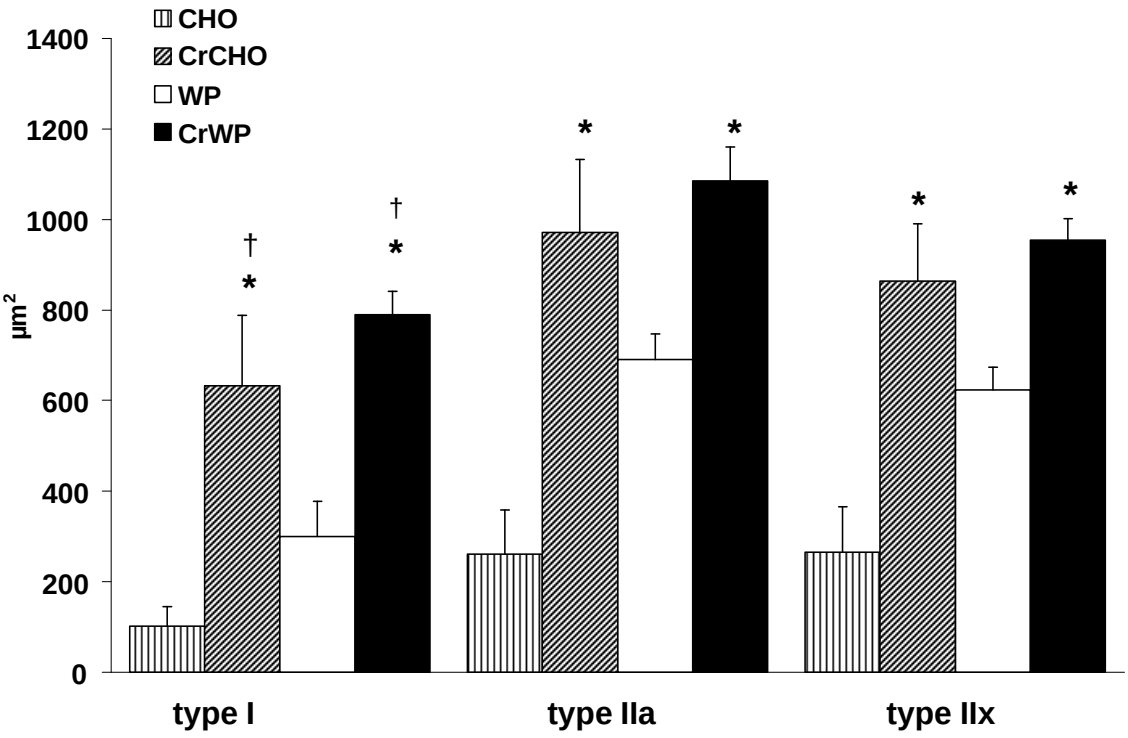
variable	CHO	WP	CrCHO	CrWP
%Type -1				
PRE	43 ± 5.9	49.9 ± 2.6	43.9 ± 2.5	41.4 ± 3.5
POST	41 ± 4.5	44.6 ± 4.3	46.7 ± 3.5	43.2 ± 3.2
%Type-IIa				
PRE	38.3 ± 5.3	30.0 ± 3.1	38.3 ± 3.3	36.9 ± 2.8
POST	39.0 ± 4.0	35.3 ± 4.0	36.7 ± 4.0	33.7 ± 2.5
%Type-IIx				
PRE	18.7 ± 2.8	18.0 ± 1.7	17.8 ± 1.8	21.6 ± 2.4
POST	20.2 ± 2.5	17.7 ± 2.7	16.5 ± 1.4	23.1 ± 1.4

Table 3.6 Muscle fibre CSA and contractile protein (mean ± SE)

variable	CHO	WP	CrCHO	CrWP
Type 1 (μm^2)				
PRE	3662 ± 103	3423 ± 39	3656 ± 210	3699 ± 316
POST[#]	3763 ± 116	3723 ± 94	4288 ± 272*[†]	4489 ± 288*[†]
Type IIa (μm^2)				
PRE	4674 ± 303	4430 ± 100	4673 ± 234	4458 ± 375
POST[#]	4901 ± 318	5220 ± 133	5644 ± 281*	5543 ± 398*
Type IIx (μm^2)				
PRE	4253 ± 248	4220 ± 100	4354 ± 344	4057 ± 247
POST[#]	4499 ± 251	4843 ± 101	5218 ± 362*	5011 ± 257*
Contractile protein (mg/g)				
PRE	45.8 ± 2.1	46.6 ± 2.4	47.0 ± 2.0	49.7 ± 1.0
POST[#]	53.4 ± 3.4	65.6 ± 2.7*	74.8 ± 3.7*	77.7 ± 1.4*

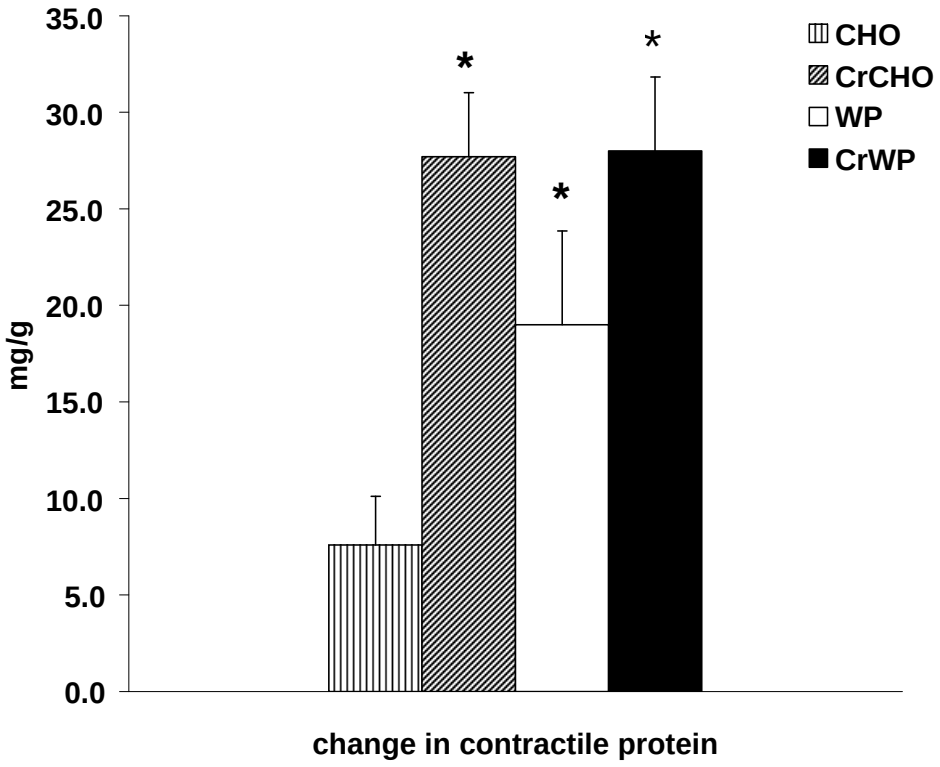
*Greater increase than CHO group ($P < 0.05$). [†]Greater increase than WP group;
[#] training effect all groups ($P < 0.05$)

Figure 3.2 Changes in CSA (μm^2) of muscle fibre types I, IIa and IIx



*greater increase than CHO group; [†]greater increase than WP group ($P < 0.05$) (table 3.6)

Figure 3.3 Changes in contractile protein (mg/g of muscle)



*Greater increase compared to the CHO group ($P < 0.05$) (table 3.6)

Table 3.7 presents muscle metabolite and glycogen data from vastus lateralis muscle samples taken before and after the training program. No group x time differences were detected for ATP, PCr or glycogen, but a group difference ($P < 0.05$) was detected for the Cr-treated groups in total Cr (PCr + Cr). Both the CrCHO and CrWP groups showed a higher ($P < 0.05$) concentration (mmol/kg dry weight) of total Cr compared to the WP and CHO group after the training program but there was no difference between these groups.

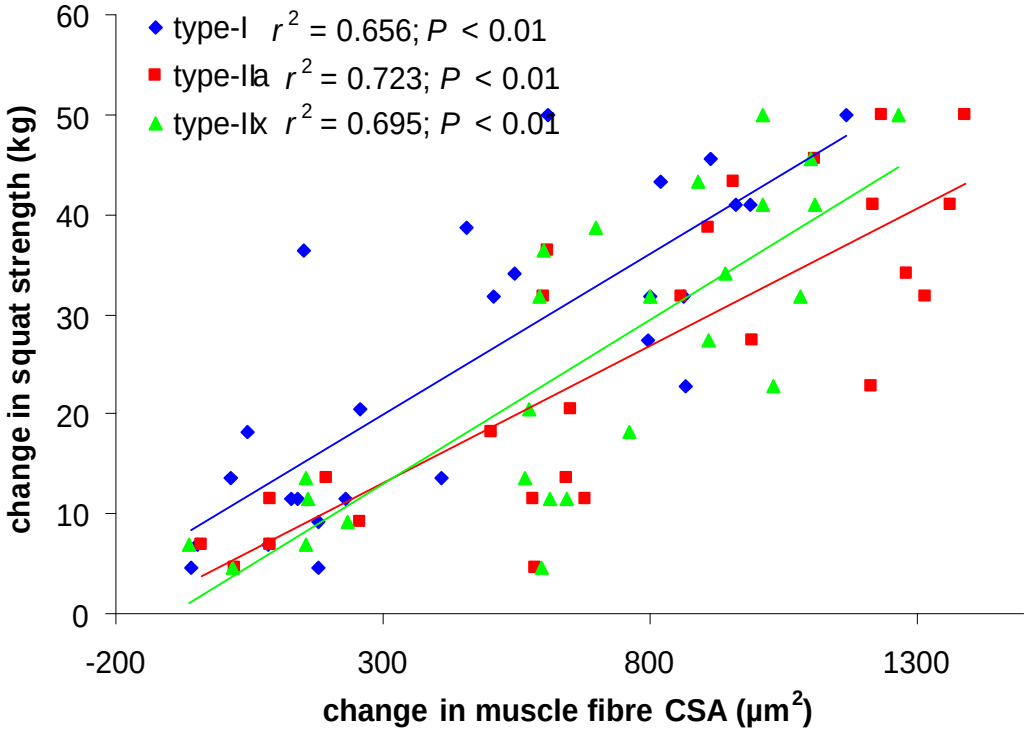
Table 3.7 Muscle metabolites and glycogen (mean ± SE)

Variable (mmol/kg dry wt)	**CHO**	**WP**	**CrCHO**	**CrWP**
ATP				
PRE	19.5 ± 1.5	20.1 ± 1.7	19.8 ± 1.6	19.5 ± 2.8
POST	20.0 ± 1.1	19.9 ± 2.1	20.0 ± 1.1	21.3 ± 3.4
PCr				
PRE	79.1 ± 9.3	80.9 ± 7.5	82.5 ± 8.1	75.5 ± 12.4
POST	72.4 ± 4.6	74.0 ± 7.5	73.4 ± 7.0	83.5 ± 14.4
Total Cr (Cr + PCr)				
PRE	94.2 ± 10.1	107.1 ± 8.7	103.6 ± 8.3	109.0 ± 16.6
POST	95.3 ± 10.5	100.5 ± 9.5	113.0 ± 24.1*†	125.3 ± 19.6*†
Glycogen				
PRE	400.0 ± 56.7	382.4 ± 90.6	409.1 ± 90.3	449.7 ± 60.9
POST	334.8 ± 43.0	340.0 ± 43.4	393.4 ± 59.4	343.7 ± 57.2

*change compared to CHO group; †change compared to WP group ($P < 0.05$)

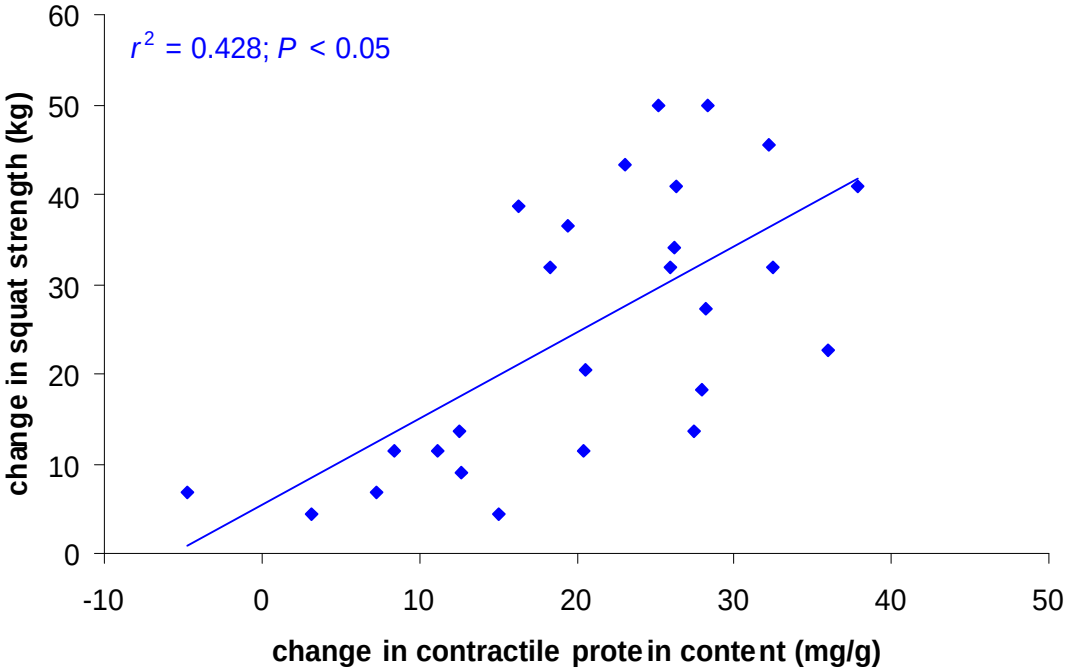
For all participants combined, positive correlations ($P < 0.01$) were detected between changes in muscle fibre CSA (in all fibre types) and strength gained in the 1RM squat exercise (type-I $r = 0.810$; type-IIa $r = 0.850$; type-IIx $r = 0.833$) (figure 3.4). A positive correlation ($P < 0.05$) was also detected between the change in contractile protein (mg/g) and (1RM) strength improvements in the squat ($r = 0.654$) (figure 3.5). Additionally, positive correlations ($P < 0.01$) were detected between the increase in contractile protein and increase in muscle fibre CSA, in all fibre types (type-I $r = 0.757$; type-IIa $r = 0.855$; type-IIx $r = 0.871$) (figure 3.6).

Figure 3.4 Relationship between changes in muscle fibre CSA and 1RM strength changes in the squat



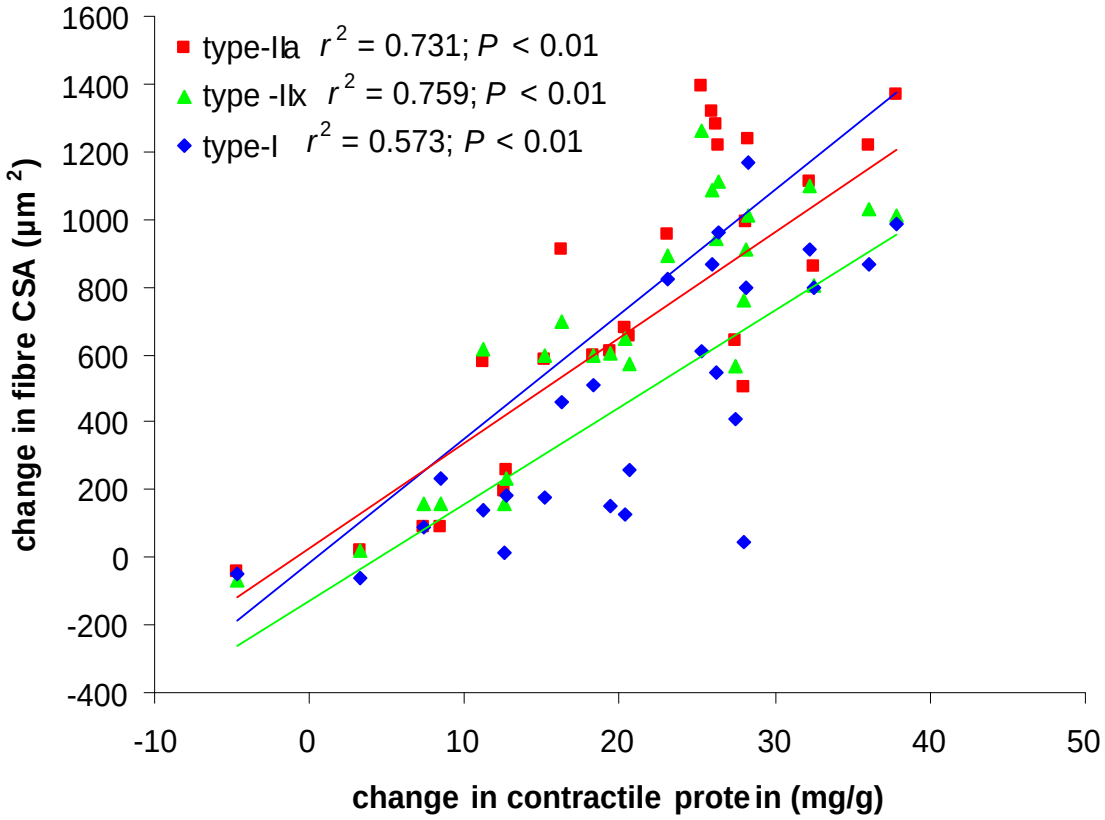
For all participants combined, a correlation ($P < 0.01$) was detected between changes in vastus lateralis muscle fibre CSA (type-I $r = 0.810$; type-IIa $r = 0.850$; type-IIx $r = 0.833$), and 1RM strength improvements in the squat exercise.

Figure 3.5 Relationship between changes in contractile protein content and 1RM strength changes in the squat



For all participants combined, a correlation (r = 0.654, P < 0.05) was detected between the changes in myofibrillar content (mg/g) in muscle taken from the vastus lateralis and 1RM strength changes in squat exercise (1RM).

