

**The Role of Nutritional Supplementation Following Resistance
Exercise in Humans**

by

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This thesis is dedicated to the Memory of
Major John A. Roy (1942-1994),
my Father.

He taught me the value of pride, honor and hard work,
this thesis is a testament to his success.

ABSTRACT

The purpose of this thesis was to investigate the effects of nutritional supplementation following resistance exercise on protein metabolism, muscle glycogen resynthesis rate, hormonal responses and training status through two unique investigations. The purpose of the first investigation was to determine the effect of post-resistance exercise glucose supplementation upon skeletal muscle fractional synthetic rate (FSR), urinary urea excretion, and whole body and myofibrillar protein degradation (WBPD and MPD, respectively). Eight healthy young males performed unilateral knee extensor resistance exercise (8 sets/~10 reps/~85% 1RM) such that the non-exercised limb served as a control. They received a carbohydrate (CHO) supplement (1g/kg) or placebo (PL) immediately ($t=0$ h) and 1 h ($t=+1$ h) following exercise. FSR was determined for both exercised (EX) and control (CON) limbs by incremental L-[1- 13 C]leucine enrichment of biopsy samples of vastus lateralis over ~10 hours post-exercise. Plasma insulin and glucose were determined at $t= -1.5, 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, \sim 10,$ and ~ 10.5 h post-exercise. MPD and WBPD were estimated from 24 hr urinary 3-methylhistidine (3-MH) and L-[1- 13 C]leucine flux, respectively, and whole body net protein balance was estimated from 24 hr urinary urea excretion. Plasma insulin concentration was greater ($p<0.01$) at 0.5, 0.75, 1.25, 1.5, 1.75 and 2 h in the CHO compared to PL condition, as was plasma glucose at 0.5 and 0.75 h ($p<0.05$). FSR was 36.1% greater in the CHO/EX leg than in the CHO/CON leg ($p=N.S.$) and 6.3% greater in the PL/EX leg than in the PL/CON leg ($p=N.S.$). 3-MH excretion was lower in the CHO

(110.43 ± 3.62 $\mu\text{mol/g}$ creatinine) than PL condition (120.14 ± 5.82)($p < 0.05$) as was urinary urea nitrogen(8.60 ± 0.66 g/g creat vs. 12.28 ± 1.84)($p < 0.05$). These findings suggest that CHO supplementation (1g/kg) immediately and 1h following resistance exercise can significantly decrease myofibrillar protein breakdown and urinary urea excretion, thus resulting in a more positive muscle and whole body protein balance.

The purpose of the second investigation was to determine the effect of various nutritional supplements upon whole body protein synthesis, urinary urea excretion, and whole body and myofibrillar protein degradation (WBPD and MPD respectively). Ten healthy young male resistance athletes performed a whole body circuit set workout (9 exercises/3 sets/80% 1Repetition Maximum). Exercises for the legs were performed unilaterally so that the non-exercised leg served as a control. They received a carbohydrate (CHO) supplement (1g/kg), a mixed CHO/PRO/FAT supplement (isoenergetic to CHO supplement)(68% CHO, 22% PRO, 10% FAT) or placebo (PL) immediately ($t=0\text{h}$) and 1 h ($t=+1\text{h}$) following exercise. Immediately following exercise muscle glycogen was significantly lower($p < 0.05$) in vastus lateralis of the exercised leg than in the control leg immediately post-exercise in all three conditions. Both the CHO and CHO/PRO/FAT supplements resulted in significantly greater increases ($p < 0.05$) in plasma insulin and glucose post-exercise than PL. The CHO and CHO/PRO/FAT also resulted in significantly greater($p < 0.05$) rates of muscle glycogen resynthesis vs. Placebo. No significant differences were observed between the three conditions for plasma testosterone and cortisol concentration

post-exercise. Similarly, no differences were observed between the three conditions for urinary creatinine, and 3-MH and urea nitrogen excretion. Thus, nutritional supplements do not appear to decrease myofibrillar protein degradation as indicated by 3-MH and urea nitrogen excretion in highly trained resistance athletes. Taken together, the two studies suggest that highly trained resistance athletes and untrained individuals both benefit from nutritional supplementation following resistance exercise, but may do so through different mechanisms.

PREFACE

The following is a list of abbreviations and operational definitions used throughout this manuscript:

MPS - muscle protein synthesis	AMP - adenosine mono-phosphate
MPD - muscle protein degradation	cAMP - cyclic-adenosine mono-phosphate
FSR - mixed muscle protein fractional synthetic rate	GLUT - glucose transport protein
ATP - adenosine tri-phosphate	G6P - glucose-6-phosphate
CAT - computer axial tomography	UDPglucose - uridine diphosphate glucose
MRI - magnetic resonance imaging	CHO - carbohydrate
NMR - nuclear magnetic resonance spectroscopy	PRO - protein
MVC - maximal voluntary contraction	Ra - rate of appearance
fCSA - muscle fiber cross-sectional area	Rd - rate of disappearance
CSA - total muscle cross-sectional area	NOLD - non-oxidative leucine disposal
ST - slow twitch muscle fibers	CO ₂ - carbon dioxide
CP - creatine phosphate	3-MH - 3-Methylhistidine
FT - fast twitch muscle fibers	IGF-1 - insulin like growth factor-I
MVD - mitochondrial volume density	FGF - fibroblast growth factor
hGH - human growth hormone	TNF - tumour necrosis factor
GH-RH - growth hormone releasing hormone	APE - atom percent excess
min - minute	α -KIC - alpha ketoisocaproic acid
h - hour	WBPS - whole body protein synthesis
d - day	PL - placebo
SE - standard error	EX - exercise
	CON - control

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CHAPTER I

RESISTANCE EXERCISE: MUSCLE GLYCOGEN, PROTEIN BALANCE AND THE INFLUENCE OF POST-EXERCISE NUTRITIONAL SUPPLEMENTS

1.1 Introduction

Skeletal muscle has a remarkable homeostatic ability to adapt to the physical demands that are imposed upon it. For example, resistance exercise training results in muscle hypertrophy and endurance training results in various metabolic adaptations within the muscle. The plasticity of muscle is mediated via a series of signals, which upregulate the expression of specific genes, and eventually result in the translation of certain proteins. Determination of which proteins are translated is dependent upon the signal initiating the transcription process. The synthesized proteins then dictate the various characteristics of the muscle.

Skeletal muscle hypertrophy is one such adaptation that is defined as an increase in the cross-sectional area of muscle fibers (MacDougall, 1986). It is well documented that resistance training results in muscle hypertrophy and increased strength (Narici and Kayser, 1995; Sale et al. 1990; MacDougall, 1986). Muscle protein balance is a function of both muscle protein synthesis (MPS) and muscle protein degradation (MPD). For muscle hypertrophy to occur, the individual must be in a net positive muscle protein balance (i.e. $MPS > MPD$)

(Waterlow et al. 1978). Muscle protein balance can be influenced by MPD (Young and Munro, 1978), MPS (Waterlow et al. 1978), hormonal status (Florini, 1987), nutritional status (Tarnopolsky et al. 1992) and exercise (Biolo et al. 1995a; Chesley et al. 1992). The potential interactions of these factors produces a very complex control system.

Resistance exercise results in both an increase in mixed protein fractional synthetic rate (FSR) (indicator of MPS) (Biolo et al. 1995a; Chesley et al. 1992), and MPD (Biolo et al. 1995a). It has been postulated that the increased MPS associated with resistance exercise is a result of a repair process occurring in response to exercise (Darr and Schultz, 1987). Muscle tissue damage is evident following isolated bouts of resistance exercise, especially that containing an eccentric component (Gibala et al. 1995; Fridén and Lieber, 1992). The amount of damage observed following a bout of eccentric exercise has been significantly correlated to FSR, but no such correlation was observed with concentric resistance exercise (Interisano et al. unpublished). Ultimately, this form of training must be initiating a signal to upregulate MPS for hypertrophy to occur. It has been hypothesized that mechanical tension, such as that produced with this type of training, is necessary for hypertrophy to occur (MacDougall, 1986). Thus, mechanical tension may be one of the stimuli for increasing MPS; however, other factors such as damage and repair, cytokines etc. may be involved.

Resistance exercise also results in changes in the metabolic characteristics of skeletal muscle, such as myosin isoform expression (Caiozzo et al. 1996, Abernethy et al. 1994; Staron et al. 1994), resting glycogen

concentration, creatine phosphate concentration, ATP concentration (Abernethy et al. 1994; MacDougall et al. 1977), mitochondrial volume density (Alway et al. 1988; MacDougall et al. 1982; MacDougall et al. 1979), and capillary density (Tesch et al. 1984).

Very little is known about glycogen utilization during, and resynthesis following, resistance exercise. It has been demonstrated that resistance training results in significant decreases in glycogen concentration in the exercised muscle (Robergs et al. 1991, MacDougall et al. 1988; Tesch et al. 1986). Furthermore, the rate of glycogenolysis observed with this form of exercise is similar to that observed with maximal isokinetic cycle ergometry (Robergs et al. 1991). Post-exercise muscle glycogen resynthesis has been extensively studied after endurance exercise (Shulman et al. 1995; Friedman et al. 1991; Ivy, 1991), whereas resynthesis following resistance exercise is limited to one publication (Pascoe et al. 1993).

The purposes of this introductory chapter are 1) to review the effects of resistance training on skeletal muscle; 2) to review muscle glycogen utilization during resistance training, and its post-exercise resynthesis; 3) to review muscle protein balance and the factors that influence it; and 4) to outline the possible role of nutritional supplementation post-resistance exercise upon glycogen and protein metabolism. Finally, the methodology involved in stable isotope analysis of muscle and protein turnover will be discussed.

The purpose of this thesis was to further examine the interactive effects of nutrition and resistance exercise upon glycogen and protein metabolism.

1.2 RESISTANCE TRAINING: EFFECTS ON SKELETAL MUSCLE

Resistance training functionally overloads skeletal muscle and results in adaptations at the muscle level. A considerable amount of this information has been obtained from the muscle biopsy technique. Morphology can be assessed from histochemical data such as fiber type, fiber area, and capillary density. Imaging techniques have also been used to evaluate muscle cross-sectional area. Some of these techniques include; computer axial tomography (CAT) (Sale et al. 1992a), magnetic resonance imaging (MRI) (Narici et al. 1989) and ultrasound. Biochemical properties such as enzyme activity and substrate concentrations can also be determined. More recently, the use of stable isotope tracers has provided another tool in the evaluation of muscle plasticity and function. ^{31}P Nuclear magnetic resonance spectroscopy has allowed a detailed examination of ATP and creatine phosphate turnover within the working muscle without implicit tissue sampling.

1.2.1 Muscle hypertrophy

Skeletal muscle hypertrophy has been demonstrated to occur in response to resistance training in both humans (Alway et al. 1988; MacDougall et al. 1980; MacDougall et al. 1979; Narici and Kayser. 1995; Sale et al. 1992a; Narici et al. 1989) and animals (Mikesky et al. 1991; Wong and Booth, 1988). In humans, cross-sectional and longitudinal training studies have both been used to investigate muscle hypertrophy. Cross-sectional studies have demonstrated

larger muscle fiber areas and greater absolute muscle strength in populations that undertake regular resistance training (bodybuilders and power lifters) (Alway et al. 1988). A 250% increase in muscle cross-sectional area and a 55% increase in maximal voluntary contraction (MVC) strength has been observed in strength trained persons compared to age matched sedentary controls (Alway et al. 1988). Similar results have been observed when comparing strength to endurance trained individuals (Tesch et al. 1984). In longitudinal training studies, similar results have also been observed. Resistance training has resulted in increased muscle fiber cross-sectional area (fCSA) (MacDougall et al. 1980; MacDougall et al. 1979), total muscle cross-sectional area (CSA) (Narici and Kayser 1995; Sale et al. 1992a; Narici et al. 1989) and muscle girth (MacDougall et al. 1977a). Histochemical analysis of fibers from individuals who had undertaken a 6 month heavy resistance training program demonstrated a 33% increase in the fCSA of fast twitch fibers (FT), and a 27% increase in slow twitch fibers (ST) (MacDougall et al. 1979). Similarly, using CAT scans Sale et al. (1992a) demonstrated an 11% increase in knee extensors CSA following 19 weeks of training. Shorter periods of training have demonstrated similar increases in CSA in both the knee extensors and the elbow flexors (Narici et al. 1989; Narici and Kayser, 1995). Most studies have demonstrated increased fCSA or girth with training protocols that are a minimum of 8 weeks in duration (Narici and Kayser, 1995; Sale et al. 1992a; Narici et al. 1989; MacDougall et al. 1979; MacDougall et al. 1979).

Increased muscle fCSA does not solely correlate with the observed increases in strength that occur with resistance training. This suggests that other adaptations occur such as changes in neural factors (Sale, 1992b). Some believe that this inconsistency provides indirect evidence that muscle fiber hyperplasia also occurs in response to resistance training (Mikesky et al. 1991; Antonio and Gonyea, 1993). There is no direct evidence that this type of muscle growth occurs in humans, but it has been documented in animal models (Antonio and Gonyea, 1993; MacDougall, 1992). For a more detailed discussion of this topic the reader can consult two different reviews (Antonio and Gonyea, 1993; MacDougall, 1992).

Collectively these results demonstrate that: 1) the magnitude of muscle hypertrophy is variable; 2) the growth process is slow; 3) increased muscle size does not account solely for increased muscle strength and 4) muscle hypertrophy can be achieved through a variety of resistance training protocols.

1.2.2 Muscle Protein Synthesis

Muscle fiber hypertrophy implies an increase in the protein content of the muscle. Thus, there must either be an increase in MPS or a decrease in MPD. Both resistance exercise and endurance exercise result in increased MPS and MPD (Biolo et al. 1995a; Carraro et al. 1990; Chesley et al. 1992; Yarasheski et al. 1993). With respect to resistance exercise, it has been demonstrated that MPS is elevated 50% at 4 and 109% at 24 hours following an exercise bout (Chesley et al. 1992). Biolo et al (1995a) and Yarasheski et al (1993) used a

different muscle group from that used by Chesley et al (1992) and MacDougall et al (1995), however, similar responses were observed with respect to MPS. Biolo et al (1995a), using an A-V difference model across the leg observed a 108% increase in muscle protein synthetic rate ~3 hours after an acute resistance training bout. Yarasheski et al (1993) observed a ~55% increase in muscle protein synthetic rate following 2 weeks of a resistance training program. It was unclear in the paper by Yarasheski (1993) as to the duration of time between the last resistance training bout and the assessment of MPS, but it appears that the assessment of MPS was made within 24 hours after the last training bout. When the resting synthetic rates or control values for MPS are compared for the above mentioned studies, the values are similar. However, it is difficult to compare the post-exercise values between studies due to: 1) different intensities and duration of exercise; 2) varied stable isotope models; 3) varied muscle group models; and 4) varied study designs (i.e. time point of MPS assessment). More recently, work from the same lab using similar subjects and the same protocol demonstrated that MPS is back to basal levels 36 hours after a bout of resistance exercise (MacDougall et al. 1995). Thus, in resistance trained individuals, an isolated bout of resistance exercise results in a rapid increase in MPS that is approximately doubled 24 hours after the exercise bout. Followed by a decline back to almost basal levels by 36 hours post-exercise. In addition, MPS responses in untrained individuals are similar to those in trained individuals (Biolo et al. 1995a; Yarasheski et al. 1993).

In summary, resistance exercise stimulates increased MPS that returns to basal levels by ~36 hours post-exercise. In addition, similar resting or basal rates of MPS have been observed in both highly resistance trained individuals and untrained individuals. It is difficult to draw more general conclusions due to the methodological differences in the literature and the paucity of studies.

1.2.3 Muscle Ultrastructure

It is well documented that resistance exercise leads to disruption of myofibrils (Gibala et al. 1995; Interisano, unpublished). Both the eccentric and the concentric components of the exercise contribute to this disruption; however, the relative contribution is far greater for eccentric exercise (Gibala et al. 1995). The observed myofibrillar disruption is characterized by z-band streaming in needle biopsy samples studied using electronmicroscopy. The exact mechanism of the z-band streaming is unknown, but disruption of the myofibrillar cytoskeleton has been implicated (Fridén et al. 1992) and this disruption is significantly increased following isolated bouts of resistance exercise (Gibala et al. 1995).

Staron and colleagues (1992) have investigated the effects of resistance exercise on muscle damage in response to an 8 week resistance training program in both men and women. Almost all of the baseline samples were free of indicators of ultrastructural damage. For the duration of the investigation, biopsies were collected every 2 weeks from both the control and training groups. Both groups showed evidence of damage and regeneration; however, this was

greater for the training group (9%) as compared to the control group (2%). Samples collected from the training group demonstrated distinct z-line streaming and myofibrillar disruption, whereas these were not present in the untrained group. It was concluded that resistance training may delay the muscle repair process and that the training may also result in additional damage to the myofibril.

The separated contribution of the concentric and eccentric component of resistance exercise on muscle ultrastructure was investigated by Gibala and colleagues (1995). They investigated the effect of these 2 types of muscle actions by having each subject perform the concentric action with one arm and the eccentric action with the contralateral arm. Following the acute bout of resistance exercise, muscle biopsies were collected from both arms immediately and 48 hours post-exercise. Analysis of ultrastructural properties showed that the eccentric arm had significantly more myofibrillar disruption at both time points (82% vs. 33% @ Immed., 80% vs. 37% @ 48 h). In addition, it was shown that the eccentric arm had more severely disrupted fibers at both of the examined time points. Clearly, resistance exercise resulted in ultrastructural damage to the myofibril and eccentric actions result in more damage than a comparable volume of concentric muscle actions.

1.2.4 Muscle Metabolic Characteristics

Resistance training results in several biochemical and metabolic adaptations in skeletal muscle. Effects can be divided into acute changes and

chronic adaptations. Acute changes reflect the metabolic stress of the exercise and indicate the energy source. Specifically, the metabolites and muscle substrates that are most often studied with respect to resistance exercise include: adenosine triphosphate (ATP), creatine phosphate (CP), glycogen, lactate, and creatine.

It has been demonstrated that an acute bout of resistance exercise can significantly decrease: ATP, CP, and muscle glycogen concentration (Tesch et al. 1986; MacDougall et al. 1988). In addition, the byproducts of flux through the aforementioned pathways are also increased. Specifically, increased free creatine (Tesch et al. 1986) and muscle lactate (Tesch et al. 1986; MacDougall et al. 1988) have been observed with acute resistance exercise. Tesch and colleagues (1986) demonstrated that an acute bout of knee extension exercise resulted in decreased ATP (~20%), CP (~50%), and muscle glycogen (~40%) in the vastus lateralis immediately post exercise. Similarly, MacDougall et al (1988) observed decreased ATP (~29%), CP (~57%), and muscle glycogen (25%) in biceps brachii. It should be noted that the larger decrease in muscle glycogen observed in the study by Tesch et al (1986) was likely due to a larger volume of exercise as compared to the study by MacDougall et al (1988) (~180 vs. ~30 contractions). Both groups observed similar decreases in CP. Both groups also observed significantly increased muscle lactate, which indicates increased flux of glycogen and glucose through glycolysis. The greater intramuscular lactate observed by MacDougall et al (1988) as compared to Tesch et al (1986) was likely due to the different muscle groups used in the two studies. Biceps brachii

tends to have a greater percent of FT fibers as compared to the vastus lateralis; consequently, the biceps would likely have a greater flux rate and a higher concentration of lactate accumulation per kg of muscle. In both studies it was concluded that resistance type exercise relies on both CP hydrolysis and glycogenolysis as sources of energy delivery. Based on the results from these two studies, it appears that the initial few contractions per set rely on CP hydrolysis, while the latter are more dependent on glycolysis.

Chronic resistance training programs also result in changes in the metabolic characteristics of the muscle. Several different metabolic adaptations have been observed with resistance training programs. Some of the adaptations include: decreased mitochondrial volume density, decreased capillary density, increased resting ATP, CP, creatine and increased muscle glycogen concentration. Both cross-sectional and long-term training studies have been used to investigate the chronic metabolic adaptations to resistance training.

One of the most consistent changes with resistance exercise is decreased mitochondrial density (Alway et al. 1988; MacDougall et al. 1982; MacDougall et al. 1979). This adaptation has been observed in both training (MacDougall et al. 1979) and cross-sectional studies (Alway et al. 1988; MacDougall et al. 1982). MacDougall and colleagues (1979) observed significantly increased fiber areas and decreased mitochondrial volume density (MVD) (26%) with 6 months of training. The authors concluded that the decreased MVD was due to the hypertrophy of the muscle fibers with no increase in the number of mitochondria, therefore potentially decreasing the muscle oxidative capacity per mass. The

proposed decrease in oxidative potential was made assuming that MVD reflects the oxidative capacity of the muscle. Alway et al (1988) observed that strength trained individuals had significantly lower MVD (~30%) as compared to endurance athletes, active and sedentary controls in the lateral gastrocnemius and soleus. Similarly, MacDougall et al (1982) observed significantly lower MVD in highly trained body builders as compared to untrained controls. The untrained subjects then underwent a heavy resistance training protocol. Following training significant decreases were observed in MVD for sedentary subjects such that they were no longer different from the elite body builders. It was concluded that the decreased MVD was a result of muscle hypertrophy, with no increase in mitochondria number. In summary, heavy resistance exercise results in muscle hypertrophy with no increase in mitochondrial number, which results in decreased MVD. This decreased mitochondrial volume density likely indicates a decrease in the oxidative potential of the muscle.

The effects of resistance training on muscle fiber capillary density have also been assessed. Tesch et al (1984) undertook a cross-sectional study, with three different subject groups; elite weight/power lifters, endurance trained athletes, and untrained controls. They found that the endurance trained athletes had significantly greater capillary to fiber ratios (Vastus lateralis) as compared to the other two groups. The elite weight/power lifters had similar ratio values to the control group; however, they had significantly larger fiber areas as compared to the other two groups. It was therefore concluded that resistance training leads to muscle hypertrophy and a decrease in capillary density. This decreased

capillary density may result in a decreased O₂ delivery and impaired removal of metabolic byproducts (decreased oxidative capacity).

In addition to mitochondrial and capillary density changes, changes have been observed in metabolite and substrate concentrations with resistance training. Resistance training results in increased resting concentrations of ATP, CP, and muscle glycogen (MacDougall et al. 1977a; Tesch et al. 1986; Abernethy et al. 1994). MacDougall et al (1977a) demonstrated these biochemical adaptations with a long-term training/disuse model. The training resulted in increased elbow extensor strength and cross-sectional area. Biochemical analysis of the biopsies taken pre/post training and pre/post immobilization demonstrated that the training resulted in significant increases in ATP, glycogen, and CP (18%, 66% and 22% respectively); while decreases in CP (25%) and glycogen (16%) were observed following immobilization. It was concluded that resistance training increases muscle energy reserves. Similar results have been observed by others. Tesch et al (1986) observed 50-100% greater resting muscle glycogen concentrations in trained individuals compared to reported values in untrained populations. These increases are likely related to the increased stress placed on these systems with resistance exercise. Because muscle glycogen is an important substrate during to resistance exercise (MacDougall et al. 1988; Tesch et al. 1986; Robergs et al. 1991), it is likely that a compensatory supercompensation occurs (Tesch et al. 1986).

In summary, resistance training results in several biochemical adaptations at the muscle level. Acute bouts of resistance exercise lead to decreases in

substrates such as CP and muscle glycogen. Continued training appears to decrease muscle oxidative capacity due to decreased mitochondrial density and capillary density. In addition, increased CP and muscle glycogen would provide a greater energy reserve for the muscle.

1.2.5 Effects on hormones

Resistance exercise results in changes in the endocrine responses and the sensitivity of various tissues to these hormones. Several of these hormones may be involved in modulating the adaptations at the muscle level. Some of the observed endocrine changes after an acute bout of resistance exercise include increased catecholamines (Nieman et al. 1995; Kraemer, 1988; Kjaer, 1992), and human growth hormone (hGH) (Kraemer et al. 1990; 1992; 1995). Other hormones, such as cortisol, have been reported to increase or remain unchanged with resistance exercise (Kraemer, 1988; Nieman et al. 1995). Similar conflicting reports exist with respect to testosterone (Kraemer et al. 1992; Kraemer et al. 1990).

Both dynamic and static exercise have been demonstrated to significantly increase circulating catecholamines (Kjaer, 1992). However, these two forms of exercise have different effects for the specific catecholamines, epinephrine and norepinephrine. Dynamic forms of exercise lead to a norepinephrine response 3 times greater than that of static exercise; comparatively, static forms of exercise lead to a greater epinephrine response than dynamic exercise (Kjaer, 1992). The increases in catecholamines are related to: the quantity of active muscle

mass (Kjaer, 1992), force of muscular contraction, and the duration of the exercise (Kraemer, 1988). Nieman et al (1995) had 10 male subjects perform an acute bout of heavy leg resistance exercise. Immediately post exercise they observed a 133% increase in epinephrine concentration, and a 465% increase in norepinephrine concentration. By 2 hours post-exercise both epinephrine and norepinephrine levels were back to baseline levels. This increase of catecholamines is not likely a stimulus for muscle growth (Kraemer, 1988), but rather functions to increase both hepatic glucose mobilization and the overall cellular rate of metabolism (Guyton, 1996).

Human growth hormone (hGH) is a single chain polypeptide that is released from the anterior pituitary through stimulation from the hypothalamus via growth hormone releasing hormone (GH-RH) (Kraemer, 1988). Acute resistance exercise also leads to increased hGH concentration (Kraemer et al. 1992; Kraemer et al. 1990; Kraemer et al. 1995). hGH can promote linear growth and has been shown to stimulate protein synthesis in adults (Moxley, 1994), however another study found no effect upon MPS (Yarasheski et al. 1993). Thus, hGH may be a stimulus for increased muscle growth following resistance exercise (discussed in a later section). Kraemer and colleagues (1990) investigated the effects of various resistance exercise bouts on hGH concentration. The same 8 exercises were used in each protocol, but one protocol was termed a "strength workout" (5 sets/5RM per exercise, 3 min rest between sets) and the other was termed a "hypertrophy workout" (3 sets/10RM per exercise, 1 min rest between sets). The hypertrophy workout resulted in

more work being performed (~49,161 J for strength vs. ~59,859 J for hypertrophy). It was observed that the "hypertrophy workout" resulted in the largest increases in hGH concentration and in the area under the hGH curve. It was concluded that different resistance training paradigms involve variable hGH responses. Kraemer et al (1992) observed similar increases in hGH with 3 sets of 4 different exercises at 10RM. Values increased during exercise, and peaked at ~25 min post-exercise. A similar pattern was observed by Kraemer et al (1990) with the highest value observed ~15 min post-exercise. This same group used a similar training stimulus to the "hypertrophy workout" referred to above and observed peak hGH values immediately post-exercise. hGH increases are transient and concentrations are back to resting levels by 90 min post-exercise (Kraemer et al. 1990; Kraemer et al. 1992). In summary, acute bouts of resistance exercise result in increases in hGH concentration that peak immediately or soon after completion of the exercise bout and return to resting levels within ~90 min.

Cortisol response to resistance exercise is not as consistent as that seen for hGH and catecholamines. Conflicting reports in the literature have reported both increased (Kraemer, 1988) and unchanged (Nieman et al. 1995) cortisol concentration following resistance exercise. However, training studies demonstrate that there may be long-term adaptations in cortisol response with resistance training (Staron et al. 1994). Cortisol is a steroid hormone secreted from the adrenal cortex in response to various stresses (Guyton, 1996). Cortisol is a member of the glucocorticoid family and accounts for ~95% of glucocorticoid

activity (Guyton, 1996). Its general effects include: stimulation of gluconeogenesis, decreased cellular glucose utilization, reduction in protein synthesis, stimulation of proteolysis, decreased amino acid transport into peripheral tissue, mobilization of fatty acids, and anti-inflammatory effects (Guyton, 1996).

No changes in post-exercise cortisol concentration were found following an acute bout of resistance exercise, in experienced weightlifters (Nieman et al. 1994). In fact, by 2 hours post-exercise cortisol concentration was below the initial baseline values. Others have found increased cortisol following bouts of resistance exercise (Kraemer, 1988). However, it is likely that the observed differences were due to circadian differences, blood draw techniques and psychological factors (Kraemer, 1988). In addition, it is possible that there is a training adaptation in cortisol response to resistance training. Staron et al (1994) conducted a resistance training study that involved the lower limbs for 8 weeks in both males and females. They observed a significant decrease in resting cortisol levels in the males but not females during the 7th week of training and even further decreases occurred following 9 weeks of training. Unfortunately, acute cortisol responses to the exercise were not determined. However, a decreased resting cortisol concentration may decrease the basal rate of protein degradation. It is clear that further work is required as to the response of cortisol to both acute bouts of resistance exercise and longer periods of resistance training.

Testosterone is a steroid hormone released from the testes in males and is a member of the androgen family of hormones (Guyton, 1996). One of the primary functions of testosterone is that of protein anabolism and muscular development (Guyton, 1996). There are differing reports as to the acute effects of exercise upon testosterone concentration. Kraemer and colleagues (1992) did not find an increased testosterone concentration following a bout of resistance exercise. An earlier study did find significant increases in testosterone both during the exercise bout and for up to 30 min post-exercise (Kraemer et al. 1990). These findings support the suggestion by Kraemer (1988) that testosterone concentration is influenced by; intensity and volume of exercise, and the amount of muscle mass used. Thus, in the study by Kraemer et al (1992) it was likely that one or a combination of these variables were not adequate to stimulate increased testosterone concentration.

With respect to the chronic adaptations of testosterone concentrations to resistance training, the responses are also unclear. Some studies have demonstrated that resting testosterone concentrations increase following 5 weeks of a resistance exercise training protocol (Staron et al. 1994), while others have observed trends for increased resting values following 10 weeks of training (Bhasin et al. 1996). Both of these studies suggest that resting testosterone concentrations increase with long-term resistance training. However, there are other studies that have observed no changes following training studies (Kraemer, 1988). With respect to females, there have been no observed changes in testosterone concentrations, both following acute bouts of resistance exercise

(Kraemer, 1988) and following 8 weeks of a training protocol (Staron et al. 1994). Further work is required to determine the response of testosterone to long-term resistance training.

Resistance exercise also results in changes in the sensitivity of various tissues to insulin (Miller et al. 1994; Fluckey et al. 1994; Craig et al. 1989). It has been observed that acute resistance exercise increases insulin clearance (Fluckey et al. 1994), while long-term resistance training results in an increase in insulin sensitivity (Miller et al. 1994; Craig et al. 1989). Fluckey et al (1994) observed an increase in insulin clearance in both controls and patients with insulin dependent diabetes mellitus following a bout of whole body resistance exercise. It was concluded that a single bout of resistance exercise can significantly enhance insulin clearance. Longer training studies have revealed that glucose disposal is higher and resting insulin concentrations are lower than pre-training resting concentrations (Miller et al. 1994). In addition, Miller et al (1994) also observed a significantly lower insulin response to an oral glucose tolerance test, suggesting that resistance training increased insulin sensitivity. Similar decreases in resting insulin concentrations were also observed by Craig et al (1989) following 12 weeks of whole body resistance training. It can be concluded that resistance exercise results in increased insulin sensitivity. However, further work is required to determine the exact cause of this increase. It is possible that the chronic adaptation may be related to increased translocation of glucose transporter proteins (discussed in a later section).

