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EFFECT OF β-HYDROXY- β-METHYLBUTYRATE (HMB) SUPPLEMENTATION ON THE BODY-COMPOSITION AND MUSCLE POWER OUTPUT OF NON COMPETITIVE SPORTING MALES BETWEEN 19 AND 24 YEARS WHO PERFORMED RESISTANCE TRAINING THREE TIMES A WEEK FOR 8 WEEKS

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TABLE OF CONTENTS

CHAPTER 1

Exercise & Resistance training: How does it influence muscular physiology of the skeletal muscle system?

CHAPTER 2

Beta-hydroxy-beta-methylbutyrate (HMB): a complete literature review

CHAPTER 3

Effect of beta-hydroxy-beta-methylbutyrate (HMB) supplementation on the body-composition and muscle power output of non competitive sporting males between 19 and 24 years who perform resistance weight training three times a week for 8 weeks.

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ABBREVIATIONS

3MH	=	3 – Methylhistidine
ACSM	=	American College of Sport Medicine
ADP	=	Adenosine diphosphate
AT	=	Aerobic Threshold
ANOVA	=	Analysis of Variance
ATP	=	Adenosine triphosphate
BMR	=	Basal Metabolic Rate
BP	=	Blood Pressure
Bpm	=	Beats per minute
BCAA	=	Branched Chain Amino Acid
Ca^{2+}	=	Calcium ions
CHO	=	Carbohydrates
CK	=	Creatine Kinase / Creatine Phosphokinase
CNS	=	Central Nervous System
CO	=	Cardiac Ouptut
CO_2	=	Carbon Dioxide
CrP	=	Creatine Phosphate
CSH	=	Cholesterol Synthesis Hypothesis
DOMS	=	Delayed Onset Muscle Soreness
EMG	=	Electromyography
ETP	=	Electron Transport
$FADH_2$	=	1,5-dihydro-flavin adenine dinucleotide
FT	=	Fast twitch fibers
FFA	=	Free Fatty Acids
GGT	=	Gamma glutamyl transpeptidase
H_2CO_3	=	Carbonic Acid
H_2O	=	Water
$HCO3^{-}$	=	Bicarbonate ion
HMB	=	β-Hydroxy- β-Methylbutyrate
HR	=	Heart Rate
IMP	=	Inositol monophosphate
In vivo	=	In the cell
Kg	=	Kilograms

KIC	**=**	**α-Ketoisocaproate**
Km	**=**	**Michaelis-Menten constant**
LDH	**=**	**Lactate Dehydrogenase**
LDL	**=**	**Low Density Lipoproteins**
LT	**=**	**Lactate**
Na^+	**=**	**Sodium ions**
NAD	**=**	**Nicotinamide-adenine dinucleotide**
NADH	**=**	**Reduced form of NAD**
O_2	**=**	**Oxygen**
PCr	**=**	**Phosphocreatine**
P_i	**=**	**Inorganic phosphate**
RM	**=**	**Repetition Maximum**
SGOT	**=**	**Serum glutamic oxaloacetic transaminase**
SGPT	**=**	**Serum glutamate pyruvate transaminase**
SPSS	**=**	**Statistical Package for the Social Sciences**
SR	**=**	**Sarcoplasmic reticulum**
ST	**=**	**Slow twitch fibers**
SV	**=**	**Stroke Volume**
VE	**=**	**Ventilation**
VLDL	**=**	**Very Low Density Lipoproteins**
VO_2	**=**	**Oxygen ventilation / oxygen uptake**
VT	**=**	**Ventilatory threshold**
W	**=**	**Work rate / Watt**

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FIGURES

TABLES

ABSTRACT

Physically active men and woman may be less likely than their inactive peers to become overweight. Exercise has a favourable effect on body fat distribution, with a reduction in waist-to-hip ratio with increased exercise. Exercise is especially important in maintaining weight loss in overweight individuals. Physical activity can directly affect both total energy intake and total energy expenditure. Physical activity can also affect fat balance and it is becoming clear that imbalances in total energy are largely due to imbalances in the distribution of fat. Exercise also has additional, beneficial effects on most of the metabolic risk factors for cardiovascular disease and non-insulin dependent diabetes mellitus. Exercise testing provides a basis for the design of training programs and allows for monitoring progress throughout the training program. Used properly, testing and monitoring is useful to both trainers and subjects. Therefore, exercise in conjunction with an appropriate diet is beneficial to overweight individuals and provided that feasible methods and motivation are available, we recommend exercise as an important part of a weight control program. The aim of this study was to determine whether HMB supplementation will increase the Lean Body Mass (LBM) and muscle power output (measured as the load a subject can bench press) of males who gym for recreational purposes, after a combination of resistance weight training, eating a balanced set diet and supplementation with HMB for 8 weeks. Two homogenous groups of 20 males were evaluated for initial strength capabilities and body composition. For 8 weeks the subjects lifted weights three times a week and followed a balanced diet. Creatine-kinase activity decreased with HMB supplementation. Gains in muscle power output were greater in the experimental group, and fat percentage decreases were recorded with HMB supplementation.