Figure 3.6 Relationship between change in contractile protein content and muscle fibre hypertrophy (CSA)



For all participants combined, a correlation (P < 0.01) was detected between the increase in contractile protein and changes in muscle fibre CSA (type-I r = 0.757; IIa r = 0 .855; IIx r = 0.871).

3.4 Discussion

This investigation is the first to compare the effects of CrM and WP supplementation on strength and body composition changes alongside alterations at the cellular level (i.e., fibre type specific hypertrophy) and sub-cellular level (i.e., contractile protein content). The most pertinent finding of this investigation was that despite the consumption of a protein-rich diet by all groups, and no differences between the groups at the start of this study, supplementation with CrCHO, WP and CrWP resulted in a significantly greater hypertrophy response (in at least 1 of the three assessments) and 1RM strength improvements (all in three assessments). These findings only partly support the hypotheses proposed. That is, treatment with CrCHO or CrWP provided greater improvements in strength and muscle hypertrophy when compared to CHO but not WP. Additionally, no greater benefit was observed from combining CrM and WP when compared to the combination of CrM and CHO. However, it is possible that small *n* of some groups may have reduced the capacity to adequately detect some differences between the groups, particularly in major variables of interest such as changes in LBM.

For example, in this study, although the WP (3.7%) CrCHO (5.5%) and CrWP (5%) groups each demonstrated relatively large changes in LBM compared to the CHO (1.1%) group, the only change in LBM deemed significantly greater than the CHO group was the CrCHO group. The *n* required to achieve a statistical power of 0.8 to detect a significant group x time interaction in LBM in studies involving RE and supplementation, has previously been reported to be 19 participants per group (Chromiack et al., 2004). In the present study, a significant group x time interaction was detected for LBM. However, the *n* of the some of the groups that finished the study was much lower than recommended as adequate (Chromiack et al., 2004). Additionally, a rather large variability in LBM changes was observed in this study (between 9.5 to 0.3kg) that was not anticipated based on our previous work (Cribb et al., 2006) and work by others (Volek et al., 1999) that have utilized CrM supplementation and RE-trained participants. This aspect, combined with a lower than desired finishing *n* in some of the groups, probably reduced the capacity to detect differences between the groups in LBM. The low sample size of the groups is acknowledged as an important limitation of this study. However, a number of significantly different changes were detected between the groups at the cellular and sub-cellular levels.

Few studies have used matched placebo-treated groups and quantified the extent of specific muscle fibre (i.e., type-I, IIa, IIx) hypertrophy in response to training and supplementation (Volek et al., 1999; Tarnopolsky et al., 2001; Andersen et al., 2005). Volek et al. (1999) utilized RE-trained

participants, a similar RE and CrM supplementation protocol to the present study, and reported comparable results. That is, after the 12 week training period, CrM supplementation (n = 9) resulted in a significantly greater gain in LBM, 1RM squat strength and muscle fibre hypertrophy in all fibre types assessed compared to a matched placebo-treated group (n = 10) (Volek et al., 1999). Similarly, after 14 weeks of RE, Andersen et al. (2005) reported significantly greater hypertrophy of both the type-I and II fibres as well as squat jump height in a group of men (n = 11) that received a pre- and post-workout protein supplement (25g each serving) as opposed to CHO (25g) (n = 11). The study by Tarnopolsky et al. (2001) actually used a protein-glucose supplement as a placebo and therefore, this study is more appropriately discussed in the next chapter. The results obtained in the present study showed no change in fibre type distribution in response to training or supplementation (table 3.5) but this is characteristic of RE-trained individuals (Staron et al., 1994). However, differences in muscle fibre hypertrophy were detected across all fibre types. For example, the Cr-treated groups demonstrated a greater increase in CSA in the type-I, IIa and IIx fibres compared to the CHO group as well as a greater increase in CSA in the type-I compared to the WP group. However, no differences in muscle fibre hypertrophy were detected between the WP, CrWP and CrCHO groups. Unlike previous studies that have examined strength and muscle fibre CSA changes in response to training and supplementation, this study was able to confirm the changes in CSA with assessment of contractile protein content.

The CrCHO, CrWP, WP groups each showed greater increases in contractile protein content (mg/g of muscle) compared to the CHO group after the training program. These changes in contractile protein reflected the changes in CSA that were observed, particularly in the CrCHO and CrWP groups and to a lesser extent the WP group. That is, a trend (P < 0.09) for greater hypertrophy in the type-IIa and IIx fibres was observed in the WP group compared to the CHO group. Although no significant differences were detected between the WP, CrCHO and CrWP groups in LBM gains or muscle fibre hypertrophy, a trend (P < 0.09) for a greater increase in myofibrillar protein content was detected in the CrCHO and CrWP groups compared to the WP group. An increase in muscle fibre CSA underlines most of the improvements in force production and strength observed in RE-trained participants (Shope et al., 2003). An increase in contractile protein is thought to be an important stimulus that results in an increase in muscle fibre CSA (Phillips, 2000). When all participants were combined, a strong relationship between changes in muscle fibre CSA (across all fibre types) and strength improvements in the squat exercise were evident (figure 3.4). A similar relationship between changes in contractile protein content and strength improvements in the squat was also detected (figure 3.5). Additionally, a strong relationship between changes in contractile protein content and muscle fibre hypertrophy (for all types) was observed (figure 3.6). The R^2 values obtained suggest that a very substantial portion (50-

76%) of the strength improvements observed across all groups could be attributed to the changes in skeletal muscle morphology. The barbell squat exercise was the focus of correlation assessments between muscle morphology and functional improvements simply because, unlike the bench press and pulldown exercise, the vastus lateralis is recruited heavily during this exercise (Sale 1992). These correlations observed reflect a direct relationship between muscle adaptations and a functional improvement. Therefore, while differences in hypertrophy between the groups were less evident with regard to body composition assessment, some statistically significant differences (and strong trends) were detected between the groups in muscle fibre hypertrophy and contractile protein accrual. It was these alterations in skeletal muscle morphology that appeared to be largely responsible for the improvements in strength in an exercise that heavily involves the vastus lateralis. While the results suggest a cause and effect relationship between muscle hypertrophy and strength, this study did not attempt to provide a mechanistic explanation for these results.

Willoughby & Rosene (2001) used healthy participants involved in RE training and managed to link an enhanced hypertrophy response from supplementation (i.e., increase in strength, LBM and thigh volume) to alterations at the molecular level that may explain these benefits. This study demonstrated that supplementation with CrM (6g/day for 12 weeks of RE) resulted in greater (relative) strength, LBM and thigh volume. These adaptations were observed alongside increases in the mRNA (type-I, IIa, and IIx), and protein (type-I, IIa and IIx) expression of the MHC isoforms (Willoughby & Rosene 2001) as well as myogenin and MRF-4 mRNA (and protein) expression (Willoughby & Rosene 2003). It is apparent that CrM treatment enables muscle to work at a higher capacity during RE (Rawson & Volek 2003). Therefore, enhanced muscle gene expression that resulted in greater strength and hypertrophy during RE may be a contributor to, or be a product of, CrM's ability to enhance weightlifting performance. This is a tempting mechanistic explanation for the greater adaptations observed in the Cr-treated groups in the present study; the improvements in strength and muscle hypertrophy demonstrated by the Cr-treated groups support this theory. However, it does not explain the significantly different adaptations detected in the WP-supplemented group as compared to the CHO group.

Although previous studies have shown that WP supplementation (1.2 to 1.5g/kg/d) results in greater LBM and strength compared to an equivalent dose of CHO (Burke et al., 2001) or casein (Cribb et al., 2006), this study is the first to report changes in skeletal muscle morphology in response to RE training and WP supplementation. The WP group demonstrated greater ($P<0.05$) 1RM strength improvements than the group given CHO. Based on the correlations observed in this study, the superior strength improvements demonstrated by the WP group can (mostly) be attributed to the changes in muscle fibre hypertrophy and contractile protein content. WP supplements such as

whey isolate contain a higher concentration of the EAA than other protein sources (per 100g of protein)(Bucci & Unlu 2000). The EAA are crucial to stimulating a high rate of protein synthesis in muscle (Bohe et al., 2003). Their presence is also shown to reduce muscle degradation (Rennie et al., 2002). A WP meal is shown to provide a higher stimulation of protein synthesis (Dangin et al., 2001) and net postprandial protein gain compared to isonitrogenous casein meals (Dangin et al., 2003). However, WP supplements also contain a 3- to 4-fold higher concentration of Cys compared to other protein sources (Bucci & Unlu 2000); supplementation is shown to increase circulating cyst(e)ine concentrations in the blood and influence GSH status (Lands et al., 1999; Marriotti et al., 2004; Middleton et al. 2004). As discussed in chapter 1, (sections 1.2 & 1.8) this would have a positive influence on whole body protein metabolism. Additionally, our laboratory has previously shown that WP supplementation after a single bout of high overload RE resulted in lower CK levels and a faster return to maximal (isokinetic) force production compared to CHO treatment (Cooke et al., 2004). For these reasons, the frequent consumption of WP throughout an RE training program may have a positive influence on muscle and whole body protein metabolism that resulted in a greater accretion of contractile protein. While the findings with WP supplementation in this study are consistent with this theory, the mechanisms that may underline the benefits obtained from WP during RE are yet to be elucidated. The ability of the WP group to achieve similar strength gains without the large increase in LBM as seen with CrCHO and CrWP supplementation may have important sports-specific implications for individuals that compete in weight-restricted events. Therefore, further studies on the chronic effects of WP during RE are warranted, particularly at the molecular level.

Based on the mechanistic explanations that have been proposed, one may expect an additive effect from combining CrM and WP on muscle strength and hypertrophy. However, in this study, no greater effect was observed from this supplement combination compared to the combination of CrM and CHO. One explanation for this may be the influence of the CHO (contained in CrCHO but not the CrWP supplement). For example, all groups consumed a high protein intake aside from supplementation and the results of at least two longitudinal studies suggest that once dietary protein requirements appear to be met, it is the energy content of the diet that has the largest effect on hypertrophy during RE (Rozenek et al., 2002; Ahtiainen et al., 2005b). In other words, when CrM is consumed in the presence of a high protein diet, the addition of CHO may be more beneficial than extra protein. However, the results also suggest that the consumption of CrM with either WP or CHO provide similar benefits. This may have important implications for populations that desire improvements from exercise but the consumption of large amounts of glucose is undesirable, such as those at risk of type-II diabetes. However, this is the only study that has compared the effects of two different CrM-containing supplements. In light of the small numbers in some of the groups that

completed this study, definitive conclusions with regard to the efficiency of combining CrM with CHO or WP are difficult to make. Based on the results observed, further study (involving larger groups) is obviously warranted.

3.5 Conclusion

In summary, this study examined the effects of four matched groups of resistance-trained males that supplemented with CrCHO, CrWP, WP or CHO (1.5g/kg body wt/day) during 11 weeks of supervised RE training. Pre-post assessments demonstrated that supplementation with CrCHO, WP and CrWP resulted in significantly greater hypertrophy responses and increases in 1RM strength (in three assessments) compared to supplementation with CHO. Up to 76% of the strength improvements in the squat could be attributed to hypertrophy of muscle involved in this exercise. However, when compared to CHO, the hypertrophy response from supplementation varied among the groups CrCHO, WP and CrWP at the three levels assessed (i.e., changes in lean mass, fibre-specific hypertrophy and contractile protein content). While the results of this trial clearly show that supplementation with WP and/or CrM promotes greater strength gains and muscle hypertrophy during RE training, the small number of participants within the groups makes it difficult to draw conclusions with regard to the effects of the different supplement combinations used in this study, and thus warrants further investigation.

Chapter 4

The addition of creatine to a protein-carbohydrate supplement improves muscle fibre hypertrophy, strength & body composition during resistance training

4.1 Introduction

As discussed previously in chapter 1 (section 1.9) supplementation with CrM during RE training is shown to enhance LBM and strength gains compared to placebo-treated groups. However, in most cases, the CrM-supplemented group was not compared with a group who received a placebo that was equivalent in nitrogen and energy content (Earnest et al., 1995; Vandenberghe et al., 1997; Kreider et al., 1998; Volek et al., 1999). Only one RE training study has compared the effects of a CrM-containing supplement (10g CrM, 75g CHO) with a supplement containing a similar nitrogen and energy content (10g milk protein, 75g CHO) (Tarnopolsky et al., 2001). This study reported that CrM treatment provided no greater gain in strength, LBM or muscle fibre hypertrophy (Tarnopolsky et al., 2001). However, this study utilized inactive males (exercised less than twice a week). While the influence of training status on the effects of supplementation is unknown, it has been speculated (Rawson & Volek 2003) that training might increase muscle Cr uptake as exercise training is associated with improved insulin sensitivity (Henriksen, 2002). Also, muscle Cr uptake is enhanced by the presence of insulin (Streenge et al., 1998) and exercise (Harris et al., 1992; Robinson et al., 1999). Based on this information, RE-trained individuals could theoretically experience greater adaptations from CrM supplementation during RE than untrained participants.

Therefore, the aim of this study was to use a group of RE-trained participants to examine the effects of a CrM-containing protein-carbohydrate (PRO-CHO) supplement in comparison to a supplement containing a similar amount of nitrogen and energy on strength, body composition and muscle fibre characteristics during 10 weeks of RE training. The hypothesis was that in RE-trained individuals, a CrM-containing PRO-CHO supplement would provide greater benefits (i.e. muscle strength and hypertrophy) compared to the consumption of PRO-CHO supplement without CrM containing a similar amount of nitrogen and energy.

4.2 Methods and Procedures

Thirty-four (34) recreational male bodybuilders met the requirements (outlined in chapter 2) to commence this study that involved pre-post assessments and supplementation (3 groups) during 10 weeks of RE training. After baseline testing the participants were matched for maximal strength (1RM) in three weight lifting exercises (see strength assessments, Chapter 2) and then randomly assigned to one of three supplement groups in a double-blind fashion; protein only (PRO), carbohydrate-protein (CHO-PRO), or the same carbohydrate-protein supplement that contained CrM (Cr-CHO-PRO).

To compare the effects of CrM, Tarnopolsky et al., (2001) utilized previously sedentary participants and a supplement that contained 10g CrM + 75g CHO and 1252kJ (or 300kcal) of energy and compared this to a placebo supplement that contained 10g protein + 75g CHO and provided 1420kJ (or 340kcal) of energy. In the present study, RE-trained participants were utilized and this was determined via questionnaire. However, to ensure that the participants were trained, they all underwent a structured RE training program (that was supervised but did not include personalized training) for almost 3 months prior to commencing this study. When the trial started, the Cr-CHO-PRO group consumed the exact same supplement (whey isolate and glucose) as the CHO-PRO that also provided a dose of CrM (0.1g/kg/day) (see Appendix for completed nutrient composition of supplements). For example, since all participants consumed 1.5g of supplement per kg of body mass per day, an 80kg participant in the PRO-CHO group consumed 120g/day of a supplement that contained 52g protein, 59g carbohydrate, <0.6g fat and 1877kJ (449 kcal). An 80kg participant in the Cr-CHO-PRO group consumed 120g/day of a supplement that supplied 48g protein, 53g carbohydrate, <0.6 fat, 8.4g CrM and 1710kJ (409 kcal). Another matched group (PRO) were provided a (whey isolate) supplement (1.5g/kg/day) that provided an 80kg participant (120g dose) with 103g protein, <6g carbohydrate, <1.2g fat and 1864 kJ (447 kcal).

The participants were asked to consume this dose in three equal servings throughout the day. For example the participants were asked to consume one serving mid-morning, one serving as soon as they finished each workout in the afternoon (or similar time on non-training days), and one serving in the evening before sleep. Participants were given approximately a one-week supply of the supplement at the start of each week and asked to return the container before they received the next weeks supply as an act of compliance to the dosing procedure. In addition to having to return the container, the participants were asked to document the time of day they took the supplement in their nutrition diaries that were provided. All pre- and post-training assessments were completed in

the week before and after the RE program these included; 1RM strength in three exercises (barbell bench press, squat and cable pulldown); body composition; and muscle biopsies for histochemical determination of muscle fibre type, CSA, contractile protein content and metabolite concentrations (as described in Chapter 2).

Three (3) participants pulled out of the trial through reasons unrelated to the study. Another 10 completed all other requirements except the final biopsy. Therefore, the *n* of the groups for strength and body composition assessments were; 10 PRO; 11 PRO-CHO; 10 Cr-PRO-CHO, while for muscle analyses $n = 7$ PRO; 7 PRO-CHO; 7 Cr-PRO-CHO. Starting characteristics of these participants are presented in table 4.1. Statistical evaluation of the data in this study was accomplished by factorial ANOVA; groups x time with repeated measures on time. Post-hoc between-group differences were identified by Tukeys analysis. Deltas for each variable were analysed with a one-way ANOVA. Simple regression was used to determine significant relationships among the deltas for selected variables. A P value < 0.05 was considered statistically significant.

Table 4.1 Baseline Characteristics

Characteristics	PRO	PRO-CHO	Cr-PRO-CHO
Age (yrs)	25 ± 4	26 ± 3	26 ± 5
Training age (yrs)	4 ± 2	4 ± 1	4 ± 5
Height (cm)	177 ± 4	177 ± 4	179 ± 5
Body mass (kg)	88 ± 11	83 ± 13	90 ± 21
Fat mass (kg)	16 ± 5	13 ± 5	16 ± 9
CSA type-I (μm^2)	2895 ± 511	3079 ± 1365	3129 ± 718
CSA type-IIa (μm^2)	4519 ± 639	4662 ± 1326	4528 ± 1014
CSA type-IIx (μm^2)	3798 ± 734	4370 ± 1405	3905 ± 901
1RM Bench (kg)	110 ± 13	112 ± 20	108 ± 13
1RM Squat (kg)	120 ± 15	127 ± 29	122 ± 24
1RM Pulldown (kg)	105 ± 9	108 ± 13	108 ± 15

4.3 Results

Baseline characteristics (table 4.1) There were no significant differences between the groups prior to the training/supplementation program.