In summary, various hormonal adaptations occur with resistance training. These changes appear in most cases to be related to intensity and the volume of exercise, and to the amount of muscle mass used. However, many hormonal adaptations require further investigation to determine the influence of both an acute bout of exercise and long-term resistance training.

1.3 Muscle Glycogen

Glucose enters the muscle and is rapidly phosphorylated by hexokinase to form glucose-6-phosphate. This molecule is then either converted to glucose-1-phosphate and eventually stored as glycogen or is converted to fructose-6-phosphate and continues down the glycolytic pathway. Glycogen is a long glucose polymer that can have a molecular weight in excess of 5 million or greater (Guyton, 1996). Muscle glycogen can comprise 1-2% of the total weight of the muscle cell (Guyton, 1996). Glycogen has long been known as an important fuel source for prolonged exercise (Friedman et al. 1991), but more recently it has been also recognized as a primary fuel source in resistance exercise (MacDougall et al. 1988; Tesch et al. 1986; Robergs et al. 1991). It has also been observed that glycogen depletion results in decreased isokinetic strength over a number of repeated contractions (Jacobs et al. 1981). Thus, muscle glycogen concentration may influence strength performance.

1.3.1 Glycogen Metabolism

When there is an increase in the energy requirement of the cell, glycogen is hydrolyzed to glucose-6-phosphate, which in turn increases glycolytic flux to ATP formation. The first step in the process is the phosphorylation of glycogen to glucose-1-phosphate, this step is catalyzed by the enzyme phosphorylase. In resting muscle, glycogen phosphorylase is in an inactive form (a), but with stimulation from AMP, cAMP and hormones such as epinephrine it is quickly transformed into its active state (b) (Itamar et al. 1991). Resistance exercise can lead to significant reductions in muscle glycogen (Robergs et al. 1991; Tesch et al. 1986; MacDougall et al. 1988; Pascoe et al. 1993). Robergs et al (1991) had subjects perform two different protocols on separate days of different intensities (35% of 1RM vs. 70% of 1RM). Both exercise protocols resulted in significant decreases in glycogen (~188 mmol/kg D.W. for 70%; ~186 mmol/kg D.W. for 35%). These differences represent a ~39% decrease in muscle glycogen over resting values. Since the number of contractions was double in the 35% condition versus the 70%, the actual rate of glycogenolysis was doubled in the 70% versus the 35% condition. It was concluded that the total amount of muscle glycogenolysis is dependent on the magnitude of muscle force development and the rate of glycogenolysis was dependent on the intensity and frequency of the exercise. Similar decreases in muscle glycogen were observed in bodybuilders by Tesch et al (1986) with knee extensor exercises. In addition, similar post-exercise muscle lactate concentrations were observed in these two studies. These data suggest that the exercise intensity was similar for the two protocols.

Pascoe et al (1993) observed a 30% decrease in muscle glycogen in untrained subjects following an acute bout of resistance exercise. Finally, with bodybuilders MacDougall et al (1988) observed a 13% decrease in muscle glycogen following the first set of exercise and a 25% decrease at the completion of 3 sets of exercise. It can be concluded that glycogenolysis is a significant energy source for resistance exercise. In addition, it appears that the degree of glycogen degradation is dependent on the intensity and duration (number of reps and sets) of the exercise.

1.3.2 Glycogen Resynthesis

Briefly, for glycogen synthesis, glucose is transported from the intercellular fluid across the muscle cell membrane by glucose transport proteins (GLUT) and is phosphorylated to glucose-6-phosphate (G6P) by hexokinase. The glucose-6-phosphate is then transformed to glucose-1-phosphate, which is then converted to uridine diphosphate glucose (UDP glucose). The UDP glucose is then added to the growing chain of muscle glycogen in the presence of glycogen synthase. The two rate limiting steps in glycogen resynthesis are glucose transport into the muscle and the transformation of UDP-glucose to glycogen by glycogen synthase.

It is essential to ensure that muscle glycogen stores are sufficient, otherwise there may be an impairment of force production (Jacobs et al. 1981). In addition, many athletes also perform cross training (i.e. mixed endurance and

resistance exercise) or perform weight training as a training form for other sports (i.e. football, rugby, etc.) where glycogen depletion may be further compromised and performance impaired. Although factors influencing glycogen resynthesis following endurance exercise have been well documented and reviewed (Ivy, 1991; Friedman et al. 1991) there is limited information on glycogen resynthesis following resistance type exercise (Pascoe et al. 1993). Several factors have been implicated in the resynthesis of glycogen, these include: type of nutritional supplement consumed post-exercise, timing of supplement ingestion, amount of supplement consumed, and form of the supplement.

1.3.2.1 Glycogen Synthase

Glycogen synthase is a rate limiting enzyme in the synthesis of glycogen (Friedman et al. 1991; Katz and Westerblad, 1995; Friis Bak, 1994). This enzyme is found in two forms within the muscle: an active dephosphorylated form (I-form) and a phosphorylated inactive form (D-form) (Alonso et al. 1995; Friis Bak, 1994; Friedman et al. 1991). It is bound to a protein termed glycogenin (Alonso et al. 1995; Friis Bak, 1994). Glycogenin acts as a primer for the formation of glycogen. Thus, a glycogen molecule contains a glycogen synthase molecule (on average one), a glycogenin molecule and glucose residues. As the molecule grows in size through the addition of glucose residues, it will eventually reach a size such that it is termed proglycogen (up to 60,000 residues) (Alonso et al. 1995; Friis Bak, 1994). For the molecule to grow to such a large size the addition of glucose residues must occur in the presence of

a branching enzyme, these enzymes transfer the glucose residues from 1-4 linkages to 1-6 linkages (Friis Bak, 1994). The molecule can continue to grow to form macroglycogen, which is much larger than the proglycogen (Alonso et al. 1995; Friis Bak, 1994). It is believed that the glycogen synthase molecule must be dephosphorylated even further to form the macroglycogen (Alonso et al. 1995). It should also be noted that the branching as well as debranching enzymes are also associated with the glycogenin molecule (Friis Bak, 1994). For a detailed review, the reader is referred to two excellent reviews (Friis Bak, 1994; Alonso et al. 1995).

Glycogen synthase activity is influenced by a number of factors. For example, glucose-6-phosphate (G-6-P) is a powerful allosteric regulator of glycogen synthase, such that an increase in G-6-P leads to increased glycogen synthase activity (Shulman et al. 1995; Friis Bak, 1994). Two additional factors influencing glycogen synthase are exercise and insulin (Friis Bak and Pedersen, 1990; Richter et al. 1989; Ebeling et al. 1993; Ren et al. 1994; Rossetti and Hu, 1993; Friis Bak, 1994; Yan et al. 1992). The fractional activity of glycogen synthase is positively correlated to the duration of dynamic exercise (Yan et al. 1992). Friis Bak and Pederson (1990) observed significant decreases in the concentration of muscle glycogen in the vastus lateralis; in addition, they observed significant increases in the fractional velocity of glycogen synthase as well as an increased I-form of the enzyme following a bout of endurance exercise. These increases were observed with no change in glycogen synthase phosphatase activity. As well, there was no change in insulin binding following

the exercise protocol. It was concluded that exercise increased glycogen synthase activity and that this increase was due to dephosphorylation of the enzyme; however, the dephosphorylation was not due increased insulin binding or glycogen synthase phosphatase activity. It appears that exercise alone can stimulate the conversion of the inactive form of glycogen synthase to the active form independent of other stimuli such as insulin.

Insulin is also a powerful stimulus for the activation of glycogen synthase. Not only can insulin lead to an increase in the transport of glucose into the muscle cell via glucose transporters, it also initiates a second messenger cascade of phosphorylation-dependent kinases and the eventual dephosphorylation of glycogen synthase (Friis Bak, 1994). There is also an insulin independent phase of glycogen resynthesis occurring soon after the completion of exercise (Price et al. 1994). Using ^{13}C nuclear magnetic resonance spectroscopy (NMR), this group investigated the effect of varied intensity heel raises on glycogen content and resynthesis of the gastrocnemius. In addition, the effect of insulin on resynthesis was assessed both with and without an infusion of somatostatin. They observed that once muscle glycogen was depleted to below a critical level (<140 mmol/kg D.W.), synthesis was not affected by low insulin levels, but synthesis ceased when glycogen levels were above 140 mmol/kg D.W. and insulin was not present. It was concluded that after glycogen depleting exercise, glycogen resynthesis is insulin independent and glycogen dependent, which suggests local control. It appears that glycogen depleting exercise results in local changes at the muscle level that aid in an initial

attempt by the muscle to rapidly increase resynthesis of glycogen. To continue to resynthesize glycogen, increased insulin is required to stimulate the activation of glycogen synthase or to increase the availability of substrates by increasing glucose transport into the muscle.

It has also been suggested that the transport of glucose into the cell and the conversion of glucose to glucose-6-phosphate in the presence of hexokinase is more important than glycogen synthase (Shulman et al. 1995). Shulman et al (1995) provide a lengthy review in this area and propose a model of glycogen synthesis control based on data obtained from studies using NMR. They propose that glycogen synthase is not the controlling step in the resynthesis of glycogen, but rather control of flux is primarily done through the glucose transport and hexokinase step and that the primary regulator of glycogen synthase is G-6-P. The authors also note that much further work is required in this area to further determine other controlling factors in the process of glycogen resynthesis.

In summary, glycogen synthase is important in the resynthesis of muscle glycogen following exercise. It may not be the rate limiting step in the resynthesis process, but it is essential that this enzyme is dephosphorylated to a more active state to ensure increased muscle glycogen formation.

1.3.2.2 Glucose Transport

For glycogen synthesis to occur there must be an adequate supply of substrate. Glucose is the substrate for the glycogenesis pathway. Because the

glucose molecule is hydrophilic it cannot cross the sacrolemma passively, and a carrier protein is required. These carrier proteins allow the facilitated diffusion of glucose into the cell. A number of different glucose transport proteins have been identified in different tissues throughout the body (Barnard and Youngren, 1992; Rodnick et al. 1992; Assimacopoulos-Jeannet et al. 1991). The two primary glucose transporters that are found in skeletal muscle are termed GLUT1 and GLUT4 (Barnard and Youngren, 1992; Rodnick et al. 1992; Assimacopoulos-Jeannet et al. 1991). GLUT4 proteins are much more abundant in skeletal muscle and other insulin sensitive tissues than GLUT1 proteins (Barnard and Youngren, 1992; Assimacopoulos-Jeannet et al. 1991; Rodnick et al. 1992). It is believed that the primary role of GLUT1 is basal glucose transport, since it is primarily found on the plasma membrane during rest and is unresponsive to exercise and/or insulin (Barnard and Youngren, 1992; Assimacopoulos-Jeannet et al. 1991; Rodnick et al. 1992). At rest GLUT4 is primarily found intracellularly. Various stimuli have been identified that initiate the translocation of the GLUT4 proteins from their intracellular site to the plasma membrane. The most notable stimuli for this translocation are insulin (Barnard and Youngren, 1992; Assimacopoulos-Jeannet et al. 1991; Rodnick et al. 1992; Gulve et al. 1990; Marette et al. 1992; Ploug et al. 1993), and exercise (Douen et al. 1989; Douen et al. 1990; Megeney et al. 1993; Barnard and Youngren, 1992; Assimacopoulos-Jeannet et al. 1991; Rodnick et al. 1992; Ploug et al. 1993; Ren et al. 1994).

Insulin is the most important hormone in stimulatory glucose transport in skeletal muscle. It has been demonstrated that insulin can stimulate an increase in the GLUT4 protein content of the plasma membrane in rat muscle (Marette et al. 1992; Ploug et al. 1993). Marette and colleagues (1992) used a fractioning technique to isolate the plasma membrane, intracellular membranes and t-tubule membrane components of rat muscle cells. Prior to killing the rats, insulin was administered and the hindlimb rapidly removed. The differing membrane fractions were assayed for immunodetectable GLUT4. They observed that insulin increased GLUT4 protein content of the plasma membrane by ~86% and decreased the GLUT4 protein content of a unique intracellular organelle source by ~40%. These investigators also observed significant increases (~180%) in the GLUT4 content of the transverse tubule membranes. It was concluded that insulin increased the GLUT4 content of both the plasma membrane and transverse tubule membranes in rat skeletal muscle, and these translocated GLUT4 proteins appeared to be recruited from an intracellular location. These results indicate that the t-tubule is also important in the transport of glucose into the cell, since the t-tubules comprise a major component of total surface membrane of the muscle cell. The methods used by Marrette et al (1992) have limited application to the human model, because a large tissue sample (10-15g) is required. It is assumed that the response observed in the rat model is similar to that observed in the human model. Further work is required to determine the influence of insulin on GLUT4 translocation in human muscle.

Exercise also results in a translocation of GLUT4 from the interior of the cell to the plasma membrane (Douen et al, 1989; Douen et al. 1990; Barnard and Youngren, 1992; Assimacopoulos-Jeannet et al. 1991; Rodnick et al. 1992; Ploug et al. 1993; Ren et al. 1994). Most of the work in this area has utilized the rat model to investigate the effects of exercise (Douen et al, 1989; Douen et al. 1990; Ploug et al. 1993; Ren et al. 1994). These studies have also used different modes of exercise including swimming (Ren et al. 1994), treadmill exercise (Douen et al, 1989; Douen et al. 1990), and electrical stimulation (Ploug et al. 1993). Most of these studies demonstrated that exercise resulted in an increase in GLUT4 number on the sarcolemma (Douen et al, 1989; Douen et al. 1990; Ploug et al. 1993). In addition, some studies have also observed increased GLUT4 expression following exercise (Ren et al. 1994). Exercise and insulin result in similar changes in the translocation of GLUT4 to the plasma membrane; however, it appears that these changes involve unique mechanisms and possibly recruit GLUT4 from different intracellular sources (Douen et al, 1989; Douen et al. 1990; Ploug et al. 1993). Resistance exercise should also result in an increase in GLUT4 translocation, but it has yet to be assessed. Further work is required to investigate the effect of resistance exercise on GLUT4 translocation and to determine the exact mechanisms of insulin and exercise stimulated GLUT4 translocation.

There has been one study in humans that has correlated the rate of glycogen resynthesis following exercise and the GLUT4 protein content of the muscle (McCoy et al. 1996). They observed a significant positive correlation

between these two factors, indicating that GLUT4 also plays an important role in glycogen resynthesis in the human model.

Thus, exercise or the resulting depletion of glycogen as a result of the exercise appear to initiate the beginning steps in the glycogen resynthesis pathway. It should also be noted that the increase in GLUT4 with exercise likely increases the flux of glucose through the glycolytic pathway. At the completion of exercise, the glucose transport likely continues to replenish any glycogen depletion that may occur as a result of the exercise. The increase in glucose transport observed following exercise is limited by the availability of glucose in the systemic circulation. Thus, substrate availability could be rate limiting in the resynthesis of glycogen. Nutritional supplementation post-exercise is a simple solution to the limited availability of substrate.

1.3.2.3 Nutritional Supplementation.

It has been well documented that the rate of glycogen resynthesis is much greater following glycogen depleting exercise when carbohydrate (CHO) is consumed within the first hour following exercise (Blom et al. 1987; Zawadzki et al. 1992; Ivy et al. 1988b; Pascoe et al. 1993; Ivy, 1991; Friedman et al. 1991). The majority of work in this area has utilized endurance type exercise as the stimulus for glycogen depletion (Blom et al. 1987; Burke et al. 1995; Zawadzki et al. 1992; Reed et al. 1989; Ivy et al. 1988a; Ivy et al. 1988b; McCoy et al. 1996). There has been limited work in the area of glycogen resynthesis following high intensity cycling sprints (MacDougall et al. 1977b) and resistance exercise

(Pascoe et al. 1993). These intense forms of exercise result in post-exercise hyperglycemia, thus allowing early resynthesis of glycogen (MacDougall et al. 1977b). However, if hepatic glycogen depletion was significant (i.e. fasting), the transient hyperglycemia would rapidly decrease to a hypoglycemic or basal glucose state due to the rapid uptake of blood glucose by the muscle that was involved in the exercise (increased GLUT4 as previously discussed). Thus, supplementation would also be required following these brief higher intensity forms of exercise.

Ivy et al (1988a, 1988b) and Reed et al (1989) provided a series of studies that gave considerable insight into the influences of post-exercise CHO supplementation. In one study they investigated the influence of the timing of CHO supplementation on glycogen resynthesis (Ivy et al. 1988a). They had subjects perform a glycogen depleting ride on a cycle ergometer and then provided 2g CHO/kg body weight either immediately post-exercise or 2 hours post-exercise. They observed that the rate of glycogen resynthesis between 2 and 4 hours post-exercise (CHO @ +2 h) was ~45% lower than between 0 and 2 hours post-exercise (CHO @ immediate). In another paper, the same group investigated the effect of various amounts of CHO on the resynthesis of glycogen (Ivy et al. 1988b). They observed similar rates of glycogen resynthesis with a high (3g/kg) and a low (1.5g/kg) dose of CHO supplement given immediately and 2 hours post-exercise. As expected, glycogen resynthesis for the two CHO conditions were significantly greater than when no CHO was consumed post-exercise. A third study from this group investigated the influence of the form of

the CHO supplement on glycogen resynthesis (Reed et al. 1989). Using a similar depletion protocol as to the previous work (Ivy et al. 1988b), they investigated the effects of CHO (3g/kg) in a liquid or solid form, or by intravenous infusion on muscle glycogen resynthesis. Plasma glucose and insulin concentrations were significantly greater for the infusion condition, but there were no differences in glycogen resynthesis rates between any of the three conditions.

Blom et al (1987) investigated the effects of various post-exercise sugar diets on muscle glycogen resynthesis. They observed that the supplements composed of glucose and sucrose resulted in a greater post-exercise glycogen resynthesis rate than the fructose supplement. They suggested that the fructose was likely metabolized in the liver, and that glucose and sucrose are the carbohydrates of choice when attempting to maximize the rate of muscle glycogen resynthesis. From these studies it can be concluded that CHO supplementation in any form should occur rapidly following the completion of exercise and the dose does not need to exceed 1.5 g of CHO/kg of body weight.

Addition of other macronutrients to CHO supplements may benefit muscle glycogen resynthesis. Zawadzki et al (1992) observed significantly greater rates of muscle glycogen resynthesis with the consumption of a combined CHO and protein (PRO) complex (112g CHO, 47g PRO) compared to the CHO (112g) and PRO (47g) alone. It should be noted that the total energy consumption would have been greater (~42%) for the CHO-PRO condition; therefore, accounting for the greater rate of glycogen resynthesis. In addition, they observed significantly

greater insulin responses to the CHO-PRO supplement, but again this may be explained by the greater total energy intake in this condition. Burke et al (1995) had subjects in one condition consume a diet that was 100% CHO for the 24 hours following 2 hours of cycle ergometry and in another consume a diet that was isoenergetic to the CHO but also had fat and protein (1.6g/kg fat; 1.2 g/kg protein). They found no significant differences in the amount of glycogen resynthesis for the 24 hours following the exercise bout. It is unknown if the rates of glycogen resynthesis were different during the first 4 hours post-exercise, since biopsies were only collected immediately post-exercise and 24 hours post-exercise. In conclusion, following exercise early CHO feeding is essential for the rapid resynthesis of glycogen, and additional macronutrients may also be important; however, the timing of the feeding may be critical (Burke et al. 1995).

1.4 Muscle Protein Balance

Protein turnover is a dynamic process in every living organism. Certain physiological conditions such as growth and injury result in increased protein turnover. As previously discussed, protein balance is a function of both protein synthesis and degradation. There have been a number of different techniques used to assess these two components of protein balance. Some of the models used to assess protein balance have included whole body turnover, A-V amino acid balance, tracer incorporation, by-product excretion, and nitrogen balance. Many of these techniques have been used to assess the effects of: various

hormones, exercise, and nutritional modifications on protein balance and/or one of its components.

1.4.1 Methods for Assessing Protein Balance

Protein turnover can be assessed using isotopic tracers. It should be noted that the data obtained in this area of study are estimates, and there is no certainty of the accuracy of the measurements since no method of validation exists (Wolfe, 1992; Waterloo, 1995). In addition, there are a number of assumptions that must be made when using tracers. These assumptions will be discussed later.

A simplistic method of calculating protein turnover is that of stochastic analysis (Wolfe, 1992; Waterloo, 1995). In this type of analysis, the body is considered to be a single pool, whereas in more complex methods the body is divided into separate compartments and the exchange between these individual tissue pools is measured (Wolfe, 1992; Waterloo, 1995). The most common tracer has traditionally been L-¹³C-leucine (Wolfe, 1992; Tarnopolsky et al. 1991; Gibson et al. 1996; Phillips et al. 1993; El-Khoury et al. 1995). Isotope is given as a primed constant infusion to achieve an isotopic steady state (Wolfe, 1992). This is the time at which the rate of appearance (Ra) of the unlabelled amino acid (tracee) into the sampling pool is equal to the rate of disappearance (Rd) of the tracee out of the sampling pool. Ra in the single pool model represents endogenous production and exogenous intake, while Rd represents the sum of the rate of irreversible tissue uptake (i.e. protein synthesis) and loss by other

routes such as oxidation of the selected amino acid (Wolfe, 1992). The single pool model describes protein turnover by the equation: $Q=S+C=B+I$; where Q = flux rate; S = the rate of incorporation of label into protein (non-oxidative leucine disposal (NOLD)); C = the rate of catabolism of amino acid or oxidation of the chosen amino acid (O); B = the rate of protein breakdown or endogenous rate of appearance (R_a), and I = exogenous amino acid intake (i.e. food). As previously indicated, this model is used for basic calculations of whole body protein turnover, by using an appropriate tracer. If expired CO_2 is collected, estimates of both whole body protein synthesis and whole body protein degradation can be determined.

Another model of protein turnover is the limb amino acid balance (A-V balance) model (Wolfe, 1992). This model involves a greater compartmentalization of the limb segment. In this type of model there is a minimum of three compartments; the arterial compartment, the muscle or tissue compartment and the venous compartment (Wolfe, 1992). This model includes the quantification of protein turnover from both muscle and skin (Biolo et al. 1994). The A-V model has been used to study physiological perturbations including the assessment of exercise on protein balance across the leg (Biolo et al. 1995a), and the influence of insulin on protein turnover across the leg (Moller-Loswick et al. 1994; Biolo et al. 1995b) and arm (Moller-Loswick et al. 1994; Newman et al. 1994). There are some limitations to this type of model including; quantitation of blood flow, choice of specific model, and it under estimates the true number of pools. In addition these models are quite invasive. With respect

to the quantification of blood flow, there is large variance in its determination and it is assumed that the total blood flow is going from the arterial pool through the compartment being studied and then to the venous pool. The most commonly used methods for calculation of blood flow in these types of models is an infusion of indocyanine green dye (Biolo et al. 1995a, 1995b) and plethysmography (Newman et al. 1994). Differences across some limbs such as the forearm can be very small and difficult to accurately quantify (Wolfe, 1992). However, with the accuracy and sensitivity of modern instrumentation this is less of a limitation. As previously stated, the A-V model generally assumes a 3 compartment model, which underestimates the total number of limb compartments. The different tissues that are not accounted for in some limb models include both skin and adipose tissue (Wolfe, 1992). It should be noted that Biolo et al (1994) estimated the relative contribution of skin to an A-V model of turnover in the dog. They determined that skin protein turnover accounted for only ~10-15% of the total leg protein kinetics. A final limitation of the A-V models is one of invasiveness. These models typically involve the simultaneous cannulation of multiple veins and arteries in different limbs, thus increasing the risk of possible blood clot formation and tissue damage. Despite these limitations, the A-V technique is a useful model for obtaining valuable information.

A third method of measuring protein turnover, specifically protein synthesis, is the incorporation technique. Essentially this technique involves measuring the amount of tracer that is incorporated into the target tissue over a period of time. There have been a large number of studies that have utilized

this technique (Chesley et al. 1992; MacDougall et al. 1995; Biolo et al. 1995a, 1995b; Carraro et al. 1990; Welle et al. 1993; Yarasheski et al. 1993). There are two primary methods that are used in the administration of the tracer in this technique; the flooding dose and the primed constant infusion. The advantages and disadvantages of these techniques are discussed in detail in a number of reviews and chapters (Rennie et al. 1994; Garlick et al. 1994; Wolfe, 1992, Waterlow, 1995). Briefly, the flooding dose technique involves a bolus injection of the tracer and the measurement of the decay response kinetics by sampling the studied amino acid in the selected compartment. The primed constant infusion involves a constant infusion of a tracer preceded by a priming dose to establish an isotopic steady state. These techniques are both used in the determination of MPS; however, the primed constant infusion is the more common approach since it is unlikely to disturb endogenous turnover (Chesley et al. 1992; MacDougall et al. 1995; Biolo et al. 1995a, 1995b; Carraro et al. 1990; Welle et al. 1993; Yarasheski et al. 1993). These methods are also limited by a number of assumptions that are similar to the assumptions pertaining to the previously discussed techniques (Tracer assumptions discussed in a later section).

Another method to indirectly assess protein turnover (specifically, protein degradation or breakdown), is the determination of myofibrillar by-products in urine and plasma. 3-Methylhistidine (3-MH) is found almost exclusively in the actin and myosin of skeletal muscle (Sjölin et al. 1989a, 1989b; Young and Munro, 1978; Lukaski et al. 1981; Kasperek et al. 1992). It is formed by the

post-translational modifications of certain histine residues. 3-MH is a by-product of myofibrillar protein degradation, it cannot be reutilized for protein synthesis or metabolized oxidatively, and is almost exclusively excreted in urine (Sjölin et al. 1989a, 1989b; Young and Munro, 1978; Lukaski et al. 1981; Kasperek et al. 1992). Sjölin et al (1989a) has suggested a number of advantages of using 3-MH as an indicator of myofibrillar protein degradation: total 24 hour breakdown is integrated, steady state is not necessary, the method is noninvasive and only one measurement is required. Some concerns about the validity of 3-MH excretion as an indicator of myofibrillar protein degradation have been raised. These concerns are related primarily to the contribution of non-muscle sources (skin/splanchnic/dietary) to the 3-MH pool (Millward et al. 1980; Rennie et al. 1983). Sjölin et al (1989a, 1989b) have demonstrated that in humans with infection, the contribution to the 3-MH pool from splanchnic sources is relatively small even during periods of extensive catabolism. Others have used 3-MH as an indicator of myofibrillar protein degradation (Lukaski et al. 1981; Hickson et al. 1986; Pivarnik et al. 1989). For 3-MH to be a valid indicator of degradation certain dietary restrictions are required. The most important restrictions is the consumption of a flesh free diet for a minimum of 3 days prior to any evaluation (Lukaski et al. 1981). Lukaski et al (1981) had subjects consume a flesh free diet for a full week and evaluated 3-MH excretion for those seven days. They observed that 3-MH excretion decreased for each day until the third day of the flesh free diet. It was concluded that 3-MH could be used as a valid marker to study *in-vivo* total body MPD provided that the necessary dietary restrictions are

observed. Thus, it appears that 3-MH is a suitable marker of myofibrillar protein degradation within a group.

The final method of assessing protein turnover is the nitrogen balance technique. This method involves the exact determination of the total nitrogen intake of an individual and the total nitrogen excretion of the individual. If the individual is excreting more nitrogen than is being consumed the person is in a negative nitrogen balance or a catabolic state. And if more nitrogen is being consumed than being excreted, the individual is in a positive nitrogen balance. This method has been used extensively in the determination of protein requirements for athletes (Tarnopolsky et al. 1988, 1992; Phillips et al. 1993; Meredith et al. 1989). Tarnopolsky et al. conducted a number of studies to investigate whether endurance athletes and strength athletes have different protein requirements than normal sedentary individuals (Tarnopolsky et al. 1988, 1992). Both of these studies demonstrated that both strength athletes and endurance athletes required a greater protein intake to maintain a positive nitrogen balance compared to sedentary individuals. Specifically, they found that strength athletes require 1.41 g protein /kg body weight/ day to maintain a nitrogen balance of 0, a value which was significantly higher than the daily recommended allowance (Tarnopolsky et al. 1992) . Similarly, in their first paper (Tarnopolsky et al. 1988), they observed that 1.08 g protein /kg body weight/ day was required to maintain a nitrogen balance of 0 in elite body builders. The notable discrepancy in these two calculated values was attributed to training status and exercise differences between the 2 groups of subjects. Others have

also found that athletes require protein intakes that are above the recommended daily intake to maintain a positive nitrogen balance (Meredith et al. 1989; Phillips et al. 1993). The nitrogen balance technique has also been used in combination with tracers (single pool model) to give an indication of whole body protein synthesis and degradation in both strength (Tarnopolsky et al. 1992) and endurance athletes (Phillips et al. 1993).

In summary, all of these techniques can provide important information with respect to protein turnover in the body. All have distinct advantages and disadvantages; however, all methods only give an estimate of protein turnover in the body. In addition, if more of these methods were combined in individual studies much more information could be gained as to the comparability of these different methods and much more learned about protein turnover in the body.

1.4.2 Hormonal Influences

Certain hormones and local growth factors appear to have some control of protein turnover. Many different hormones and growth factors have been identified as having significant effects on protein synthesis and protein degradation. Some of the more studied hormones and growth factors with respect to protein turnover include; growth hormone (hGH), insulin like growth factor-I (IGF-I), testosterone, insulin, and other growth factors. It should be noted that these hormones do not regulate the entire process, but likely contribute to providing an environment that influences the magnitude of different aspects of protein turnover.

1.4.2.1 Human growth hormone

Many different stimuli can result in increased growth hormone secretion including; resistance exercise (previously discussed), other forms of exercise, amino acid ingestion, physical stress, psychological stress, and other hormones (Macintyre, 1987; Guyton, 1996). It has been observed that hGH administration to normal young individuals results in increased MPS, but does not affect whole body protein synthesis (Fryburg et al. 1992, 1993). Fryburg et al (1992) investigated the effects of an intra-arterial infusion of hGH and insulin on forearm protein turnover in healthy young individuals. When hGH was infused, a significant increase in both phenylalanine and leucine balance across the forearm occurred, compared to an infusion of saline. The increased leucine and phenylalanine balance appeared to be mediated through an increased rate of disappearance, suggesting an increase in protein synthesis across the forearm. In a similar study, Fryburg et al (1993) infused hGH and measured protein synthesis across the forearm and also calculated whole body protein synthesis using the reciprocal pool model. They observed similar increases in forearm tissue synthesis as in the previous study, but did not observe any changes in whole body protein synthesis. It was concluded that muscle, but not whole body protein turnover is acutely and specifically influenced by hGH. hGH administration in both clinical and normal populations has also been demonstrated to acutely increase nitrogen balance, suggesting that this hormone can increase lean tissue mass (Yarasheski, 1994). However, it appears that

these increases are not maintained with prolonged hGH treatment (Yarasheski, 1994). The combined effects of hGH treatment and resistance training have also been investigated in resistance trained individuals (Yarasheski et al. 1993). Yarasheski et al (1993) investigated the effects of 14 days of hGH treatment on whole body protein turnover as well as fractional muscle protein synthetic rate in the vastus lateralis. They observed that there was no change in whole body protein turnover or fractional synthetic rate with the hGH treatment. They concluded that resistance exercise may render the muscle unresponsive to the potential anabolic effects of hGH administration. Yarasheski et al (1992) also investigated the effects of resistance training with hGH treatment in previously sedentary individuals with a randomized double-blind placebo controlled trial. They observed that the hGH administration and exercise led to significant increases in whole body protein synthesis as compared to resistance training alone. However, no significant differences were observed with respect to MPS. It should also be noted that there were no differences in limb circumference or strength between the group that received the hGH and the group that did not. These data suggested that the increased whole body protein synthesis was contributing to lean tissue other than skeletal muscle (Yarasheski et al. 1992). To summarize, it appears that short term administration of this hormone leads to a more positive nitrogen balance; however, conflicting data exists within the tracer work. The A-V models have demonstrated increased MPS, while the incorporation techniques have not. Clearly, from the whole body estimates of protein turnover and the nitrogen balance data, there are definite changes in

protein turnover occurring. It is necessary to isolate the specific sites of this turnover to give a better understanding of interaction of hGH and protein turnover. There appears to be little benefit in the combination of hGH treatment and resistance training. Further examination of the interaction of hGH, exercise, and protein turnover are required.