Keywords: Exercise, overweight, diet, HMB

OPSOMMING

Fisies aktiewe mans en vroue mag dalk minder geneig wees om gewig op te tel as individue wat nie aktief is of oefen nie. Voldoende en gereelde oefening het 'n gunstige effek op die liggaamsvet verspreiding van individue asook 'n verlaging in die "waist-to-hip" (maag-tot-heup) verhouding. Oefening is veral belangrik in die handhawing van 'n gewenste liggaamsmassa of gewigsverlies by oorgewig individue. Fisiese oefening kan 'n direkte effek hê op die totale energie innname en totale energie verbruik van individue. Fisiese oefening kan ook die vet balans van 'n persoon affekteer en dit wil voorkom of energie wanbalanse gekoppel kan word aan wanbalanse in vet verspreiding. Oefening het bykomende voordelige effekte op meeste van die metaboliese risiko faktore wat verantwoordelik is vir kardiovaskulêre siektes en insulilen onafhanklike diabetes mellitus. Om hierdie redes kan oefenings toetse die grondslag gee vir die ontwikkeling van oefen programme en monitering. Die prosesse van evaluering en opvolg kan dus bruikbaar wees vir beide atlete en afrigters, mits die proses selfstandig toegepas word. Diëet, gepaard met 'n oefen program, kan voordelig wees vir 'n oorgewig persoon as die nodige motivering en uitvoerbare metodes van oefen beskikbaar is. Die effek van beta-hydroksie-beta-metielbuturaat (HMB) supplementasie op die liggaamssamestelling en spierkrag van nie kompeterende jong mans tussen die ouderdom van 19 en 24 jaar, wat weerstands oefeninge doen 3 keer per week vir 8 weke lank, is getoets. Twee homogene groepe van 20 mans was geevalueer om hulle oorspronklike kragkapasiteit en liggaamssamestelling te toets. Vir 8 weke het elkeen in die steekproef drie keer 'n week met gewigte in 'n gimnasium geoefen en 'n gebalanseerde dieet gevolg. Creatine-kinase aktiwiteit het verlaag met HMB supplementasie. Verlagings in vetpersentasie is ook in die eksperimentele groep waargeneem.

CHAPTER 1

EXERCISE AND RESISTANCE TRAINING: WHAT IS IT? HOW DOES IT INFLUENCE SKELETAL MUSCLE PHYSIOLOGY?

1.1   INTRODUCTION

Resistance training is very popular among teenagers and young adults. The definition of exercise in Taber's Cyclopaedic Medical Dictionary is: "A performed activity of the muscles" (Thomas, 1989) or in simple terms: contraction of any of the muscles can be classified as exercise according to scientific terms.

Young men especially train to enhance their physical appearance, but young woman also seem to be taken with the idea of looking physically more appealing, since the health and wellness trend has taken the world by storm (Aulin, 1995; Bryner, 1997; Kraemer, 1997). For example woman's magazines used to focus on style, fashion and cosmetics, but there are a few magazines that are starting to give attention to physical fitness and wellness. An example of such a magazine is "SHAPE". Plenty of these "health-and-fitness-lifestyle-focused-people's" social and recreational time is spent in gymnasiums or on sports grounds among friends.

The two primary objectives of resistance training among men are to increase the strength of skeletal muscles and to enhance or improve their size.

The muscular system is affected by resistance training (Brown, 1985; Armstrong, 1986; Evans & Cannon, 1991; Evans 1995; Garrow, 1995; Fielding, 1995; Hill, 1996; Fox, 1999) and to understand the relationship between resistance training, muscle growth and strength, close attention needs to be given to the physiological aspects that play a key role in the structure, functioning and regulation of the muscular system. For example, the muscle cells (fibres) must be able to produce and utilize adenosine triphosphate (ATP) to provide the energy for contraction and force production (Korzeniewski, 1998). This process requires that the digestive, respiratory, endocrine and cardiovascular systems operate effectively to provide the muscle cells with the oxygen and nutrients they require to produce the energy.

1.2   PHYSICAL EXERCISE

Understanding how physical exercise affects the muscular system will provide the necessary background information on how HMB supplementation can affect muscular physiology.

Physical exercise can be defined as a general term for any sort of muscular effort, but especially the kind intended to train, condition, or increase flexibility of the muscular and skeletal systems of the body according to the "Taber's Cyclopaedic Medical Dictionary" (Thomas, 1989). It can also be defined as a physiological state of well being that provides the foundation for the tasks of daily living; a degree of protection against hypokinetic disease and a basis for participation in sport (Plowman *et al.,* 1991). A person's physical fitness can be described by a set of factors. These factors are cardio-respiratory endurance, muscular endurance, muscular strength, body composition and joint flexibility. Physical fitness can be acquired by exercising regularly.

Exercise is a physical activity that is planned, structured, and repetitive with the aim to reach a physical objective. Exercise can therefore be also defined as bodily exertion or muscular activity that requires an expenditure of energy above resting level that in most, but not all, cases results in voluntary movement (Plowman *et al.,* 1991). In other words, even lifting a single heavy object and transferring it from one point to another will require higher than normal energy utilisation. Therefore in some instances exercise can even be defined by a single acute bout of physical exertion depending on the objective of the training regime. One can train for endurance purposes (running, cycling) or to improve strength (weight lifting). Exercise itself is a form of science and therefore a few basic principles need to be followed. To apply these principles, knowledge of how the body reacts to exercise is needed and therefore it is of vital importance to understand muscular function, dynamics, and metabolism of muslces (Van de Graaff & Fox, 1989).

Muscle accounts for almost half of the body's weight. Skeletal muscle mass of the body ranges between 32% and 40% respectively for women and men (Van de Graaff & Fox, 1989). Similar to the other physiological systems in the body, skeletal

muscles can be classified as part of the muscular system which is responsible for movement of the whole body.

Regular exercise benefits the health of a human being (Brown *et al.,* 1985). Research suggests that moderate regular exercise should be considered as a worthwhile means of treating depression, anxiety, as well as poor mental and physical self-perceptions (Fox, 1999).Physical work can be classified according to the various types of work: (a) positive dynamic work: work that is performed by alternatively contracting and relaxing muscle (e.g. walking uphill); (b) negative dynamic work: work is performed to counteract the stretching muscle groups, in alternation with contraction under zero external load (e.g. walking downhill); (c) static (postural) work (e.g. standing still) (Despopoulos & Sibernagl, 1991).