Dietary Analyses The average of three day written dietary recalls for energy (kJ/kg/day), carbohydrate and protein (g/kg/day) are presented in table 4.2. Weeks 1 and 10 data does not include supplementation. No differences were identified between the groups or across time with regard to energy, protein and carbohydrate intake.

Table 4.2 Dietary analyses (values are mean ± SD)

variable	PRO	PRO-CHO	Cr-PRO-CHO
Energy intake (kJ/kg/day)			
before	135.7 ± 15.1	137.3 ± 15.5	138.2 ± 17.6
week 1	126.0 ± 14.2	137.3 ± 13.0	126.0 ± 15.5
week 10	126.0 ± 8.0	131.5 ± 14.7	123.1 ± 10.5
Carbohydrate (g/kg/day)			
before	3.0 ± 0.8	3.3 ± 0.6	3.1 ± 0.7
week 1	3.0 ± 0.5	3.3 ± 0.7	2.8 ± 0.6
week 10	2.8 ± 0.5	3.2 ± 0.5	3.0 ± 0.4
Protein (g/kg/day)			
before	2.3 ± 0.5	2.0 ± 0.8	1.8 ± 0.3
week 1	1.7 ± 0.2	1.7 ± 0.2	1.6 ± 0.3
week 10	1.7 ± 0.2	1.8 ± 0.6	1.6 ± 0.2

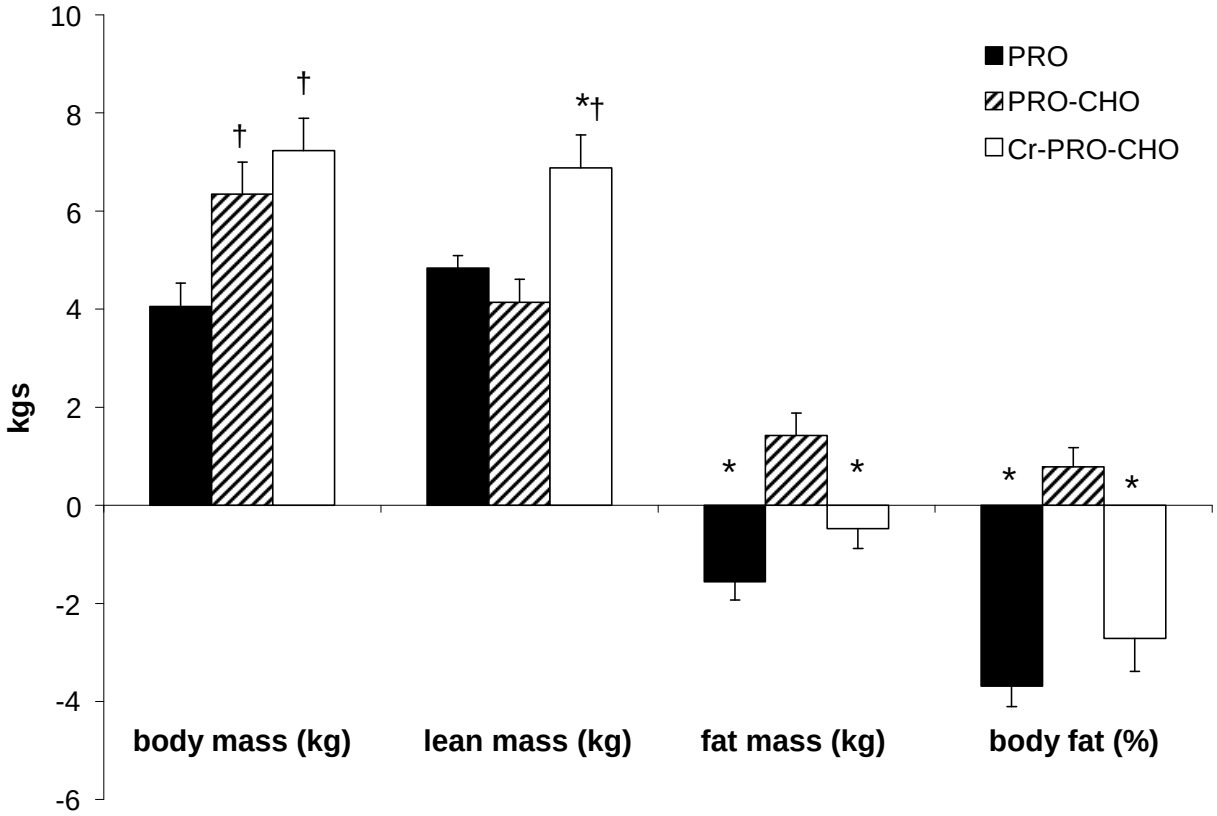
Body composition Body mass and DEXA determined body composition are presented in table 4.3. Body composition changes are presented in figure 4.1. While all groups demonstrated an increase ($P < 0.05$) in body mass after the training program, a group x time interaction ($P < 0.05$) was detected; the PRO-CHO and Cr-PRO-CHO groups demonstrated a greater gain in body mass (post hoc $P < 0.05$) compared to the PRO group. All groups demonstrated an increase ($P < 0.05$) in lean mass (LBM) after the training program. However, a group x time interaction ($P < 0.01$) for LBM was detected; the Cr-PRO-CHO group showed a greater gain in LBM compared to the PRO and PRO-CHO groups (post hoc $P < 0.05$). Additionally, a group x time interaction ($P < 0.05$) for fat mass and body fat percent was observed; when compared to the PRO-CHO group, the PRO and Cr-PRO-CHO groups demonstrated a decrease in fat mass and body fat percent (post hoc $P < 0.05$).

Table 4.3 Body mass and composition (mean ± SE)

variable	PRO	PRO-CHO	Cr-PRO-CHO
Body mass (kg)			
PRE	88.0 ± 3.6	82.0 ± 4.0	89.6 ± 6.5
POST[#]	92.2 ± 3.5	88.8 ± 3.9[†]	96.7 ± 2.7[†]
Lean mass (kg)			
PRE	69.1 ± 2.5	66.5 ± 2.8	69.6 ± 3.8
POST[#]	74.0 ± 2.5	70.6 ± 2.9	76.5 ± 4.2*[†]
Fat mass (kg)			
PRE	16.2 ± 1.7	12.7 ± 1.4	19.9 ± 2.8
POST	14.6 ± 1.5*	14.0 ± 1.2	15.4 ± 2.5*
Fat %			
PRE	17.2 ± 1.5	15.1 ± 1.1	16.3 ± 0.9
POST	13.6 ± 1.2*	15.9 ± 0.8	14.1 ± 1.4*

*Greater change than PRO-CHO; [†]Greater change than PRO; [#] training effect all groups ($P < 0.05$)

Figure 4.1 Changes in mass and body composition



*Greater change than PRO-CHO; †Greater change than PRO ($P < 0.05$) (table 4.3)

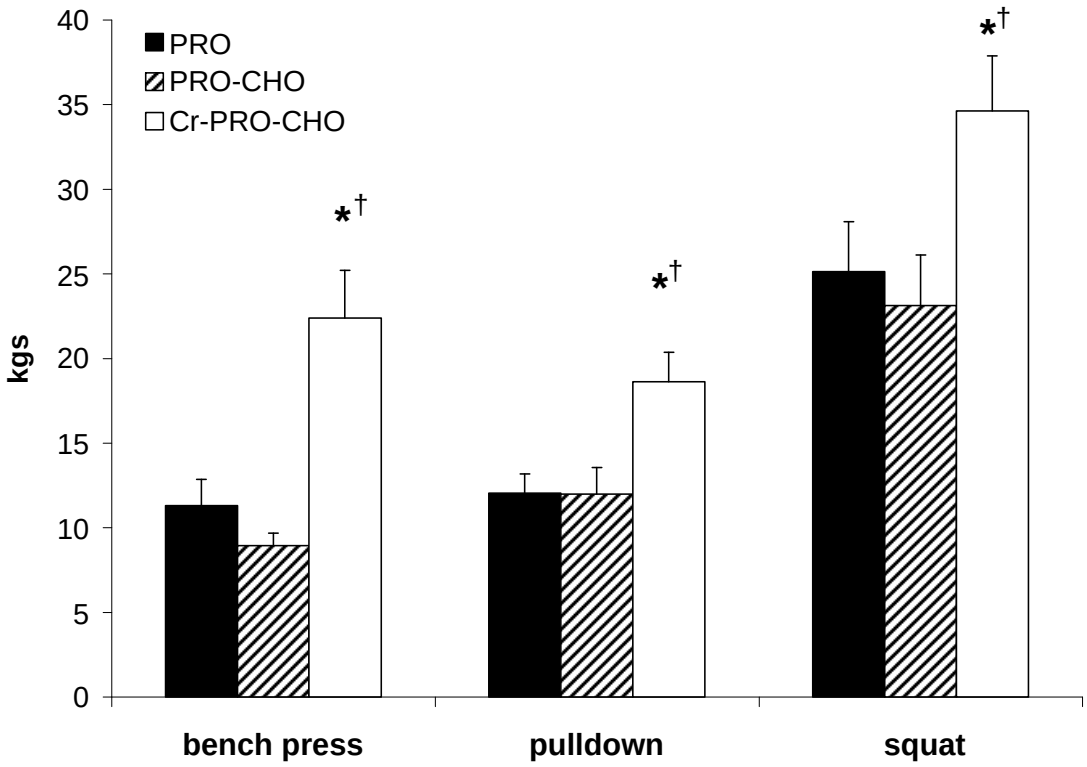
Strength Table 4.4 presents the results of 1RM strength assessments in three exercises (barbell squat, bench press and cable pulldown). All groups demonstrated an improvement ($P < 0.05$) in strength in each exercise after the training program. However, a group x time interaction ($P < 0.05$) was detected for each exercise. The Cr-PRO-CHO group demonstrated a greater gain in strength in each exercise compared to the PRO and PRO-CHO groups (post hoc $P < 0.05$) (figure 4.2). No other differences between the groups were detected.

Table 4.4 1RM Strength (mean ± SE)

variable	PRO	PRO-CHO	Cr-PRO-CHO
Squat (kg)			
PRE	119.6 ± 4.9	126.7 ± 8.7	122.2 ± 7.6
POST[#]	144.8 ± 4.7	149.8 ± 9.8	156.9 ± 9.6*[†]
Bench press (kg)			
PRE	110.3 ± 4.0	112.0 ± 6.0	108.3 ± 4.0
POST[#]	121.6 ± 4.1	121.0 ± 6.0	130.7 ± 5.3*[†]
Pulldown (kg)			
PRE	105.2 ± 2.9	107.8 ± 3.8	108.4 ± 4.8
POST[#]	117.3 ± 3.0	119.9 ± 4.8	127.1 ± 4.9*[†]

*Greater change than PRO-CHO; [†]Greater change than PRO; [#] training effect all groups ($P < 0.05$)

Figure 4.2 Changes 1RM strength



*Greater change than PRO-CHO; †Greater change than PRO ($P < 0.05$) (table 4.4)

Muscle characteristics There were no changes between the groups or across time with regard to fibre type proportions (table 4.5). While all groups demonstrated an increase in CSA across all muscle fibre types ($P < 0.05$) after the training program, a group x time interaction ($P < 0.05$) in CSA of both type-II fibre subgroups was detected (table 4.6). The Cr-PRO-CHO group demonstrated a greater increase in CSA in the type-IIa and IIx fibres compared to the PRO and PRO-CHO groups (post hoc $P < 0.05$) (figure 4.3). A group x time interaction ($P < 0.05$) was also observed for contractile protein content. The Cr-PRO-CHO group showed a greater increase in contractile protein content compared to the PRO and PRO-CHO groups (post hoc $P < 0.05$) (figure 4.4).

Table 4.5 Muscle fibre type (%) (mean ± SE)

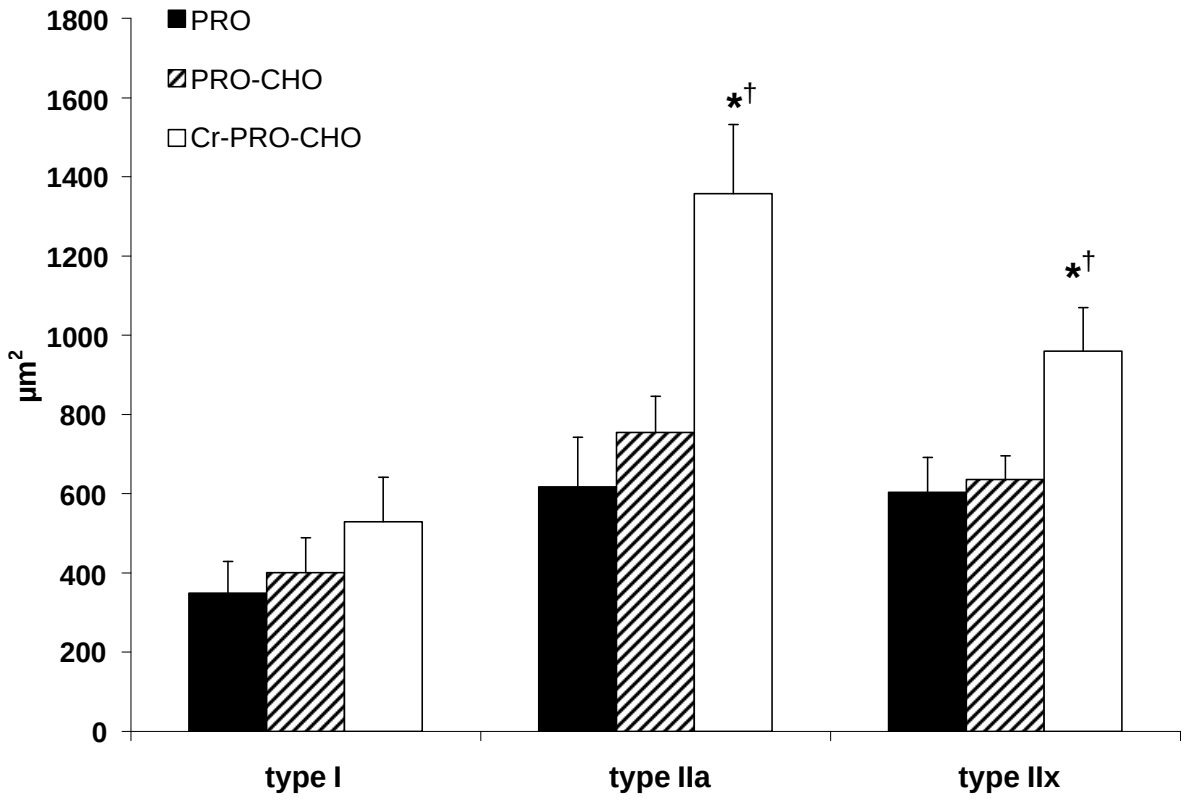
variable	PRO	PRO-CHO	Cr-PRO-CHO
%Type -1			
PRE	40.9 ± 1.9	37.9 ± 2.9	40.3 ± 1.6
POST	40.0 ± 0.9	35.7 ± 3.0	38.5 ± 2.0
%Type-IIa			
PRE	44.0 ± 1.4	45.6 ± 2.1	47.4 ± 2.2
POST	45.1 ± 1.6	48.6 ± 2.2	51.4 ± 3.5
%Type-IIx			
PRE	15.1 ± 1.0	16.5 ± 1.0	13.8 ± 1.0
POST	15.0 ± 1.0	15.7 ± 1.0	14.1 ± 1.0

Table 4.6 Muscle fibre CSA and contractile protein (mean ± SE)

Variable	PRO	PRO-CHO	Cr-PRO-CHO
Type 1 (μm^2)			
PRE	2895 ± 193	3079 ± 516	3129 ± 271
POST$^{\#}$	3244 ± 213	3480 ± 497	3659 ± 208
Type IIa (μm^2)			
PRE	4519 ± 242	4662 ± 501	4529 ± 383
POST$^{\#}$	5136 ± 231	5416 ± 518	5886 ± 315*†
Type IIx (μm^2)			
PRE	3798 ± 277	4370 ± 531	3905 ± 403
POST$^{\#}$	4402 ± 261	5007 ± 486	4864 ± 316*†
Contractile protein (mg/g)			
PRE	57.8 ± 2.9	55.8 ± 2.0	57.1 ± 1.3
POST$^{\#}$	78.4 ± 31	76.3 ± 1.0	89.1 ± 1.5*†

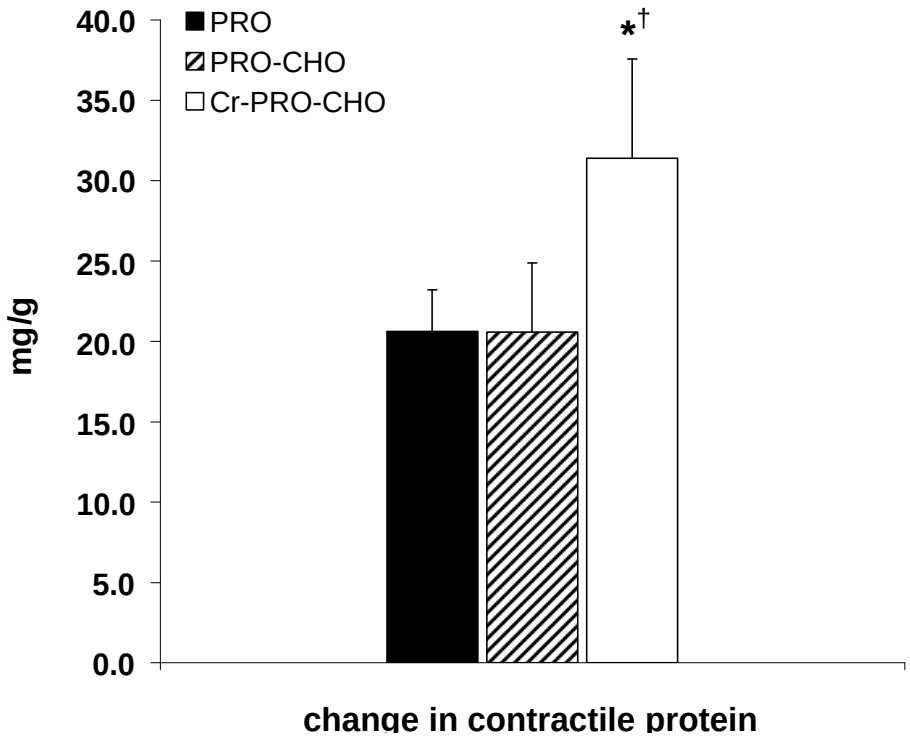
*Greater change than PRO-CHO; †Greater change than PRO; $^{\#}$ training effect all groups ($P < 0.05$)