1.4.6.2 Insulin like growth factor-I

The IGF-I molecule is similar in structure to the proinsulin molecule. Circulating IGF-I is believed to be produced primarily in the liver, but it has been demonstrated to be synthesized in skeletal muscle cells (Florini et al. 1991). One of the possible mechanisms of hGH action is through increased IGF-I concentration. It has been observed that both natural and pharmacologically elevated hGH concentrations lead to increased circulating IGF-I concentrations. The entire IGF family has been demonstrated to be important stimulators of many anabolic processes in muscle (Florini, 1987). The effects of IGF-I on protein turnover are similar to those observed with hGH. Using the reciprocal pool model Russell-Jones et al. (1994) determined the effects of an infusion of IGF-I on healthy young subjects at rest. They observed that IGF-I resulted in increased whole body protein synthesis (~20% increase) with no change in whole body protein degradation. Mauras and Beaufre (1995) observed a ~14% increase in whole body protein synthesis with normal healthy young subjects during an IGF-I infusion. The results of the study by Mauras and Beaufre (1995) are quite similar to those observed by Russell-Jones et al.

(1994). The slight difference between the two studies maybe accounted for by the greater infusion rate of IGF-I used in the study by Russell-Jones et al (1994). Fryburg (1994) observed similar increases in MPS across the forearm in normal subjects with an infusion of IGF-I as was observed following hGH infusion (Fryburg et al. 1993). They also investigated the effects of various doses of IGF-I on protein turnover. They observed that all 3 doses (1.8, 6.0, 10.0 $\mu\text{g/kg/h}$) resulted in a significantly more positive phenylalanine balance across the forearm. In addition, a significant decrease in the Ra of the phenylalanine was observed (decrease in whole body protein degradation). It was concluded that IGF-I exhibited growth hormone like effects at all test doses. This supported the concept that the anabolic actions of growth hormone are mediated through IGF-I. However, to this author's knowledge there has not been an investigation into the effects of an IGF-I infusion on the fractional synthetic rate of muscle protein (FSR). If the FSR response to IGF-I is similar to the FSR response observed with hGH (Yarasheski et al. 1992) it is unlikely that the increased forearm balance observed by Fryburg (1994) is related to increased contractile protein. Clearly, further work is required to determine the specific effects of IGF-I on protein turnover, and to isolate the specific sites of these changes (contractile protein vs. skin vs. connective tissue etc.).

1.4.2.3 Testosterone

Testosterone is a steroid hormone that is produced primarily in the testes, in males with a small amount being produced in the adrenals (Guyton, 1996).

Testosterone increases are observed in males at puberty, peak at ~20 years of age and then gradually decline with increasing age (Guyton, 1996). Exogenous testosterone administration has been demonstrated to increase muscle size, and strength (Bhasin et al. 1996), lean body mass (Forbes et al. 1992) and the muscle fractional synthetic rate (Griggs et al. 1989).

In a recent study, Bhasin et al (1996) administered testosterone to a sedentary group and a resistance training group. They also had 2 control groups that did not receive testosterone, a sedentary group that performed no resistance training and another resistance training group. They observed that the testosterone with the resistance training resulted in significant increases in body weight (~8%), fat free mass (~9%), strength (~30%), and muscle cross-sectional area (~14%). The testosterone alone resulted in significant increases in body weight (~4%), muscle cross-sectional area (~9%), and strength in one of the two measured exercises (leg squats) (~12.6%). The increases observed for the testosterone only condition were very similar to those observed for the exercise-placebo group. This suggested that testosterone influenced protein turnover, by increasing fat free mass, and possibly increasing contractile protein content. It is unclear as to the direct influence of this dose of testosterone on protein turnover (increase in MPS/whole body protein synthesis, a decrease in protein degradation, or a combination of the two).

Similar increases in fat free mass have been observed by others (Forbes et al. 1992). Forbes et al (1992) observed a 12% increase in fat free mass and 27% decrease in body fat relative to baseline values with 12 weekly injections of

testosterone. This study did not include control groups or a placebo condition, thus these results may be skewed. In addition, no strict controls were in place to control for exercise and as a result, it is unclear if these results are solely due to the intervention. However, with the dramatic changes in body composition it is difficult to dispute that the testosterone had an impact.

The same group investigated the effects of a similar 12 week period of testosterone administration on muscle mass, whole body protein turnover, and MPS in non-exercising males (Griggs et al. 1989). They observed a significant 27% increase in MPS, but no changes in whole body protein synthesis. In addition, there were no changes in leucine flux (indicator of whole body protein degradation); however, they did observe a significant increase in muscle mass and lean body mass as estimated by creatinine excretion and total body potassium (^{40}K counting). No direct or indirect measures of MPD were made, thus it is difficult to fully interpret the effect of the testosterone. Clearly, if MPS had been elevated 27% for the duration of the 12 week study there should have been much greater increases in body weight and lean muscle mass. There must have been a simultaneous increase in protein degradation as well as MPS.

It is clear that testosterone has a significant effect on protein turnover and lean body mass. As well, with recent indirect evidence it appears that testosterone also has an effect on contractile protein content as demonstrated by increased strength. Further work is required to determine testosterone's specific mechanisms and interactions upon protein turnover.

1.4.2.4 Insulin

Insulin is a well studied hormone with respect to protein turnover in humans. It has been demonstrated to influence protein turnover in several different ways. It can alter MPS (Biolo et al. 1995b; Newman et al. 1994) and degradation (Denne et al. 1991; Fukagawa et al. 1985; Heslin et al. 1992), and whole body protein degradation (Castellino et al. 1987; Flakoll et al. 1989; Tessari et al. 1986, 1987; Shangraw et al. 1988; Pacy et al. 1989; Denne et al. 1991; Fukagawa et al. 1986; Heslin et al. 1992; Moller-Loswick et al. 1994).

The most consistent effect of insulin on protein turnover is a suppression of whole body protein degradation (Castellino et al. 1987; Flakoll et al. 1989; Tessari et al. 1986, 1987; Shangraw et al. 1988; Pacy et al. 1989; Denne et al. 1991; Fukagawa et al. 1986; Heslin et al. 1992; Moller-Loswick et al. 1994). The majority of studies in this area have found decreased protein degradation using either the reciprocal pool model of protein turnover and/or an A-V balance model across a limb. For example, Heslin et al (1992) administered an insulin infusion that resulted in a hyperinsulinemic condition. They observed a 17% reduction in whole body leucine Ra (indicator of protein degradation) and a 37% reduction in leucine Ra across the forearm. They did not observe any changes in whole body protein synthesis or protein synthesis across the forearm. Denne et al (1991) observed similar results using a whole body model in addition to an A-V balance model across the leg. Denne et al (1991) observed a similar decrease in protein degradation across the leg as Heslin et al (1992) observed across the forearm. This effect of suppression of protein degradation appears to be related to the

insulin concentration. Tessari et al (1986) observed a dose response effect for insulin on whole body protein breakdown. This dose response effect has also been observed by others (Fukagawa et al. 1985). Clearly, insulin results in decreased protein degradation.

Some groups have attempted to investigate the specific source of the decreased protein degradation in skeletal muscle. The most common method for assessing the effects of insulin on muscle protein turnover is the A-V balance model. In this model it is assumed that the major contributor to protein degradation and synthesis across the limb is skeletal muscle. Unfortunately, skin and fat also contribute to these calculated differences. Both Heslin et al (1992) and Denne et al (1991) reported significant decreases in the Ra across the limbs, suggesting a decrease in skeletal MPD. Fukagawa et al (1985) observed significant decreases in circulating 3-MH during an insulin infusion, suggesting a decrease in MPD. Further work is required to better understand the exact role that insulin plays in these decreases in MPD. It has been demonstrated that insulin can inhibit lysosomal protein degradation, and can also antagonize the catabolic actions of glucocorticoids (the reader is referred to the following reviews) (Biolo and Wolfe, 1993; Goldberg, 1979; Nair and Schwenk, 1994; Kimball and Jefferson, 1988; Goldberg et al. 1980). These two factors may be mechanisms by which protein degradation is decreased, but further work is required.

Others have demonstrated that insulin also has an anabolic effect by increasing protein synthesis (Biolo et al. 1995b; Newman et al. 1994) and that

this anabolic effect is affected by the availability of amino acids in the systemic circulation (Biolo et al. 1995b). There is considerable support for this concept from studies involving insulin infusions with elevated amino acid levels (Newman et al. 1994; Bennet et al. 1990; Castellino et al. 1987). Newman et al (1994), Bennet et al (1990), and Castellino et al (1987) all observed significantly increased protein synthesis and decreased protein degradation across the forearm and leg respectively with the infusion of an insulin and amino acid combination. As previously discussed, many studies have observed this decreased protein degradation (Flakoll et al. 1989; Tessari et al. 1986, 1987; Shangraw et al. 1988; Pacy et al. 1989; Denne et al. 1991; Fukagawa et al. 1986; Heslin et al. 1992; Moller-Loswick et al. 1994), but very few have reported increased protein synthesis without a simultaneous amino acid infusion (Newman et al. 1994; Bennet et al. 1990; Castellino et al. 1987). Increased MPS is likely a function of the increase in amino acid transport that results from insulin stimulation (Biolo et al. 1995b) and an increase in peripheral blood flow (Baron, 1994; Shoemaker and Bonen, 1995). Both of these mechanisms are likely related to the increase in MPS that was observed by Biolo et al (1995b). Further work is required to determine the specific mechanisms by which MPS is stimulated by insulin infusion.

It should be noted that insulin is not required for muscle hypertrophy to occur as demonstrated in diabetic rats (Goldberg, 1975). Muscle hypertrophy occurred to the same extent in diabetic and normal rats. Thus, insulin may not be essential for hypertrophy to occur in rats, but it appears from the influence of

insulin on both MPS and MPD in humans that it would likely accentuate the hypertrophy process.

Insulin has several different effects on protein turnover. Most of the work in the area of insulin and protein turnover has utilized insulin infusions. It is unclear what the possible role is of diet induced increases in insulin concentration on protein turnover post-exercise.

1.4.2.5 Other growth factors

A number of growth factors have been identified in the last few years that may have a role in the regulation of protein turnover. These growth factors include Fibroblast growth factor (FGF) and Tumour necrosis factor (TNF). Both of these growth factors have been implicated in influencing different components of protein turnover.

Fibroblast growth factor has been identified in the extracellular matrix of rat skeletal muscle and is believed to influence the activation of satellite cells and possibly the replication of these cells, which ultimately results in muscle hypertrophy (Yamada et al. 1989). It is possible then that this growth factor may provide a paracrine up-regulation of MPS that is required for the hypertrophy of skeletal muscle fibers.

Tumour necrosis factor (TNF) has also been implicated in the regulation of protein turnover. However, it appears it is primarily involved in the regulation of protein turnover in the gut (Evans et al. 1993). The influence of TNF on protein metabolism in humans has yet to be evaluated.

With the improvement in the ability to identify previously undetectable compounds in the body, it is likely that more growth factors will be identified. With the exception of IGF-1 there has been very little work into the role of the various growth factors on protein turnover. Again, much work is required to determine the exact influences of these growth factors on the different factors involved in protein turnover; in addition, the interactions of these growth factors and various other hormones and the overall effect on protein turnover still need to be investigated.

In summary the control of protein turnover is very complex and involves a number of different factors. Some of the more important factors include different endocrine hormones, growth factors, and exercise (see below). Much is known as to how these hormones individually affect protein turnover, but little is known as to the complex interactions that are likely occurring.

1.4.3 Influence of Exercise

Physical activity has a dramatic effect on protein turnover. Endurance exercise (Devlin et al. 1990) has been demonstrated to increase whole body protein synthesis following the completion of exercise, however a similar increase was not found for up to 2 hours post-resistance exercise in fed humans (Tarnopolsky et al. 1992). It has also been noted that both forms of exercise result in a chronic elevation of whole body protein synthesis (Tarnopolsky et al. 1991; Lamont et al. 1990). Exercise has also been associated with elevated protein degradation (Pivarnik et al. 1989; Booth and Watson, 1985; Booth et al.

1982). As previously discussed, resistance and endurance exercise also result in increased MPS (Chesley et al. 1992; Biolo et al. 1995a; Carraro et al. 1990). Specifically, resistance exercise results in increases in both MPS and MPD, but the increased synthesis is greater than the increased degradation (Biolo et al. 1995a). The specific causes for these changes in protein turnover are likely quite different for the different forms of exercise. With respect to resistance exercise, these changes likely occur in the contractile proteins since; (1) muscle hypertrophy occurs with this form of exercise, (2) there is increased 3-MH excretion, and (3) this type of exercise results in disruption of the myofillaments. For endurance exercise, the increased protein turnover is likely due to different factors as compared to resistance exercise. The changes with endurance exercise likely result from; (1) increased amino acid oxidation, (2) increased mitochondrial volume, and (3) increased fiber capillary density and myoglobin content.

1.4.4 Nutritional Supplementation

Dietary supplementation plays an important role in both resistance exercise and endurance exercise. Both forms of exercise result in significant decreases in muscle glycogen. Supplementation within the first 4 hours of completion of exercise results in the most rapid rate of resynthesis of muscle glycogen (Ivy et al. 1988a). Thus, consumption of a carbohydrate supplement post-exercise is important in maximizing glycogen resynthesis.

Nutritional supplementation may also have an important role with respect to protein turnover. For example, consumption of a carbohydrate supplement following a resistance exercise bout would increase circulating levels of insulin (Ivy et al. 1988a, 1988b; Zawadzki et al. 1992). Increased insulin could potentially decrease the amount of protein degradation that occurs with this form of exercise. In addition, it may increase protein synthesis to a greater extent than that induced by exercise alone. Also, if protein were included with the carbohydrate, the substrate self-limiting effect that occurs with insulin and protein synthesis may be avoided.

Finally, it has been observed that consumption of nutritional supplements following resistance exercise can provide a hormonal environment that may be favorable for muscle growth (Chandler et al. 1994). Chandler et al (1994) observed significantly increased hGH 6h following consumption of a carbohydrate and protein supplement after the completion of a resistance training bout as compared to the placebo condition. In addition, the authors also suggested a possible increase in the clearance of testosterone with the nutritional supplement conditions. No measures of protein turnover were made, so it is unclear if these changes in the hormonal environment had any direct effects on protein turnover. However, the nutritional supplementation may have provided a suitable hormonal environment to promote protein synthesis.

Clearly, further work is required as to the influence of nutritional supplementation on protein turnover post-exercise. It appears that

supplementation may lead to a more positive protein balance through changes in the hormonal environment.

1.5 Stable Isotope Methodology

Stable isotopes have been used to study protein turnover for the last 50 years (Wolfe, 1992). A stable isotope is an isotope of a given element having the same atomic number (protons), but differing in atomic mass (different number of neutrons). Stable isotopes have been used with greater frequency with the technological advances in gas chromatography/mass spectrometry (GC/MS) and the availability of ^{18}O , ^2H , ^{13}C , and ^{15}N . The most commonly used stable isotopes to label amino acids are ^{13}C and ^{15}N and their natural abundance's are 1.11% and 0.37% respectively (Wolfe, 1992). Infusion of an isotope tracer into the venous circulation will increase both the plasma pool and body pool enrichment of the chosen label above the natural background enrichment. The increases in enrichment are usually expressed in atom percent excess (APE). For example, a primed constant infusion (~90 min) of L- ^{13}C Leucine results in APE values of ~4 APE (Tarnopolsky et al. 1991). This indicates that 4 out of every 100 amino acid molecules studied is labeled with the tracer above the natural abundance. Several kinetic terms have already been discussed; rate of appearance of a labeled amino acid in the plasma pool (Ra) and the rate of disappearance of the labeled amino acid out of the plasma pool (Rd) into tissues for either protein synthesis or oxidation. Both of these factors

are expressed as mass of substrate per kg body mass per unit of time (i.e. $\mu\text{mol/kg/h}$).

The use of stable isotopes to measure whole body protein turnover and MPS is based on a number of assumptions. These assumptions include: (1) the labeled tracer is biologically indistinguishable from the naturally occurring tracee, (2) the administration of the tracer dose will not disturb the kinetics of the system, (3) the tracer is rapidly and evenly distributed through all body pools, and (4) during a continuous infusion protocol measurements of kinetics are only made after an isotopic steady-state has been achieved (Wolfe, 1992). These assumptions have been tested and evidence to support them has been presented in a number of other papers (Wolfe, 1992; Waterlow et al. 1978).

The most common method for the administration of a labeled (^{13}C) amino acid is the primed-constant infusion technique. Briefly, this technique involves the administration of a priming dose (1 mg/kg) followed by a constant infusion (1 mg/kg/h) of a labeled amino acid. The prime serves to rapidly increase the enrichment in the different body pools, thus achieving an isotopic steady state more rapidly. The isotopic steady state is achieved when the $R_a=R_d$ (Wolfe, 1992). With this model it is essential that the measurement of protein turnover is only made when $R_a=R_d$, otherwise errors in protein synthesis and degradation calculations could occur. In addition, infusions of greater than 24 h can result in recycling and an underestimation of flux (Wolfe, 1992). Isotope recycling occurs when the labeled amino acid is incorporated into protein and then later released during the infusion. This would interfere with the calculations of R_a and R_d , thus

leading to underestimation of protein synthesis. However, infusion duration in most studies is not very long (~3-10 h) compared to the turnover rates of myofibrillar protein (Waterlow et al. 1978).

There are a number of factors that determine the choice of labeled amino acid and the position of the chosen label. These factors include: (1) the amino acid concentration gradient between the plasma and the muscle, (2) the intracellular free amino acid pool size, (3) the concentration of the amino acid bound in protein, and (4) the cellular metabolism of the amino acid (Smith and Rennie, 1990). Tyrosine and phenylalanine are not metabolized in skeletal muscle, and are only incorporated and released from protein. Leucine is also one of the few amino acids that is primarily oxidized in muscle (Odessey et al. 1972). Thus, when infusing L-¹³C leucine the recovery of expired breath will allow the determination of the ¹³C content of expired CO₂ allowing the determination of leucine oxidation. Leucine is an essential amino acid; therefore, the only sources of this amino acid are diet and protein degradation. It is beneficial to use leucine for protein turnover and protein synthesis studies since the intracellular concentration of leucine only accounts for ~0.41% of the total free intracellular amino acid pool (Smith and Rennie, 1990). However, muscle leucine accounts for approximately 8-10% of the total bound amino acid pool (Smith and Rennie, 1990). Therefore, it is easier to label the small amino acid pool for leucine and observe changes in the muscle due to the slower turnover rate of muscle protein (Waterlow et al. 1978).

The reciprocal pool model of leucine metabolism is an example of stochastic analysis. Leucine is rapidly transaminated to α -ketoisocaproic acid (α -KIC) once in the muscle. This reaction is readily reversible and the α -KIC can rapidly be reaminated back to leucine. The α -KIC can also be decarboxylated at the α -carbon. Leucyl-tRNA is the true precursor to synthesis of muscle protein, and it is desirable to express measures of synthesis relative to the precursor. However, due to the large sample size required (>1 g) it is difficult to isolate leucyl-tRNA and other aminoacylated t-RNA in human studies. Due to these limitations α -KIC has been used as an estimate of leucyl-tRNA labeling. Plasma α -KIC has been demonstrated to closely represent intracellular leucine enrichment by a number of different groups (Smith and Rennie, 1990; Watt et al. 1991; Vazquez et al. 1986). The validity of this assumption has been demonstrated in studies of patients undergoing elective back surgery (Watt et al. 1991). There are a number of reasons for the use of α -KIC, they include: (1) α -KIC can readily pass across the muscle membrane such that an equilibrium is achieved between the intracellular pool and the extracellular pool, (2) α -KIC can only be formed from leucine intracellularly and therefore should reflect intracellular leucine metabolism, and (3) α -KIC can be isolated and measured using gas chromatography / mass spectrometry (Ford et al. 1985).

Interpretation of the data obtained from the various stable isotope techniques to measure protein turnover must be done with caution, especially when comparing data from different techniques. For example, increased MPS

(FSR) observed using the incorporation technique may not always translate into changes in whole body protein synthesis. MPS only accounts for approximately 25% of whole body protein synthesis (Nair et al. 1988). Tarnopolsky et al (1991) observed no significant increases in whole body MPS following a bout of resistance training, but Chesley et al (1992) observed significant increases in MPS following an isolated bout of resistance training. Increases in MPS then may not be reflected in measurements of whole body protein synthesis.

1.6 Summary

From the information presented, it is clear that resistance exercise results in several different adaptations including muscle fiber hypertrophy, changes in protein turnover, muscle ultrastructural changes, muscle fiber metabolic characteristics and endocrine hormone concentrations. In addition, it is also clear that muscle glycogen is an important substrate for resistance exercise and nutritional supplementation is important in maximizing the resynthesis of this substrate following exercise. Various factors that influence protein turnover were also discussed, including insulin which suppresses protein degradation and possibly increase MPS. And finally, nutritional supplements and there influence on plasma insulin were discussed in relation to the possible changes in protein turnover that could occur.

1.7 Purpose / Hypothesis

The purpose of the two studies in this thesis are: 1) To investigate the influence of various nutritional supplements on protein metabolism following an isolated bout of heavy resistance exercise; 2) To determine the influence of various nutritional supplements on the resynthesis of muscle glycogen following a bout of resistance exercise; 3) To investigate the hormonal response to various nutritional supplements following heavy resistance exercise.

We hypothesize that: 1) Glycogen resynthesis will be greater with CHO and the CHO/PRO/FAT supplement given post-exercise compared to Placebo; 2) There will be a more favourable hormonal milieu for protein synthesis with the consumption of the supplements (both CHO and CHO/PRO/FAT) (increased testosterone/Insulin, decreased cortisol); 3) Both supplements will result in a decrease in indicators of protein degradation, with the CHO/PRO/FAT leading to a greater suppression of these indicators (urinary urea and 3-MH excretion, leucine flux); and 4) Nutritional supplementation should also result in an increase in protein synthesis (NOLD, FSR), with a greater increase with the CHO/PRO/FAT supplement.

CHAPTER II

THE EFFECT OF ORAL GLUCOSE SUPPLEMENTATION ON PROTEIN METABOLISM FOLLOWING RESISTANCE TRAINING

2.1 INTRODUCTION

Muscle growth in adult humans results from muscle fibre hypertrophy (MacDougall. 1992). Such hypertrophy is the result of an increased net muscle protein balance [i.e. fractional muscle protein synthetic rate (FSR) - muscle protein degradation (MPD)]. Both FSR (Biolo et al. 1995a; Chesley et al. 1992; Yarasheski et al. 1993) and MPD (Biolo et al. 1995a) can be stimulated by heavy resistance exercise in humans. It is also known that amino acid transport is increased after resistance exercise (Biolo et al. 1995a). Further understanding of the factors influencing net protein balance may allow the ability to maximize FSR and minimize MPD, thus maximizing the rate and amount of muscle hypertrophy.

Research in the area of resistance training and its effects on FSR and MPD is limited. Recent work from independent laboratories has shown that FSR was elevated after a bout resistance training in humans (Chesley et al. 1992; Biolo et al. 1995a). Net protein balance, however, although more positive, was still negative following resistance exercise in the fasted state (Biolo et al. 1995a). In addition, it has been demonstrated that in the fed state, strength trained

individuals have a net positive whole body protein balance, elevated whole body protein synthesis (WBPS) and amino acid flux compared to sedentary individuals (Tarnopolsky et al. 1992). Although there has been considerable work into the effects of insulin on FSR and MPD, the results are inconclusive. While some animal-model studies have demonstrated that insulin has no effect on FSR (Baillie and Garlick, 1991a; Baillie and Garlick, 1991b; Watt et al. 1992), they did not examine MPD, and thus the effect on net protein balance is unknown. Conversely, there have been several studies in animals that have observed an insulin stimulated increase in FSR (Garlick et al. 1983; McNulty et al. 1993).

In humans, two studies have demonstrated that insulin can both increase FSR and decrease MPD (Newman et al. 1994; Biolo et al. 1995b). Other research has indicated that insulin may have no effect on FSR, but rather an inhibitory effect on MPD (Pacy et al. 1989; Goldberg, 1979). Therefore insulin can be expected to decrease MPD, and possibly to increase FSR (Moller-Loswick et al. 1994; McNurlan et al. 1994).

The literature to date has not addressed the potential interaction of resistance exercise and insulin/nutritional state on leucine turnover/protein balance. Since insulin may cause a decrease in MPD, and a possible increase in FSR, and resistance exercise is known to increase FSR, it is possible that insulin could decrease MPD and increase FSR synergistically following a bout of resistance exercise. If the latter occurs in combination with the increase in FSR due to the exercise (Chesley et al. 1992; Biolo et al. 1995a), the net protein balance would be even more positive, thus resulting in a greater net accretion of

myofibrillar protein. The consumption of a carbohydrate supplement is a simple method of increasing insulin concentrations following exercise (Ivy et al. 1988a, Ivy et al. 1988b, Zawadzki et al. 1992).

We hypothesized that a carbohydrate supplement consumed immediately after resistance exercise would result in: (a) decreased urinary 3-MH excretion (a marker of MPD), (b) increase muscle ^{13}C -leucine incorporation (increased FSR), and (c) decreased urea N excretion (net positive protein balance).

2.2 METHODOLOGY

2.2.1 Subjects

Eight healthy young (20-25yrs) males who had been participating in a resistance training program for at least a year prior to the investigation (~2x per week) were recruited as subjects (Table 1). The experimental procedures, possible risks and benefits were explained to each volunteer before written consent was obtained. The study was approved by the McMaster University Human Ethics committee.

2.2.2 Design

Each subject participated in a placebo-controlled randomized double blind trial with a carbohydrate supplement (CHO) and a placebo (NutraSweet®) (PL) trial. They performed unilateral knee extensor exercise such that the muscles of the non-exercised limb served as a control (exercise (EX) and rest (CON) leg). 1 week prior to the two trials, the subjects' single maximum repetition (1RM) strength was determined for knee extension and leg press and their body density

was determined by hydrostatic weighing. In addition, subjects completed 4 day diet records which were analyzed using a nutritional analysis software package (Nutritionist III, First Data Bank, San Bruno, CA.). From this, a dietary checklist was created for each subject. The diets were isoenergetic, isonitrogenous, flesh-free and were controlled for the 3d prior to each trial (dietary checklist). On the day of the trial, all food was supplied in a pre-packaged form and consumed in 3 distributed meals. For the CHO trial, the subjects received the drink (glucose 1g/kg) immediately and 1 h after the exercise bout and consumed placebo with breakfast. In the control condition, the subjects consumed a drink (2 g/kg glucose) with their breakfast and the placebo post-EX at the same times as CHO. Daily energy and nitrogen consumption was constant between the two trials.

The subjects refrained from any resistance exercise with the legs for 3d prior to each trial and any form of exercise for the 2d prior to each trial. They consumed meals at 0700h/1100h/1400h and then reported to the laboratory at 1700h (t=-120 min.). A 20-gauge catheter was inserted into an antecubital vein for tracer infusion (as described by Tarnopolsky (1991)). A second catheter was placed into a contralateral dorsal hand vein for "arterialized" blood sampling (hot box at 65 ± 5 °C) (Nauck et al. 1992). A primed (0.972 ± 0.03 mg/kg) constant (0.977 ± 0.02 mg/kg/hr) infusion of L-[1-¹³C] leucine (99% AP, Masstrace, Somerville, MA.) was used to calculate fractional mixed protein synthetic rate (FSR) as previously described (Chesley et al. 1992; Tarnopolsky et al. 1992; Yarasheski, 1992). The infusate was passed through an anti-bacterial filter (0.2

µm Acrodisc) immediately prior to infusion. At ~1830h (t=-30 min.) subjects performed the prescribed weight training bout (4 sets each of unilateral leg press and knee extension, 85% of 1RM, 8-10 reps). The 90 min between the start of the infusion and the onset of exercise was to ensure that an isotopic plateau had been achieved (Tarnopolsky et al. 1992). Immediately following this (~1900h) (t=0) a blood sample drawn, a muscle biopsy sample taken from each leg (post-EX₀, post-CON₀) using the Bergström needle biopsy technique (Bergström, 1962) and the glucose (1g/kg) or Nutrasweet[®] drink was consumed. Blood samples (4 mL) were collected every 15 minutes for the next hour and immediately centrifuged and stored at -50 °C. At ~2030h (t=+1h) a second CHO (1g/kg) or PL drink was administered. Blood samples were again collected every 15 minutes for the next 1.5 hours and again at ~0400h, ~0430h and ~0500h the next morning. Final biopsy specimens were taken at ~0500h (post-EX₁₀, post-CON₁₀) (~10h incorporation time). The subjects also collected all urine excreted during the 24 h period (~12 pre-exercise, ~12 post-exercise) for subsequent creatinine, 3-methylhistidine (3-MH) and urea nitrogen determination. Sample collection began in the morning (0600h) of the trial (first urination not collected), and continued through to the following morning (0600h). The subjects did not leave the laboratory until the final urine sample was collected.

2.2.3 Analysis

Muscle biopsy samples were quenched in liquid nitrogen immediately following collection and stored at -70 °C. The [¹³C]-Leucine enrichment in mixed muscle protein was determined using gas-chromatography / combustion /

isotope ratio mass spectroscopy (GC/C/IRMS) as described by Yarasheski (1992). Blood was analyzed for plasma glucose (Sigma Diagnostics kit# 315, St. Louis MO.) (coefficient of variation (CV) =3.9%) and insulin concentration (radioimmunoassay) (Diagnostic Products Corporation, Los Angeles, CA.) (CV=2.9%). Plasma ^{13}C α -ketoisocaproic acid prepared as the trimethyl-silyl-quinoxalinol derivative, its enrichment was determined using electron impact ionization capillary gas chromatography / mass spectrometry (GC/MS), and monitoring mass/charge ratio with selected ion monitoring (SIM) of 233/232. Urinary urea nitrogen and creatinine excretion were determined from aliquots of the 24 h urine collections using colorimetric methods as described by Tarnopolsky (1992) (kit 640 and kit 555, Sigma Diagnostics, St. Louis, MO) (CV=4.7% and CV<1%, respectively). 3-MH concentration was determined using an automated amino acid analyzer and was normalized to the 24 h urine creatinine excretion (Beckman Instruments, Palo Alto, CA).