A number of normal daily and athletic activities require isometric or static exercise (Longhurst & Stebbins, 1997). Power athletes use weight lifting and other high-resistance activities to gain isometric strength and skeletal muscle bulk. These exercises, the predominant activities used in power training, also increase blood pressure, heart rate, myocardial contractility (contraction of the cardiac muscles) and cardiac output during the exercise session (Longhurst, 1997 & Wright, 1999). These changes during static exercise occur under control of central command from the higher brain centres and the central nervous system (CNS), as well as spinal reflexes triggered from the statically contracting muscle (Longhurst, 1997; Paasuke *et al.,* 1999).

Blood pressure appears to be one of the regulated variables in resistance training. Increased arterial pressure provides blood flow into muscles where the arterial inflow is restricted as a result of increases in intramuscular pressure created by skeletal muscle contraction (Longhurst, 1997 & Paasuke, 1999). The pressure load on the heart during static exercise can be differentiated from the hemodynamic response to dynamic (isotonic) exercise, which involves a volume load to the heart (Longhurst, 1997). Physical training with static exercise (i.e. power training) leads to concentric cardiac (particularly left ventricular) hypertrophy, and training with dynamic exercise leads to eccentric hypertrophy. Neither systolic nor diastolic pressure is altered by the hypertrophic process associated with static exercise training (Longhurst, 1997).

Much of the energy requirements during severe levels of static exercise, are met by anaerobic glycolysis, because the blood flow to the contracting muscle is reduced (Longhurst, 1997). This enables the muscles to provide a quick efficient supply of adenosine triphospate (ATP) to the necessary contractile components within a muscle. Maximal oxygen consumption (VO_{2max}) is increased minimally, if at all, in power athletes training with repetitive static exercises (Longhurst, 1997). Peripheral cardiovascular adaptations, for example a decrease in resting arterial blood pressure, a reduced increase in sympathetic nerve activity during a given workload; enhanced baro-reflex function; increases in muscle capillary-to-fiber ratio; improvements in lipid and lipoprotein profiles and increases in glucose and insulin responsiveness, can occur in response to training with regular static exercise (Longhurst, 1997; Iellamo *et al.,* 1999).

The manner in which resistance training is performed may dictate the extent to which these adjustments take place in both healthy individuals and those with cardiovascular disease. Differences in duration, frequency and intensity of physical activities may create considerable variations in total energy expenditure (Van Baak, 1999). For moderate (below lactate threshold) exercise, and also with a rapidly increasing workload, oxygen uptake (VO_2) increases as a linear function of work rate (Poole, 1997). In heavy muscular work the muscles have to be supplied with up to 500 times more oxygen (O_2) than when the body is in a resting state, and at the same time, larger quantities of carbon dioxide (CO_2) and lactate have to be removed (Despopoulos & Sibernagl, 1991). The metabolic and morphological adaptations of resistance and endurance exercise are quite different. A subject's response to endurance exercise is determined by the resultant response of several interrelated control systems. This response (performance) is finally determined by a ratio between oxygen transfer to and CO_2 removal from the exercising cells, and secondly (not of less importance) the cells ability to produce ATP aerobically (Weber *et al.,* 1984b; Brown, 1985; Sue, 1989).

1.2.1   ATP-supply during exercise

A continuous supply of O_2 to all tissues is necessary for the efficient production of ATP and this supply is considered sufficient when aerobic metabolism is maintained.

Non-healing wounds, necrotizing infections, radiation-induced necrosis, crush injury, and decompression illness all exhibit impaired tissue oxygenation (Robertson & Hart, 1999). Deficient ATP supply could result in degeneration of neurons, especially those with high energy requirements (Das *et al.,* 1999). The capacity of an individual to endure exercise can therefore be calculated if the above responses, namely oxygen transfer to and CO_2 removal from the exercising cells and the cells ability to produce ATP aerobically, can be measured. For quantitative analysis of metabolic and transport processes associated with ATP production during exercise, a model (Cabrera *et al.,* 1999) was recently developed that links cellular metabolism and its control, to whole body responses at rest. The model suggested that the continuous stimulation of the ATP synthesis process during moderate exercise is mainly due to a higher ADP:ATP relation and not to a higher NADH:NAD (NAD is the abbreviation for Nicotinamide-adenine dinucleotide, NADH is the reduced form of NAD) relation (Cabrera *et al.,* 1999). ATP synthesis rates were measured (in vivo) in the human medial gastrocnemius muscle during high intensity exercise (30 s maximal voluntary rate exercise using localised 31P-magnetic resonance spectroscopy (31P-MRS)). The results support the observation that glycogenolytic and glycolytic rates are elevated, in vivo, in the presence of elevated inorganic phosphate (Pi) levels and do not support the hypothesis that glycogenolysis follows Michaelis-Menten kinetics with an apparent Michaelis-Menten constant (Km) for [Pi] in vivo (Walter *et al.,* 1999).

The results also indicated that measured (in vivo) ATP utilization rates and the initial dependence on phosphocreatine (PCr) and glycolysis were similar to those previously reported in sito studies involving short duration, high intensity exercise. This experimental approach presents a non-invasive, quantitative measure of peak glycolytic rates in human skeletal muscle (Walter *et al.,* 1999).

Baldwin *et al.* (1999) showed that although intramuscular glycogen content was reduced (P<0.05) in both trained [peak oxygen uptake (VO_{2peak}) = 65.8 ± 2.4 ml. kg $^-$^{1}. min $^{-1}$] and untrained individuals [(VO_{2peak}) = 46. 2 ± 1.9 ml. kg $^{-1}$. min^{-1}], at fatigue, inositol monophosphate (IMP), a marker of a mismatch between ATP supply and demand, was only elevated (p<0.01) in untrained muscle (fourfold higher) at fatigue. In a study by Korzeniewski *et al.* (1998) a dynamic computer model of oxidative phosphorylation was used to study the question of how ATP production

rate, via oxidative phosphorylation, is adjusted to meet energy demands during muscle contraction. The results showed that the parallel direct activation of actinomyosin-ATPase and oxidative phosphorylation by an external effector, Ca^{2+}, is the main mechanism responsible for fitting of ATP production to ATP consumption, while an increase in ADP concentration (decrease in ATP:ADP) is responsible for the negative feedback (Korzeniewski, 1998; Kavanagh, 2000). Most of the oxidative phosphorylation steps should be directly activated in order to explain the observed changes in the respiration rate and ADP:ATP ratio (and also in other parameters) during muscle contraction, suggesting that a universal external activator/regulatory mechanism should exist which causes a parallel stimulation of different enzymes/processes (Korzeniewski, 1998).