Figure 4.3 Change in CSA (μm^2) of the type I, IIa and IIx fibres



*Greater increase than PRO-CHO; †Greater change than PRO ($P < 0.05$) (table 4.6)

Figure 4.4 Change in contractile protein (mg/g of muscle)



*greater increase than PRO; †greater change than PRO ($P < 0.05$) (table 4.6)

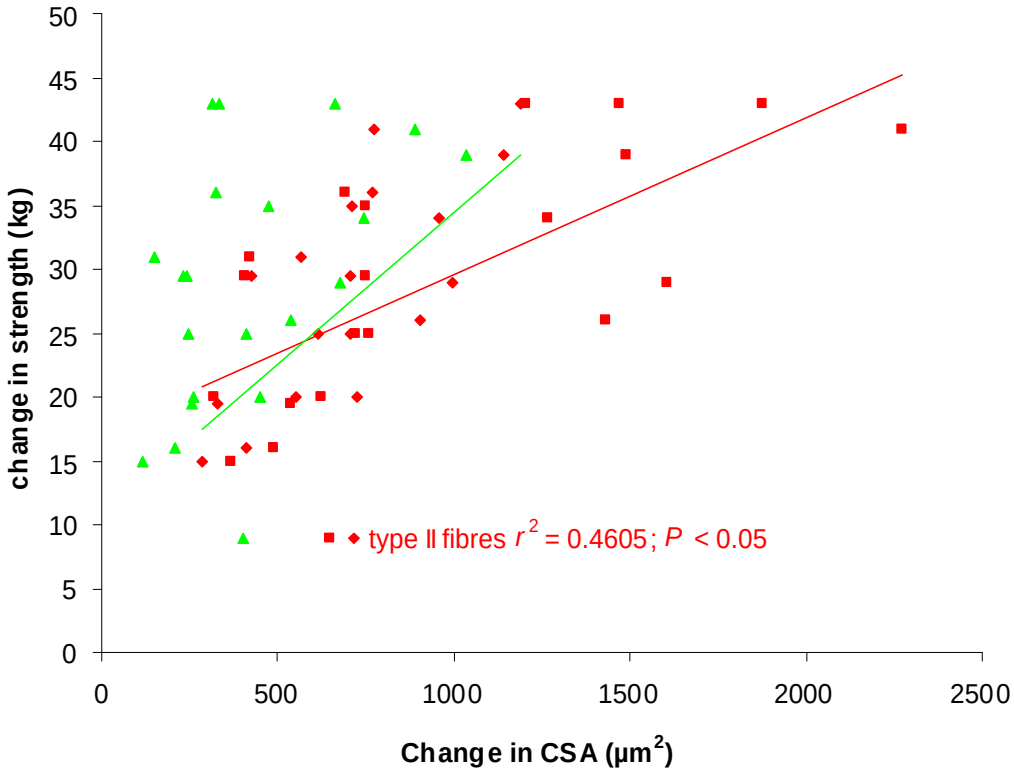
Table 4.7 presents muscle metabolite and glycogen data from samples taken before and after the training program. No differences between the groups or across time were detected for ATP, PCr, total Cr (PCr + Cr) or glycogen.

Table 4.7 Muscle metabolites and glycogen (mean ± SE)

Variable (mmol/kg dry wt)	**PRO**	**PRO-CHO**	**Cr-PRO-CHO**
ATP			
PRE	23.2 ± 1.0	23.8 ± 1.0	22.0 ± 1.0
POST	22.7 ± 1.4	22.4 ± 1.4	21.6 ± 1.1
PCr			
PRE	77.0 ± 2.0	80.7 ± 3.9	74.0 ± 2.0
POST	71.9 ± 4.9	69.6 ± 5.1	68.8 ± 4.6
Total Cr (Cr + PCr)			
PRE	117.5 ± 2.1	119.5 ± 4.7	115.8 ± 4.4
POST	111.2 ± 6.8	109.6 ± 7.1	119.8 ± 4.2
Glycogen			
PRE	342.0 ± 21.1	342.7 ± 10.1	328.7 ± 27.1
POST	335.3 ± 27.5	326.1 ± 23.5	290.3 ± 31.0

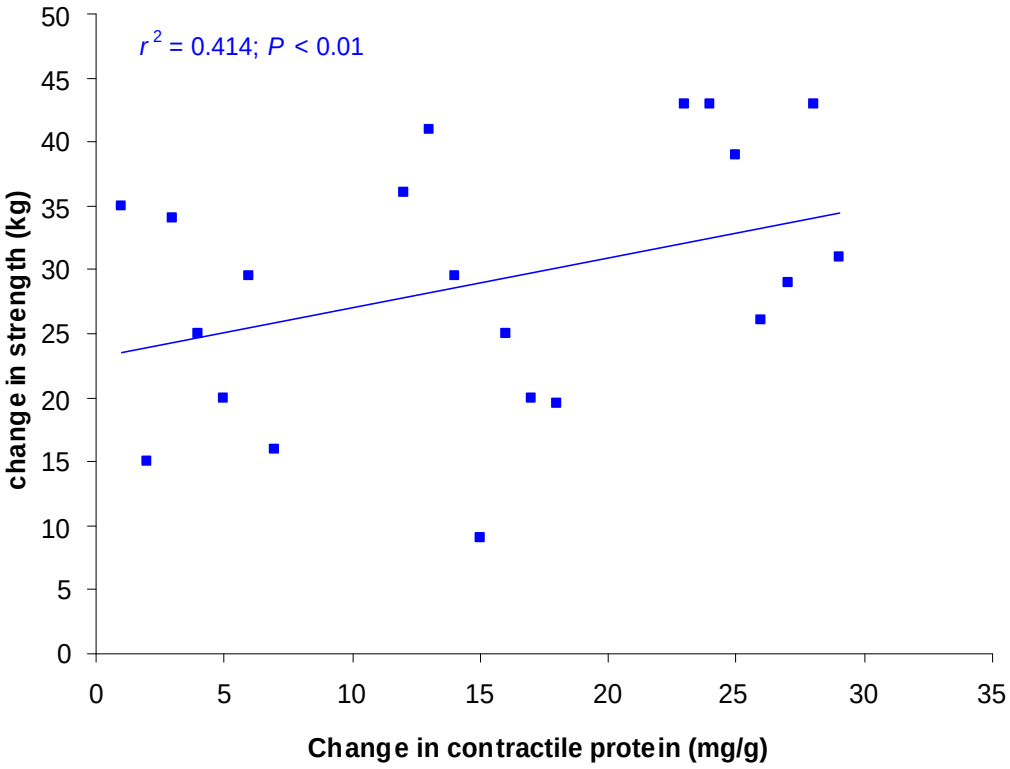
For all participants combined, positive correlations ($P < 0.05$) were detected between changes in CSA in the type-II fibres and strength gained in the 1RM squat exercise ($r = 0.677$) (figure 4.5). A correlation was also detected between the changes in contractile content (mg/g) and strength gained in squat exercise (1RM) ($r = 0.643$) ($P < 0.01$) (figure 4.6). For all participants combined, a positive correlation was also detected between the change LBM and strength (1RM) improvements in the squat ($r = 0.661$) ($P < 0.01$) (figure 4.7).

Figure 4.5 Relationship between change in muscle fibre CSA and 1RM strength changes in the squat



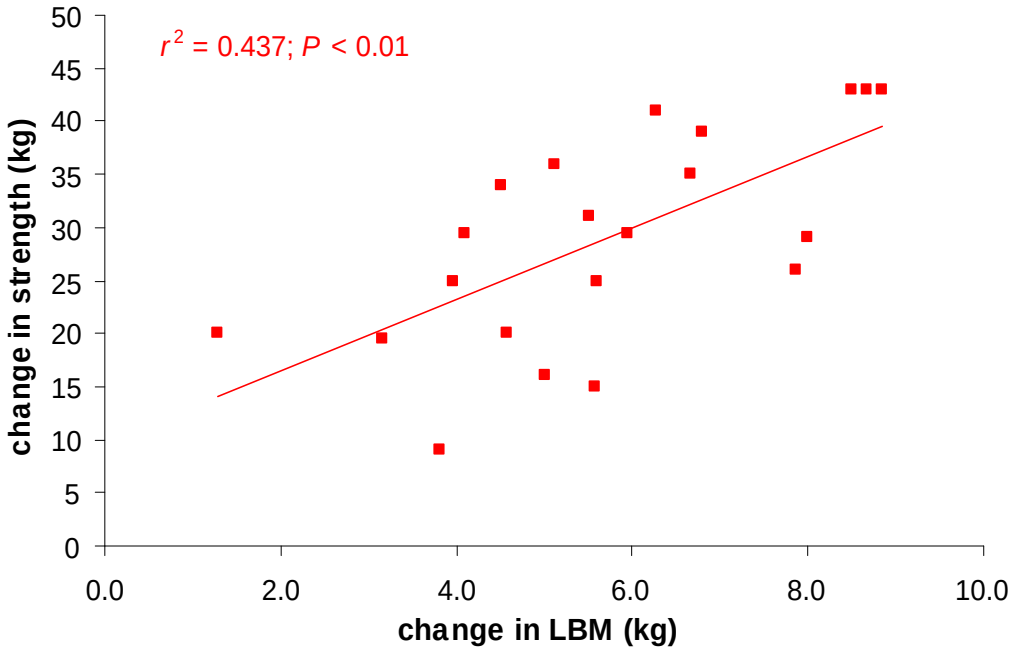
For all participants combined, a correlation ($P < 0.05$) was detected between changes in vastus lateralis CSA in the type-II fibres (IIa and IIx pooled) ($r = 0.677$) and 1RM strength improvements in the squat exercise

Figure 4.6 Relationship between change in contractile protein content and 1RM strength changes in the squat



For all participants combined, a correlation (r = 0.643) (P < 0.01) was detected between the changes in myofibrillar content (mg/g) in muscle taken from the vastus lateralis and 1RM strength changes in squat exercise (1RM).

Figure 4.7 Relationship between change in LBM and 1RM strength changes in the squat



For all participants combined, a correlation ($P < 0.01$) between the change LBM and strength (1RM) improvements in the squat was detected ($r = 0.661$).

4.4 Discussion

The results of this study demonstrated that a CrM-containing PRO-CHO supplement provided significantly greater gains in 1RM strength (in three assessments), LBM and muscle fibre hypertrophy (of the type-II fibres) compared to supplementation with an equivalent dose of PRO-CHO or PRO after 10 weeks of training. The greater improvements in 1RM strength demonstrated by the CrM-treated group are supported by the significantly greater hypertrophy responses at three different levels; LBM, muscle fibre CSA and contractile protein content. Therefore, these results support the hypothesis that, in RE-trained individuals a CrM-containing PRO-CHO supplement provides greater adaptations than a PRO-CHO supplement containing a similar amount of nitrogen and energy.

At least five RE training studies have reported greater increases in strength and LBM in participants who consumed CrM as compared with a placebo (Earnest et al., 1995; Vandenberghe et al., 1997; Kreider et al., 1998; Volek et al., 1999). However, only one has compared the effects of a CrM-containing supplement with a supplement containing a similar nitrogen and energy content (Tarnopolsky et al., 2001). When compared in this manner, Tarnopolsky et al. (2001) concluded that CrM supplementation provided no greater benefit in strength, LBM gains or muscle fibre hypertrophy. However, unlike the study by Tarnopolsky et al. (2001), the present study utilized RE-trained participants, and demonstrated significantly greater improvements in strength and muscle hypertrophy from treatment with CrM. It is generally believed that untrained participants experience strength and lean mass changes that are of greater magnitude compared to RE-trained athletes (Lemon et al., 1992; Earnest et al., 1995). However, the influence of training status on the effects of supplements such as CrM is unknown. It has been speculated that exercise training might increase muscle Cr uptake (and thus ensure greater benefits from supplementation) as regular exercise is associated with improved insulin sensitivity (Henriksen, 2002). Also, muscle Cr uptake is shown to be enhanced by the insulin response to macronutrient consumption (Streenge et al., 1998) and post-exercise supplementation (Robinson et al., 1999). The results of this trial would appear to support the suggestion that CrM supplementation may provide greater benefits in RE-trained individuals. However, as we did not assess insulin sensitivity, we can only speculate that improved Cr uptake by muscle was the mechanism underlying these differences.

Despite the clear beneficial effect of CrM that was observed in this study and the study presented previously (Chapter 3), metabolite assessments revealed only a modest increase (chapter 3) or, (as in the present study) no change in Cr content after the training/supplementation program.

The beneficial effects from CrM are thought to be dependant on the extent that Cr accumulates within muscle (Harris et al., 1992; Hultman et al., 1996). Others (Pearson et al., 1999; Willoughby & Rosene 2001) have shown significant improvements in LBM and strength performance using a small daily dose (5-6g/day) of CrM with no loading phase. This is why a "maintenance-only" dose of 0.1g/kg/day was used in this study. However, based on the metabolite analyses from this study and others (Volek et al., 1999), it may be that small doses of CrM (5-10g/day) for a prolonged duration (10 weeks) are not the most effective strategy for maintaining high muscle Cr concentrations during intense RE training. For instance, even with a one week loading phase completed by the CrM groups in the previous study (chapter 3), only a small increase (~10%) in muscle Cr was detected after the training program. This is in agreement with others, (Vokek et al., 1999) who have shown only a small (~10%) increase in resting Cr concentration in muscle after 12 weeks of RE training and supplementation. An insightful study by Van Loon et al. (2003) showed that a small maintenance daily dose (2-3g for 6 weeks) in sedentary individuals failed to maintain high Cr muscle concentrations that were achieved by a CrM loading phase. In fact, after the 6 week maintenance phase, muscle Cr levels had returned to pre supplementation values (Van Loon et al., 2003). Therefore, although the CrM dosing strategy used in this investigation did appear to enhance muscle hypertrophy, significantly, it may not be the most effective strategy for maintaining high muscle Cr concentrations during intense RE training. Due to the important benefits that CrM supplementation is capable of providing to a wide sector of the population, further studies should investigate strategies that create and maintain high muscle Cr concentrations during exercise training.

Few studies that have involved CrM supplementation during RE training have assessed strength and LBM alongside fibre-specific (type-I, IIa and IIx) hypertrophy and changes in contractile protein content. In this study, the CrM-treated group demonstrated a significantly greater increase in LBM (figure 4.2) and muscle fibre hypertrophy (in the type-IIa and IIx fibres) (figure 4.3) and these changes were reflected by the changes in myofibrillar protein content that were detected (figure 4.4). In a similar fashion to the previous study, although of lesser magnitude, some correlations (coefficient of determination) were detected that suggest a substantial portion (almost 50%) of the strength improvements observed in the squat exercise were due to the changes in skeletal muscle morphology (figures 4.5; 4.6; 4.7). Strength performance in compound exercises such as the squat, appear to be a function of LBM. Meaning, 1RM performance in this exercise is likely to be influenced by changes in LBM (Brechue & Abe 2002). However, it is equally as important to attempt to identify what factors might be responsible for the other "half" of the strength gains observed. For example, alterations in muscle architecture appear to play an important role in expression of strength. Greater fascicle length in muscles such as the triceps and vastus

lateralis are associated with greater LBM accumulation and 1RM performance in exercises that involve these muscles (i.e., the bench press and squat respectively) (Brechue & Abe 2002). The theoretical benefit of greater fascicle length would be an increase in force per CSA; while changing the pennation angle allows for greater sarcomere packing per CSA, force per CSA can be reduced when hypertrophy occurs with increased pennation angle (Kearns et al., 1998). Greater fascicle length is an adaptation that limits the changes in pennation angle associated with muscle hypertrophy (Kearns et al., 1998; 2000; Kumagai et al., 2000), thus maintaining the increase in force per CSA. To underline the influence of fascicle length in strength performance, a study of experienced weight-lifters demonstrated that 1RM performance in the barbell squat, deadlift and bench press correlated with greater fascicle lengths in the triceps and vastus lateralis (r values ranged from 0.63 to 0.54; $P < 0.01$)(Brechue & Abe 2002). More importantly, the extent of this adaptation appears to be closely associated with the extent of LBM accretion (Brechue & Abe 2002). As each group in the present study gained a substantial amount of LBM from the training program, an increased fascicle length may have been a substantial physiological contributor to the strength improvements observed. However, as this aspect was not measured, the extent of this adaptive response can only be speculated.

Aside from morphological adaptations, the improvements in 1RM strength observed in this trial (and the others presented within this dissertation) can also be attributed to the benefits of personalized coaching and supervision. Although the participants in our study were experienced in RE training, none had ever received personal coaching by a qualified instructor (the personal training only occurred during the 10 week trial, and not in the training leading up to the study). Personal training would provide more consistent feedback that result in more rapid development of skill and improved lifting technique. An improvement in technique in complex weight lifting exercises is indicative of neurological adaptations that are thought to contribute significantly to strength improvements in RE-trained individuals (Sale, 1992; Hakkinen et al., 1988; 1998b). While, it is obvious that both hypertrophy and neural factors contributed to the strength gains observed, it is difficult to ascertain the level of interplay between these factors in what they may have contributed to the development of strength as only pre- and post-training assessments were obtained. Previous work on untrained participants (Staron et al., 1994; Abe et al., 2000) has obtained data on hypertrophy and strength changes throughout an RE program. However, no training studies have attempted similar time course observations in RE-trained participants. Nonetheless, personalized instruction of the participants was a major strength of the trials presented within this dissertation as this level of supervision is shown to provide better control of workout intensity and greater strength improvements during training (Mazzetti et al., 2000). In lieu of the general hypothesis that was presented in chapter 1 (section 1.11), this level of supervision was

important as it would ensure the best chance of enhanced physiological adaptations from an interaction between training and supplementation. This is based on the premise that those treated with CrM would be capable of training at a higher intensity level and progressing at a faster rate. It is important to remember that the two instructors were blinded to the supplement groups, yet in this study, the Cr-PRO-CHO group demonstrated significantly greater gains in 1RM strength across all three assessments; thus supporting the hypothesis presented in the introduction at the start of this chapter.