2.2.4 Calculations

Muscle fractional synthetic rate was calculated according to the equation:

$$\text{FSR} = (\text{Le}_m \times 100) / (\text{K}_{\text{ep}} \times t)$$

where FSR is the fractional muscle protein synthetic rate (%/h), t is the incorporation time (in h) between muscle samples taken from the same leg, Le_m is the increment in ^{13}C abundance in leucine from mixed muscle protein obtained between the muscle samples removed from each leg, and K_{ep} is the mean

plasma ^{13}C - α -KIC enrichment for $t = 2.5$, ~ 10 , and ~ 10.5 h blood samples (corrected for enrichment from the $t = 0$ h blood sample).

Leucine flux (Q) was calculated using the reciprocal pool model (Horber et al. 1989), at isotopic plateau:

$$Q = I[(E_i/E_p)-1]$$

where, $I = L\text{-}[^{13}\text{C}]\text{leucine infusion rate } (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1})$, E_i = enrichment of the infused leucine, and E_p = enrichment of the plasma α -KIC (APE) and the term “-1” corrects for the contribution of the infused isotope to Q. The rate of whole body protein degradation (WBPD) was estimated from the leucine flux based on the equation:

$$Q = B + I$$

where Q is the total leucine flux, B is the rate of appearance of endogenous leucine, and I is the dietary leucine intake. Since the subjects had no dietary intake of leucine during the infusion, $I = 0$, thus $B = Q$.

2.2.5 Statistical Analysis

Muscle and blood data were analyzed using repeated measures analysis of variance (time $\times$ treatment) (GB-STAT V5.30, Dynamic Microsystems Inc.). When a significant interaction occurred, Tukey's post-hoc analysis was used to locate the pairwise differences. Area under the curve (insulin, glucose) was calculated with a custom made software package. Urine and area under the curve data were analyzed using paired T-tests. $P < 0.05$ was selected as being indicative of statistical significance. Values are expressed as means $\pm$ SE.

2.3 RESULTS

There were no differences in plasma insulin concentrations at the beginning ($t=-1.5$ h) and end of the infusion ($t\sim 10$ h) ($CV=2.9\%$). Plasma insulin concentrations were significantly higher for the CHO compared to the PL condition at the +0.5 h, +0.75 h, +1.25 h, +1.5 h, +1.75 h and +2.0 h time points (Figure 1) ($p<0.01$). The area under the insulin curve over the first 2.5h was $\sim 4\times$ greater for the CHO condition compared to PL ($65.2 \pm 12.1 \mu\text{IU}\cdot\text{h/L}$ for CHO, $15.2 \pm 2.1 \mu\text{IU}\cdot\text{h/L}$ for PL) (Figure 2) ($p<0.01$).

Plasma glucose concentrations were not significantly different between CHO and PL prior to the beginning of ($t=-1.5$ h) and at the end of the isotope infusion (Figure 3). At the completion of exercise, plasma glucose levels were greater than baseline values for both the CHO and PL conditions (6.26 ± 0.40 mmol/L to 6.73 ± 0.54 mmol/L for CHO, 5.70 ± 0.55 mmol/L to 6.50 ± 0.25 mmol/L for PL) ($p<0.05$). Plasma glucose concentration was also significantly higher ($p<0.01$) at +0.5 h and +0.75 h in the CHO condition compared to PL. The area under the curve for glucose in the first 2.5h was significantly greater for CHO compared to PL ($p<0.01$) (CHO = 7.21 ± 0.43 mmol·h/L, PL = 5.88 ± 0.16 mmol·h/L) (Figure 4).

Twenty four h urinary creatinine excretion was not significantly different between the two conditions ($n=7$) (1.76 ± 0.15 g/24h for CHO, 1.70 ± 0.09 g/24h for PL). Since these values were not significantly different, the remainder of the urinary results were expressed relative to the creatinine values. 3-methylhistidine excretion was significantly lower for the CHO condition vs. PL

($p < 0.05$) ($n=7$) ($110.43 \pm 3.62 \mu\text{mol/g creat.}$ and $120.14 \pm 5.82 \mu\text{mol/g creat.}$ respectively) (figure 5a). A similar difference was observed for urine urea nitrogen ($p < 0.05$) ($n=7$) (CHO = $8.60 \pm 0.66 \text{ g/g creat.}$ and PL = $12.28 \pm 1.84 \text{ g/g creat.}$) (Figure 5b).

Enrichment for plasma L-[1- ^{13}C]- αKIC at each sampling point is shown in Figure 6 ($n=7$). Isotopic equilibrium was achieved for each individual subject (C.V.= $<10\%$, slope=N.S.) and maintained for the duration of the infusion as expected from previous work (Tarnopolsky et al. 1991).

Compared to the control leg muscle, FSR in the exercised muscle was elevated by 36.1% in the CHO condition and by 6.3% in the PL condition (non-significant) (Figure 7) ($n=6$). No significant differences were observed for leucine flux between the two conditions ($n=7$) ($115.37 \pm 5.65 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for CHO, $113.07 \pm 4.05 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for PL).

2.4 DISCUSSION

The purpose of this investigation was to determine the effect of glucose supplementation immediately following a bout of resistance exercise upon FSR, MPD, WBPD, and urinary urea excretion. A glucose supplement of 1mg/kg (immediately and +1 h post-exercise) resulted in a significant increase in plasma glucose and insulin concentration as seen by others (Ivy et al. 1988a; Ivy et al. 1988b; Zawadzki et al. 1992). This was associated with less 3-MH and urinary urea nitrogen excretion with no difference

in FSR or WBPD. The net effect was anabolic and should result in a more positive net muscle protein balance.

Most of the work in the area of insulin and its effects on protein turnover has involved the use of insulin and glucose infusions (Biolo et al. 1995b; McNurlan et al. 1994; Moller-Loswick et al. 1994; Newman et al. 1994;). The present study is the first report in humans of the influence of oral glucose supplementation upon post-resistance-exercise protein metabolism and has practical implications for athletes and persons performing therapeutic exercise. Supplementation was achieved from a simple redistribution of the timing of the subject's habitual caloric intake.

The administration of a CHO drink led to a significant decrease in urinary 3-MH excretion over the day of the study. We interpret this as a reduction in MPD. This finding is supportive of some (Goldberg, 1979; Moller-Loswick et al. 1994; McNurlan et al. 1994; Pacy et al. 1989) but not all (Biolo et al. 1995b) previous studies of the effect of insulin on MPD. An advantage of 24h urinary 3-MH excretion over the A-V balance technique is the length over which the determination occurs. The longer collection duration for the urinary excretion method is advantageous in assessing MPD over the entire post-exercise recovery period and thus is more useful in determining the impact of an intervention upon whole body protein degradation.

Concerns about the validity of 3-MH excretion as an indicator of MPD relate primarily to the contribution of non-muscle sources (skin/splanchnic/dietary) to the 3-MH pool (Millward et al. 1980; Rennie et al.

1983). It has been demonstrated in humans with infection that the contribution to the 3-MH pool from splanchnic sources is relatively small during periods of extensive catabolism (Sjölin et al. 1989a; Sjölin et al. 1989b). In the present study, the subjects were on a controlled flesh free diet for 3 days prior to the trials and a prepackaged flesh free diet the day of each trial (Lukaski et al. 1981). Furthermore, we used a cross-over repeated measures design and therefore the individual variation in contribution from skeletal muscle vs. non-skeletal muscle protein to urinary 3-MH would be constant. Others have also considered 3-MH to be indicative of MPD (Virgili et al. 1994; Carraro et al. 1990). Thus, since the present study included dietary controls, and was a repeated measures design, 3-MH was likely a valid indicator of directional changes in MPD.

Differences in WBPD were not observed between conditions. It appears the CHO treatment did not provide enough of a reduction in MPD to influence WBPD. However, FSR contributes about 25% to WBPS (Nair et al. 1988) and one could estimate that only about 7% of the total muscle mass was active during the exercise. Therefore, changes in MPD (3-methylhistidine) may have contributed too little to influence WBPD due to a dilutional effect. Alternatively, MPD in non-skeletal muscle tissue (i.e. splanchnic) may have changed in an opposite direction and attenuated the influence of MPD on WBPD (Williams et al. 1996). If a similar protocol were employed using a whole body exercise stimulus (vs. single leg) it is possible that an effect of MPD on WBPD could have been demonstrated.

The decrease in 3-MH excretion was accompanied by significantly lower urine urea nitrogen excretion which suggested a reduction in amino acid transamination and oxidative deamination, since urine urea excretion is determined by the concentration of urea in the plasma and the glomerular filtration rate (GFR) (Guyton and Hall, 1996). We assumed that the GFR for each subject was similar between trials, since dietary energy and fluid intake and exercise was identical for each condition. Thus, assuming that sweat and fecal loss did not differ between the two trials (Tarnopolsky et al. 1988; Phillips et al. 1993), whole body nitrogen balance would be more positive for the CHO condition.

We found that the rates of FSR remained unchanged in response to the administration of CHO. A trend was observed in that the CHO condition led to a non-statistically significant 36% increase in the difference between the exercise leg and the rest leg (Figure 7). A positive effect of insulin upon FSR has been described by others (Biolo et al. 1995b; Newman et al. 1994).

Unforeseen sampling errors led to a decrease in the sample size for the FSR analysis ($n=6$). Therefore a type II error may have occurred. In addition, the exercise stimulus might not have been sufficient to stimulate an increase in protein synthesis in the vastus lateralis. We have previously reported an increase in FSR using a greater volume of training in a fusiform muscle (biceps brachii) following training (Chesley et al. 1992). Since the vastus lateralis is a pennate muscle that contributes to both knee stabilization and extension, the force/unit area may have been less than in our previous study using the biceps

brachii. A study of female swimmers also found no effect of resistance exercise upon FSR using a muscle that is difficult to fully activate (posterior deltoid) (Tipton et al. 1995). This same group, however, found an increase in FSR in the vastus lateralis using an almost identical intensity and volume of resistance exercise (Biolo et al. 1995a). It should be noted however that the subjects in the latter study were untrained (Biolo et al. 1995a) and it is possible that this may partially explain the discrepant results. A third possibility is that our measurement of FSR over a 10 h period immediately following exercise may not have included the time points over which FSR is maximal. We know from previous studies that FSR appears to peak at approximately 24 h following exercise (MacDougall et al. 1995).

In summary, our results indicate that consumption of a 1 g/kg CHO supplement immediately and 1 hour following completion of a resistance training bout significantly decreased myofibrillar protein breakdown and urine urea nitrogen excretion, and slightly increased FSR, resulting in a more positive protein balance. This suggests that a glucose supplement consumed following resistance exercise that increases insulin concentration may enhance muscle protein balance and muscle hypertrophy.

CHAPTER III

INFLUENCE OF VARIOUS NUTRITIONAL SUPPLEMENTS ON MUSCLE GLYCOGEN RESYNTHESIS, WHOLE BODY PROTEIN SYNTHESIS, AND MUSCLE PROTEIN DEGRADATION FOLLOWING RESISTANCE TRAINING

3.1 INTRODUCTION

Nutritional supplementation following endurance exercise is known to increase the rate of muscle glycogen resynthesis (Ivy, 1991; Friedman et al. 1991). However, there has been very little investigation into the role of nutritional supplementation following resistance exercise (Pascoe et al. 1993). Similar to endurance exercise, resistance exercise results in significantly decreased muscle glycogen (MacDougall et al. 1988; Tesch et al. 1986; Robergs et al. 1991). The magnitude of the decreases are not as great as that observed with endurance exercise. The influence of carbohydrate (CHO) supplementation following resistance training has been investigated following resistance exercise (Pascoe et al. 1993). It was found that CHO supplementation resulted in similar glycogen resynthesis rates as those observed following endurance exercise (Pascoe et al. 1993). However, others have observed greater rates of muscle glycogen resynthesis and higher plasma insulin levels with the consumption of a CHO/protein (PRO) supplement following endurance exercise (Zawadzki et al.

1992). Non-isoennergetic supplements made the interpretation of these latter findings difficult; however, they suggested that PRO and CHO supplements were more efficacious in promoting glycogen resynthesis than CHO alone.

Resistance exercise also results in changes in the rate of protein turnover. Specifically, resistance exercise results in simultaneous increases in muscle protein synthesis and muscle protein degradation, resulting in a more positive but still negative net protein balance in individuals in the fasted state (Biolo et al. 1995a). Since muscle protein balance is determined by both muscle protein synthesis (MPS) and muscle protein degradation (MPD), influencing one or both of these factors change muscle protein balance. Any changes in muscle protein balance could be expected to result in changes in muscle size.

Although the presence of insulin is not necessary for exercise induced hypertrophy to occur (Goldberg, 1979) it may influence rates of protein synthesis and/or degradation. There is evidence supporting an anabolic effect of insulin on protein metabolism through a stimulation of protein synthesis and a decrease in muscle protein degradation (Newman et al. 1994; Biolo et al. 1995b). There have been additional studies that have only observed decreased MPD with no effect on MPS. Thus, it appears that insulin would have an anabolic effect by decreasing MPD and possibly increasing MPS.

In addition, it has been demonstrated that insulin can increase the cellular uptake of amino acids (Biolo et al. 1995b). The same study also demonstrated an insulin mediated increase in MPS (Biolo et al. 1995b). It was concluded that insulin can stimulate protein synthesis, but the increase in synthesis is self-

limiting due to the decrease in available substrate (circulating amino acids). It is possible that insulin might be most beneficial as an anabolic agent when accompanied by increased circulating amino acids from an exogenous source.

There is a paucity of literature examining the role of insulin on muscle protein balance in response to nutritional interventions. The administration of a post-exercise CHO or CHO/PRO supplement could result in a more positive protein balance by stimulating an increase in circulating insulin (Pascoe et al. 1993; Zawadzki et al. 1992). In addition, a supplement that contained both CHO and PRO increase PRO synthesis to a greater extent due to the exogenous supply of amino acids. Finally, consumption of nutritional supplements following resistance exercise has been demonstrated to provide a hormonal environment that may be favorable for muscle growth (Chandler et al. 1994). ^{which is CHO}

The literature has not addressed the potential role of combined nutritional supplementation upon protein and CHO metabolism following resistance exercise. The possible interaction of these two factors may lead to a more anabolic state due to the combined influence of the insulin (increase in PRO synthesis, decrease in PRO degradation) and the increase in PRO synthesis due to the exercise stimulus.

The purpose of this investigation was: (1) to determine the effects of various nutritional supplements on the rate of glycogen resynthesis following a typical resistance training session, and (2) to determine the influence of the different nutritional supplements on whole body PRO synthesis, muscle PRO degradation and endocrine responses following resistance exercise.

3.2 METHODOLOGY:

3.2.1 Subjects:

Ten young (19-21yrs) males were recruited and screened to ensure that they were healthy and had been participating in a resistance training program for at least two years prior to the investigation (~4x per week) (Table 2). The experimental procedures, risks and benefits were explained to each subject before written consent was obtained after approval from the McMaster University Human Ethics Committee.

3.2.2 Design:

The subjects participated in a placebo controlled randomized trial with a carbohydrate/protein/fat supplement (CHO/PRO/FAT), a carbohydrate only supplement (CHO) and a placebo (PL) trial. Prior to the experimental trials, the subjects' 1RM was determined for 8 different exercises (see below). In addition, subjects collected 4 day diet records which were analyzed using a nutritional analysis software package (Nutritionist IV, First Data Bank, San Bruno, CA.). From this, individual diets were designed for each subject, for both the three days prior to each trial (diet checklist) and for the trial day itself (pre-packaged diet). The diets were isoenergetic, isonitrogenous and flesh free. The pre-packaged diet composition varied according to the post exercise supplement that was administered. It was only the timing of nutrient delivery that varied between the CHO, CHO/PRO/FAT, and PL trials with the energy content remaining constant (Table 3).

The subjects refrained from any resistance exercise for 3 days and any form of exercise for 2 days prior to each trial. They consumed meals at 0800h and 1100h (Table 4) and then reported to the laboratory at ~1400h ($t=-1.25h$). A 22-gauge catheter was inserted into an antecubital vein to allow for blood sampling. A blood sample was collected and an expired gas collection was performed ($t=-1.25h$) (Figure 8). The subjects then performed the exercise bout under supervision. Exercise consisted of a full body circuit set workout using a Global gym multi-station training apparatus. Each workout consisted of 3 sets of each of the following exercises; bench press, sit-ups, knee extension, latissimus pull-downs, bicep-curls, leg press, tricep-press, military press, and an additional series of knee extensions. All 3 leg exercises were performed unilaterally so that during the placebo trial the muscle of the non-exercised limb served as a control (exercise (EX) and rest (RST) leg). Subjects performed 20 sit-ups and for all other exercises 3 sets of ~10 repetitions were performed at ~80% of the individual's one repetition maximum (1RM). During the exercise bout of the first trial, the subjects were allowed to drink water ad libum, but were required to consume exactly the same quantity for the ensuing 2 trials. Upon completion of the exercise ($t=0h$), a blood sample was collected, a muscle biopsy was obtained from the vastus lateralis of the EX-leg using the Bergström needle biopsy technique (Bergström, 1962), and a supplement was given (CHO/PRO/FAT isoenergetic to CHO (1g/kg)). Blood samples were then collected every 20 minutes for the next 2 hours and 40 minutes. Additional blood samples were collected at 4h, 4.25h and 4.5h post-exercise. A second supplement was given

at the 1h post-exercise point and a second muscle biopsy was collected at 4h post exercise time point. Expired gas collections were also performed at 4h, 4.25h and 4.5h post exercise. At the 2h post exercise time point a second catheter was inserted into a contralateral dorsal hand vein to allow “arterialized” blood sampling (hot box at 65 ± 5 °C) (Nauck et al. 1992). A priming dose of L-[1- 13 C]leucine (0.949 ± 0.004 mg·kg $^{-1}$) (99% atom %; C.I.L. Andover, MA.) and [13 C]sodium bicarbonate (0.310 ± 0.003 mg·kg $^{-1}$) (99% atom %; Mass Trace, Woburn, MA.) was administered over 1 minute, followed by a constant infusion of L-[1- 13 C]leucine (0.999 ± 0.006 mg·kg $^{-1}$ ·h $^{-1}$) for 2.5 h. Under aseptic conditions the isotopes were diluted into sterile saline immediately prior to infusion. Prior to infusion, the infusate was passed through an anti-bacterial filter (0.2 μ m Acrodisc).

All blood samples were collected into heparinized tubes, centrifuged immediately, and the plasma stored at -50 °C for later analysis. All biopsy samples were dissected free of visible connective tissue, quenched in liquid nitrogen, and stored at -50 °C for later analysis. Expired gas samples were collected into a computerized closed gas analysis system and then into a 50L meteorological balloon (CO $_2$ analyzer: Hewlett Packard 78356A , O $_2$ analyzer: Ametek S-3A/I, Pneumotachometer: Ametek R-1). Expired O $_2$ and CO $_2$ concentrations and ventilatory volumes were determined for subsequent calculation of $\dot{V}O_2$, $\dot{V}CO_2$, and RER. Duplicate samples were drawn from the collected expired gas and injected into evacuated 10 mL Vacutainer tubes (Becton Dickinson, Mississauga, ON.). Within 72h of collection, the CO $_2$ of the

expired gas samples was cryogenically extracted and sealed in a glass tube for later determination of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio by gas-isotope ratio mass spectrometry. The subjects also collected a 24h urine samples on the day of each trial. Sample collection began the morning of the trial (first urination not collected), and continued through to the following morning (first urination collected).

3.2.3 Analysis:

a) Breath Analysis:

The CO_2 was separated from the collected breath samples by a series of freeze/thaws in liquid nitrogen and methanol/dry ice slush. It was then condensed into a glass collection tube, which was then sealed by melting the glass. On a later date, the isotopic enrichment of the CO_2 samples was determined by gas-isotope ratio mass spectrometry (VG Isogas, SIRA 10, Cheshire, England). Values are expressed atom percent excess (APE) relative to the baseline samples.

b) Plasma Analysis:

All collected plasma samples were assayed for Insulin by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA.). The intra-assay CV's were 3.2%. In addition, glucose concentrations were also determined for every sample (Sigma Diagnostics, Kit# 135, St. Louis, MO.), with an intra-assay CV of 3.5%.

Plasma samples collected at the t=BSL, 0h, 1h, 2h, and 4h time points were assayed for cortisol and total-testosterone by radioimmunoassay

(Diagnostic Products Corporation, Los Angeles, CA.). The intra-assay CV's for cortisol and testosterone were 4.8% and 5.4% respectively.

Plasma alpha-ketoisocaproic acid ^{13}C enrichment was determined by the method described by Wolfe et al (1982) and adapted by Tarnopolsky, et al. (1991). Briefly, the proteins in 500 mL of plasma were precipitated by the addition of absolute ethanol (5 mL x 2) and then centrifuged (10 min @ 10,000 rpm at 4 °C.). The supernatant was evaporated under dry N_2 at 50 °C and then resuspended in 1 mL of H_2O . 1 mL of 2% o-phenylenediamine solution was added (2% in 4 N HCl), after which the solution was heated at 100 °C for 1 hour and then cooled. The derivative was then extracted with 2.5 mL of methylene chloride and centrifuged (2-3 min @ 3000 rpm). The lower layer was removed and placed in a clean tube and the procedure was repeated. The lower layer was then transferred to a screw top tube and evaporated under a stream of dry N_2 . Finally, 75 μL of BSTFA + 1% TMCS was added and then the solution was heated at 100 °C for 30 min.

The α -KIC enrichment was determined using GC / MS (Hewlett Packard Model 5890 GC; VG-Trio-2 quadrupole mass spectrometer (VG, Cheshire, England)). Approximately 0.3 μL of derivitized plasma was injected directly onto a 15 m fused silica capillary column (DB5 J.W. Scientific, Rancho Verda, CA). The GC oven was initially set at 120 °C, then increased to 160 °C at 8 °C $\cdot\text{min}^{-1}$, and then increased to 300 °C at a rate of 20 °C $\cdot\text{min}^{-1}$ and held at 300 °C for 3 min to drive off later eluting compounds.

Ionization was achieved through electron impact (70 eV, trap current 170 (A, source current 1.6 mA, ion source temp. 200 °C). Helium was used as the carrier gas (32 cm·sec⁻¹). The enrichments of the 233.1/232.1 amu species (enriched/unenriched) were monitored by scanning the machine over a narrow scan range (228 to 238 amu) and the listing of the mass spectra acquired at the apex of the chromatographic peak was used to determine sample enrichment.

Plasma lactate concentration was determined for the baseline sample and for the immediately post-exercise sample. Plasma lactate was determined in duplicate using a YSI 23L Lactate Analyzer (Yellow Springs Instruments, Yellow Springs, OH.).

c) 24 Hour Urine Sample Analysis:

The volume of each 24 h collection was determined and three aliquots of urine were taken and frozen at -50 °C for later analysis. Urea nitrogen and creatinine were calculated from the 24 h urine collections by colorimetric methods (Sigma Diagnostics, kit# 640 and 555 respectively, St. Louis, MO.). Urinary 3-methylhistidine (3-MH) concentration was determined by a method similar to that described by Yarasheski, et al. (1993). Briefly, to prepare the urine sample; 25 µL of SSA (30%) was added to 100 µL of the urine and vortexed. The solution was then centrifuged at 3,000 rpm for 3 min. 100 µL of the supernatant was mixed with 100 µL of an internal standard, and then the solution was placed into a 1 mL syringe fitted with a millipore filter. The solution tube is then rinsed with 100 µL of LiS dilution

reagent, which is then also transferred to the 1 mL syringe. The liquid contents of the syringe were then forced through the millipore filter and subsequently frozen. At a later date the prepared urine sample 3-MH concentration was then determined using an automated amino acid analyzer (Beckman Inst., Palo Alto, CA.) by comparison of the area under the internal standard spike to the spike of the sample.

d) Muscle Glycogen Analysis:

All visible fat and connective tissue was dissected free from the biopsy samples, prior to quenching in liquid nitrogen. They were then stored at -50 °C for later analysis. Prior to analysis samples were lyophilized, and powdered, and any visible remaining blood or connective tissue removed before weighed. Glycogen concentration was determined by the method adapted from the method described by Bergmeyer (1983). Briefly, 160 μ L of NaOH was added to ~2.0-4.0 mg of dry muscle tissue and mixed thoroughly. Following incubated at 80 °C for 10 min, 640 μ L of a combined acid-buffer solution (HCl-Citrate) was added to neutralize the sample. 40 μ L of amyloglucosidase (Sigma Chemical, St. Louis, MO.) was then added, and the sample mixed and allowed to sit for 80 min. Then 80 μ L of a reagent solution (Triethanolamine 375 mmol/L; KOH 150 mmol/L; Mg (Ac)₂-2H₂O 112.5 mmol/L; EDTA-Na₂-2H₂O 3.75 mmol/L), 5 μ L of an ATP solution (45 mmol/L), 5 μ L of a DTT solution (60 mmol/L), and 10 μ L of a NAD solution (30 mmol/L) was added. Background absorbance measures were then made with a UV-spectrophotometer at 340 nm (Shimadzu, Model 1201,

Japan). A combined G6-PDH / HK solution was then added (4 μ L) (200 units of G6-PDH, 200 units HK, dissolved in 800 μ L DDI, Sigma Chemical, St. Louis, MO.). Absorbance at 340 nm was then measured 15 min after the addition of the enzyme solution. Background absorbance was then subtracted from the reaction absorbance, and the obtained value plotted against a glycogen standard curve for determination of glycogen concentration. Muscle glycogen concentrations are presented as mmol of glycogen per kg of dry muscle weight.

3.2.4 Calculations:

Leucine flux (Q) was calculated using the reciprocal pool model (from [13 C] α -KIC values) (Horber et al. 1989), at isotopic plateau:

$$Q = i (E_i/E_p - 1)$$

where, i = L-[13 C]leucine infusion rate (μ mol $\cdot$ kg $^{-1}\cdot$ h $^{-1}$), E_i = enrichment of the infused leucine (% 13 C), and E_p = enrichment of the plasma α -KIC (APE) and the term "-1" corrects for the contribution of the infused isotope to Q. Whole body protein degradation (WBPD) was estimated from leucine flux based on the equation:

$$Q = B + I$$

where Q is the total leucine flux, B is the rate of appearance of endogenous leucine, and I is the dietary leucine intake. Since the subjects had no dietary intake of leucine during the infusion, $I = 0$, thus $B = Q$.

Leucine oxidation was calculated from the equation (Wolfe, 1992):

$$\text{Total leucine oxidation} = [(IECO_2/c)/IE_{\alpha\text{-KIC}}] \cdot \dot{V}CO_2$$

where; IE_{CO_2} = enrichment of expired CO_2 (APE), $IE_{\alpha-KIC}$ = enrichment of plasma α -KIC (APE), $\dot{V}CO_2$ = volume of carbon dioxide evolved ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), and c = bicarbonate retention factor. Since exercise is known to affect the retention of CO_2 in the body (Wolfe, 1992), the bicarbonate retention factor was determined in 2 subjects 1 week prior to the experiment using an identical experimental design with a primed-continuous infusion of [^{13}C]sodium bicarbonate as described by Kien (1989):

$$c = \dot{V}CO_2 \cdot (IE_{\text{bicarbonate } CO_2} \cdot F^{-1})$$

where; $\dot{V}CO_2$ = volume of carbon dioxide evolved ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), $IE_{\text{bicarbonate } CO_2}$ = isotopic enrichment of expired CO_2 at plateau (corrected for background CO_2 enrichment), and F = the infusion rate of the [^{13}C]sodium bicarbonate ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). The individual c was used for each of the 2 studied subjects and the mean of these values was applied to the other 8 subjects in the calculation of leucine oxidation. In addition, 2 subjects for each of the different supplements were studied under identical study conditions with no isotope infusion to account for changes in background CO_2 enrichment caused by the protocol and supplements (Wolfe, 1992), to further correct the leucine oxidation values.

Muscle glycogen resynthesis rate was calculated by;

$$\text{Rate} = (G_{\text{post}} - G_{\text{pre}}) / t$$

where G_{pre} is the muscle glycogen concentration immediately post exercise, G_{post} is the muscle glycogen concentration ~4h post exercise, and t is the time between the two biopsies.

3.2.5 Statistical Analysis:

Muscle and blood data were analyzed using repeated measures analysis of variance (time x treatment) (Statistica, V. 5.0, StatSoft Inc. 1995). Integrated area under the curve calculations were done using a custom made software package. Urine and area under the curve data were analyzed using a one way repeated measures ANOVA (Statistica, V. 5.0, StatSoft Inc. 1995). When a significant interaction occurred, Tukey's post-hoc analysis was used to locate the pairwise differences. $P < 0.05$ was selected as being indicative of statistical significance. Values are expressed as means $\pm$ SD.

3.3 RESULTS

The exercise stimulus resulted in similar increases in plasma lactate for all three conditions (Figure 9).

Significantly higher baseline plasma glucose concentrations were observed for both the CHO/PRO/FAT and CHO trial ($p < 0.05$). In all three conditions exercise resulted in slight (non-significant) increased plasma glucose (Figure 10). Consumption of the CHO/PRO/FAT beverage resulted in significantly greater glucose at 20, 40, 80, 100, 120, 140, and 160 min post-exercise as compared to the placebo condition ($p < 0.01$) (Figure 10). The CHO trial resulted in a similar glucose response to the CHO/PRO/FAT trial, with significant increases at 20, 40, 60, 100, 120, and 140 min post-exercise versus the PL condition ($p < 0.01$) (Figure 10). The area under the glucose curve was not significantly different between the CHO/PRO/FAT condition and the CHO

condition (CHO/PRO/FAT = 5.87 ± 0.27 mmol·h/L, and CHO = 5.59 ± 0.35 mmol·h/L), but these were greater when compared to PL ($p < 0.01$) (Figure 11).

There were no differences in plasma insulin concentrations between trials at the beginning of exercise ($t = -1.5$ h), the end of exercise ($t = 0$ h), the beginning ($t = 2.5$ h) and end of the infusion ($t = 4$ h). Plasma insulin concentrations were significantly higher for the CHO/PRO/FAT versus the PL condition at the +20 min, +80 min, +100 min, and +120 min post-exercise time points (Figure 12) ($p < 0.01$). Similarly, the CHO condition significantly elevated plasma insulin concentrations at +20 min, +40 min, +60 min, +80 min, +100 min, +120 min, and +140 min as compared to the placebo (Figure 12) ($p < 0.01$). The area under the insulin curve was ~3x greater for both the CHO/PRO/FAT condition and the CHO condition versus the PL ($p < 0.01$) (28.9 ± 2.7 $\mu\text{IU}\cdot\text{h/L}$ for CHO/PRO/FAT, 33.6 ± 4.6 $\mu\text{IU}\cdot\text{h/L}$ for CHO, 10.1 ± 1.2 $\mu\text{IU}\cdot\text{h/L}$ for PL) (Figure 13).

No significant differences were observed for pre-exercise plasma cortisol concentration between any of the three conditions (Figure 14). No significant differences were observed between the 3 conditions for any of the measured time points. Similarly, no differences were observed with respect to the area under the cortisol curve for all 3 conditions (CHO/PRO/FAT = 367.7 ± 46.8 nmol·h/L, CHO = 349.17 ± 26.3 nmol·h/L, PL = 489.07 ± 73.3 nmol·h/L) (Figure 15).