Three energy-producing systems play an important role in metabolic conversion during physical activity, e.g. breakdown of creatine phosphate (CrP), anaerobic glycolysis and aerobic glycolysis (Martin, 1997).

Like ATP, CrP contains high energy phosphate bonds. During the breakdown of CrP a high-energy ATP is split into ADP and inorganic phosphate with the release of energy. TH energy required to resynthesize ATP from ADP and phosphate is supplied by CrP; when ATP is split, energy is transferred from CrP to ADP by means of the high energy phosphate group and ATP is quickly reformed (Meyer *et al.,* 2002). The reaction is catalysed by the enzyme creatine kinase. The chemical reaction can be seen below.

A maximal effort (100 m sprint) over a brief period (10-25 seconds) can be managed entirely by available stores of CrP in muscle (Despopoulos & Sibernagl, 1991). Creatine, a natural nutrient, is synthesised from the amino acids glycine, arginine and methionine in the kidneys, liver and pancreas, and is predominantly found in skeletal muscle, where it exists in 2 forms. Approximately 40% is in the free creatine form (free Cr), and 60% is in the phosphorylated form, phosphocreatine (PCr) (anonym for creatine phosphate) (Demant, 1999). The daily turnover rate (2 g per day) is equally met via exogenous intake and endogenous synthesis. Although free Cr concentration is higher in fast twitch muscle fibres than in slow twitch fibres, due

to their increased aerobic capacity, they have a greater resynthesis capability (Demant, 1999).

The functions in which creatine are involved during performance are temporal energy buffering, spatial energy buffering, proton buffering and glycolysis regulation (Demant, 1999). Research suggests that oral creatine monohydrate supplementation has resulted in significant increases in total Cr (free Creatine) and CrP for some individuals but not others, suggesting that there are 'responders' and 'non responders' (Williams, 1998; Demant, 1999). Increased concentrations of both free Cr and CrP are believed to enhance performance by providing more short term energy, as well as increasing the rate of resynthesis during rest intervals (Williams, 1998; Demant, 1999), but creatine supplementation does not appear to aid endurance and incremental type exercises. The only side effect associated with creatine supplementation appears to be a small increase in body mass in males, which is due to either water retention or increased protein synthesis (Williams, 1998; Demant, 1999). If the resynthesis of CrP could be sped up it could be hypothesized that an increase in enzyme activity of the enzymes that synthesize CrP should result in quicker recovery from muscle fatigue and ATP production from the resynthesized CrP.

High intensity work carried out over a longer time period (20 seconds to 4 minutes) need far more energy than provided by aerobic metabolism. A person will therefore produce the extra energy via anaerobic glycolysis, using carbohydrates as the fuel source (Despopoulos & Sibernagl, 1991). Carbohydrates (CHO) are used preferentially with rising exercise intensity because of increased CHO uptake, progressive recruitment of type II fibres and sympathetic stimulation of metabolism (Mercier, 1997). Anaerobic metabolism is self-limiting because the accumulation of lactic acid shuts down the metabolic system. As the time period lengthens from about 4 min to 90 min, it becomes possible for the metabolic O_2 demands to be better met by ongoing O_2 intake (aerobic metabolism). Glycogen and triglycerides are available as fuel sources for aerobic metabolism (aerobic glycolysis and lipolysis). Lipid oxidation rises with the duration of exercise and falls with increasing intensity. Proteins can also provide an important source of energy during exercise (Dohm, 1983). Rico-Sanz (1998) examined aerobic and anaerobic muscle energy

production during supra maximal repeated exercise. Their data suggests that oxidative metabolism does not compensate for the reduction of anaerobic glycolysis during repeated fatiguing exercise. Aerobic glycolysis, by proliferating cells, might be a way to minimize oxidative stress during the phase of the cell cycle when maximally enhanced biosynthesis and cell division occur (Brand, 1997).

The maximal capacity for oxygen consumption is usually expressed as the VO_{2max}, which signifies the maximal cardiopulmonary potential (Arts, 1994; Sue 1994). By determining the anaerobic threshold (AT), the aerobic ATP-production limit can be approximated. The mechanisms responsible for lactate production with increased intensity of muscle contraction are controversial. While some investigators suggest that the mitochondrial functions are O_2-limited, others suggest that lactate production occurs when O_2 delivery to the mitochondria is adequate and that the increased lactate production is due to a mass-action effect when pyruvate production exceeds the pyruvate oxidation rate (Heigenhauser, 1999). Pyruvate dehydrogenase plays a central role in the integration of carbohydrate and fat metabolism and in the entry of pyruvate into the tri carboxylic acid cycle, whereas the lactate dehydrogenase enzyme plays a key role in lactate production (Heigenhauser, 1999). At higher exercise intensities, which are more reliant on glycogen as substrate, the rate of pyruvate production exceeds the catalytic activity of pyruvate dehydrogenase, resulting in lactate production (Heigenhauser, 1999).