An interesting finding from this study was the influence of the different supplements on body composition. While all groups demonstrated a gain in body mass after the training program, the Cr-PRO-CHO group demonstrated a significantly greater gain in body mass compared to the PRO group but not the PRO-CHO group. However, there were differences in the composition of these changes in mass. Compared to the PRO-CHO group, the Cr-PRO-CHO group and the PRO groups demonstrated a significant decrease in fat mass and body fat percent (figure 4.1). The exact reasons for these different responses to the various supplements are not clear. However, a decrease in body fat and/or body fat percent in response to WP supplementation (6-10 weeks) is a phenomenon that has been reported previously in rodents (Bouthegourd et al., 2002) and healthy humans undertaking exercise testing (Lands et al., 1999) or RE training (Cribb et al., 2006). WP supplementation has been shown to induce greater lipid oxidation during and after exercise compared to casein and CHO; a response that resulted in a greater utilization of body fat for fuel and a reduction in body fat (Bouthegourd et al., 2002). WP supplementation is also shown to increase circulating cyst(e)ine concentrations in the blood and influence GSH status (Lands et al., 1999; Marriotti et al., 2004; Middleton et al. 2004); a response that can result in a change in body composition in humans (see sections 1.2 & 1.8). While these findings provide some explanation for the body composition changes observed in the PRO group, it does not explain the contrasting body composition changes observed in the Cr-PRO-CHO and PRO-CHO groups.

Both the Cr-PRO-CHO and PRO-CHO groups consumed the same supplement; the only difference being the relatively small amount of CrM present in the Cr-PRO-CHO supplement which was approximately 7% (see Appendix). However, the Cr-PRO-CHO group demonstrated a significant reduction in fat mass and body fat percentage when compared to the PRO-CHO group. CrM does not appear to provide any benefit with regard to fat metabolism (Huso et al., 2002). Therefore, the improvement in body composition observed from CrM-supplementation may have been due to the large accretion of LBM that was observed in this group, which was (on average) over 6kgs of lean mass. This extra muscle mass would almost certainly have had a positive influence on resting metabolic rate and therefore, fat metabolism, particularly in active individuals

that consume the same relative energy intake (per kg of body mass) for a prolonged period of time (Poehlman & Melby 1998), as was the case in this study. If the addition of CrM to a PRO-CHO supplement does enhance LBM gains and improve body composition (as observed in this study), this may have specific implications for some populations. For example, those that desire an increase in LBM, maximum strength and muscle hypertrophy without an increase in fat mass may benefit from a CrM-containing PRO-CHO supplement during RE training. However, in those that desire a gain in body mass in general, CrM may not be required. Alternatively, those that desire strength and muscle hypertrophy with a relatively modest increase in body mass should opt for supplementation with whey protein only.

4.5 Conclusion

In summary, this study used a group of RE-trained participants to examine the effects of a CrM-containing (0.1g/kg/day) PRO-CHO supplement in comparison to the same PRO-CHO supplement during 10 weeks of RE training. Although the Cr-PRO-CHO and PRO-CHO supplements were similar in energy and nitrogen content, the group who received CrM in their PRO-CHO supplement demonstrated greater gains in 1RM strength in three assessments and these improvements were supported by a greater hypertrophy response that was apparent at three different levels; LBM, muscle fibre CSA and contractile protein content. Therefore, in RE-trained individuals, the presence of CrM in a PRO-CHO supplement results in significantly greater adaptations during RE training than supplementation without CrM.

Chapter 5
Supplement-timing improves muscle fibre hypertrophy, strength & body composition during resistance training

5.1 Introduction

As discussed in chapter 1 (section 1.6) oral supplementation with PRO and CHO before and/or after RE promotes a better anabolic response (i.e. a higher stimulation of protein synthesis and a positive net protein balance) compared to RE and placebo treatments. Therefore, it has been suggested that supplement-timing (i.e., the consumption of a PRO-CHO supplement immediately before and after RE) may provide the ideal anabolic conditions for muscle hypertrophy (Volek 2004). In chapter 1 (section 1.7), it was also identified that some longitudinal studies have reported greater muscle hypertrophy from supplement-timing. However, the participants in these studies were not permitted to consume any other nutrients other than the designated supplement for up to 3 hours before and after each workout. Therefore, the results can be attributed to the presence or absence of macronutrients, such as protein. However, as normal eating patterns where inhibited, these effects could not be attributed to supplementation *per se*. Additionally, no studies have examined whether this supplement-timing strategy may provide greater benefits in terms of muscle hypertrophy or strength development compared to the consumption of the same supplement at other times during the day.

Therefore, the aim of this study was to examine the chronic adaptations from supplement-timing in a group of RE-trained individuals as they consumed their habitual diet during a RE training program. It was hypothesized that supplement-timing would provide greater chronic adaptations (i.e., greater increases in LBM, strength and muscle fibre hypertrophy) compared to supplementation in the hours not close to RE. Based on the results of the study presented in the previous chapter, the supplement combination; CrM-PRO-CHO was utilized in this study to examine the effects of supplement-timing during 10 weeks of RE in comparison to supplementation at times not close to the workout.

5.2 Methods and Procedures

Twenty three (23) recreational male bodybuilders met the requirements (outlined in chapter 2) to commence this study that involved pre-post assessments and supplementation (2 groups) during 10 weeks of RE training. After baseline testing the participants were matched for maximal strength (1RM) in three weight lifting exercises (see strength assessments, Chapter 2) and then randomly assigned to one of two supplement groups. As we wanted to examine the effects of supplement-timing specifically, the participants were instructed to consume the supplement only on training days. The PRE-POST group consumed their supplement immediately before and after each workout (4 times per week for 10 weeks). The MOR-EVE group consumed the same supplement in the morning, before breakfast and in late evening before sleep, every training day. These times were at least 5 hours outside of the workout (see figure 5.1).

All participants were prescribed 1g of the supplement/kg of body mass, to be consumed twice on training days only. The supplement contained (per 100g), 40g protein (from whey isolate), 43g carbohydrate (glucose), <0.5g fat, 7g CrM. This dose was used as it would provide an 80kg participant with 32g protein, 34.4g carbohydrate, <0.4 fat and 5.6g dose of CrM in each serving (a total of 1124kJ). This quantity is characteristic of the dose consumed by this population (Marquart et al., 1998) and is not dissimilar to other studies that have examined the effects of supplement-timing during RE (Chromiak et al., 2004; Andersen et al., 2005). However, unlike other studies that have involved supplement-timing during RE, the participants were instructed to maintain their habitual daily diet during the trial (figure 5.1). The MOR-EVE group consumed the supplement with breakfast, performed RE (for 1 hour) between 3-6PM, consumed their normal evening meal approximately 1-2 hours after the workout and then consumed their second supplement dose before sleep. The PRE-POST group ate and trained at similar times to the MOR/EVE group but this group took their supplement servings immediately before and after each workout. The participants signed a consent form stipulating that they would follow their habitual daily diet (as determined by dietary records), take the supplement only as prescribed and not consume any other type of CrM, PRO or CHO supplement during the study. Participants were given a one-week supply of the supplement at the start of each week and asked to return the container before they received the next weeks supply as an act of compliance to the dosing procedure.

Obviously, an individual in this study was not blinded to the group that he was in. However, the researcher and personal trainer involved in the trial were blinded to the participants group. After baseline testing the participants were handed a sealed envelope by an individual not

involved in the study. This envelope contained a letter notifying the participant of their group allocation and instructions on how to consume their supplement dose. To maintain this blinded procedure, the participants were asked not to discuss the supplement with others and to consume their supplement whilst not in the presence of others involved in the study. Ability to comply with this request was made very easy. Each participant was supplied with several identical opaque drink bottles in which they consumed water ad libitum during each workout; the MOR-EVE group mixed and consumed their supplement with water in one of these bottles at home, whereas participants in the PRE-POST group carried their supplement servings in these dry bottles that were kept in lockers at the facility, and consumed discreetly away from others just before commencing each workout and again as soon as the workout was completed.

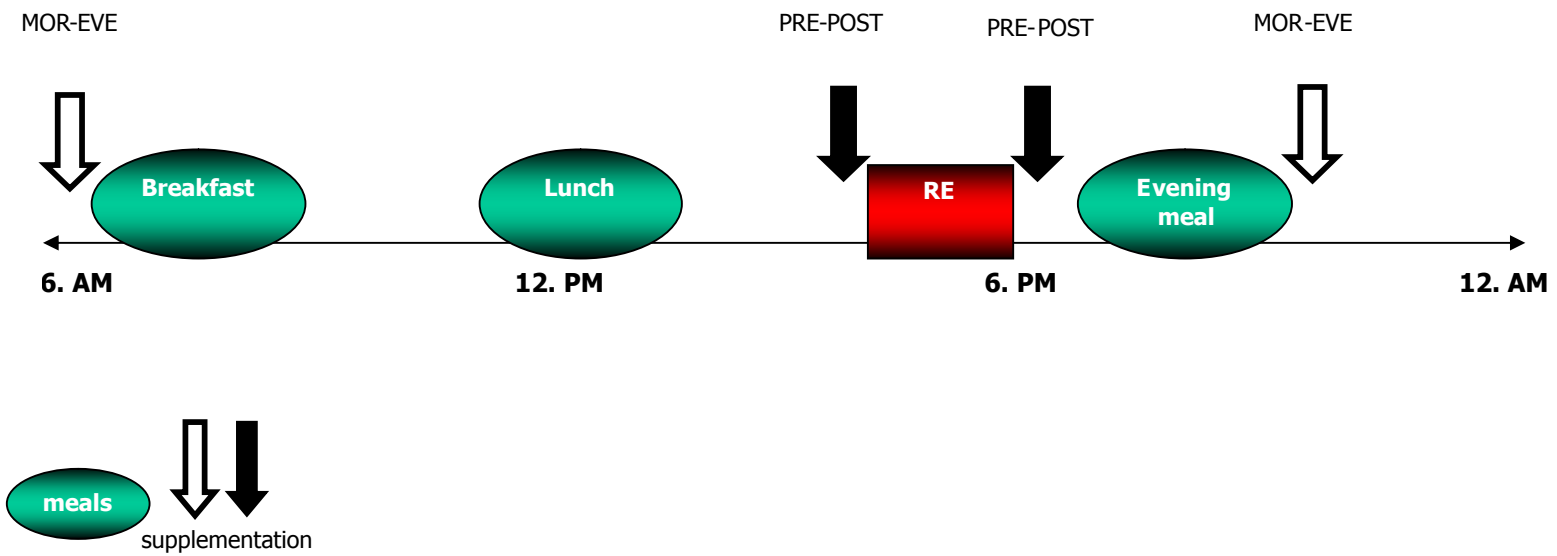
Nutritional intake was monitored via written dietary recalls as described in Chapter 2. Strength and body composition (assessments as in chapter 2) occurred in the week before, and after the 10 week RE program. Muscle biopsies for histochemical determination of muscle fibre type, CSA and contractile protein content as well as metabolite concentrations (as described in Chapter 2), were taken within 30 minutes after a leg workout that was completed on the Monday of the first and last week of the RE program.

Six participants did not complete the trial for reasons unrelated to the study. All other participants completed all requirements. Therefore, data obtained from 17 individuals (n = 8 PRE-POST; n = 9 MOR-EVE) is presented in the following tables and figures. Statistical evaluation of the data was accomplished by factorial ANOVA; groups x time with repeated measures on time. When an interaction was identified, between-group differences were determined by independent t-tests. A P value < 0.05 was considered statistically significant.

Table 5.1 Baseline Characteristics (values are means ± SD)

Characteristics	PRE-POST	MOR-EVE
Age (yrs)	21 ± 3	24 ± 4
Training age (yrs)	3 ± 2	3 ± 2
Height (cm)	178 ± 5	178 ± 2
Body mass (kg)	82 ± 9	78 ± 5
Fat mass (kg)	12 ± 4	13 ± 4
CSA type-I (μm^2)	3206 ± 389	2887 ± 382
CSA type-IIa (μm^2)	4604 ± 590	4491 ± 584
CSA type-IIx (μm^2)	4507 ±613	4360 ± 594
1RM Bench (kg)	127 ± 22	121 ± 14
1RM Deadlift (kg)	153± 18	142 ± 19
1RM Squat (kg)	148 ± 24	148 ± 26

Figure 5.1 Training day diet and supplementation schedules of the groups



Representation of eating and supplementation schedule during each training day of the 10 week program. The PRE-POST group (n = 8) consumed a supplement dose (1gm/kg) immediately before and after RE. The MOR-EVE group (n = 9) consumed the same dose of the same supplement before breakfast and sleep (at least 5 hours outside of the workout period). Training lasted for 1 hour and was completed between 3-6PM. The participants were instructed to maintain their habitual daily diet (that included no other use of supplements) and consume the supplement only on training days, at the time prescribed.

5.3 Results

Baseline characteristics (table 5.1) There were no significant differences between the groups prior to the training/supplementation program.

Dietary Analyses The average of three day written dietary recalls for energy (kJ/kg/day), carbohydrate and protein (g/kg/day) are presented in table 5.2. Weeks 1 and 10 data does not include supplementation. No differences were identified between the groups or across time with regard to energy, protein and carbohydrate intake.

Table 5.2 Dietary analyses (mean ± SD)

variable	PRE-POST	MOR-EVE
Energy intake (kJ/kg/day)		
before	183.0 ± 27.6	185.9 ± 20.1
week 1	184.6 ± 28.9	179.6 ± 17.2
week 10	179.2 ± 27.6	176.7 ± 11.7
Carbohydrate (g/kg/day)		
before	4.88 ± 1.3	4.63 ± 0.7
week 1	4.86 ± 1.0	4.62 ± 0.8
week 10	4.79 ± 0.9	4.50 ± 0.7
Protein (g/kg/day)		
before	1.84 ± 0.4	2.08 ± 0.4
week 1	1.91 ± 0.4	2.17 ± 0.3
week 10	1.92 ± 0.4	2.11 ± 0.3

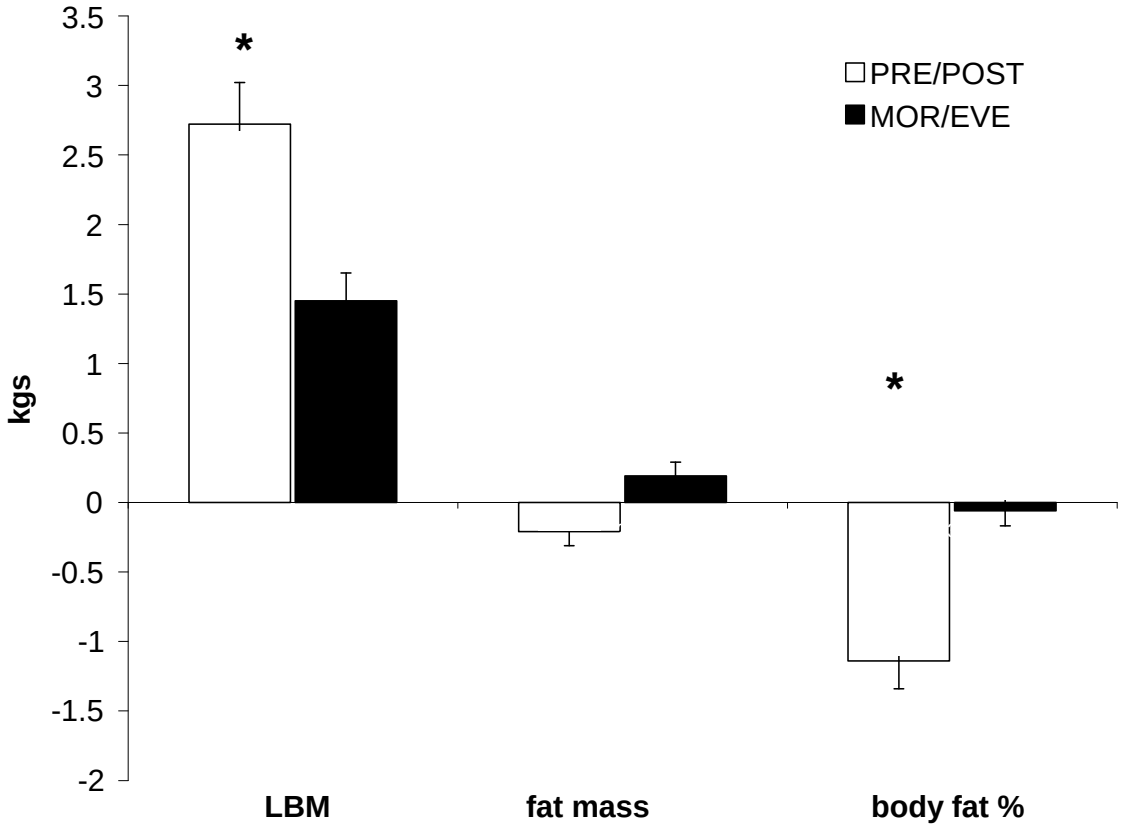
Body composition Table 5.3 presents body mass and DEXA determined body composition data. Figure 5.2 presents the body composition changes. Both groups demonstrated an increase ($P < 0.05$) in body mass and LBM after the training program. However, a group x time interaction ($P < 0.05$) for both body mass and LBM was detected. The PRE-POST group demonstrated a greater gain in body mass and LBM (post hoc $P < 0.05$) compared to the MOR-EVE group. No interaction for fat mass was detected however, a group x time interaction ($P < 0.05$) for body fat percentage was observed. The PRE-POST group demonstrated a decrease in body fat percentage compared to the MOR-EVE group (post hoc $P < 0.05$).