Plasma total testosterone concentrations were not different between the 3 conditions for any of the observed time points (Figure 16). In addition, area under the testosterone curve was similar for the three conditions (Figure 17)

(CHO/PRO/FAT = 17.9 ± 1.4 nmol·h/L, CHO = 18.1 ± 1.2 nmol·h/L, PL = 18.9 ± 1.2 nmol·h/L).

Immediately post-exercise muscle glycogen concentrations in all 3 conditions were significantly lower than in the unexercised control leg ($p < 0.05$) (CHO/PRO/FAT = 220.3 ± 25.1 mmol/kg D.W., CHO = 235.1 ± 27.7 mmol/kg D.W., PL = 247.6 ± 22.9 mmol/kg D.W., Cont. = 366.1 ± 33.5 mmol/kg D.W.) (Figure 18). In both the CHO/PRO/FAT and CHO condition muscle glycogen concentrations were significantly higher after 4 h than at the immediate post-exercise time point ($p < 0.05$) (Figure 18) (CHO/PRO/FAT: 220.3 ± 25.1 mmol/kg D.W. $\rightarrow$ 312.2 ± 21.8 mmol/kg D.W., and CHO: 235.1 ± 27.7 mmol/kg D.W. $\rightarrow$ 312.2 ± 25.1 mmol/kg D.W.). The placebo treatment, however, did not result in any increases in muscle glycogen concentration after 4 h in either the exercise or the control leg (Figure 18) (PL: Exer.: 247.6 ± 22.9 mmol/kg D.W. $\rightarrow$ 255.7 ± 27.5 mmol/kg D.W., Cont.: 366.1 ± 33.5 mmol/kg D.W. $\rightarrow$ 367.5 ± 40.8 mmol/kg D.W.). Both the CHO/PRO/FAT condition and the CHO condition led to significantly greater rates of muscle glycogen resynthesis as compared to both the Placebo conditions ($p < 0.05$) (Figure 19) (CHO/PRO/FAT: 45.0 ± 8.5 mmol/kg/h, CHO: 38.5 ± 11.0 mmol/kg/h, PL: Exer. 4.1 ± 27.5 mmol/kg/h, Cont. 7.6 ± 27.5 mmol/kg/h).

24 h urinary creatinine excretion was not significantly different between the three conditions (Figure 20) (CHO/PRO/FAT: 1.82 ± 0.09 g/24h, CHO: 1.77 ± 0.12 g/24h, PL: 1.62 ± 0.15 g/24h). Since these values were not significantly

different, the remainder of the urinary values were expressed relative to the creatinine values. 3-methylhistidine excretion was not significantly different between the three conditions (Figure 21) (CHO/PRO/FAT: $84.2 \pm 10.4 \mu\text{mol/g creat.}$, CHO: $86.9 \pm 8.1 \mu\text{mol/g creat.}$, PL: $95.4 \pm 7.8 \mu\text{mol/g creat.}$). Similarly, no differences were observed in urinary urea nitrogen excretion for the three conditions (Figure 22) (CHO/PRO/FAT: $7.27 \pm 0.36 \text{ g/g creat.}$, CHO: $6.93 \pm 0.39 \text{ g/g creat.}$, PL: $6.68 \pm 0.77 \text{ g/g creat.}$).

The CHO treatment led to significant decreases in the enrichment of expired $^{13}\text{CO}_2/^{12}\text{CO}_2$ at 4 h, 4.25 h, and 4.5 h post-exercise as compared to both the CHO/PRO/FAT condition and the Placebo condition ($p < 0.01$) (Figure 23). There was also a significant decrease in ^{13}C enrichment at 4.5 h during the CHO/PRO/FAT trial as compared to the other time points for the same trial ($p < 0.01$) (Figure 23).

Due to unforeseen technical problems plasma α -KIC enrichments could not be determined at this time, but are derivitized and awaiting analysis at the time of submission of this paper. They will be completed and included in the manuscript that will be submitted for publication. Since α -KIC enrichments were not determined leucine flux and whole body protein synthesis could not be calculated at the present time but will also be included in the future manuscript. However, assuming the α -KIC enrichments were similar for the three conditions whole body protein synthesis may have been elevated for the CHO condition. This is assuming that the non-oxidative leucine disposal (NOLD) is elevated

based on the significantly lower ^{13}C enrichment in the expired breath and represents whole body protein synthesis.

3.4 DISCUSSION

The purpose of this investigation was to determine the effects of nutritional supplements of various composition on indicators of protein metabolism, muscle glycogen, and various hormones following a bout of whole body resistance exercise. As previously demonstrated (Roy et al. 1996), a CHO supplement (1 mg/kg, given @ 0h and +1h post-exercise) resulted in significantly increased plasma glucose and insulin. Similarly, a supplement of mixed composition (~66%CHO, ~23%PRO, ~12%FAT), CHO/PRO/FAT, led to similar increases in both plasma glucose and insulin. The increases observed in the present study were similar to those observed by others (Ivy et al. 1988a; Ivy et al. 1988b; Zawadzki et al. 1992) and similar to the increases we observed in our first study (Chapter 2) (Roy et al. 1996).

Significant decreases in muscle glycogen were demonstrated in the current study following resistance exercise. The 3 sets of the 3 different knee extensor exercises in the current protocol resulted in an ~36% decrease in muscle glycogen. Others have also observed significant decreases in muscle glycogen following resistance exercise (Robergs et al. 1991; Tesch et al. 1986; MacDougall et al. 1988; Pascoe et al. 1993). The decrease in the present study was similar to that seen by others when both intensity and volume of the exercise is considered. For the 4 h time period after completion of the exercise,

there was very little resynthesis of muscle glycogen in the placebo condition. This indicates that nutritional intake is important in the resynthesis of muscle glycogen following resistance exercise. It also demonstrates that over this time there was only a very small contribution to glycogen resynthesis from gluconeogenic precursor delivery to the liver and subsequent hepatic glucose release.

Both the CHO/PRO/FAT beverage and the CHO beverage resulted in similar rates of muscle glycogen resynthesis. These rates were considerably higher than those observed for both the PL condition and the Control condition. The rates of resynthesis in the current study were similar to those observed following endurance exercise (Zawadzki et al. 1992). In the study by Zawadzki (1992), a number of different beverages were given, but the combined CHO/PRO beverage resulted in the greatest rate of muscle glycogen resynthesis. However, this combined CHO/PRO beverage was the individual CHO beverage added to the PRO beverage, thus resulting in a beverage with a 42% greater energy content. The finding that the isoenergetic supplements in the present study resulted in similar rates of muscle glycogen resynthesis suggests that total energy content of the supplement is an important factor in the resynthesis of glycogen. The attenuated rates of muscle glycogen resynthesis observed with the placebo condition demonstrated the importance of energy and macronutrient intake following resistance exercise.

The exercise stimulus in the current study did not result in an increased plasma cortisol concentration. Others have also reported that plasma cortisol

remained unchanged following a bout of heavy resistance exercise in trained individuals (Nieman et al. 1995). It is likely that a training adaptation occurred with the cortisol response since others have observed significant decreases in resting cortisol concentrations with 7 weeks of resistance training in previously untrained individuals (Staron et al. 1994). Cortisol response was similar in all 3 conditions, with a trend for the placebo condition to result in a slightly higher response. It is likely that the slightly greater response was a function of the 2 additional muscle biopsies that were collected from the control leg during this trial.

There have been reports that heavy resistance exercise such as that used in the current study results in significant increases in testosterone concentration immediately post-exercise (Chandler et al. 1994; Kraemer et al. 1990). In the current study, only a significant increase from the baseline value to the immediately post-exercise value was observed in the placebo condition. However, both of the other conditions demonstrated trends towards an increase immediately post-exercise. No significant differences were observed between the three experimental conditions in the current study. Following completion of the exercise, testosterone concentrations were below baseline levels for both the CHO/PRO/FAT and CHO conditions similar to values observed by Chandler et al (1994). It has been suggested that the decreased testosterone may reflect an increase in clearance from the circulation if no changes are observed in circulating levels of luteinizing hormone (Chandler et al. 1994). If this decline in

testosterone was a result of increased uptake by muscle, it could lead to an increase in protein synthesis in the muscle.

The testosterone/cortisol ratio (T/C ratio) has been suggested by others as an indicator of the favourable hormonal milieu for the promotion of anabolic processes (Kraemer, 1988). Others have demonstrated that long-term resistance training results in increases in the T/C ratio (Hakkinen et al. 1985; Guezennec et al. 1986). With an acute bout of exercise in highly trained individuals, we observed no significant changes in the ratio (Figure 24). In addition, there were no differences between the 3 experimental conditions. The response of the T/C ratio observed with long-term training is likely a function of the decrease in resting cortisol that has been observed with training (Staron et al. 1994).

The insulin/cortisol ratio has also been suggested as a possible measure of the hormonal milieu for anabolic processes (Kraemer, 1988). However, there has yet to be substantiated in the literature. The significant increase in this ratio for the CHO/PRO/FAT and CHO conditions in the current study was due to the large increases in insulin concentrations observed during these two trials (Figure 25). If insulin antagonized the action of cortisol during the current study, a decrease in protein catabolism may have occurred. However, it is unclear what contribution cortisol may have had on protein catabolism, thus it is difficult to conclude if the I/C ratio was a valid indicator of the hormonal milieu for anabolic processes.

Unlike the first study (Chapter 2) the increased insulin concentrations were not associated with decreased 3-MH and urea nitrogen excretion. The current study involved similar dietary controls (isoenergetic/isonitrogenous/flesh free) and a similar repeated measures design as the first study. It should be noted that the relative differences in 3-MH excretion were similar between the two studies, but there was more inter-subject variability in the current study which likely contributed to a lack of statistical power (Type II error). Furthermore, the absolute levels of 3-MH excretion was slightly lower in the current study, even though the total muscle mass used during the exercise was much greater. This suggests that training status may influence the amount of myofibrillar damage that occurs with an isolated bout of resistance exercise. Others have also demonstrated that previous damage, such as that caused by resistance exercise, causes the muscle to become more resilient to future damage (Clarkson et al. 1992). Similarly, Interisano et al (unpublished) observed significantly less damage in highly trained subjects using the same protocol and relative intensity as that used by Gibala et al (1995) with untrained individuals. The subjects of our first study (Chapter 2) were not as highly trained as the subjects in the current study, and the adaptation to muscle damage that occurs with regular training likely inhibited or prevented any significant increase in myofibrillar breakdown. However, there was a trend for the 3-MH excretion to be slightly elevated in the placebo condition of the current study. Another measure of myofibrillar protein degradation such as the A-V balance technique may be a

more sensitive indicator of the effects of nutritional supplementation on myofibrillar protein degradation in highly trained resistance athletes.

Urea nitrogen excretion remained unchanged between the three conditions. Assuming that sweat and fecal loss of nitrogen was similar for the three conditions, this suggested that protein turnover was also similar. However in our first study (Chapter 2), the CHO treatment significantly decreased the amount of urea nitrogen excretion. This suggested that there may be a training adaptation in protein turnover during the initial stages of a weight training program. Lemon et al (1992) and Tarnopolsky et al (1988) both demonstrated that individuals who are initiating a resistance training program require a greater daily intake of protein as compared to elite strength athletes. The urinary urea excretion in the current study was lower for all conditions as compared to the first study; suggesting that protein oxidation was not as great in the more highly trained individuals.

In summary, our results indicate that consumption of a 1 g/kg CHO supplement or a CHO/PRO/FAT supplement (isocaloric to CHO) immediately and 1 hour following completion of a resistance training bout significantly increased the rate of muscle glycogen resynthesis over the first 4 hours after the completion of the exercise. In addition, the same supplements do not appear to decrease myofibrillar protein degradation as indicated by 3-MH and urea nitrogen excretion in highly trained resistance athletes. However, it appears that an acute bout of resistance exercise in highly trained strength athletes does not increase 3-MH and urea nitrogen excretion to the same extent as observed in

untrained individuals. This suggests that the disruptive effect of resistance exercise on myofibrillar protein may not be as great in highly trained resistance athletes compared to untrained individuals. Finally, nutritional supplements of carbohydrate or carbohydrate/protein/fat do not influence the testosterone and cortisol response to a bout of resistance exercise.

CHAPTER IV

OVERALL CONCLUSIONS

4.1 CONCLUSIONS

This research has focused on the role of the consumption of nutritional supplements following resistance exercise. It was hypothesized that consumption of a supplement that increased plasma insulin levels would decrease myofibrillar protein degradation associated with resistance exercise and possibly increase protein synthesis. Furthermore, it was hypothesized that consumption of a supplement would also increase the rate of muscle glycogen resynthesis following resistance exercise.

In the first study it was demonstrated that CHO supplementation (1g/kg) immediately and 1 h following the completion of a resistance training bout significantly reduced urinary 3-MH excretion and urea nitrogen excretion. This provided evidence of a more positive protein balance. In addition, the CHO supplement led to a non-significant increase in muscle protein synthetic rate following the exercise bout. Both of these observations demonstrated that CHO supplementation following resistance training in untrained, but physically active individuals may aid in maintaining a more positive protein balance and prevent the degradation of myofibrillar protein.

In the second study CHO supplementation did not appear to attenuate protein catabolism following exercise by highly trained resistance athletes.

However, it should be noted that the tracer component of the second study has not yet been completed, and when completed will provide more information as to the influence of supplementation on protein turnover following resistance exercise. Muscle glycogen resynthesis rate following resistance exercise in highly trained strength athletes was greater for CHO and CHO/PRO/FAT as compared to PL. Both of these studies also demonstrate that the timing of meals is important in PRO and CHO metabolism in resistance athletes.

Clearly, there is benefit in the consumption of energy immediately following resistance exercise for both the untrained and highly trained individual. However, further work is required to determine the benefit of this energy intake in individuals of different training status.

This information is useful for both the athlete and coach, especially for athletes who incorporate multiple training sessions into a single day. For example, an athlete may include a heavy aerobic workout in the morning and then include a resistance training session in the evening. Failure to replenish muscle glycogen stores following a workout may hinder the evening workout.

4.2 Future Research

This work has added to the growing area of information relating to the importance of nutrition and its effects on protein balance and muscle glycogen storage following resistance exercise. There still are many questions that remain unanswered in this growing area of interest.

1. What is the influence of nutritional supplementation on protein balance with chronic resistance training? Would nutritional supplementation following resistance exercise lead to a more rapid accretion of lean body mass?

This could be assessed with a longitudinal study where one group is given a supplement immediately post-exercise and another group could be given a supplement in the morning. A number of measures could be assessed including: fCSA, limb girth, strength, lean body mass, etc.

2. Does nutritional supplementation immediately post-exercise improve performance for the next training session or next day's training?

For the athlete that cross trains(i.e. aerobic training and resistance training) within the same day, nutritional supplementation may allow for increased performance in the following training session. This question could be addressed through a simple study where an athlete would perform a resistance training bout in the morning and then a aerobic training bout in the afternoon. Simple performance measures could be assessed such as time to exhaustion.

3. How would nutritional supplementation affect the response of deconditioned rehabilitation patients to physical therapy and training?

The possible advantage of nutritional interventions in this population should be investigated. It is possible that nutritional supplementation following therapy and training could decrease the hospitalization period of these individuals and decrease the costs of prolonged rehabilitation. This could be investigated by randomizing patients as they enter into a rehabilitation program

into either a group that receives nutritional supplements following therapy or a group that receives the supplement in the morning prior to any form of therapy.

4. What is the influence of nutritional supplementation following resistance exercise in highly trained athletes compared to sedentary controls?

This could be assessed with either a longitudinal training study where protein degradation and synthesis are assessed post-exercise both before and after a training period. It could also be assessed with a cross-sectional study such that the response of highly trained resistance athletes is compared to that of sedentary controls.

5. Is there a gender difference in protein turnover following resistance exercise, and do females respond in a similar manner as males to nutritional supplementation following resistance exercise?

To date the possible gender difference in protein metabolism following resistance exercise has not been assessed. Since there are distinct gender differences in protein and CHO metabolism with endurance exercise(Phillips et al, 1992; Tarnopolsky et al, 1995): it is thus probable that gender differences also exist following resistance exercise. This could also be assessed with either a longitudinal training study or an acute cross-sectional study. Variables of interest could include anabolic hormones, urinary indices of protein turnover, tracer assessment of both whole body protein turnover and muscle protein synthesis, and changes in muscle glycogen concentrations.

6. Would supplements of various energy composition have varying effects on protein metabolism?

In the current study all of the nutritional supplements were isoenergetic based on the CHO supplement that provided 1g/kg of CHO. As demonstrated with the current study, it appears that the total energy content of the beverage appears to be more important than the composition of the supplement. To directly assess the role of energy content and the composition of this energy content would be beneficial. The dosage of supplement could be determined by kcal/kg, with two different dosages of energy(4 kcal/kg and 6 kcal/kg) and two different supplements(CHO only and CHO/PRO).

Clearly, more work is required in this area to further understand the role of nutritional supplementation following resistance exercise.

TABLES AND FIGURES

Table 1: Subject Characteristics (Study 1).

Subject	Age (y)	Mass (kg)	Height (cm)	%Body Fat	Energy (kcal/d)	%CHO	%Fat	%Pro
JM	24	73.5	176	16.8	2990	68	22	10
SI	27	77	175	11.2	2863	63	25	12
RP	21	76.3	174	15.6	2674	64	23	12
CV	19	79.6	172	18.1	2533	65	24	12
FF	21	73.8	182	21.4	3176	62	26	12
ST	18	63	167	12.7	3091	61	27	13
SC	21	87.4	187	17.8	2593	69	20	11
KC	21	69.2	173	18.4	2056	74	16	10
mean	21.5	75.0	175.8	16.5	2747	65.8	22.9	11.5
st. dev.	2.8	7.2	6.2	3.3	363.9	4.3	3.6	1.1

Table 2. Subject's descriptive data and habitual diet characteristics (Study 2).

Subject	Age	Mass (kg)	Height (cm)	Density (g/cm ³)	Years Trn	Energy (kcal)	%CHO	%PRO	%Fat
1	19	75.4	186	1.064	3.5	2767	55	12	33
2	20	77.5	174.5	1.091	2.5	1793	48	18	34
3	19	85.4	184.8	1.091	3.5	3105	45	15	39
4	20	75.5	176	1.086	7	2342	49	17	34
5	19	104	190	1.064	5.5	5203	49	23	28
6	19	95.4	198.5	1.071	5	4011	35	24	41
7	19	90	180	1.083	5	1967	46	17	37
8	20	85	178	1.06	4	3157	51	18	31
9	20	97.5	181.7	1.066	3	3394	61	20	19
10	21	82.2	173	1.062	5	2560	58	17	24
Mean	19.6	86.8	182.3	1.07	4.4	3030	50	18	32
SEM	0.22	3.10	2.48	0.004	0.43	320.80	2.31	1.12	2.14

Table 3. Daily nutritional intake for each trial day (Study 2).

	Breakfast (0800h)	Lunch (1100h)	Snack (1400h)	Post-Ex
PL	CHO/PRO/FAT + B	L	S	PL
CHO	PRO/FAT + B	L	S	CHO (1g/kg)
CHO/PRO/FAT	PL + B	L	S	CHO/PRO/FAT

Table 4. Distribution of nutritional supplements for each trial (Study 2).

Trial	Energy(kcal)	%CHO	%Protein	%Fat
PL	3008	48.8	17.2	31.8
CHO	3036.2	52.6	18.4	28.7
CHO/PRO/FAT	3010	51.2	19.4	28.3
Habitual	3029.9	49.7	18.1	32
P	N.S.	N.S.	N.S.	N.S.

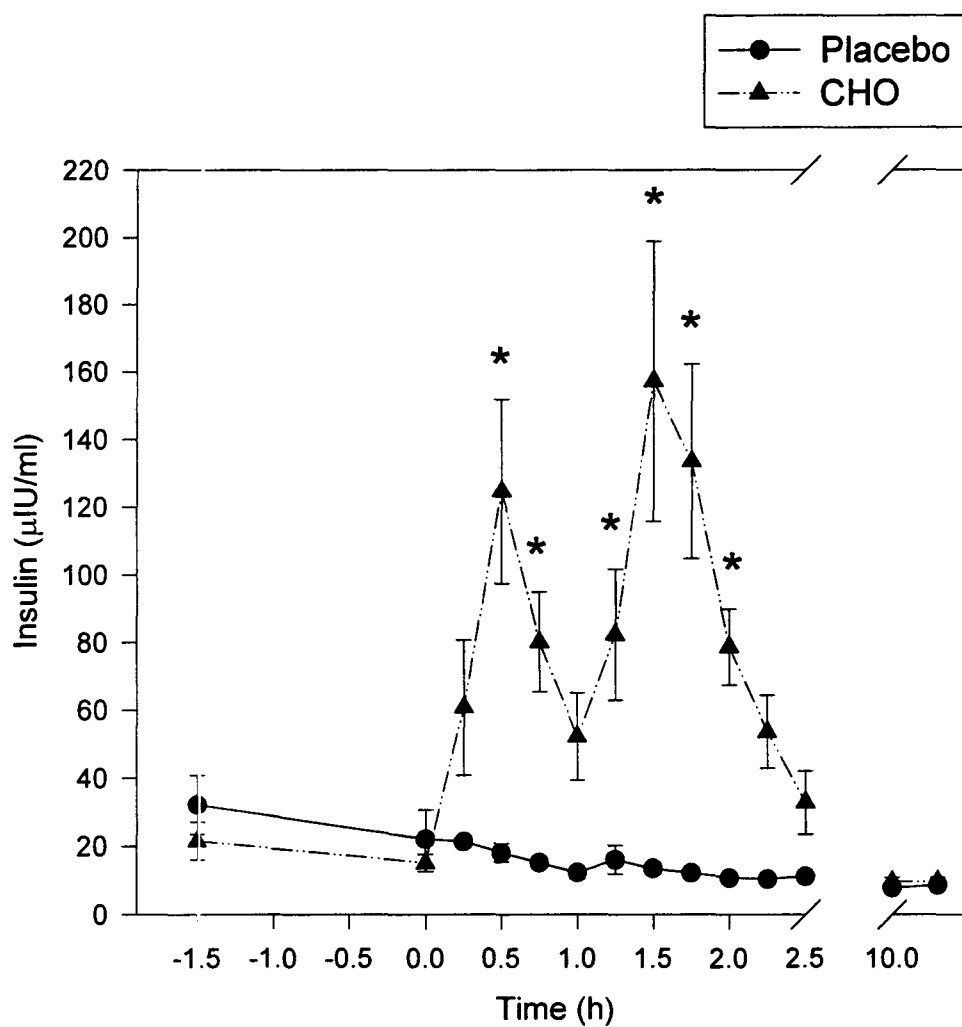


Figure 1. Plasma Insulin values for both CHO and PI (Placebo) with respect to time (mean $\pm$ SE). * $p < 0.01$ between the two conditions

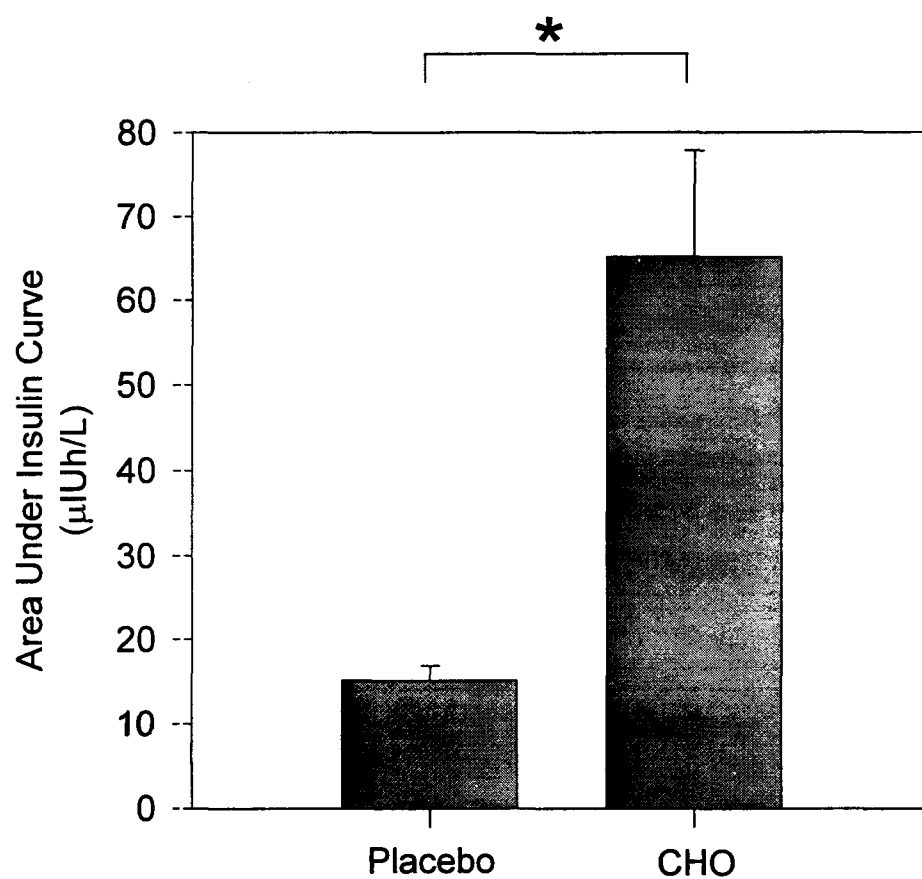


Figure 2. Area under the insulin curve for CHO and PL (Placebo) (mean $\pm$ SE)

for the first 2.5 post-exercise. * $p < 0.01$ between the two conditions

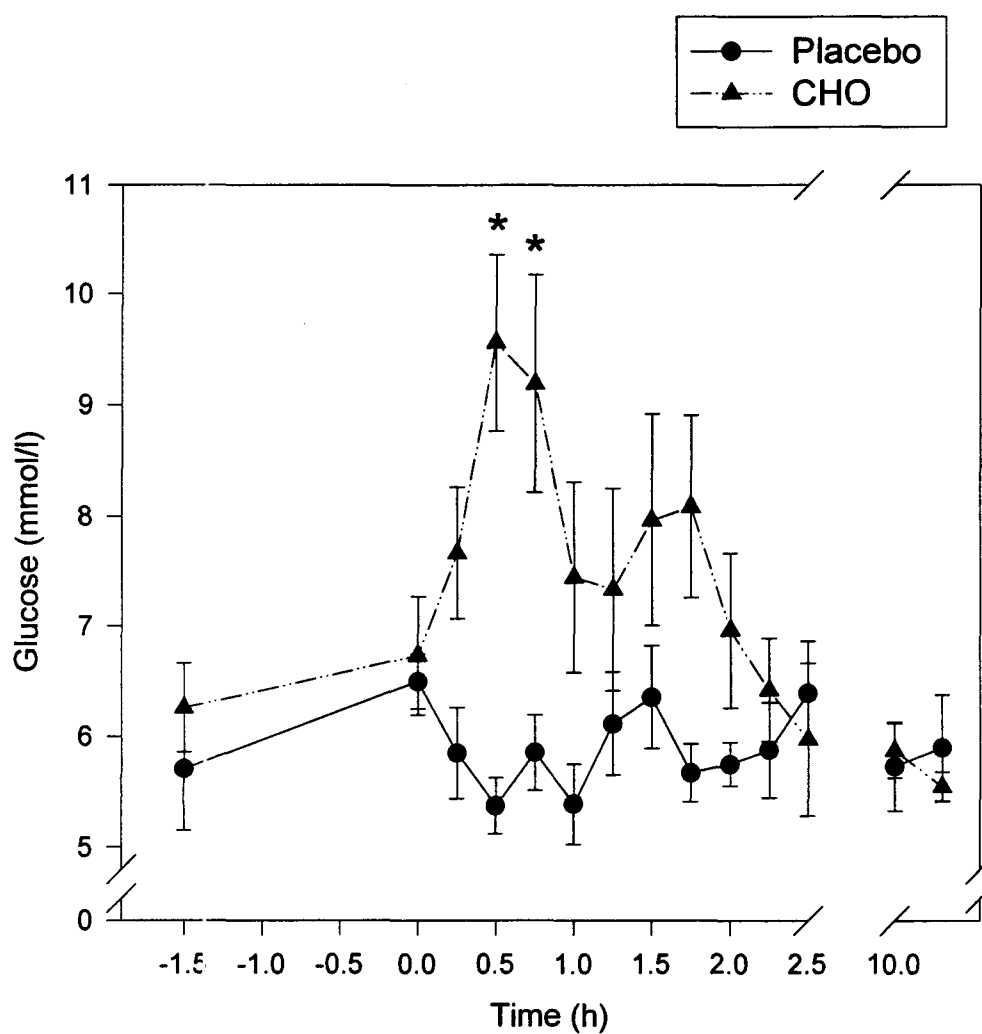


Figure 3. Plasma Glucose values for both CHO and PL (Placebo) with respect to time (mean $\pm$ SE). * $p < 0.05$ between the two conditions