The absolute blood lactate concentration at rest, and during exercise, appears to be affected by factors like free fatty acid concentrations in blood (Yoshida, 1986), acid-base changes (Kowalchuk, 1984), glycogen content in muscles (Heigenhauser, 1983), diet (Yoshida, 1986) and hypoxic conditions (Yoshida, 1986). (Figure 1.1 & 1.2)
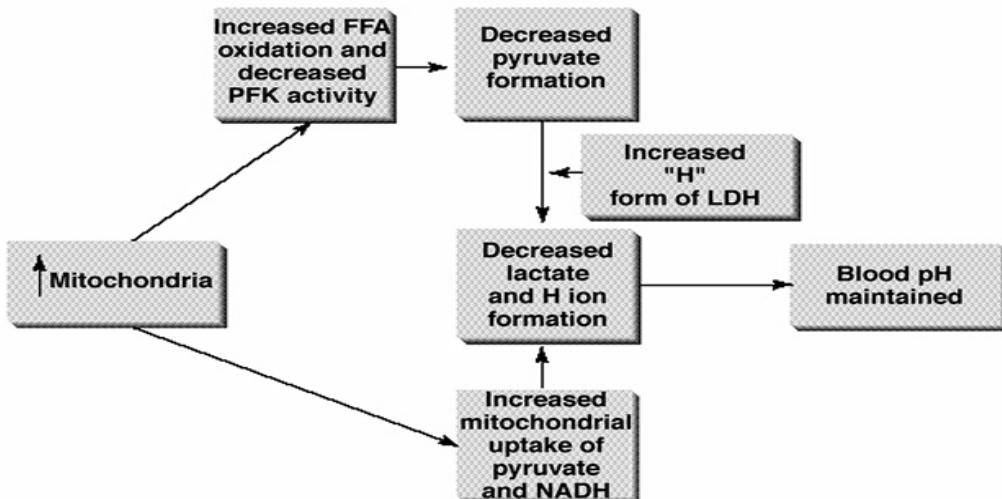
Figure 1.1: Mitochondrial and biochemical adaptations and blood pH (Yoshida, 1986)
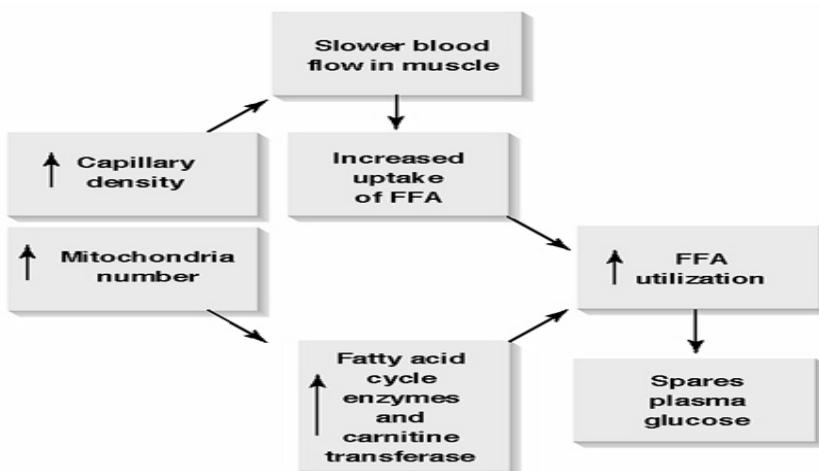
Figure 1.2: Interrelationship between fatty acids and glucose in metabolism (Martin, 1997)

The anaerobic threshold is not only dependent on the biochemical processes involved in aerobic ATP-production, but also on the VO_{2max} (Van de Wolle, 1987; Sue, 1994). The relevance of the anaerobic energy system to human performance and physical fitness throughout the age spectrum is that the anaerobic energy system is involved in providing energy for all forms of physical activity (Cahill, 1997). The intensity of the exercise is an important factor that determines whether anaerobic or aerobic metabolism prevails. For anaerobic exercise to occur, the exercise must be of high intensity and performed to near-exhaustion and can be indirectly assessed by performance tests such as a vertical jump or stair climb, or

more directly by supra maximal bicycle tests (Cahill, 1997). Recent research showed that the elderly respond positively to anaerobic training so that their independence and quality of life are improved (Cahill, 1997). Therefore, appropriate application of the anaerobic system assessments and training principles are an important aspect of sports medicine practice (Cahill, 1997).

Wasserman and McIlroy (1997) first used the AT to define a particular work load at which blood lactate levels first begin to rise above their resting levels. This was accompanied by an increase in the rate of rise in expired ventilation (VE) in milliliters per minute that was greater than the rate of an ongoing increase in O_2 uptake. The term AT suggests the notion that anaerobic metabolic processes increased their role in supplementing aerobic processes to provide energy for movement. The term anaerobic literally means without O_2 (anaerobic metabolism) and the term threshold refers to a region of change (Martin, 1997).

Controversy began when scientists around the world could not confirm Wasserman's results on the anaerobic threshold (Martin, 1997). One reason for the controversy is the existence of two thresholds. The first threshold is observed with mild work, accompanied by breathing changes and a slight rise in blood lactate. The second threshold is observed with more intense exercise, with additional breathing changes and steadily accumulating blood lactate. Chicharro *et al.* (1997) investigated the relationship between the lactate threshold (LT) and the ventilatory threshold (VT) during a ramp protocol in cycle ergometry. Results showed significant differences ($p < 0.05$) between mean values of VT and LT when both were expressed either as heart rate (bpm), work rate (W), or VO_2 ($ml.kg^1.min^{-1}$), which means that LT and VT occur at different exercise intensities during ramp protocol exercise on a cycle ergo meter (Chicharro, 1997).

The change in breathing (ventilatory rise) is explainable on the basis of blood bicarbonate (HCO_3^-) buffering mechanisms:

$$Na^+ + HCO_3^- + H^+ + Lactate \leftrightarrow NaLactate + H_2CO_3 \leftrightarrow H_2O + CO_2$$

Many of the hydrogen (H^+) ions resulting from lactate production combine with HCO_3^- to form carbonic acid (H_2CO_3), limiting the rate of acidosis. After passing through the lungs, H_2CO_3 dissociates to CO_2 and H_2O, with CO_2 being excreted. The higher PCO_2 provides an additional ventilatory stimulus (Harrison, 1930; Martin, 1997).