Table 5.3 Body mass and composition (mean ± SE)

variable	PRE-POST	MOR-EVE
Body mass (kg)		
PRE	81.8 ± 3.2	78.2 ± 1.8
POST[#]	84.3 ± 3.2*	79.6 ± 1.7
Lean mass (kg)		
PRE	69.5 ± 2.3	65.2 ± 1.5
POST[#]	72.3 ± 2.3*	66.7 ± 1.5
Fat mass (kg)		
PRE	12.1 ± 1.5	12.9 ± 1.2
POST	11.9 ± 1.4	13.0 ± 1.3
Fat %		
PRE	13.7 ± 1.4	15.7 ± 1.4
POST	12.6 ± 1.3*	15.7 ± 1.5

[#] Training effect both groups; *greater change compared to MOR-EVE ($P < 0.05$)

Figure 5.2 Body composition changes



*greater change compared to MOR-EVE ($P < 0.05$) (table 5.3)

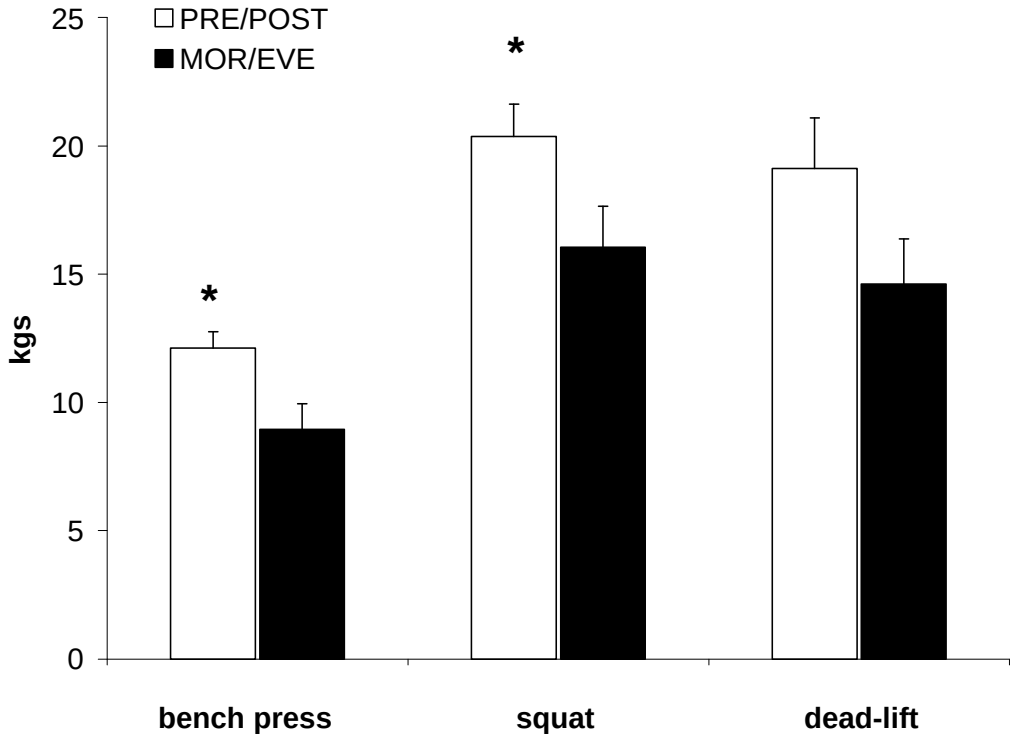
Strength Table 5.4 presents the results of 1RM strength assessments in three exercises (barbell squat, bench press and deadlift). All groups demonstrated strength improvements in each exercise after the training program ($P < 0.05$). However, a group x time interaction ($P < 0.05$) was detected for the bench press and squat. What could be considered a trend toward an interaction between treatment and training was also detected in the deadlift exercise ($P = 0.1$). The PRE-POST group demonstrated greater gains in 1RM squat and bench press strength (post hoc $P < 0.05$) (figure 5.3), and a similar trend was detected in the deadlift ($P = 0.1$).

Table 5.4 1RM Strength (mean ± SE)

variable	PRE-POST	MOR-EVE
Squat (kg)		
PRE	144.4 ± 8.2	138.3 ± 8.5
POST[#]	164.8 ± 8.6*	154.4 ± 7.9
Bench press (kg)		
PRE	126.9 ± 6.9	121.9 ± 4.7
POST[#]	139.1 ± 6.8*	130.9 ± 4.5
Deadlift (kg)		
PRE	149.7 ± 6.5	141.9 ± 6.4
POST[#]	168.1 ± 7.7	156.6 ± 6.5

[#] Training effect both groups; *greater change compared to MOR-EVE ($P < 0.05$)

Figure 5.3 Changes 1RM strength



*greater change compared to MOR-EVE ($P < 0.05$) (table 5.4)

Muscle characteristics There were no changes between the groups or across time with regard to fibre type proportions (table 5.5). While both groups demonstrated an increase in CSA across all fibre types after the program (table 5.6) ($P < 0.05$), a group x time interaction ($P < 0.05$) was detected in the type-II fibre sub-groups. The PRE-POST group demonstrated a greater increase in CSA in the type-IIa and IIx fibres compared to the MOR-EVE group (post hoc $P < 0.05$) (figure 5.4). A group x time interaction ($P < 0.05$) was also observed for contractile protein content; the PRE-POST group showed a greater increase in contractile protein content compared to the MOR-EVE group after the program (post hoc $P < 0.05$) (figure 5.5).

Table 5.5 Muscle fibre type (%) (mean ± SE)

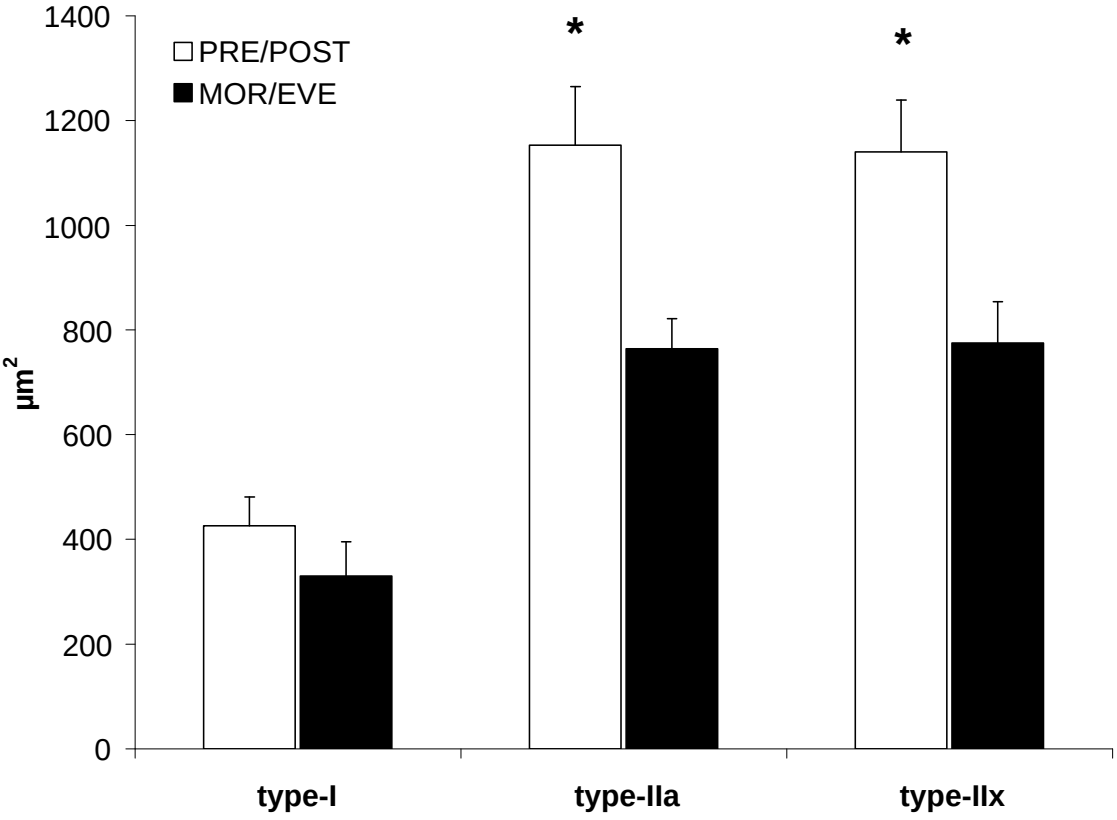
variable	PRE-POST	MOR-EVE
%Type 1		
PRE	45 ± 0.01	44 ± 0.01
POST	43 ± 0.01	45 ± 0.01
%Type IIa		
PRE	41 ± 0.02	44 ± 0.01
POST	44 ± 0.02	44 ± 0.01
%Type IIx		
PRE	14 ± 0.01	12 ± 0.01
POST	13 ± 0.01	12 ± 0.01

Table 5.6 Muscle fibre CSA and contractile protein (mean ± SE)

variable	PRE-POST	MOR-EVE
Type 1 (μm^2)		
PRE	3206 ± 138	2887 ± 127
POST[#]	3632 ± 126	3217 ± 104
Type IIa (μm^2)		
PRE	4604 ± 209	4491 ± 195
POST[#]	5757 ± 207*	5255 ± 160
Type IIx (μm^2)		
PRE	4507 ± 217	4360 ± 198
POST[#]	5647 ± 221*	5135 ± 169
Contractile protein (mg/g)		
PRE	61.2 ± 2.0	67.0 ± 2.6
POST[#]	91.5 ± 1.9*	84.6 ± 2.9

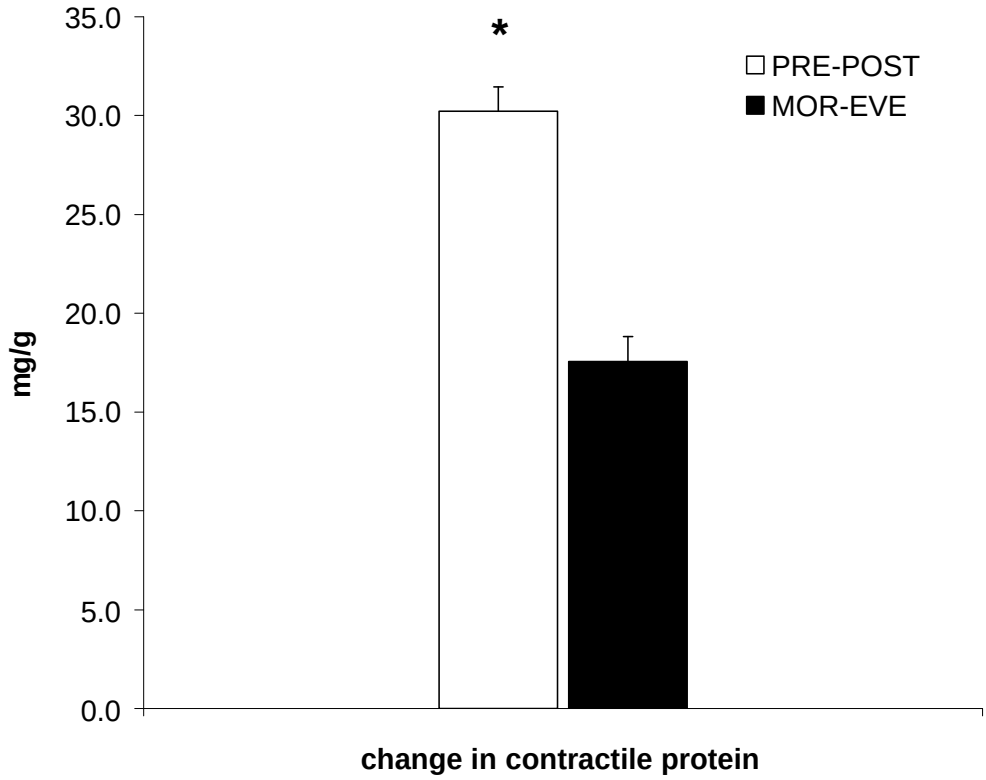
[#] Training effect both groups; *greater change compared to MOR-EVE ($P < 0.05$)

Figure 5.4 Changes in CSA (μm^2) of muscle fibre types-I, IIa and IIx



*greater increase compared to MOR-EVE ($P < 0.05$) (table 5.6)

Figure 5.5 Change in contractile protein (mg/g of muscle) (table 5.6)



*greater change compared to MOR-EVE ($P < 0.05$)

Table 5.7 presents metabolite and glycogen data from muscle samples taken before and after the training program. Both groups demonstrated an increase in total Cr (Cr +PCr) after the program ($P < 0.05$). However, group x time interaction ($P < 0.05$) was detected for total Cr and PCr as well as glycogen. The PRE-POST group demonstrated higher total Cr and PCr as well as glycogen concentrations (mmol/kg dry weight) compared to the MOR-EVE group after the program (post hoc $P < 0.05$).

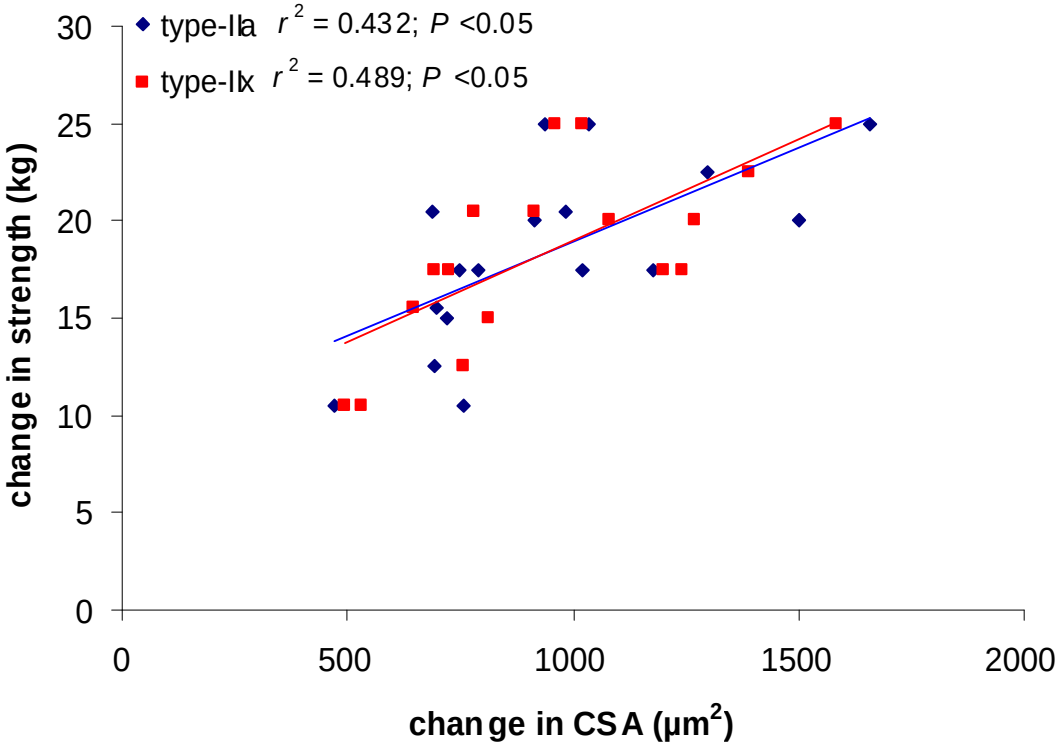
Table 5.7 Muscle metabolites and glycogen (mean ± SE)

Variable (mmol/kg dry wt)	**PRE-POST**	**MOR-EVE**
ATP		
PRE	21.6 ± 0.7	21.8 ± 0.7
POST	20.8 ± 0.6	21.8 ± 1.3
PCr		
PRE	78.1 ± 1.5	79.7 ± 2.6
POST	91.2 ± 1.4*	81.6 ± 2.7
Total Cr (Cr + PCr)		
PRE	123.0 ± 2.3	129.0 ± 3.9
POST#	153.2 ± 1.5*	138.2 ± 3.8
Glycogen		
PRE	235.1 ± 12.4	234.0 ± 4.3
POST	294.0 ± 8.0*	232.9 ± 2.8

Main effect both groups; *greater change compared to MOR-EVE ($P < 0.05$)

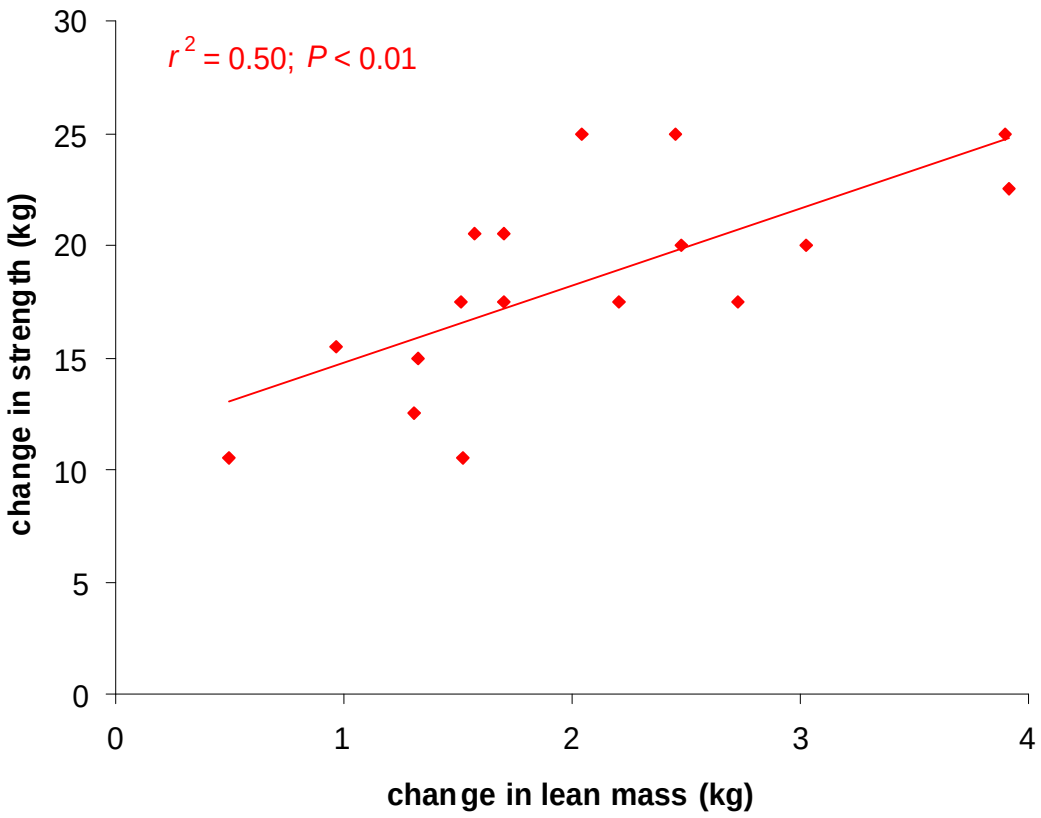
For all participants combined, a positive correlation was detected between changes in contractile protein content and strength gained in the 1RM squat exercise (r = 0.434) (P < 0.05) and also changes in type-II CSA and strength gained in the 1RM squat exercise (type-IIa r =.647) (type-IIx, r = 0.705) (P < 0.05) (figure 5.6). A positive correlation (P < 0.01) was also detected between the change in LBM and strength (1RM) improvements in the squat (r = 0.734) (figure 5.7). Finally, a positive correlation (P < 0.05) was also detected between changes in contractile protein content and changes in muscle fibre CSA (type-IIa, r = 0.624), (type-IIx, r = 0.570) (figure 5.8).