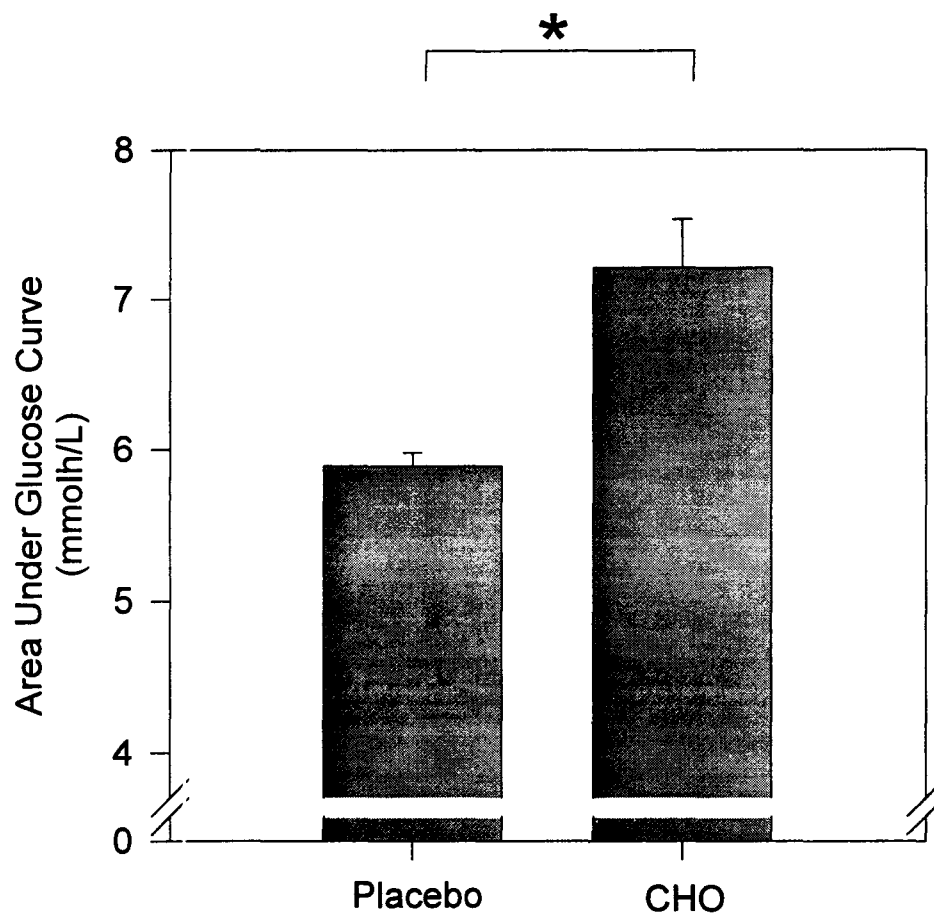


Figure 4. Area under the glucose curve for CHO and PL (Placebo) (mean $\pm$ SE)

for the first 2.5 post-exercise. * $p < 0.01$ between the two conditions

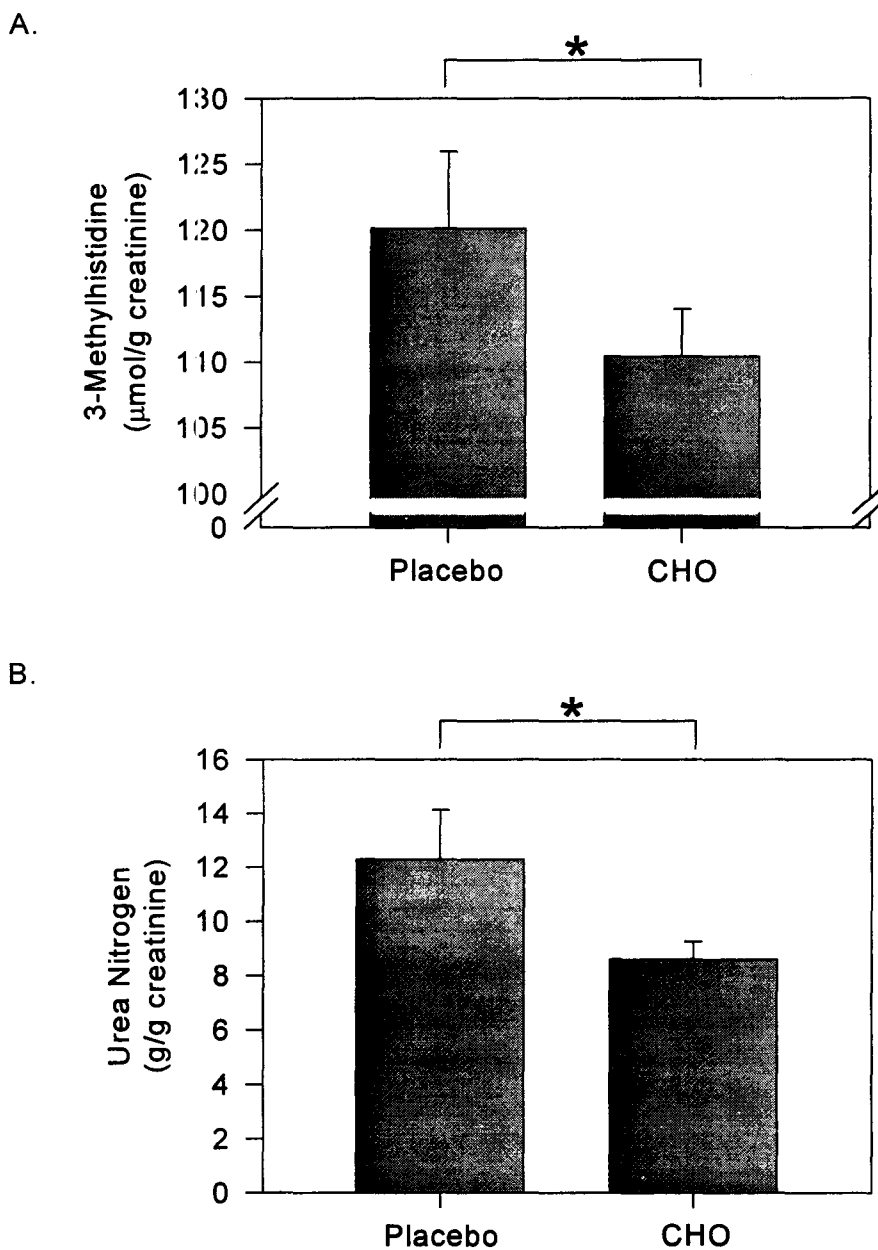


Figure 5. 24 hour Urine specimen results. (a) 3-methylhistidine for both PL (placebo) and CHO (b) urine urea nitrogen for both PL (placebo) and CHO. (mean $\pm$ SE) * $p < 0.01$ between the two conditions

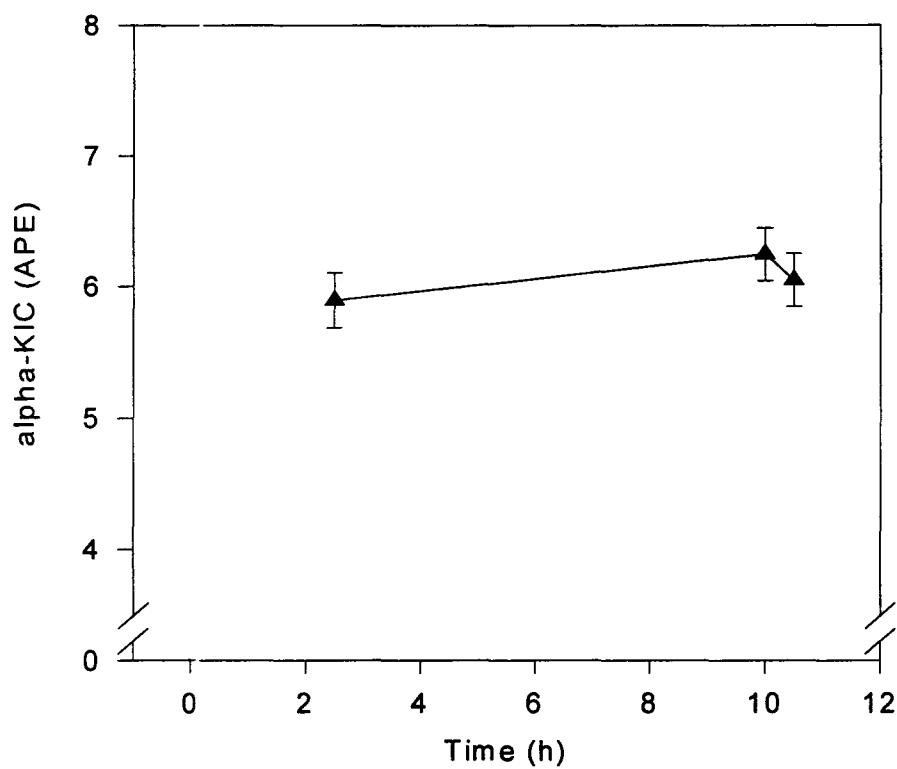


Figure 6. Plasma (-KIC enrichment with respect to time (mean $\pm$ SE).

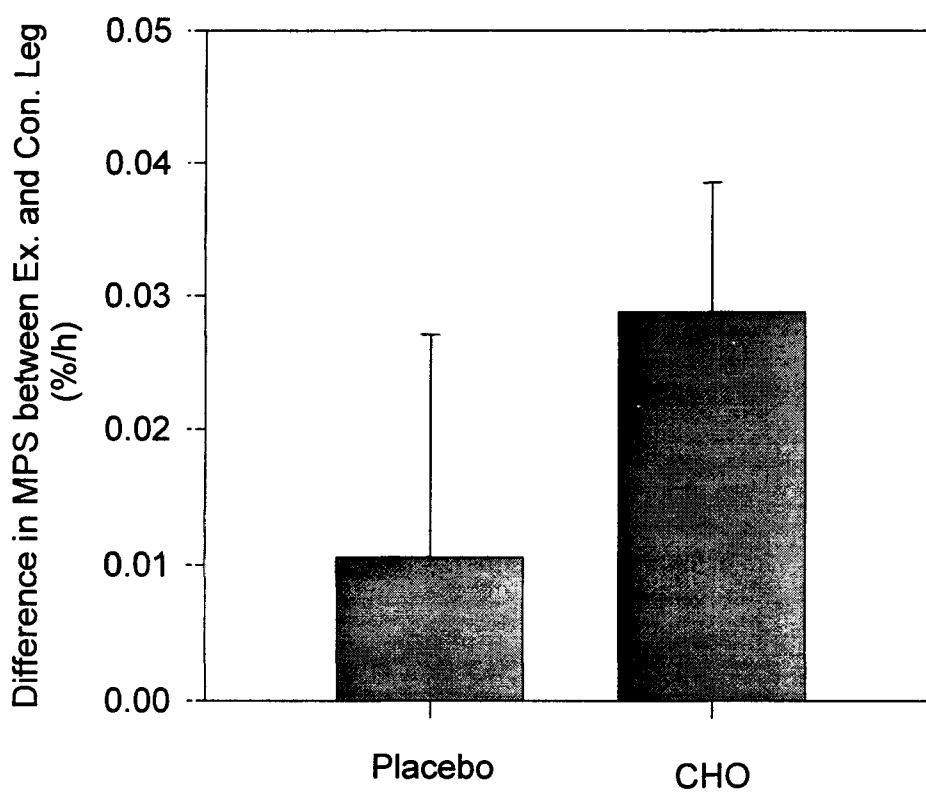


Figure 7. Muscle protein synthetic rate; difference between exercise and control leg for both PL (Placebo) and CHO (mean $\pm$ SE).

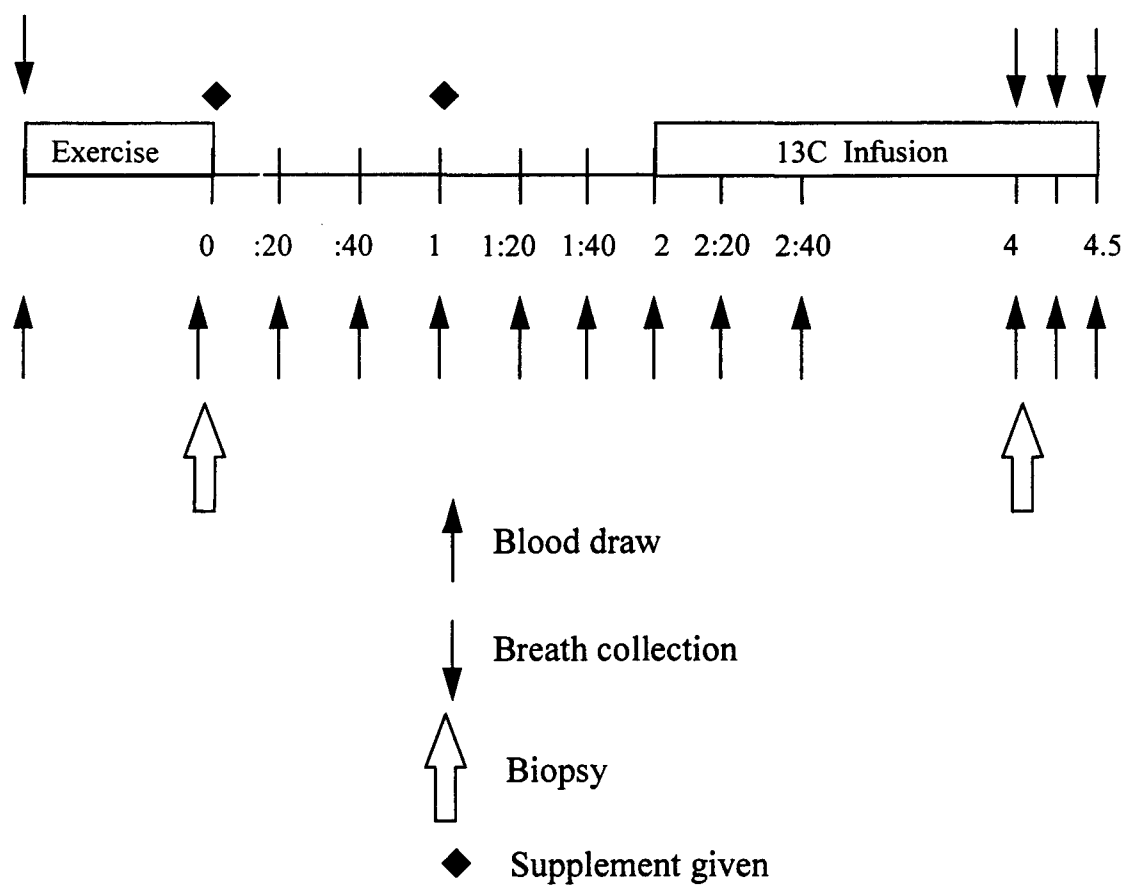


Figure 8. Study Design (Study 2).

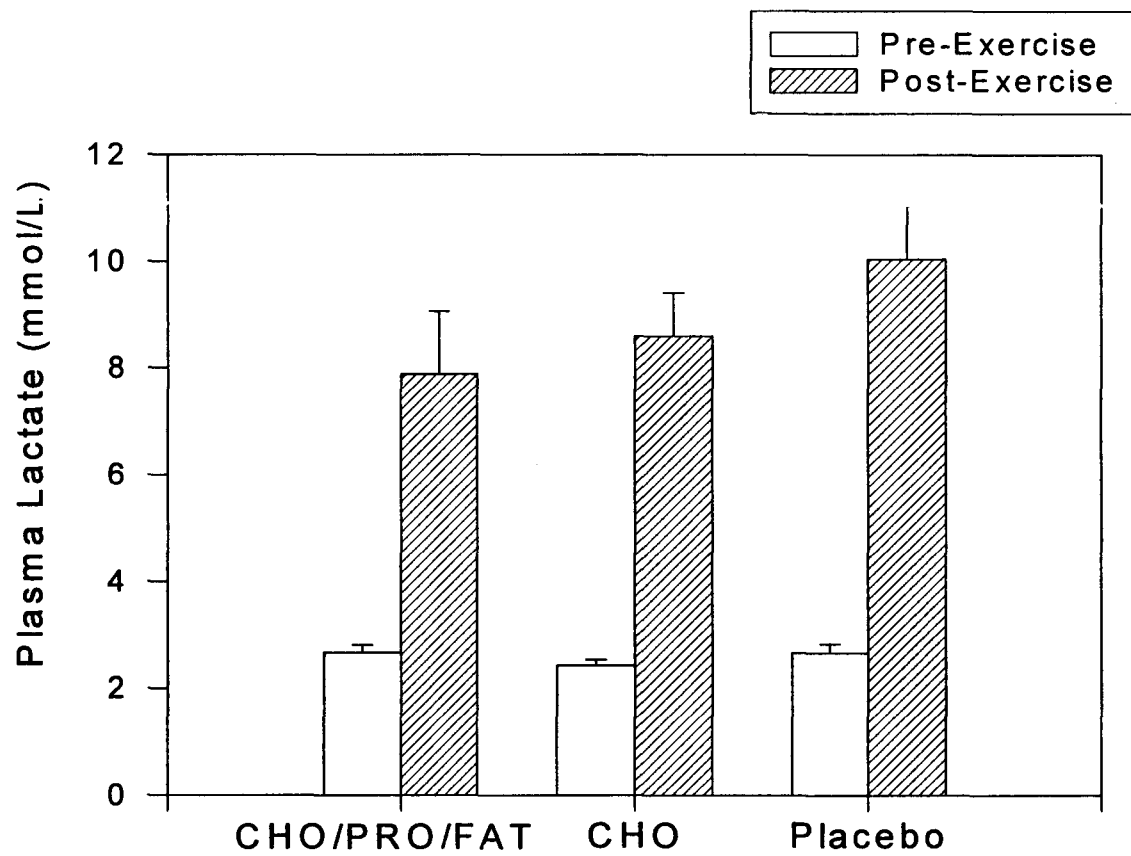


Figure 9. Pre, post-exercise plasma lactate for CHO/PRO/FAT, CHO and PL (mean $\pm$ SE).

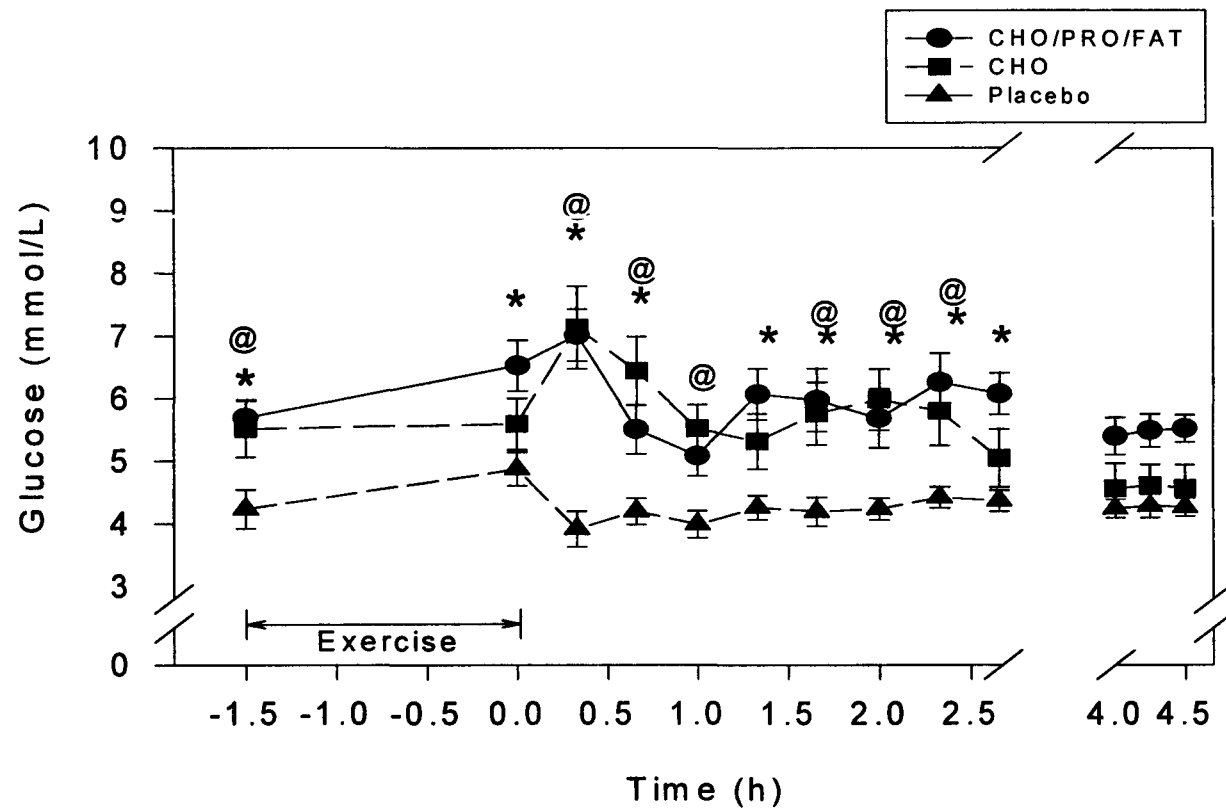


Figure 10. Plasma Glucose values for CHO/PRO/FAT, CHO and PL (Placebo) with respect to time (mean $\pm$ SE).

* $p < 0.05$ between the CHO/PRO/FAT and PL, @ $p < 0.05$ between CHO and PL.

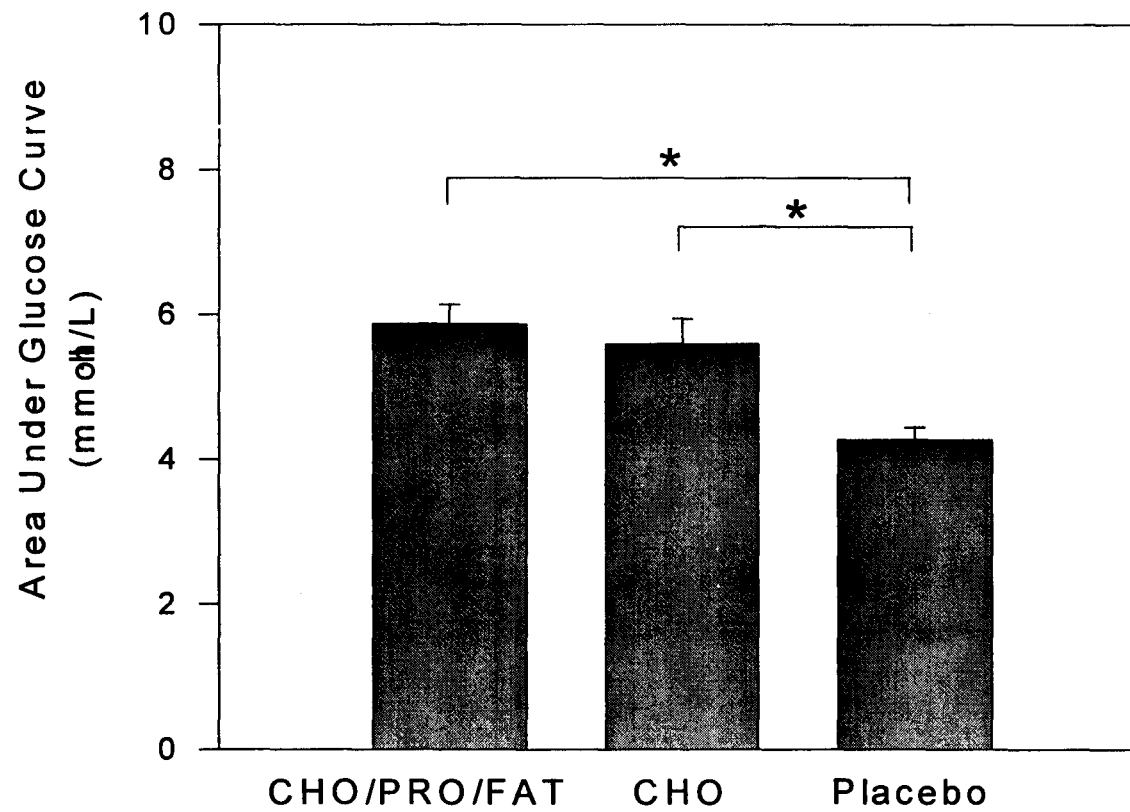


Figure 11. Area under the glucose curve for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE). * $p < 0.01$

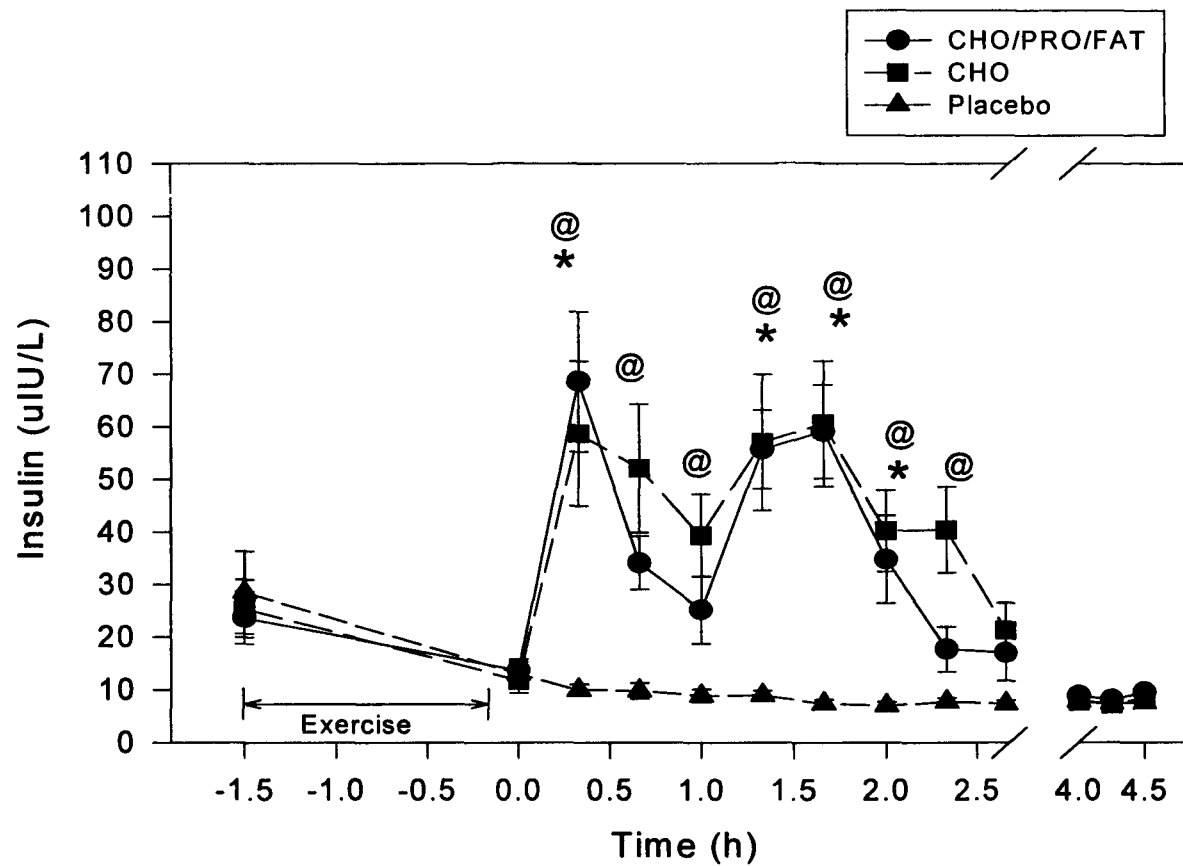


Figure 12. Plasma Insulin values for CHO/PRO/FAT, CHO and PL (Placebo) with respect to time (mean $\pm$ SE).

*p<0.01 between the CHO/PRO/FAT and PL, @p<0.01 between CHO and PL.

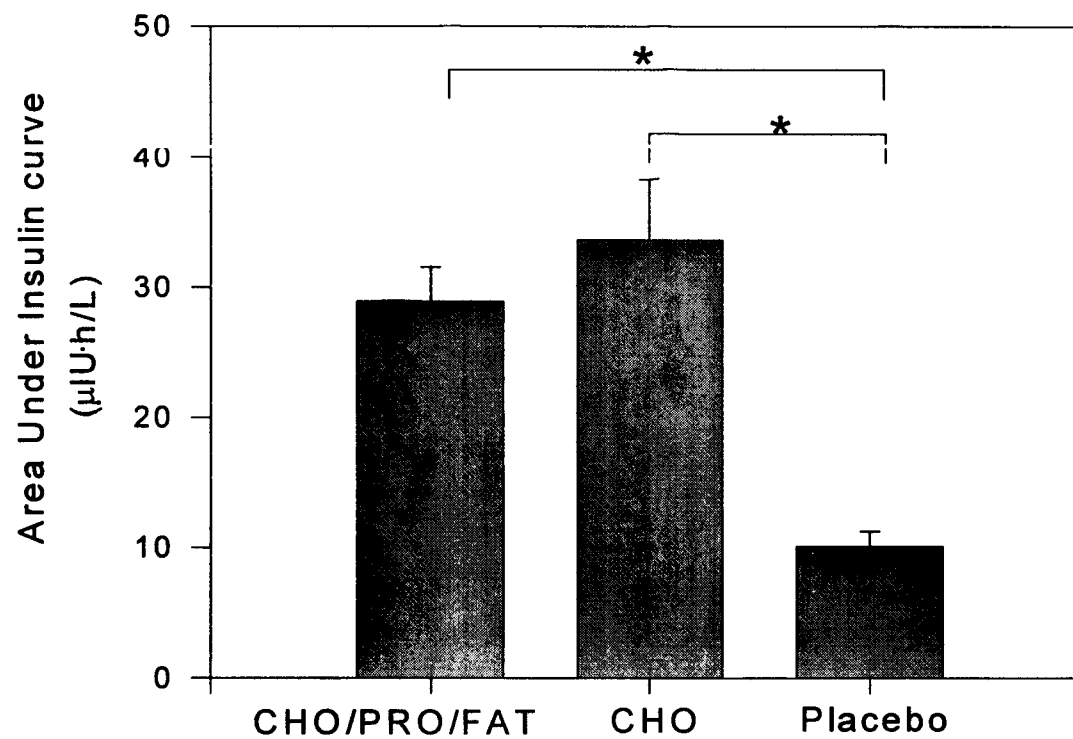


Figure 13. Area under the insulin curve for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE). * $p < 0.01$

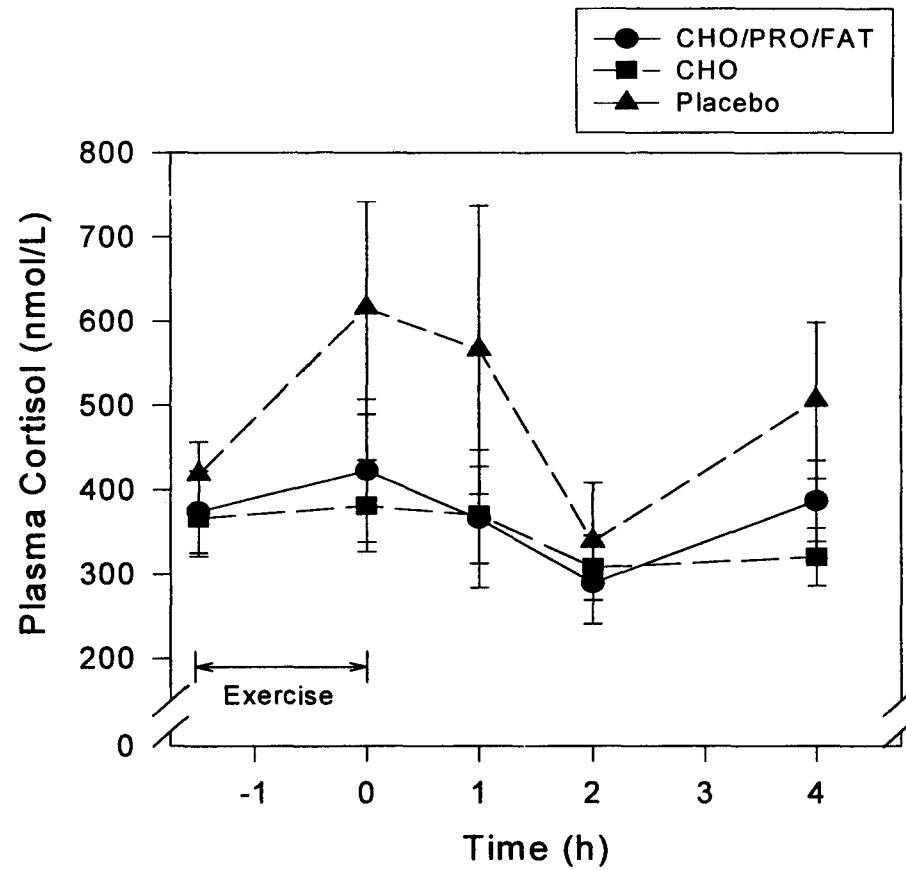


Figure 14. Plasma cortisol values for CHO/PRO/FAT, CHO and PL (Placebo) with respect to time (mean $\pm$ SE).