Lactic acid is produced during resting states and in increasing concentration as exercise intensity increases (Martin, 1997). Red blood cells, a well-known source, have no mitochondria but are capable of anaerobic glycolysis. Pyruvate and lactate diffuse out of the red blood cells into the plasma. Skeletal muscle, and the intestines, can also produce and release lactate into the blood stream. Non-exercising (and even exercising) skeletal muscle (Essen, 1975), the liver (Wahren, 1975), kidneys (Yudlen, 1975) and the heart (Welch, 1973) will metabolise lactate. Aerobic energy metabolism utilizes glucose and oxygen to satisfy the energy needs of the adult brain, while anaerobically, the brain switches to the significantly less efficient glycolytic pathway for its most basic energy requirements and other essential processes (Schurr, 1998). Recent evidence indicated that lactate, produced mainly in glial cells during a period of oxygen deprivation, becomes the only utilizable, and thus obligatory, substrate for aerobic energy metabolism upon re-oxygenation (Schurr, 1998).

This evidence also supports the hypothesis that a lactate shuttle exists between glia and neurons, as well as emphasizing its importance in the post-ischemic survival of neurons (Schurr, 1998).

1.2.2 Exercise can be influenced by endurance, metabolism and nutrition

Regular moderate intensity physical activity and a healthy diet have been recommended for the prevention of atherosclerotic diseases (Dontas, 1999; Rauramaa, 1999). The background for these guidelines is the key role of plasma lipids because hyper lipidaemia and excess of adipose tissue increase platelet aggregability and blood coagulation and decreases fibrinolysis (Rauramaa, 1999). Both regular physical activity and dietary fat reduction increases the turnover of lipid substrates with effects on their transport and disposition, resulting in a decrease in blood lipids and body fat, thereby diminishing the risk of thrombosis (Hardman, 1999; Rauramaa, 1999). Regular moderate intensity physical activity as well as a diet that

is rich in omega-3 fatty acids, decreases platelet aggregability and may even decrease plasminogen activator inhibitor-1, a possible link between insulin resistance syndrome and coronary heart disease. Thus, it can be hypothesized that moderation in physical activity and diet carries a more powerful impact on blood coagulation and fibrinolysis than either lifestyle modification alone (Hardman, 1999; Rauramaa, 1999).

An important aspect of muscle performance is the relation between endurance, metabolism and nutrition. Carbohydrate (CHO) intake plays a particularly major role in the performance of endurance athletes (Maughan, 1999). The resting glycogen concentration in the muscles of an athlete on a carbohydrate rich diet is high (Egger, 1992). Such an athlete will be able to endure exercise for a longer time than before muscle fatigue sets in. On the other hand, an athlete that makes use of a mixed diet will not endure as long (Egger, 1992). During exercise the two primary fuels that are utilized by the muscles for energy are stored lipids and carbohydrates (Berne, 1993). During light and moderate exercise (25% of VO_{2max}), fatty acids are the preferred fuel (Martin, 1997). As exercise continues (65% VO_{2max}), the role of plasma glucose and muscle triglycerides becomes more important, whilst there is a decrease in energy contribution from plasma fatty acids (Ibrahimi, 1999). Between 65% and 85% of VO_{2max}, the energy contribution of plasma glucose increases while the energy contribution from muscle triglycerides and plasma fatty acids decreases (Ibrahimi, 1999).

When the demands for energy increases with an increase in the intensity of the exercise, (85% VO_{2max}) the increased O_2 demands exceed O_2 intake capability and the preferred fuel source changes to carbohydrates (Turcotte, 1992).

Three major issues play an important role in CHO ingestion and exercise: (a) to what extent CHO ingestion influences CHO and fat stores, (b) by what mechanisms changing CHO stores alter the responses to CHO or fat ingestion, and (c) the roles of CHO in exercise performance and metabolism (Graham, 1999).

Dietary manipulation is not a simple issue. By increasing the dietary content of any specific nutrient, the entire diet composition is altered. High CHO diets (often low fat

diets), change the metabolism and storage of both fats and CHO (Martin, 1997). Acute CHO ingestion increases CHO oxidation, prior to, and during, prolonged exercise which can increase endurance. However, chronic high CHO ingestion (without exercise or an active lifestyle) leads to muscle becoming insulin-insensitive, adipose tissue processing CHO to fatty acids, and the liver increasing production of very low density lipoproteins (VLDL). Recent identification of a key enzyme, glycogenin, and two forms of glycogen (pro- and macroglycogen) may lead to a better understanding of the physiological nature of the regulation of CHO stores (Graham, 1999). Glucose can enter either skeletal muscles (for eventual breakdown) or adipose cells (for conversion to triglycerides). Fatty acids can also enter skeletal muscles, where they are metabolized for energy, or adipose cells where the fatty acids can be stored for later transport to muscle tissue (Martin, 1997).

The change in fuels (fatty acids to carbohydrates) is associated with an increased level of lactic acid in the blood, resulting in an increase in respiration, thereby increasing the amount of CO_2 expelled with each breath. Since there is no similar increase in the amount of O_2 being consumed, there is a change in the ratio between the amount of O_2 that is consumed and the amount of the carbon dioxide, which is produced (AT point) (Weber, 1984b).

The increase in the lactic acid level becomes greater as exercise becomes more intense and the muscle cells cannot meet the additional energy demands aerobically. As the demands of exercise increase further with increasing workloads, the muscles require more energy and oxygen, and the need to expel more CO_2 also increases (Weber, 1984b).

1.3   RESISTANCE TRAINING

Resistance exercise works specifically on the skeletal muscles of the human body. It can be defined as a systematic program of exercises involving the exertion of force against a load used to develop strength, endurance, and/or hypertrophy of the muscles (Davies, 1972). Resistance training isn't bonded to one sport type for example only to body building and weight lifting. It can be used towards a wide variety of different sport types and individuals. The possibilities are endless and range from people who want to improve physical appearance and/or athletic

performance; to those who need to rehabilitate injured muscles or even compete in a power lifting or body building contest as mentioned earlier (Demant, 1999).