Figure 5.6 Relationship between change in muscle fibre CSA and 1RM strength changes in the squat



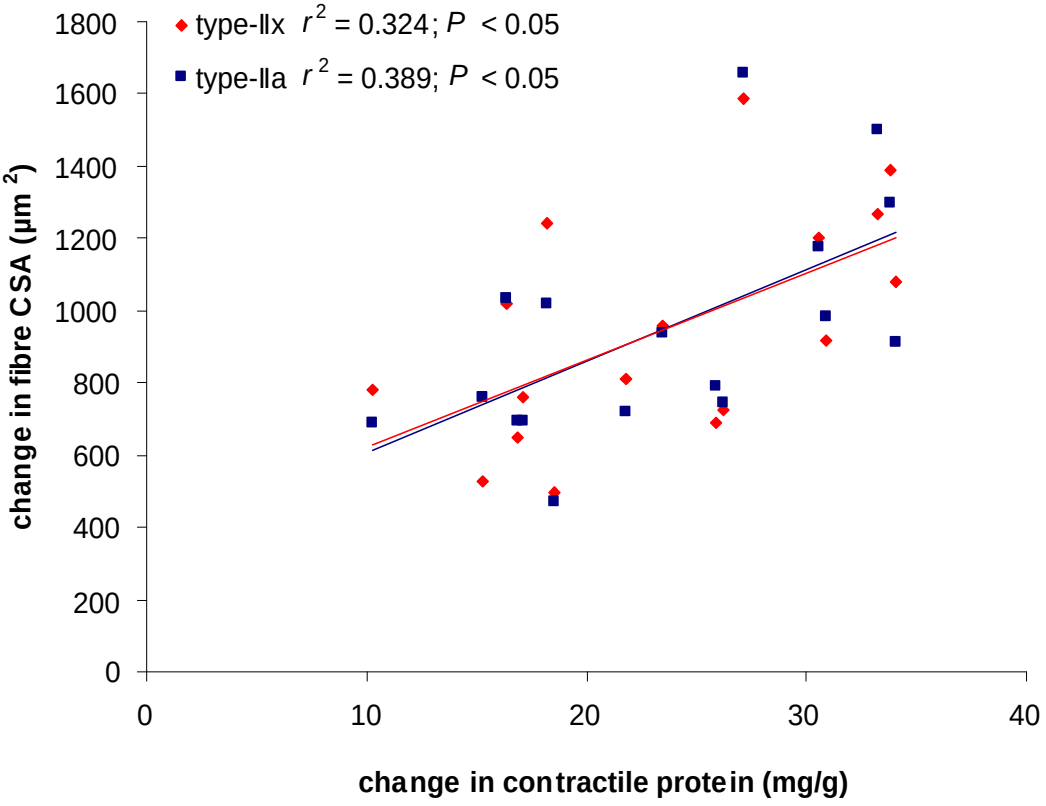
For all participants combined, correlations (P < 0.05) were observed between changes in muscle fibre CSA type IIa (r = 0.657), type-IIx (r = 0.700) and changes in 1RM squat strength.

Figure 5.7 Relationship between change in LBM and 1RM strength changes in the squat



For all participants combined, a correlation ($P < 0.01$) was detected between gains in LBM and strength improvements in the squat (1RM) ($r = 0.702$).

Figure 5.8 Relationship between change in contractile protein content and muscle fibre hypertrophy



For all participants combined, a correlation (P < 0.05) was detected between the increase in contractile protein and changes in muscle fibre CSA
type-IIa (r = 0.624) and type-IIx fibres (r = 0.570).

5.4 Discussion

The major finding of this study was that after 10 weeks of training, supplementation before and after each workout resulted in significantly greater improvements in 1RM strength and body composition (i.e., increase in LBM and decrease in body fat percentage) compared to a matched group who consumed the same supplement at times outside of the pre-post workout time frame. Significantly greater muscle hypertrophy from supplement-timing was evident at three levels. That is, the PRE-POST group demonstrated a significantly greater increase in LBM, hypertrophy of the type-IIa and IIx fibres, and contractile protein. This is an important finding as most (if not all) RE studies that report favourable changes in body composition from dietary supplementation did not assess changes in LBM alongside hypertrophy responses at the cellular (fibre type specific hypertrophy) and/or sub-cellular (contractile protein content) levels (Kreider 1996; 1998; Esmarck et al., 2001; Chromiak et al., 2004; Rankin et al., 2004; Andersen et al., 2005). While these results support the hypothesis presented at the start of this chapter, it is the design of this study that makes these findings particularly relevant to a wide sector of the population.

A number of acute-response investigations have shown that supplementation with protein (or EAA) and carbohydrate before and/or after RE will enhance the anabolic response by increasing muscle protein synthesis rates, decreasing protein degradation and providing a higher net protein balance (Miller et al., 2003; Tipton et al., 1999; 2001; 2003; 2004). With slight variations within their protocols, a number of longitudinal studies involving RE and supplementation generally provide evidence that supports the theory that supplementation before and/or after RE will enhance the chronic adaptations desired from training (i.e. muscle hypertrophy and strength) (Esmarck et al., 2001; Chromiak et al., 2004; Rankin et al., 2004; Andersen et al., 2005). However, the assessment conditions used to obtain these results may have less relevance in a real-world setting. That is, strength athletes and others that desire muscle mass gains would not usually abstain from consuming protein for up to 3 hours before and/or after exercise. Therefore, a novel aspect of the present is that the benefits of supplement-timing were demonstrated whilst the participants followed normal eating patterns in the hours surrounding exercise.

Unlike the previously mentioned studies, the effects of supplementation were assessed while the participants consumed their habitual daily diet during the trial (figure 5.1). That is, the MOR-EVE group consumed the supplement before breakfast, performed RE (for 1 hour) between 3-6PM, consumed their normal meal approximately 1-2 hours after the workout and then consumed their second supplement dose before sleep. The PRE-POST group ate and trained at similar times to the MOR/EVE group but this group took their supplement servings just before and straight after

each workout. Both groups consumed their regular meals and no other supplements that may have enhanced muscle growth during the trial. The use of bodybuilders in this trial was particularly advantageous as these athletes characteristically consume a protein-rich diet in regimented (frequent) meal patterns (Marquart et al., 1998; Leutholtz & Kreider 2000). For this reason and the fact we observed the participants exercise for up to 12 weeks prior to the start of the study, we were confident they would maintain their normal diet during the trial period. Aside from the observed results, the analyses of the nutrition diaries (table 5.2) suggest this to be true. Therefore, it can be confidently suggested that the beneficial effects of supplement-timing reported in this chapter cannot be attributed simply to the presence or absence of certain macronutrients in the hours surrounding RE. The beneficial adaptations observed were due to a specific interactive effect between high-intensity muscle contraction and the presence of an abundance of nutritional material (i.e., EAA, Cr and glucose) provided by supplement-timing.

A high concentration of EAA in plasma is shown to increase the acute stimulation of protein synthesis in muscle during RE (Biolo et al., 1997; Tipton et al., 2001) as well as provide a higher positive net protein balance over a 24 hour assessment period (Tipton et al., 2003). The presence of CHO (glucose) at this time is thought to enhance this anabolic stimulus (Miller et al., 2003; Volpi et al., 2003) probably by increasing plasma insulin concentrations (that also serve to increase protein synthesis rates when EAA are present) (Rennie et al., 2002), or by reducing myofibrillar protein breakdown after RE (Roy et al., 1997). The major source of nitrogen in the supplement used in this trial was whey isolate; a protein that is rich in EAA, particularly the BCAA, (20-26g/100g of protein) (Bucci & Unlu 2000). WP in general have rapid absorption kinetics (Dangin et al., 2001; 2003) and stimulate a high rate of muscle protein synthesis in a similar fashion to oral doses of free-form EAA (Tipton et al., 2004; Paddon-Jones et al., 2005). Supplementation with the EAA before and after RE, results in higher stimulation of muscle protein synthesis and net gain in protein over a 24 hour period (Tipton et al., 2003). This may be due to a greater phosphorylation of p70-S6k in skeletal muscles; a key (rate limiting) kinase in the signalling network controlling protein synthesis through translational initiation (Karlsson et al., 2004). The stimulation of MPS is considered to be the facilitating mechanism of muscle hypertrophy (Rennie et al., 2004). Therefore, the hypertrophy obtained from supplement-timing may be (at least partly) attributed to the abundance of EAA and glucose during high intensity muscle contraction that fundamentally provided a greater anabolic response (i.e., higher stimulation of protein synthesis and net protein balance) after each workout. However, the results obtained in Chapter 4, demonstrated clearly that the addition of CrM to a PRO-CHO supplement provided greater strength gains and muscle hypertrophy. Therefore, CrM probably contributed substantially to the improvements in strength and hypertrophy observed in both groups in this study. However,

the PRE-POST group demonstrated significantly higher muscle Cr concentrations (both PCr and total Cr) after the study. Prior to this investigation, no studies had examined the effects of CrM supplementation at different times during the day on muscle Cr concentrations and skeletal muscle morphology during RE training.

The beneficial effects of CrM on strength and LBM are purported to be largely dependant on the extent of Cr accumulation within muscle (Hultman et al., 1996; Tarnopolsky, et al., 2004). In this study, the PRE-POST group demonstrated higher muscle PCr and total Cr values after the program (table 5.7). As this group also demonstrated significantly greater gains in strength, LBM and muscle hypertrophy, the consumption of CrM (in a PRO-CHO supplement) just before and after RE could be considered a strategy that promotes more efficient accumulation of Cr within muscle. This theory is supported by others that have shown CrM retention is improved when consumed in PRO-CHO supplement (Steenge et al., 2000) after submaximal exercise (Robinson et al., 1999). Greater hypertrophy of the exercised (upper) limb from post-training CrM supplementation has also been reported (Chilibeck et al., 2004). Therefore, the findings from the present study, along with others (Robinson et al., 1999; Chilibeck et al., 2004) support the theory that supplement-timing promotes more efficient Cr accumulation within muscle. However, it is interesting to note that in this study, unlike the previous study (presented in Chapter 4) Cr-treatment resulted in a significant increase in muscle Cr stores in both groups. The reason for this may be the different dosing strategies used in these trials. For example, an 80kg individual in the present study received two distinct 6g doses of CrM each day but only on training days, whereas an 80kg participant in the Cr-PRO-CHO group in the study presented in Chapter 4 received a total of 6g via three supplement servings each day. While it is tempting to suggest that the dosing protocol used in the present study may provide a more effective means of increasing/maintaining muscle Cr stores, it should be remembered that both CrM supplementation protocols (chapter 4 and the present study) resulted in significant gains in strength, LBM and muscle fibre hypertrophy. Although well over 200 studies have examined the effects of CrM supplementation on exercise performance since 1993 (Rawson & Volek 2003), this study is the first to examine the effects of CrM supplementation at different times of the day on muscle Cr concentrations and adaptations from exercise training. Based on the results obtained from this study, further investigations in this area are warranted.

Another novel finding was that the PRE-POST group finished the study with significantly higher muscle glycogen concentrations (table 5.7). Muscle glycogen is an important fuel source during RE. A single bout of high-intensity RE can result in a significant reduction in muscle glycogen, particularly in type-II muscle fibres (Tesch, 1998). As type-II fibres are responsible for

maximum force production, low glycogen levels in these fibres may compromise performance during RE (Lambert et al., 1991; Haff et al., 1999; Leveritt & Abernethy 1999). The type (Burke et al., 1993), timing (Ivy et al., 1988a), and quantity (Ivy et al., 1988b) of CHO consumed after exercise is shown to affect glycogen resynthesis. The consumption of CHO before and/or after exercise is also shown to increase glycogen synthesis rates following exercise (Ivy et al., 1988). It has previously been shown that during high-intensity exercise (97% of VO_{2max}), blood glucose contributes only 1% of the CHO utilized, muscle glycogen provides the remainder (Katz et al., 1986b). A glycogen-sparing mechanism as a result of CHO feedings has also been reported during intense intermittent running (Nicholas et al., 1999) and during RE (Haff et al., 2000). Supplementation with glucose immediately before and/or after RE is shown to provide more efficient muscle glycogen restoration (Pascoe et al., 1993; Haff et al., 2000) which in turn appears to offer some ergogenic benefit such as increased work capacity in subsequent workouts (Haff et al., 1999). The combination of protein and glucose (1gm/kg/body mass) after exercise is shown to provide a similar beneficial effect on muscle glycogen resynthesis compared to glucose alone (Roy et al., 1998). Alternately, little glycogen resynthesis occurs in the absence of CHO consumption after exercise (Ivy, 1988). In fact, a delay in CHO intake after intense exercise by as little as 2 hours is thought to have negative impact on the amount of glycogen restored in muscle (Haff, 2003).

The pre- and post-training biopsies in this trial were taken 30 mins after the completion of a leg workout performed on Monday of the first and last week of training. On both occasions, the groups were instructed to consume the first dose of their supplement in the prescribed manner. That is, the PRE-POST group consumed their PRO-CHO dose prior to the workout. Therefore, it could be suggested that the higher glycogen and Cr values detected in the PRE-POST group were simply due to increased availability of CHO and Cr from the supplementation on the day of the biopsy. However, if this was the case then both weeks 1 and 10 values should have been higher in the PRE-POST group. The data presented in table 5.7 shows clearly, they were not. The muscle samples taken in the first week showed no significant differences between the groups in glycogen or Cr whereas the PRE-POST group showed significantly higher glycogen and Cr values in the samples obtained in week 10. Based on these findings it could be suggested that PRE-POST supplement-timing not only promoted more efficient CrM accumulation within muscle, this strategy may have also promoted more efficient muscle glycogen restoration during the RE program. In turn, these benefits may have enabled greater work capacity during subsequent workouts that helped to promote greater strength improvements and muscle hypertrophy. The participants' work capacity was not assessed in any of these trials simply because it was presumed that three 1RM strength assessments and three hypertrophy assessments would provide ample data that would support or reject the hypothesis presented. Indeed, when the metabolite results are considered along with the

strength and morphological data presented in this study, it is reasonable to suggest that the strategic consumption of whey isolate, glucose and CrM before and after each workout creates a favourable environment that results in better muscle strength and hypertrophy gains during RE training.

In line with the studies presented in the previous two chapters, both groups of RE-trained participants in this trial demonstrated significant improvements in strength (in each test). Also in a similar fashion to the studies presented previously, correlations were detected that suggest a substantial portion (in this case, almost 50%) of the strength improvements observed in the squat exercise were due to the changes in skeletal muscle morphology (figures 5.6; 5.7; 5.8). However, it is also clear that there are other factors that may contribute substantially to strength improvements in RE-trained individuals, such as neurological improvements and/or the impact of personal supervision. Based on the results presented in each of these trials, it is clear that under certain conditions, trained individuals can experience significant improvements in functional strength. Further, it is also clear that both neurological and morphological changes are contributing factors to these improvements in strength. Previous studies have utilized untrained participants to obtain data on hypertrophy and strength changes throughout an RE program. However, no training studies have attempted similar time course observations in RE-trained participants. With regard to strength development, RE training studies in the future should attempt to provide data on the interplay between neurological and morphological factors alongside assessment of factors that may augment chronic adaptations such as personal training and supplementation.

5.5 Conclusion

In summary, while there has been a sound theoretical basis for expecting a beneficial effect from supplement-timing, this is the first study to clearly demonstrate that this strategy resulted in greater strength and LBM gains as well as muscle fibre hypertrophy (type-IIa and IIx fibres) compared to the consumption of the same supplement at times outside of the workout period. Additionally, unlike previous work that has examined the effects of macronutrient consumption close to RE, these results were obtained in RE-trained participants that followed their normal eating patterns throughout the program. Therefore, it is concluded that supplement-timing with a CrM-PRO-CHO supplement represents a simple but effective strategy that enhances the adaptations that are desired from RE training.

Chapter 6

Conclusions & future directions

Strategies that promote the maintenance/increase of muscle mass would improve the health of a wide sector of the population. The focus of this dissertation was to examine physiological adaptations from intervention with two dietary supplements; whey protein (WP) and creatine monohydrate (CrM), during resistance exercise (RE) training designed to promote muscle hypertrophy in healthy adults. This thesis consisted of three separate trials. The collective aim of these studies was to examine the chronic effects of consuming WP and CrM, both separately and in various combinations as well as at strategic times of the day, during RE training. Each trial utilized groups of matched, RE-trained males that followed their normal eating patterns and consumed their prescribed supplement for the duration of each program. Each study consisted of pre- and post-training assessments that were completed within the week before and after the 10-11 weeks of RE training. Assessments included whole body DEXA scans to determine body composition (lean mass, fat mass and body fat percentage), maximum (absolute) strength in three weight lifting exercises, and vastus lateralis muscle biopsies for determination of muscle fibre proportion (type-I, IIa and IIx), cross-sectional area (CSA), energy metabolites (ATP, PCr, Cr) and glycogen concentrations as well as contractile protein content (mg/g of muscle). The most pertinent findings from these trials and possible future directions for further research are presented in this chapter.