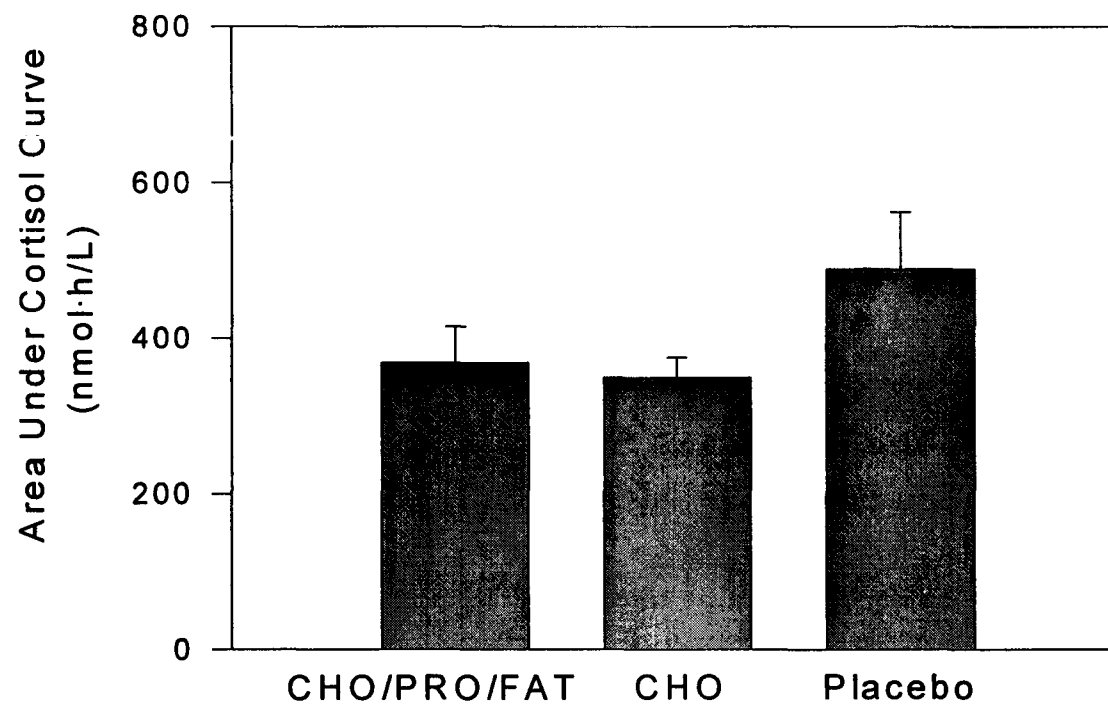


Figure 15. Area under the cortisol curve for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE).

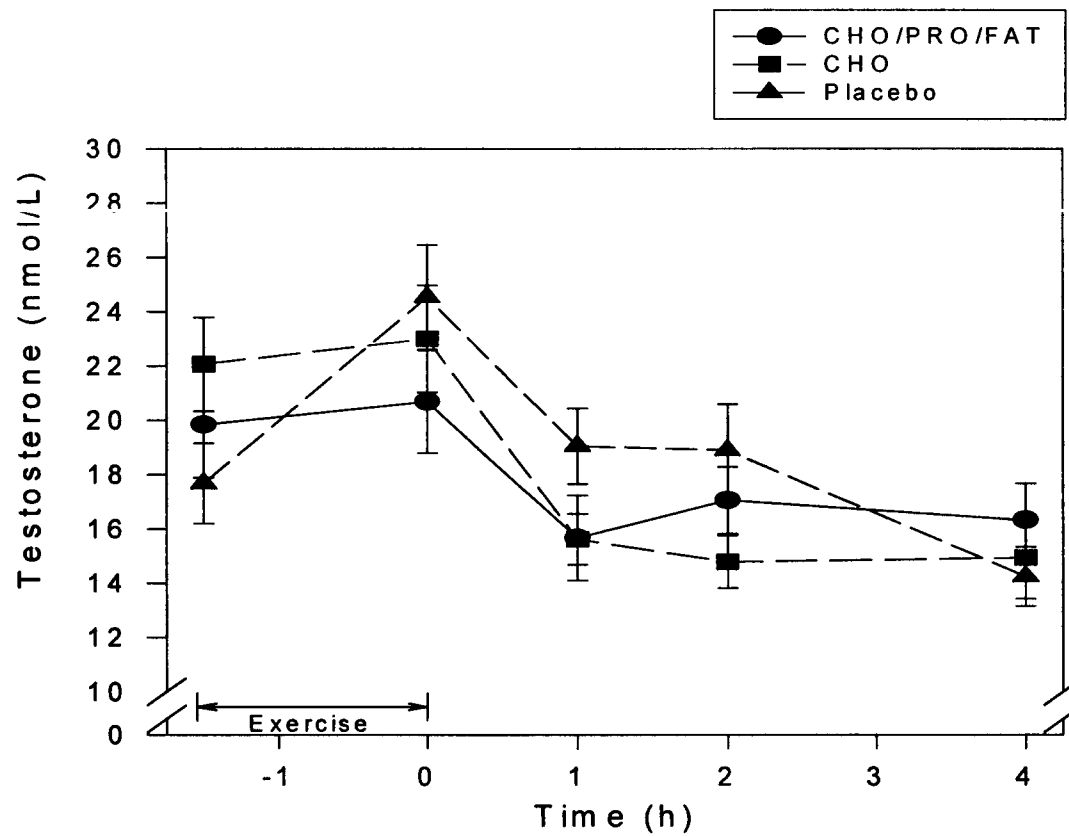


Figure 16. Plasma total testosterone values for CHO/PRO/FAT, CHO and PL (Placebo) with respect to time (mean $\pm$ SE).

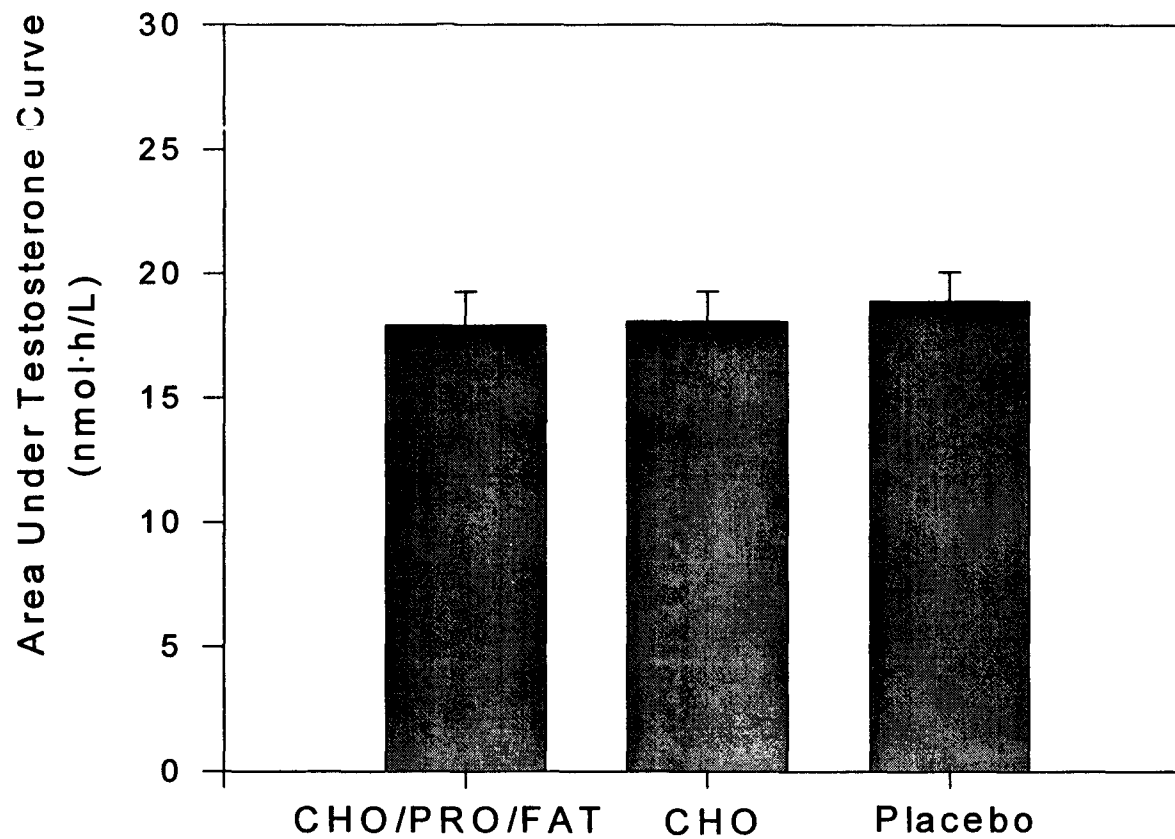


Figure 17. Area under the total testosterone curve for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE).

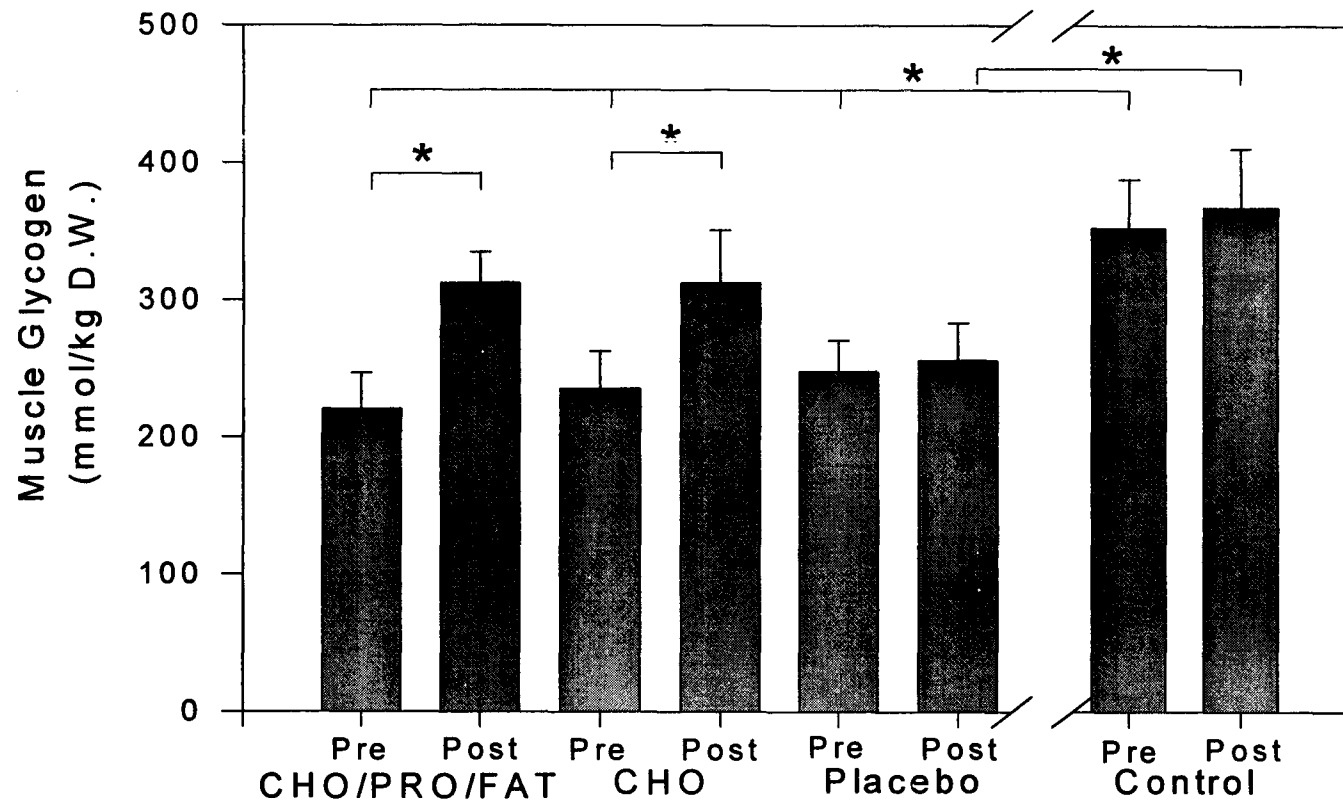


Figure 18. Muscle glycogen concentration immediately (pre) and 4h post-exercise (post) for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE). * $p < 0.05$.

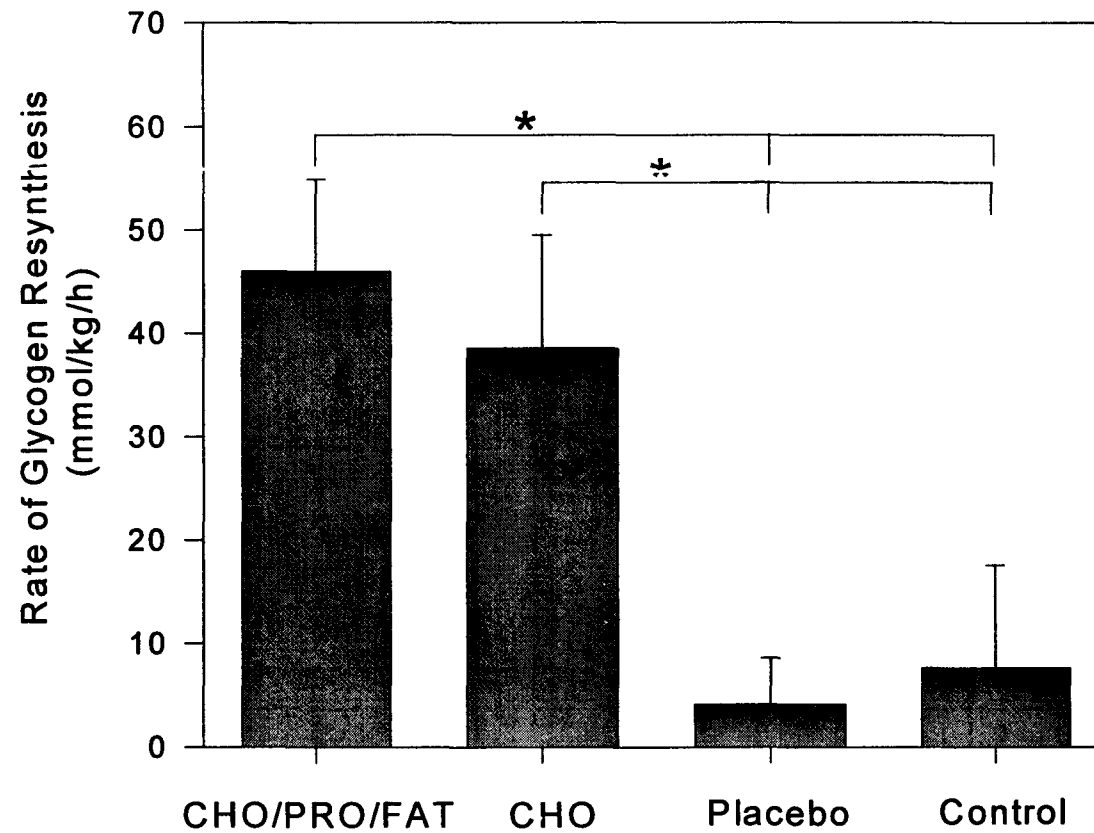


Figure 19. Rate of muscle glycogen resynthesis for first 4h following exercise for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE). * $p < 0.05$.

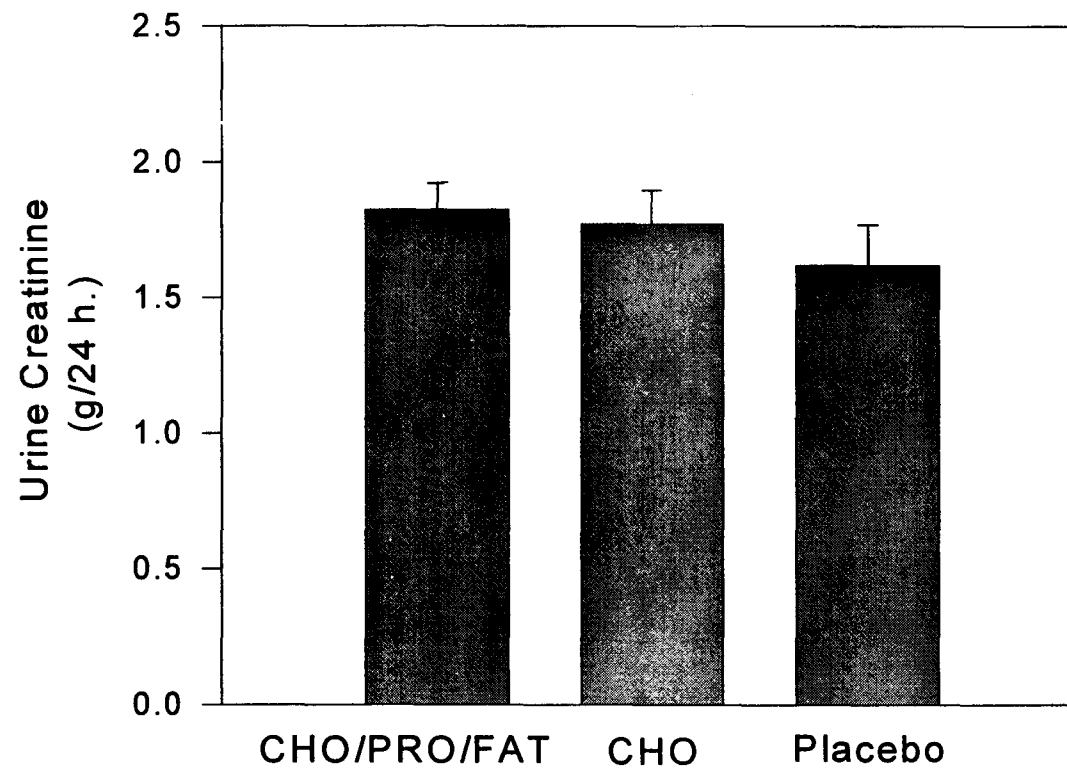


Figure 20. 24h Urine creatinine excretion for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE).

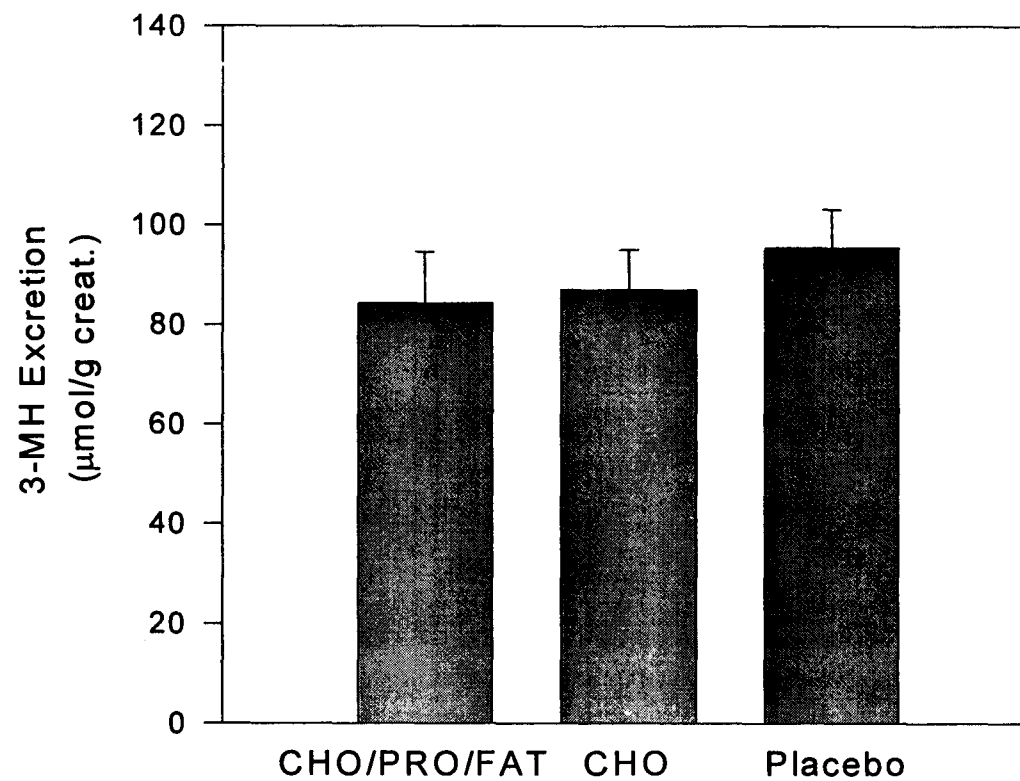


Figure 21. 24h Urine 3-methylhistidine excretion for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE).

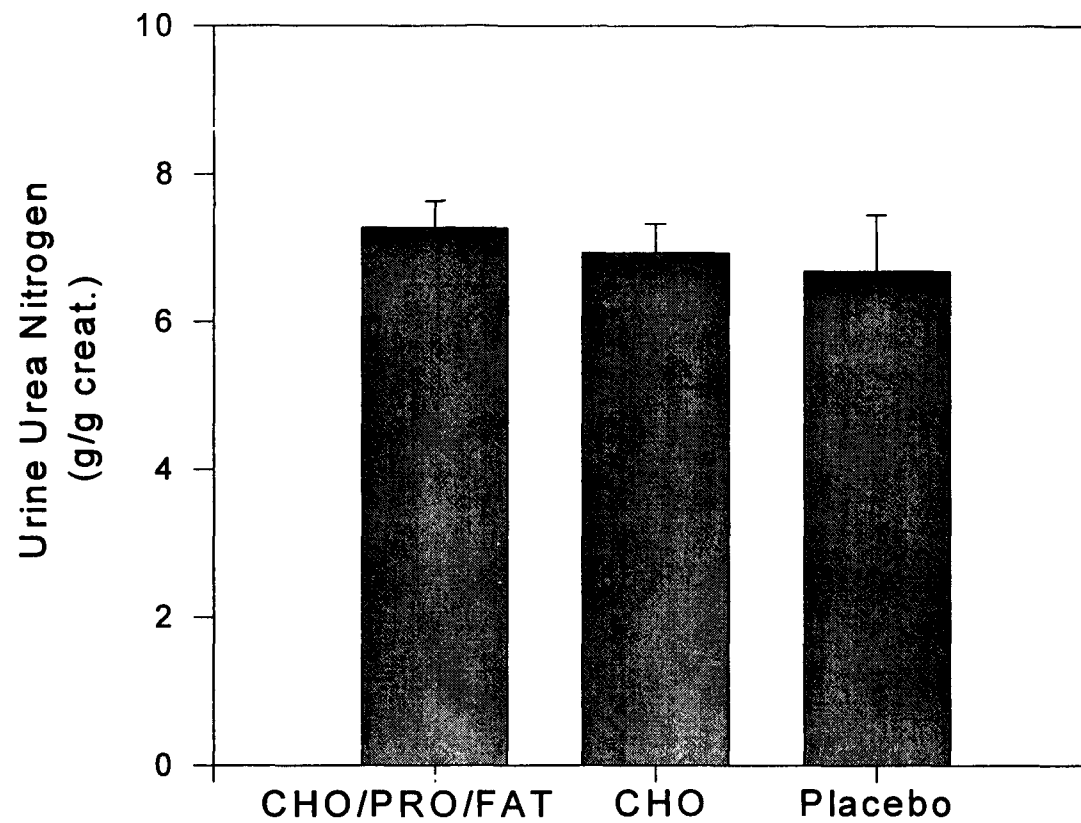


Figure 22. 24h urine urea nitrogen excretion for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE).

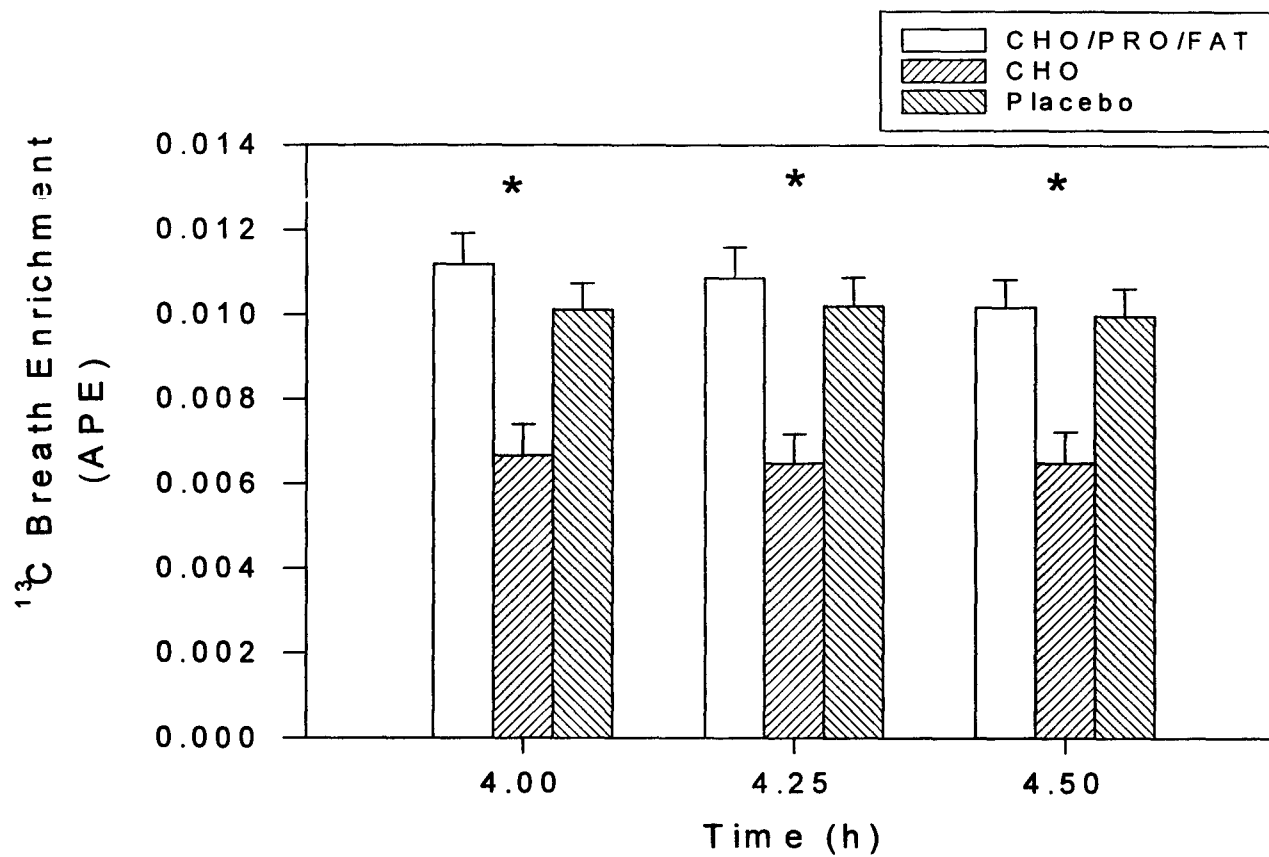


Figure 23. $[^{13}\text{C}]$ -Breath enrichment for CHO/PRO/FAT, CHO and PL (Placebo) (mean $\pm$ SE). * $p < 0.05$ for CHO vs. CHO/PRO/FAT and PL (Placebo).

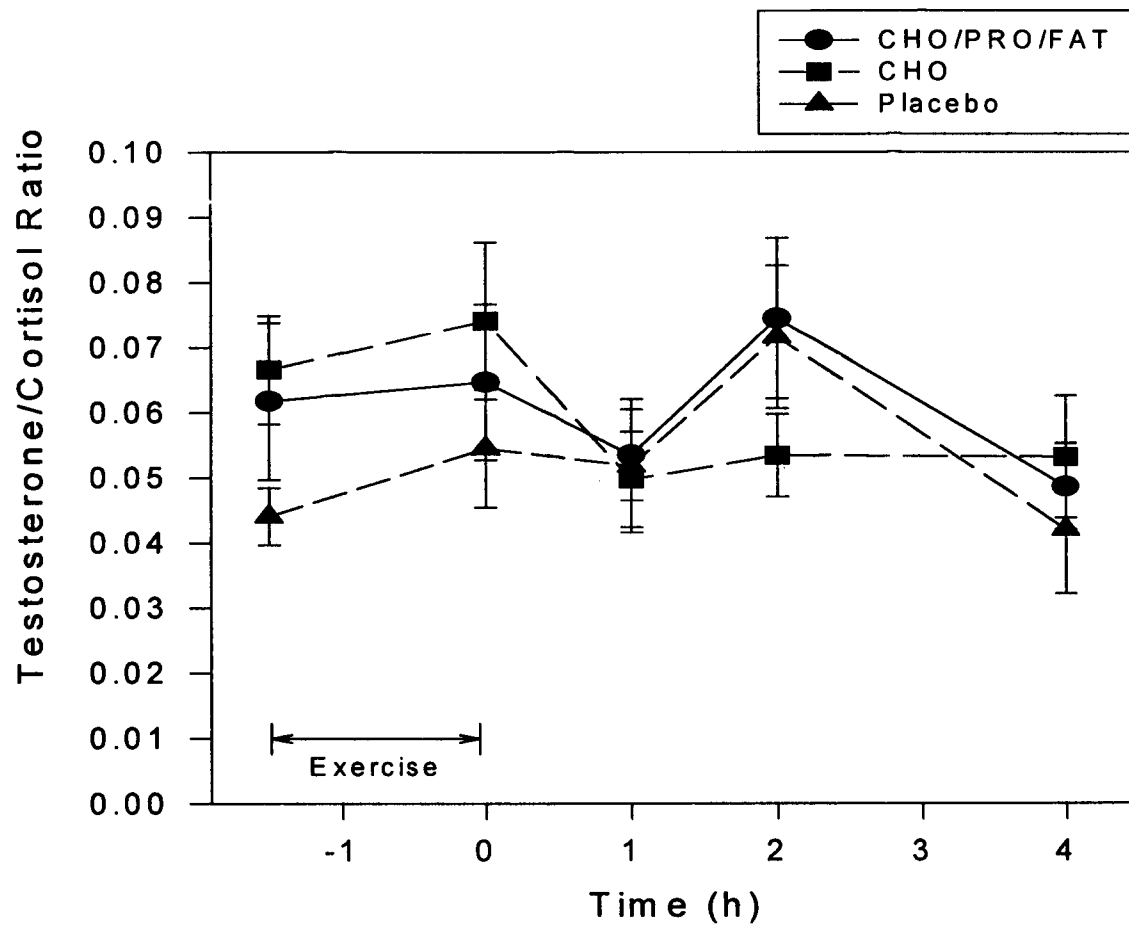


Figure 24. Testosterone/Cortisol ratio for CHO/PRO/FAT, CHO and PL (Placebo) with respect to time (mean $\pm$ SE).