If resistance training is performed correctly under certain conditions, it can be very beneficial and have a very low risk of any harm to the trainee (Plowman et al, 1991). These conditions include: supervision by a professional, the correct body-posture during each exercise, breathing, mechanics and prescribed loads.

It is important to define and declare the various aspects of resistance training due to the integral role it plays in the experimental study.

1.3.1 Principles of resistance training

The principles that govern exercise are universally applicable to all types of sport and exercise (Plowman *et al.,* 1991). These principles set the standard for planning a successful training program. The application of these principles should be used in any experimental study that involves the development of resistance exercises.

1.3.1.1 Specificity

No two people are the same or identical in any variable that can be measured and they differ in such a vast range of parameters ranging from fingerprints to their genetic composition (Demant, 1999). For this reason no two people can train exactly the same and their goals might and will also differ from one another (Demant, 1999). Some would like to enhance muscular strength; others would want to improve their cardiovascular endurance or even a combination of more than one component of physical fitness.

Muscles respond in a specific manner to different loads and therefore they also contract differently to the loads used and resistance imposed upon them (Marshall, 1980). Sport types such as track and field (sprinting, long jump, shot put) make use of training programs that apply dynamic resistance programs (Shephard, 1990), but other sport types such as swimming make use of an isokinetic program that perform exercises through a specified range of motion (Hardy, 1986).

Resistance training is also specific to the muscle group being used and resistance training program should include at least one exercise for all the major muscle groups of the body (Hubley-Kozey, 1991) it is also wise to arrange exercises in a specific order to prevent fatigue or over exertion to occur, therefore most programs alternate between upper- and lower-body exercises to allow muscles to recover from exertion and between exercises and sessions. Generally, the large muscles groups should be exercised first and then the smaller muscles groups (Hutton, 1992). The amount of stress applied to the muscle will determine the response of the muscle to that stress. A muscle that is exposed to a stress that results in a near maximal effort will develop greater strength than a muscle that is required to repeat many sub maximal contractions (Hardy, 1986; Ethyre 1987; Hutton, 1992). However, performing the greater amount of sub maximal contractions will result in a greater increase in muscular endurance than a few repetitions performed at near maximum effort.

Specificity also appears to apply to the velocity at which a muscle contracts during isokinetic exercises (Malina, 1991). Exercises performed at a slow velocity tend to increase or develop the torque that is specific for that muscle at the velocity of contraction. Training muscles at high velocities tends to increase the strength at and below the exercise velocity and is not as specific as slow velocity contractions. Furthermore, it is a misconception that the velocity of isokinetic exercise should be specific to specific events (Malina, 1991). In reality the angular velocities of joint movements found in many athletic events for example throwing, exceed what can be performed during isokinetic exercise.

1.3.1.2   Individualization

This principle goes hand in hand with the principle of specificity that relates to the differences that exist among people. The first step in individualizing a resistance training program is to determine the individual goals of the participant (Marshall, 1980). Once they are established, it is important to evaluate the current strength level of the participant (Marshall, 1980). Usually the strength level is measured by determining the person's 1 Repetition Maximum (1RM). The 1RM should be established for each of the different muscle groups that are going to be exercised (Marshall, 1980).

The final step is to determine the training cycle that will be used. This is referred to as the periodisation of training and it is found in all athletes' training schedules who use a resistance training program to ad to their own training program (Marshall, 1980). One off the simpler reasons for applying periodisation is that it keeps the participant from getting bored. Competitive athletes also use it to ensure that they peak at the correct time in their season (Marshall, 1980).

There are also a few additional considerations that need to be given thought relating to specificity and individualization. Considerations like: large muscles heal slower than smaller muscles (Hubley-Kozey, 1991); Fast or explosive movements require more recovery time than slow movements (Egger, 1992); fast twitch muscle fibres recover faster than slow twitch muscle fibres (Egger, 1992); women generally need more recovery time than men (Hubley-Kozey, 1991); older athletes generally need more recovery time than younger athletes (Hubley-Kozey, 1991); the heavier the load lifted, the longer it will take the muscles to recover (Egger, 1992).

These are just some of the many differences in athletes. All of these differences will factor into an athlete's training routine. Coaches should also be aware of these differences and not expect all the athletes on a team to perform the exact same routines.

1.3.1.3   *Overload*

The principle of overload states that a greater than normal stress or load on the body is required for training adaptation to take place (Plowman *et al.,* 1991). The body will adapt to this stimulus and once the body has adapted then a different stimulus is required to continue the enhancement of that specific muscle (Malina, 1991). In order for a muscle (including the heart) to increase strength, it must be gradually stressed by working against a load greater than it is used to (Massey, 1956). To increase endurance, muscles must work for a longer period of time than they are used to. If this stress is removed or decreased there will be a decrease in that particular component of fitness. A normal amount of exercise will maintain the current fitness level (Plowman *et al.,* 1991).

The successful application of the overload principle as it applies to resistance training necessitates the manipulation of intensity (load), frequency and duration (number of repetitions, sets and rest periods). Of these variables intensity is probably the most important and seems to have the greatest effect on the outcome of the program (Hubley-Kozey, 1991).

Historically, the development of the programs based on these variables began in 1948 when De Lorme and Watkins (Gomez, 1991) introduced progressive resistance training. Since then, considerable research has been done to estimate what the optimal amount of repetitions, workload, sets and frequency would be to develop muscular strength and endurance (Gomez, 1991). Due to all this research, various training programs have been developed (Gomez, 1991).

Multiple studies indicate that there are not any clearly defined, absolute numbers or single combination of repetitions and sets that should be performed to produce the best results for muscular enhancements in strength and growth (Egger, 1992). The principle of specificity and individualism plays a role in this debate, because the individual's goals rather determine the ideal amount of sets and repetitions. Research indicates that 3-6 repetitions per set for 3-5 sets are probably best for developing muscular strength (Hubley-Kozey, 1991). These types of sessions are performed with higher loads, but if an individual's goal is to enhance muscular endurance the individual would have to perform more repetitions of an exercise at a time.