- Study-1 (Chapter-3), examined the effects of four matched groups supplementing with CrCHO, CrWP, WP or CHO (1.5g/kg body wt/day) during 11 weeks of RE training. Despite the consumption of a high protein intake (≥1.5g/kg/day) by all groups and no differences between the groups before the study, supplementation with CrCHO, WP and CrWP resulted in significantly greater hypertrophy and 1RM strength gains (in the three assessments) compared to supplementation with CHO. Up to 76% of the strength improvements observed in the squat test could be attributed to hypertrophy of muscle involved in this exercise. However, when compared to CHO, the hypertrophy response from supplementation varied between the groups CrCHO, WP and CrWP at three levels assessed (i.e., changes in lean mass, fibre-specific hypertrophy and contractile protein content). While the results of this trial clearly showed that supplementation with WP and/or CrM promotes greater strength gains and muscle hypertrophy during RE training, the small number of participants within the groups makes it difficult to draw conclusions with regard to the effects of the different supplement combinations used in this study.

- In study-2 (Chapter-4), again, all groups in this trial consumed a high protein intake and there were no differences between the groups in any variable at the start of the program. After the training/supplementation program, all groups demonstrated significant strength improvements and muscle hypertrophy. However, the group that received a CrM-containing PRO-CHO supplement demonstrated significantly greater gains in strength in the three assessments (barbell squat, bench press and cable pulldown) compared to matched groups that received PRO-CHO or PRO only. The CrM-treated group also demonstrated a significantly greater hypertrophy response that was evident at the three levels assessed; lean mass, muscle fibre CSA (type-IIa and IIx fibres) and contractile protein content. In this trial it was concluded that a CrM-containing supplement provides greater strength and muscle hypertrophy during RE training even when compared to supplementation with a similar amount of energy and nitrogen.

- In the final study (study-3, Chapter 5), two matched groups were used to assess the effects of supplement-timing immediately before and after each workout (PRE-POST) during 10 weeks of RE in comparison to the consumption of the same supplement at times not close to the workout, such as in the early morning and late evening (MOR-EVE). The PRE-POST group demonstrated significantly greater gains in lean mass, strength (in 2 out of the 3 assessments), muscle fibre hypertrophy (type-IIa and IIx) and contractile protein. More importantly, unlike previous work that has examined the effects of nutrient consumption close to RE, these results were obtained in RE-trained participants that followed their normal eating patterns throughout the program. Therefore, it was concluded that supplement-timing represents a simple but effective strategy that enhances chronic adaptations from RE training.

CrM supplementation was a feature of this dissertation. In particular, the effects of three different CrM supplementation protocols on chronic adaptations from RE training were presented. More than any other dietary supplement, CrM has single-handedly revolutionized sports nutrition. It is one of very few nutritional ergogenic aids shown consistently in research to enhance athletic performance and training adaptations (under a variety of conditions) as well as affect certain aspects of muscle physiology that may explain its benefits. However, in comparison to the plethora of research completed on this supplement over the last decade, very few studies have examined responses to different dosing strategies in an attempt to improve its effectiveness. In the first study (Chapter 3), the effects of a traditional dosing protocol were assessed (a loading phase followed by a 10 week maintenance phase); study 2 (Chapter 4), examined the effects of a 10 week maintenance dose without a loading phase. In study 3 (Chapter 5), the effects of a strategic dosing protocol was

examined in which the CrM was consumed only on training days at different times of the day. Some of these dosing strategies provided higher muscle Cr values than others after the training program. However, what is truly fascinating is that, regardless of the supplement protocol, treatment with CrM generally resulted in greater adaptations (i.e., improvements in 1RM strength and muscle hypertrophy) when compared to matched, non-CrM treated groups. Additionally, supplement-timing with a CrM-containing supplement clearly provided greater gains in strength and muscle hypertrophy than consumption of the same supplement at times outside of the pre- and post-workout time frame.

Supplementation with CrM increases muscle Cr content and PCr availability. This is presumed to enhance the cellular bioenergetics of the phosphagen system and muscular performance during high intensity contractions. For example, Arciero et al., (2001) report that supplementation alone may contribute up to 40% of the strength gains observed from supplementation and training. Lifting heavier weight for more reps, more often is bound to influence the hypertrophy response from RE. However, supplementation with caffeine would probably produce a similar result in terms of workout performance, but I doubt that the couple of espressos I down before hitting the gym each day does anything to enhance the actual muscle hypertrophy process. In other words, unlike other some other compounds that may enhance exercise performance, CrM appears to enhance muscle strength and hypertrophy development via its ergogenic effect on energy metabolism, but also "other factors". The extent of each is unclear.

One of these "other factors" is described by Bessman & Savabi (1988). According to these researchers, increasing the availability of Cr would boost the efficiency of the Cr-P_i shuttle and the transfer of high-energy phosphates between the mitochondria and cytosol to the sites of major energy utilization; thereby enhancing the availability of energy not only for muscle contraction, but also the synthesis of muscle proteins during hypertrophy. This is an important concept that may have facilitated at least some of the benefits detected from supplementation with CrM. However, this aspect has received very little attention within the scientific community. The small amount of data that does exist suggests a clear lack of an acute anabolic effect from CrM on muscle protein turnover (namely, the stimulation of MPS). However, this should not be considered strong evidence for a lack of effect on the mechanisms of muscle hypertrophy. The relationship between energy status and protein turnover (in the short and long term) in human muscle is one that has received very little exploration.

The increase in lean and total body water from CrM during RE training appears to be mainly intracellular, that is, an increase in cell volume, this suggests that body cell (dry) mass has

increased (Poortmans & Francaux 1999; Bemben et al., 2001). CrM may promote an increase in cell volume and the subsequent activation of the anabolic mechanisms this phenomenon provides (refer back to figure 1.7). Precisely how an increase in cell volume might be linked to greater rates of protein synthesis following CrM supplementation is yet to be identified. While the signalling mechanisms that link changes in cell volume to anabolic and catabolic events is unclear, the pathway is thought to resemble that activated by growth factors such as IGF. That is, phosphorylation of the MAP kinases and c-jun (Agius et al., 1994). This is based on the recent finding that a CrM loading phase (21g/day for 5 days) is shown to increase the expression of IGF-I and IGF-II mRNA in resting muscle. The activation of this signalling cascade may also (partly) explain the influence of cell hydration on gene expression (Lang et al., 1998).

Transcriptional changes in muscle gene expression from CrM supplementation have resulted in greater strength and hypertrophy during RE. These phenotype alterations within the muscle fiber may contribute to, or be a product of, CrM's ability to enhance weightlifting performance. While it is apparent that Cr-loaded muscle is able to perform at a higher capacity during RE, only one study (Willoughby & Rosene 2001; 2003), involving healthy participants performing RE training, has managed to link an enhanced hypertrophy response from supplementation (i.e., increase in strength, LBM and thigh volume) to alterations at the molecular level that may explain these benefits. Due to the clear benefits from CrM on muscle strength and hypertrophy, further research in this area is warranted.

The improvements in strength, LBM and muscle hypertrophy that have been reported from CrM supplementation during RE appear to be a result of several mechanisms, such as improved work capacity via a more efficient supply of ATP; an increased expression of the muscle genes and regulatory factors associated with hypertrophy; an increase in satellite cell mitotic activity; or initiation of other anabolic processes which may be secondary to increased cell volume. However, no mechanistic investigations were attempted within this dissertation. Therefore, the origin of the adaptations presented must remain speculative. It is unfortunate that we could only perform pre- and post-training biopsies and therefore could not obtain any information on the extent, magnitude or timing of muscle Cr accumulation that the dosing protocols provided. Nonetheless, based on the results obtained with CrM supplementation in these studies, future research involving this supplement should focus on obtaining data on the effects that various dosing strategies have on muscle Cr concentrations alongside any physiological responses. Ultimately, this will lead to establishing the most effective way this supplement can be used to amplify the hypertrophy response from RE.

In general, RE-trained individuals are expected to achieve adaptations from training that are lesser in magnitude compared to novice participants. However, very few studies have examined the effects of training and dietary strategies that may optimize the hypertrophy response, particularly with RE-trained participants. Trained participants were used in these trials as it was presumed that adaptations would primarily reflect an interaction between supplementation and training. Aside from selecting individuals that were considered experienced in RE, in the second and third trials (chapters 4 and 5) the participants underwent a supervised RE program (without personal training) for 2-3 months prior to commencing these studies. However, despite taking these measures to ensure the participants were trained prior to each trial, all groups, across all trials still demonstrated significant improvements in strength and LBM after the training program. For example, gains in LBM in the treated groups ranged from 3-6% and muscle fiber CSA increases of up to 20%. These results are in accordance with the only other study that has assessed body composition, muscle fiber CSA during supervised RE and supplementation with trained participants (Volek et al., 1999). The study by Volek et al., (1999), demonstrated gains in LBM of approximately 6% and increases in CSA of 35% for each fiber type assessed.

Aside from morphology, a substantial portion of the improvements observed in the trials can be attributed to the benefits of personalized coaching and supervision. Personalized instruction of the participants is a major strength of the trials presented within this dissertation. Firstly, because this level of supervision is shown to provide better control of workout intensity and greater strength improvements during training. Secondly, in lieu of each hypothesis presented at the start of each trial, this level of supervision was important as it would ensure the best chance of enhanced physiological adaptations from the supplementation strategies. This was based on the premise that some supplementation strategies would enable work at a higher intensity level and progression at a faster rate. Work capacity was not measured in these trials because it was presumed that the three 1RM and three hypertrophy assessments that were completed before and after each trial would provide the clearest indication of an enhanced adaptation from training and supplementation. In most instances, the 1RM strength improvements and hypertrophy responses observed in each trial tended to support this theory.

Despite the widely held belief that trained individuals have much slower rates of improvement than untrained individuals (Kraemer et al., 2002), the results presented within this dissertation demonstrate that certain dietary and training interventions can appreciably influence the adaptations desired from RE, even in individuals that have been training regularly for a considerable period of time. In light of this information, and the fact that most RE training studies that assessed hypertrophy did not control diet or provide a level of supervision that would optimize strength

development, it may be suggested that some important variables that influence hypertrophy have been overlooked by previous investigations. If nothing else, the research presented within this dissertation demonstrates that some relatively straight-forward, easily-implemented strategies (i.e. qualified-personal supervision, a protein-rich diet and the use of certain supplements) can provide substantial improvements in functional strength and muscle hypertrophy in relatively short periods of time, even in participants whose physiology would be considered *resistant* to these changes. Therefore, the dietary and training protocols presented within this dissertation may have important implications for a wide sector of the population that are not as highly trained but still desire improvements in strength and/or body composition.

For instance, recent work has shown that a small dose of EAA (7g) may not stimulate protein synthesis in the muscles of older adults (Katsanos et al., 2005); a situation that has been attributed to a higher first-pass splanchnic extraction of AA in this population (Volpi et al., 1999). Additionally, unlike younger adults, the anabolic response to protein supplementation appears to be blunted by the addition of CHO in older adults (Volpi et al., 2000). Therefore, supplementation with protein that contains a high concentration of EAA such as WP, may provide a more calorie-efficient means of stimulating muscle anabolism in older populations (Paddon-Jones et al., 2004b). As CrM supplementation is shown to be effective for improving strength and LBM gains in older adults (Brose et al., 2003), a combination of WP and CrM (without carbohydrate) may be the most appropriate supplement to augment the benefits of RE in older populations. However, this hypothesis is yet to be tested. Other sectors of the population that would particularly benefit from this supplement combination are people living with conditions that promote muscle wasting (such as HIV, various forms of cancer and intestinal conditions such as Crohn's disease and Colitis). Obviously, CrM is effective for encouraging the maintenance of LBM; an essential component of survival in cachectic conditions (Kotler, 2000). However, WP contains a high concentration of EAA, cyst(e)ine residues and glutamine precursors (BCAA). WP's AA profile is considered alongside its rapid absorption kinetics, this protein source appears to be biochemically tailored to preserve LBM but also promote immune competence by improving GSH status, particularly in people living with cachectic conditions (Cribb, 2004). With regard to athletic populations, for those that desire gains in LBM, maximum strength and muscle hypertrophy without an increase in fat mass, supplementation with a combination of CrM, WP and CHO during RE would appear best suited to achieving this objective. Alternatively, those that desire strength and muscle hypertrophy with a relatively modest increase in body mass should opt for supplementation with WP only.

Based on the results presented in this thesis, the next logical step is to obtain a better understanding of how supplements such as WP and CrM influence the processes and pathways that

lead to changes in human muscle mass. Along this line, WP supplementation has recently been shown to enhance the activation of translation initiation during RE training (Farnfield et al., 2005). That is, after 12 weeks of training and supplementation, post-workout WP consumption resulted in significantly greater phosphorylation of p70-S6K and an increase in Pax7 gene (marker of satellite cell activation) compared to a placebo-treated group. With regard to CrM, one recent study has shown that a CrM loading phase (21g/day for 5 days) increased the expression of IGF-I and IGF-II mRNA in resting muscle. Additionally, the CrM treatment resulted in an increased phosphorylation state of the 4E-BP1 complex 24 hours after a single bout of RE (Deldicque et al., 2005b). These studies suggest that CrM and WP may enhance the activation of some of the key (rate-limiting steps) in muscle protein synthesis. The stimulation of muscle protein synthesis is considered to be the facilitating mechanism that promotes increases in muscle mass. Therefore, dietary intervention that may amplify the signalling processes that activate MPS represents an exciting area for future research.

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Appendix

Nutritional breakdown of all supplements used in these trials is based on the following information on the stock supplements.

- Carbohydrate supplement (CHO): 88.6g of carbohydrate (d-glucose), zero protein and fat/ 100g of supplement
- Protein supplement (PRO) (whey isolate): 86g of protein, < 5g carbohydrate, < 1g fat/ 100g of supplement.
- Creatine monohydrate (CrM) supplement is 99.9% CrM and therefore provided approximately (~) 100g CrM/ 100g of supplement. Creatine contains zero energy.
- 1g of protein contains 16.7 kilojoules (kJ) of energy, 1g of carbohydrate contains 16.7 kJ energy and 1g of fat contains 37.7 kJ energy

Chapter 3 (Study 1)

Each group was instructed to consume 1.5g of supplement/kg of bodyweight each day

WP group

Supplement: 100% PRO (provided 86g of protein, < 5g carbohydrate, < 1g fat /100g)

An 80kg subject consumed 120g of supplement/day that contained

103g protein, <6g carbohydrate, <1.2g fat and ~ 1864 kJ (447 kcal)

CHO group

Supplement: 100% CHO (provided 88.6g carbohydrate, 0 protein, 0 fat/100g)

An 80kg subject consumed 120g of supplement/day that contained

106g carbohydrate and 1770 kJ (424 kcal)

CrCHO group

loading phase supplement – 1st week; 80% CHO, 20% CrM

(provided ~71g carbohydrate, 0 protein, 0 fat, 20g CrM/100g)

An 80kg subject consumed 120g of supplement/day that contained

~ 85g carbohydrate, 24g CrM and 1420 kJ (340 kcal)

maintenance phase supplement – weeks 2-11; 93% CHO, 7% CrM
(provided ~82.4g carbohydrate, 0 protein, 0 fat, 7g CrM/100g)
An 80kg subject consumed 120g of supplement/day that contained
~ 98.9g carbohydrate, 8.4g CrM and 1651 kJ (396 kcal)

CrWP group

loading phase supplement – 1st week; 80% PRO, 20% CrM
(provided ~69g protein, <4g carbohydrate, <0.8g fat, 20g CrM/100g)
An 80kg subject consumed 120g of supplement/day that contained
~ 83g protein, <4.8g carbohydrate, <1g fat, 24g CrM and 1500 kJ (359 kcal)

maintenance phase supplement – weeks 2-11; 93% PRO, 7% CrM
(provided ~80g protein, <4.6g carbohydrate, <0.8g fat, 7g CrM/100g)
An 80kg subject consumed 120g of supplement/day that contained
~ 96g protein, <5.5g carbohydrate, <1g fat, 8.4g CrM and 1729 kJ (415 kcal)

Chapter 4 (Study 2)

Each group was instructed to consume 1.5g of supplement/kg of bodyweight each day

PRO group

Supplement: 100% PRO (provided 86g of protein, < 5g carbohydrate, < 1g fat /100g)

An 80kg subject consumed 120g of supplement/day that contained

103g protein, <6g carbohydrate, <1.2g fat and 1864 kJ (447 kcal)

PRO-CHO group

Supplement: 50% PRO, 50% CHO (provided ~43g of protein, 49g carbohydrate, < 0.5g fat /100g)

An 80kg subject consumed 120g of supplement/day that contained

~ 52g protein, 59g carbohydrate, <0.6g fat and 1877kJ (449 kcal)

Cr-PRO-CHO group

loading phase supplement – 1st week; 40% PRO, 40% CHO, 20% CrM

(provided ~ 34.4g protein, 37.4g carbohydrate, <0.4g fat, 20g CrM/100g)

An 80kg subject consumed 120g of supplement/day that contained

~ 41g protein, 45g carbohydrate, <0.5g fat, 24g CrM and 1455kJ (348 kcal)

maintenance phase supplement – weeks 2-11; 46.5% PRO, 46.5% CHO, 7% CrM

(provided ~ 40g protein, 44g carbohydrate, <0.5g fat, 7g CrM /100g)

An 80kg subject consumed 120g of supplement/day that contained

~ 48g protein, 53g carbohydrate, <0.6 fat, 8.4g CrM and 1710kJ (409 kcal)

Chapter 5 (Study 3)

All participants consumed 1g of supplement/kg of body mass twice a day, only on training days.

PRE-POST & MOR-EVE groups:

Supplement; 46.5% PRO, 46.5% CHO, 7% CrM (provided ~ 40g protein, 43g carbohydrate, <0.5g fat, 7g CrM /100g)

An 80kg subject consumed an 80g serving of supplement twice each training day that contained ~ 32g protein, 34.4g carbohydrate, <0.4 fat, 5.6g CrM and 1124kJ (269 kcal).