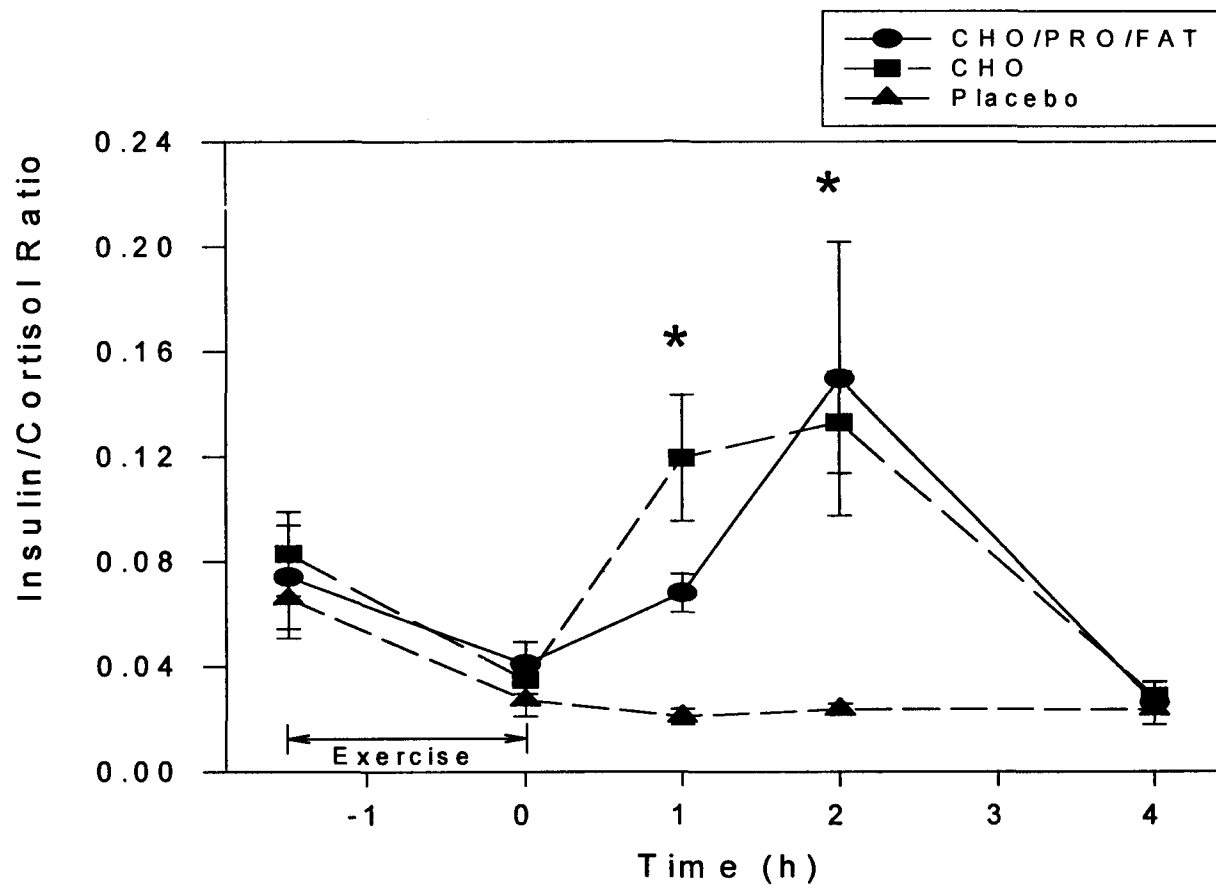


Figure 25. Insulin/Cortisol ratio for CHO/PRO/FAT, CHO and PL (Placebo) with respect to time (mean $\pm$ SE). * $p < 0.01$ for CHO/PRO/FAT and CHO vs. PL. (Placebo).

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APPENDIX I:**ANOVA SUMMARY TABLES**

STAT. GENERAL MANOVA	Summary of all Effects; design: (infusion.sta) 1-CONDITN				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	.000539	18	.001008	.534330

STAT. GENERAL MANOVA	Summary of all Effects; design: (infusion.sta) 1-CONDITN	
Effect	p-level	
1	.595074	

STAT. GENERAL MANOVA	Summary of all Effects; design: (lactate.sta) 1-CONDITIO, 2-PRE/POST				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	6.3682	18	3.095204	2.05743
2	1*	586.5627*	9*	9.205629*	63.71782*
12	2	5.9122	18	2.572907	2.29785

STAT. GENERAL MANOVA	Summary of all Effects; design: (lactate.sta) 1-CONDITIO, 2-PRE/POST	
Effect	p-level	
1	.156781	
2	.000023*	
12	.129187	

STAT. GENERAL MANOVA	Summary of all Effects; design: (glucose.sta) 1-CONDITIO, 2-TIME				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2*	95.30772*	18*	7.908935*	12.05064*
2	12*	4.67181*	108*	.648827*	7.20039*
12	24*	2.46677*	216*	.526207*	4.68784*

STAT. GENERAL MANOVA	Summary of all Effects; design: (glucose.sta) 1-CONDITIO, 2-TIME	
Effect	p-level	
1	.000477*	
2	.000000*	
12	.000000*	

STAT. GENERAL MANOVA	Summary of all Effects; design: (gluarea.sta) 1-CONDITIO				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2*	7.331363*	18*	.608380*	12.05064*

STAT. GENERAL MANOVA	Summary of all Effects; design: (gluarea.sta) 1-CONDITIO	
Effect	p-level	
1	.000477*	

STAT. GENERAL MANOVA	Summary of all Effects; design: (insulin.sta) 1-CONDITIO, 2-TIME				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2*	20215.21*	18*	871.2406*	23.20278*
2	12*	5362.92*	108*	422.1834*	12.70283*
12	24*	1690.93*	216*	236.4171*	7.15231*

STAT. GENERAL MANOVA	Summary of all Effects; design: (insulin.sta) 1-CONDITIO, 2-TIME	
Effect	p-level	
1	.000010*	
2	.000000*	
12	.000000*	

STAT. GENERAL MANOVA	Summary of all Effects; design: (insarea.sta) 1-CONDITIO				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2*	1555.016*	18*	67.01851*	23.20278*

STAT. GENERAL MANOVA	Summary of all Effects; design: (insarea.sta) 1-CONDITIO	
Effect	p-level	
1	.000010*	

STAT. GENERAL MANOVA	Summary of all Effects; design: (cortisol.sta) 1-CONDITIO, 2-TIME				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	288454.8	18	91091.13	3.166662
2	4	108164.1	36	65779.10	1.644354
12	8	25507.5	72	30518.92	.835794

STAT. GENERAL MANOVA	Summary of all Effects; design: (cortisol.sta) 1-CONDITIO, 2-TIME	
Effect	p-level	
1	.066319	
2	.184411	
12	.574234	

STAT. GENERAL MANOVA	Summary of all Effects; design: (cortarea.sta) 1-CONDITIO				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	57690.96	18	18218.22	3.166662

STAT. GENERAL MANOVA	Summary of all Effects; design: (cortarea.sta) 1-CONDITIO	
Effect	p-level	
1	.066319	

STAT. GENERAL MANOVA	Summary of all Effects; design: (testos.sta) 1-CONDITIO, 2-TIME				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	13.3885	18	29.32453	.45656
2	4*	271.3935*	36*	17.14686*	15.82759*
12	8*	41.1211*	72*	6.57808*	6.25123*

STAT. GENERAL MANOVA	Summary of all Effects; design: (testos.sta) 1-CONDITIO, 2-TIME	
Effect	p-level	
1	.640593	
2	.000000*	
12	.000004*	

STAT. GENERAL MANOVA	Summary of all Effects; design: (test.sta) 1-CONDITIO				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	2.677704	18	5.864906	.456564

STAT. GENERAL MANOVA	Summary of all Effects; design: (test.sta) 1-CONDITIO	
Effect	p-level	
1	.640593	

STAT. GENERAL MANOVA	Summary of all Effects; design: (glycog.sta) 1-CONDITIO, 2-TIME				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	3*	34353.52*	21*	11003.19*	3.12214*
2	1*	38211.45*	7*	1163.56*	32.84011*
12	3*	5458.92*	21*	1705.51*	3.20076*

STAT. GENERAL MANOVA	Summary of all Effects; design: (glycog.sta) 1-CONDITIO, 2-TIME	
Effect	p-level	
1	.047688*	
2	.000712*	
12	.044261*	

STAT. GENERAL MANOVA	Summary of all Effects; design: (creat.sta) 1-CONDITN				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	.091081	14	.058350	1.560934

STAT. GENERAL MANOVA	Summary of all Effects; design: (creat.sta) 1-CONDITN	
Effect	p-level	
1	.244364	

STAT. GENERAL MANOVA	Summary of all Effects; design: (3mh.sta) 1-CONDITN				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	272.5639	14	404.4214	.673960

STAT. GENERAL MANOVA	Summary of all Effects; design: (3mh.sta) 1-CONDITN	
Effect	p-level	
1	.525472	

STAT. GENERAL MANOVA	Summary of all Effects; design: (urea.sta) 1-CONDITN				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	.698504	14	1.667666	.418851

STAT. GENERAL MANOVA	Summary of all Effects; design: (urea.sta) 1-CONDITN	
Effect	p-level	
1	.665778	

STAT. GENERAL MANOVA	Summary of all Effects; design: (breath.sta) 1-CONDITIO, 2-TIME				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2*	.000153*	18*	.000010*	15.73550*
2	2*	.000002*	18*	.000000*	10.81412*
12	4*	.000001*	36*	.000000*	4.63289*

STAT. GENERAL MANOVA	Summary of all Effects; design: (breath.sta) 1-CONDITIO, 2-TIME	
Effect	p-level	
1	.000112*	
2	.000823*	
12	.004037*	

STAT. GENERAL MANOVA	Summary of all Effects; design: (t-cratio.sta) 1-CONDITIO, 2-TIME				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2	.000883	18	.001009	.874481
2	4	.001908	36	.000782	2.439027
12	8	.000785	72	.000468	1.676993

STAT. GENERAL MANOVA	Summary of all Effects; design: (t-cratio.sta) 1-CONDITIO, 2-TIME	
Effect	p-level	
1	.434064	
2	.064569	
12	.118846	

STAT. GENERAL MANOVA	Summary of all Effects; design: (i-cratio.sta) 1-CONDITIO, 2-TIME				
Effect	df Effect	MS Effect	df Error	MS Error	F
1	2*	.032414*	18*	.002103*	15.41098*
2	4*	.029050*	36*	.003511*	8.27490*
12	8*	.010037*	72*	.003253*	3.08520*

STAT. GENERAL MANOVA	Summary of all Effects; design: (i-cratio.sta) 1-CONDITIO, 2-TIME	
Effect	p-level	
1	.000126*	
2	.000077*	
12	.004778*	

APPENDIX II:

Consent form for Study #1:

THE EFFECTS OF GLUCOSE SUPPLEMENTATION ON MUSCLE PROTEIN SYNTHESIS FOLLOWING RESISTANCE TRAINING

INFORMATION AND CONSENT FORM

The principal investigators for this project are Dr. Duncan MacDougall, Dr. Mark Tarnopolsky and Mr. Brian Roy. They will provide you with a detailed verbal description of the procedures involved in the study. In addition, you are asked to carefully read the following information form and sign it if you wish to be a subject for this study.

A. PURPOSE:

The purpose of this study is to examine the effects of glucose supplementation on muscle protein synthesis following a session of leg weight training.

B. PROCEDURES:

The study will require you to visit the lab on a number of occasions. During your first visit to the lab, your maximal leg strength will be assessed on two different weight training exercises. On your next visit to the lab you will be required to perform the same two weight training exercises that you did on your first visit. One leg will actually perform the exercise, while the other will serve as a non-exercise control for comparison. Approximately 1.5 hours before the exercise session a small plastic catheter will be placed in a vein on the top of your hand (to allow blood sampling) and another in a forearm vein. You will then receive a injection of a small amount of the amino acid (building block of protein) leucine into a vein in your arm. The only difference between this substance and what you normally consume every day is that it is a more concentrated form(99%) of a naturally occurring non-radioactive tracer(~1.1% of all carbons) so that it can be identified as being different from the protein which is naturally found in your muscle. The tracer amino acid occurs naturally in normal diets, but in small amounts. You would naturally consume a total of about 2X this amount in a normal diet. This procedure will take approximately 12 hours, and will require an overnight stay in the lab.

Following the exercise, two samples of muscle will be taken from the vastus lateralis outer thigh) of each leg (four samples in total) using a needle biopsy procedure. This technique involves freezing the skin(using local anesthetic), making a small incision(0.5cm) in the skin, and the removal of a small amount of muscle tissue by a skilled physician using a special needle. From these samples, the amount of labeled tracer that is taken up by the muscle can be determined and a protein synthetic rate can be calculated. This entire procedure will be done on two occasions. One will involve consuming a beverage containing glucose(approximately four chocolate bars worth) immediately following the exercise bout, and the other consuming a similar beverage 6 hours prior to the exercise bout. By comparing the two situations, we will be able to determine the effect of elevated insulin levels on muscle protein synthesis in the legs.

The biopsy samples will be taken from each leg approximately 2 hours following the start of the tracer infusion, and again at the end of the infusion. During the infusion you will be required to stay overnight in the lab. During this time in the lab you can read, study or watch videos and go to sleep at your normal bed time. You will also be required to collect urine in a container for 24h on each occasion.

C. DETAILS OF THE PROCEDURE AND POSSIBLE RISKS:

(1) Labeled leucine infusion

Leucine is an amino acid which you consume daily in your food when you eat meat and dairy products. It is an essential amino acid, which means your body is not capable of producing it, thus it is ideal for tracer studies. A small plastic catheter will be placed in a vein in your forearm and sterile, non-radioactive labeled leucine will be infused into your vein over a period of approximately 12 hours using a Harvard infusion pump. There is no discomfort associated with this technique, and is expected that you will sleep through most of the procedure.

The entire procedure will be carried out by a skilled physician with strict adherence to sterile procedures. Following the infusion, there may be slight bruising around the needle area, which may persist for up three days. Since the injected tracer is non-radioactive, there are no known risks to health/fertility associated with this procedure (which has been used in studies with human subjects for over 30 years).

(2) Needle biopsy procedure

This procedure involves the local injection of an anesthetic ("freezing") into the skin of the outer thigh area, after which a small (4mm) incision will be made and a small (50-100mg) piece of muscle will be removed using a sterile hollow needle. After the procedure, a suture will be used to close the skin, and ice and pressure will be applied to minimize bruising. The procedure will be performed by a skilled physician who is thoroughly familiar with the needle biopsy technique (having performed it more than 3000 times).

Complications with the procedure are rare. However, in our experience with athletes, 1 in 3000 subjects experience a local skin infection, 1 in 400 have temporary (up to 4 months) localized loss of sensation in the skin at the site of incision, and a few subjects have mild bruising around the incision for 4-5 days. Some subjects (1 in 500) experience a small lump at the biopsy site that could last 4-6 months. There is also a very rare (one in a million) chance that you may be allergic to the local anesthetic.

D. CONFIDENTIALITY OF RESULTS:

The data collected will be used in preparation of reports to be published in scientific journals. Subjects will not be identified by name in these reports. You will have access to your own data and the group data when it is available for your own interest.

E. REMUNERATION:

You will receive an honorarium of \$150.00 for the completion of the study to help compensate for your time commitment.

F. FREEDOM TO WITHDRAW FROM THE STUDY:

You are free to withdraw from the study at any time. If after reading the above information, you are interested in participating as a subject, please read the statement below and sign in the space provided.

G. IN CASE OF EMERGENCY:

Please contact Brian Roy at either 525-9140 ext. 24625 or 525-1342, he will be able to get in contact with Dr. Tarnopolsky immediately. If you cannot get through call Dr. Tarnopolsky at 527-1295, he or a physician filling in for him will be available 24h a day throughout the duration of the study in the study.

H. QUESTIONS OR CONCERNS:

Please contact Brian Roy at either 525-9140 ext. 24625 or 525-1342.

I _____ HAVE READ AND UNDERSTAND THE ABOVE
EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT,
AND AGREE TO PARTICIPATE AS A SUBJECT.

SIGNATURE

DATE

WITNESS

DATE

Consent form for study #2:

**THE EFFECTS OF VARIOUS NUTRITIONAL SUPPLEMENTS ON
PROTEIN SYNTHESIS AND GLYCOGEN RESYNTHESIS FOLLOWING
RESISTANCE TRAINING**

INFORMATION AND CONSENT FORM

The principal investigators for this project are Dr. Mark Tarnopolsky, Dr. J. D. MacDougall and Mr. Brian Roy. They will provide you with a detailed verbal description of the procedures involved in the study. In addition, you are asked to carefully read the following information form and sign it if you wish to be a subject for this study.

A. PURPOSE:

The purpose of this study is to examine the effects of different nutritional supplements on muscle protein synthesis, whole body protein synthesis and glycogen resynthesis following a session of resistance training.

B. PROCEDURES:

The study will require you to visit the lab on a number of occasions. During your first visit to the lab, your maximal strength will be assessed on a number of different weight training exercises. On your next visit to the lab you will be required to perform the same weight training exercises that you did on your first visit. Approximately 1.5 hours before the exercise session a small plastic catheter will be placed in a vein on the top of your hand (to allow blood sampling) and another in a forearm vein. You will then receive a injection of a small amount of the amino acid (building block of protein) leucine into a vein in your arm. The only difference between this substance and what you normally consume every day is that it is a more concentrated form(99%) of a naturally occurring non-radioactive tracer(~1.1% of all carbons) so that it can be identified as being different from the protein which is naturally found in your muscle. The tracer amino acid occurs naturally in normal diets, but in small amounts(~1.1%). You would naturally consume a total of about 2X this amount over a week in a normal diet..

Following the exercise, 8 samples of muscle(total for 3 trials) will be taken from the vastus lateralis (outer thigh) of the leg using the needle biopsy procedure. This technique involves freezing the skin(using local anesthetic), making a small incision(0.5cm) in the skin, and the removal of a small amount of muscle tissue by a skilled physician using a special needle. From these samples, the amount of labeled tracer that is taken up by the muscle can be determined and a protein synthetic rate can be calculated, and in addition, glycogen concentrations will also be determined. This entire procedure will be done on 3 occasions. One will involve consuming a beverage containing glucose(approximately 4 chocolate bars worth) immediately following the exercise bout, one containing glucose and protein ("Results") and the other consuming a placebo beverage immediately following the exercise. By comparing the 3 situations, we will be able to determine the effect of the various supplements on insulin levels and protein synthesis. The biopsy samples will be taken immediately upon completion of the exercise and again 4 hours after the exercise. In addition, intermittently during the infusion expired breath samples will be

collected to allow determination of the whole body protein synthetic rate. During the infusion you will be required to stay in the lab. During this time in the lab you can read, study or watch videos. You will also be required to collect urine in a container for 24h on each occasion.

C. DETAILS OF THE PROCEDURE AND POSSIBLE RISKS:

(1) Labeled leucine infusion

Leucine is an amino acid which you consume daily in your food when you eat meat and dairy products. It is an essential amino acid, which means your body is not capable of producing it, thus it is ideal for tracer studies. A small plastic catheter will be placed in a vein in your forearm and sterile, non-radioactive labeled leucine will be infused into your vein over a period of approximately 4 hours using a Harvard infusion pump. There is no discomfort associated with this technique, and is expected that you will sleep through most of the procedure.

The entire procedure will be carried out by a skilled physician with strict adherence to sterile procedures. Following the infusion, there may be slight bruising around the needle area, which may persist for up three days. Since the injected tracer is non-radioactive, there are no known risks to health/fertility associated with this procedure (which has been used in studies with human subjects for over 30 years).

(2) Needle biopsy procedure

This procedure involves the local injection of an anesthetic ("freezing") into the skin of the outer thigh area, after which a small (4mm) incision will be made and a small (50-100mg) piece of muscle will be removed using a sterile hollow needle. After the procedure, a suture will be used to close the skin, and ice and pressure will be applied to minimize bruising. The procedure will be performed by a skilled physician who is thoroughly familiar with the needle biopsy technique (having performed it more than 3000 times).

Complications with the procedure are rare. However, in our experience with athletes, 1 in 3500 subjects experience a local skin infection, 1 in 400 have temporary (up to 4 months) localized loss of sensation in the skin at the site of incision, and a few subjects have mild bruising around the incision for 4-5 days. Some subjects (1 in 500) experience a small lump at the biopsy site that could last 4-6 months. There is also a very rare (one in a million) chance that you may be allergic to the local anesthetic.

D. CONFIDENTIALITY OF RESULTS:

The data collected will be used in preparation of reports to be published in scientific journals. Subjects will not be identified by name in these reports. You will have access to your own data and the group data when it is available for your own interest.

E. REMUNERATION:

You will receive an honorarium of \$250.00 for the completion of the study to help compensate for your time commitment.

F. FREEDOM TO WITHDRAW FROM THE STUDY:

You are free to withdraw from the study at any time. If after reading the above information, you are interested in participating as a subject, please read the statement below and sign in the space provided.

G. IN CASE OF EMERGENCY:

Please contact Dr. Tarnopolsky immediately at 525-9140 ext.27037 or 23589 or 527-1295, he or a physician filling in for him will be available 24h a day throughout the duration of the study.

H. QUESTIONS OR CONCERNS:

Please contact Brian Roy at either 525-9140 ext. 24625 or 525-1342.

I _____ HAVE READ AND UNDERSTAND THE ABOVE
EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT,
AND AGREE TO PARTICIPATE AS A SUBJECT.

SIGNATURE

DATE

WITNESS

DATE

APPENDIX III:**Assay Principals and Information**

Cotisol, Insulin, and Total testosterone:

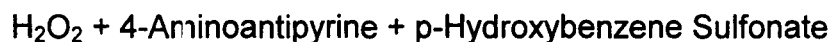
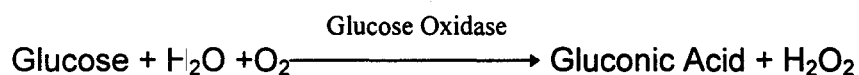
All of the above hormones determinations were done using Coat-A-Count[®] solid-phase radioimmunoassays(¹²⁵I). The kits are manufactured by Diagnostic Products Corporation, but are purchased through Inter Medico in Mississauga, 1-800-387-9643.

- * Note: When ordering these kits, ensure that you request the most recently irradiated hormones. This is important, since the ¹²⁵I hormones have a very short shelf life. Thus you should allow atleast a couple of weeks for delivery.

Plasa Glucose:

Glucose (Trinder)(Pro# 315), Sigma Diagnostics, St. Louis, 1-800-325-0250.

Principal:

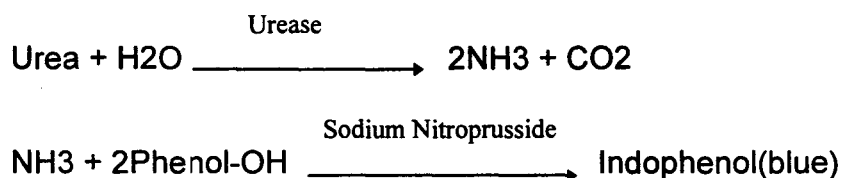


The intensity of the colour produced is directly proportional to the glucose concentration in the sample.

Urea Nitrogen:

Procedure No. 640, Sigma Diagnostics, St. Louis, 1-800-325-0250.

Principal:

Creatinine:

Procedure No. 555, Sigma Diagnostics, St. Louis, 1-800-325-0250.

Derivitization of α KIC: (Wolfe, 1992)(Adapted by Tarnopolsky et al, 1992)

Reagents and Materials

Absolute ethanol (200 proof)
O-phenylenediamine (OPDA) solution 2% in 4 m HCl
Ethyl acetate
BSTFA with 1% TMCS¹²

Procedure

1. Place 5 mL of absolute ethanol in 16 x 100 mm disposable culture tube.
2. Add 1 mL freshly drawn whole blood or 1 mL plasma. Shake vigorously and centrifuge at 2,000 rpm for 20 minutes at 4°C.
3. Remove supernatant to 13 x 100 mm screw top tube.
4. Evaporate to dry under N₂ at 50°C.
5. Dissolve the residue in 1.0 ml of d.d H₂O.

6. React the solution with 1.0 ml freshly prepared OPDA. (This solution is photosensitive, so use immediately after preparation. Solution has deteriorated when it is no longer pink.)
7. Cap the tube and heat for 1 hour at 100°C, then allow to cool.
8. Extract twice with 2.5 mL methyl chloride; centrifuge at 2,500 rpm for 2-3 minutes at 4°C. Combine the extract in 16 x 100 mm screw top tube.
9. Dry under N₂ at room temperature.
10. GCMS conditions:
 - a. Add 75 µL 1.1 BSTFA with 1% TMCS, heat at 100°C for 30 min.
 - b. Inject 0.3 µL of derivitized sample onto a 15 m fused silica capillary column(0.25 mm i.d.).
GC: 120°-160°C at 8°C/min, then ramp to 290°C at 20°C/min and hold at 290°C for 3 min.
 - c. MS: monitor 232.1, 233.1 for alpha-KICA (m + 1)

Muscle Glycogen:**GLYCOGEN EXTRACTION PROTOCOL****Reference:**

Bergmeyer, Hans Ulrich. (1983). Methods of Enzymatic Analysis. New York, Academic Press, Inc. Vol. VI. pp. 11-18.

Pre-extraction Procedure:

(1) Turn on waterbath to 80°C. Remove samples from freezer and allow to equilibrate at room temperature.

(2) Prepare solutions **G5, G6, & G7**.

(i) **G5** - 0.1 M NaOH

(ii) **G6** - 0.1 M HCl

		<u>In Solution</u>	<u>gm/100ml</u>
(iii)	G7 - 0.2 M Citric acid(F.W.- 210.1)	C ₆ H ₈ O ₇ .H ₂ O	4.2
	0.2 M Na ₂ HPO ₄ (F.W.- 142.0)	Na ₂ HPO ₄ .7H ₂ O	5.36

(iv) Amyloglucosidase (**AGS**) (Boehr. 208 469; 2g).

(3) Combine solutions **G5 & G6** in a ratio of 1:1 to see if they neutralize one another in the pH range of **6.2-7.8**. Normally, this pH range is not achieved by a 1:1 solution mix. Experiment with the volumes of HCl and NaOH to see what is needed to achieve the proper range.

(4) If **G5 & G6** neutralize at 1 : 0.74 (for example), then for every 100 µl of **G5** added, there must be 74 µl of **G6** added to neutralize the solution.

- Now prepare the **G7** solution. Mix the aforementioned amounts of solute into 75-80 ml of DDI H₂O and dilute up to 100 ml volume (or desired volume). Use 10 M NaOH to pH the solution up to 5.0.
- According to the 1 : 0.74 neutralization example in step 4, **G5** will be added to a **G6+G7** solution in a ratio of 1 : 4 **during the solution addition of the extraction procedure** (described in the chart below). Therefore, for every 100 µl of **G5** added, 400 µl of the **G6+G7** solution will be added. In this case, within the 400 µl of **G6+G7** we want: 74 µl to be solution **G6** and the remaining 326 µl to be **G7**. Therefore, **G6** and **G7** should be mixed 74 : 326 or 1 : 4.41.

- (5) Now prepare a solution of AGS + G7 with a concentration of 1mg AGS/per ml of G7 (takes time to fully dissolve, mix by inversion DO NOT VORTEX ENZYMES).

Glycogen Standards Preparation

Table I

Glycogen STD. sol'n (ul)	DDI volume (ul)	Glycogen Conc. (nmol/ul)
3	47	75
4	46	100
8	42	200
16	34	400
32	18	800
50	0	1250

- (1) Prepare the dilutions outlined in Table 1 within their respected eppendorf tubes.
 (2) Now proceed with the reaction mixture additions outlined in Table II

Glycogen powder 1 mol = 162g

- Add 81mg of glycogen powder to 5ml of DDI.
- Take 2.5 ml of this 5 ml and add to 7.5ml of DDI for a final volume of 10ml.
- Proceed with chart dilutions.

Calculations:

- $81\text{mg}/5\text{ml} = 16.2\text{mg/ml}$ therefore, 2.5 ml of a 16.2mg/ml solution contains 40.5mg/2.5ml.
- Now dilute this 40.5mg/2.5ml solution with 7.5ml to a total of 10ml. We now have 40.5mg in 10ml of sol'n which equals 4.05mg/ml.

Procedure:

Table II (Standards Preparation)

Muscle STD Solution	G5 (NaOH)(ul)	G6+G7 (HCl, Citrate)(ul)	AGS (ul)	Total (ul)
Prepared dilution (50ul total)	130	520	40	740

Table III (Muscle Preparation)

Dry Muscle(mg)	G5 (NaOH)(μl)	G6+G7 (HCl, Citrate)(μl)	AGS (μl)	Total (ul)
2.0 - 4.0	160	640	40	840

- (1) Add the appropriate amount of **G5** to muscle samples or std. solution according to the table above. **Make sure that all muscle is reached by the solution by gently vortexing**
- (2) Incubate the samples for 10 min at 80°C. This step destroys background glucose and hexose monophosphates.
- (3) Allow samples to cool for 10-15 min. and proceed to neutralize the samples by adding the appropriate amount of **G6+G7** (from table) after allowing the samples to cool. Close Eppendorf tubes and gently shake or invert.
- (4) Add **AGS** as outlined in the table, gently mix and incubate samples at room temperature for 80 min. Glycogen degradation occurs at this step.
- (5) Freeze samples at -50°C (or colder) or measure glycogen (glucose) content directly.

Glycogen (Glucose Assay)

Table IV

No.	Reagents	Wt/ Vol.	Stock Conc. mmol/ l	Cuvette Conc. mmol/l	1 rxn (ul)	20 rxns (μl)	40 rxns (μl)	60 rxns (μl)	80 rxns (μl)
G1	Triethanola mine	7g/100ml	375	100	80	1600	3200	4800	6400
	KOH	0.8g/100ml	150	40					
	Mg(Ac) ₂ .2 H ₂ O	2.4g/100ml	112.5	30					
	EDTA.Na ₂ . 2H ₂ O	0.4g/100ml	3.75	1					
	(adjust to pH 8.2 with KOH)								
G2	ATP	27.7mg/ml	45	0.75	5	100	200	300	400
G3	DTT	9.36mg/ml	60	1	5	100	200	300	400
G4	NAD	19.9mg/ml	30	1	10	200	400	600	800
DDI					150	3000	6000	9000	12ml

Enzymes:

- (1) **G6PDH** - Glucose-6-Phosphate Dehydrogenase (100 NADP units or 228 NAD units), dilute in 200 μl of DDI.
- (2) **HK** - Hexokinase (200 units), dilute in 200 μl of DDI.
- (3) After enzyme reconstitution in separate vials, take all 200ul of the G6PDH and add it to the 200ul HK vial. Next take an additional 400ul to rinse out the G6PDH vial and add it to the HK.

Procedure:

NOTE **Be sure to centrifuge incubated samples before glucose analysis to eliminate any muscle sediment from the reaction mixtures.**

- (1) Add 25 µl of sample, 25 µl of **DDI** and 250 µl of reagent to cuvette. Reagent will consist of solutions G1-G4 & DDI according to the concentrations outlined in table II. Mix by gentle inversion.
 - (2) Measure background absorbance at 340 nm.
 - (3) Add 4 µl of **HK/G6PDH** enzyme mixture to cuvette. Gently mix contents every 5 min and measure absorbance after 15 min at 340 nm. **Ensure enzyme is mixed with sample by gentle inversion.**
- If samples are frozen, allow them to thaw and then centrifuge to pull sediment to tube bottom and ensure particle free extracts for the assay.
 - **NOTE:** Given the variability in the measure of muscle glycogen content, two aliquots of a given muscle sample should be extracted and assayed separately. Acceptable variation between aliquots is 10%.
 - **Be sure to multiply final glycogen concentrations by 2 to account for the 2X dilution of the muscle samples in step 1 from above.**

Expectations:

- Normal glycogen values range from **50-600 mmol/kg D.W. = 50-600 nmol/mg D.W.**

REAGENT STABILITIES

G5, G6, G7 - All stable in solution at room temperature for max of 3 months.

G1 - Can be stored at room temperature for several months.

G2 (ATP) - Can be solvated with DDI, frozen and stored at -50°C for several months. Avoid excessive freeze thaw cycles.

G3 (DTT) - Can be solvated with DDI, frozen and stored at -50°C for several months. Avoid excessive freeze thaw cycles.

G4 (NAD)- Same as above two solutions.

Enzymes:

- G6PDH-** Can be frozen for two months at conc. of 1mg/ml. No more than one freeze thaw cycle is recommended. Can be frozen for 2 months, it is suggested to make up fresh each time.
- Hexokinase-** Can store frozen for 30 days at -50°C with repeated freeze thaw cycles.