Depending on whether a person is looking to enhance is strength or muscle endurance, training for gains in strength would have to be performed at 60% of the maximum or higher (Massey, 1956). Gains in endurance can be achieved with an intensity level of 30% of maximum if the muscle group is exercised until fatigued (Ethyre, 1987). Figure 1.3 depicts a theoretical continuum for the development of muscular strength, power and muscle endurance (Hubley-Kozey, 1991).

Characteristics of training Program

Weight	Heavy		Light
Power output	Medium		Low
Intensity	High		Low

Expected Outcome

Muscular strength gains	High		Low
Muscular endurance gains	Low		High
	Low		High

3   6   10   12   20   25

Number of repetitions
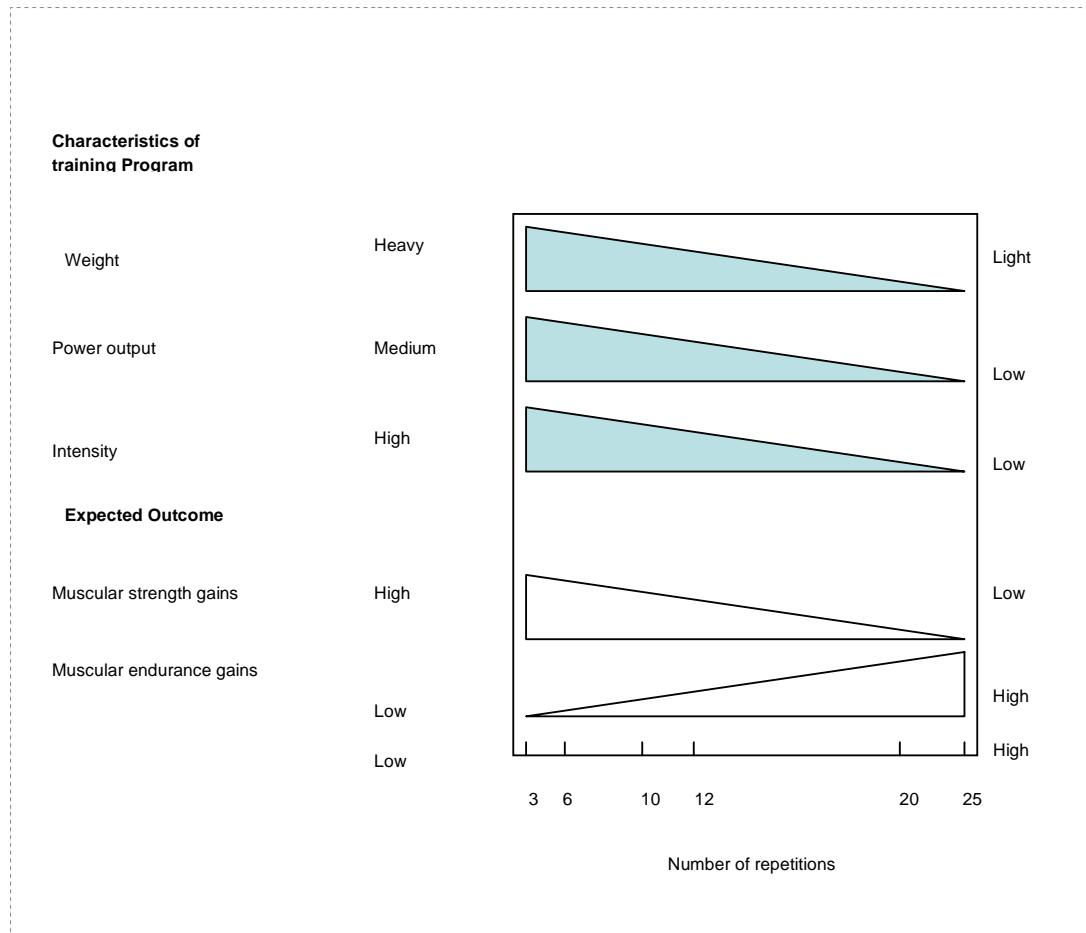
Figure 1.3: Theoretical continuum presenting characteristics of a training program and expected outcomes (Hubley-Koze, 1991)

For isokinetic exercises the duration of a single exercise appears to be more important than the number of repetitions being completed. Rest periods between sets play a role in the overload principle. For gains in strength the rest periods should be relatively long (several minutes) between sets, but if endurance is the primary goal for the trainee, shorter (less than 1 minute) rest periods should be used between sets (Hubley-Kozey, 1991). Even the frequency of resistance training needs to vary along with personal goals of the individual. A professional competing athlete would train much more than someone who is purely training to enhance physical fitness for recreational purposes. The American College of Sport Medicine (ACSM) currently recommends that strengthening exercises should be done two to three times per week to achieve the health related benefits of such exercises (Alter, 1988). Twice a week is considered the minimum requirement necessary to improve muscular strength.

The duration (amount of time sent in the gym) will largely be determined by the number of repetitions, sets and exercises being performed (Massey, 1956). The average duration is 20-30 minutes per session although many people spend more time than that in the gym. The volume for a given training session is defined as the number of sets multiplied by the number of repetitions multiplied by the load (Massey, 1956). Larger amounts of time spent in the weight room are important for both gains in strength and decreases in percentage body fat.

It is very important to start at a low level of intensity and then when the body has adapted to the exercise, to increase the load steadily and repeat the same process.

1.3.1.4   Adaptation

The body adapts to stress in a highly specific way (Wilmore, 1974; Cureton, 1988; Tesch, 1992). Adaptation is the way the body 'programs' muscles to remember particular activities, movements or skills. By repeating that skill or activity, the body adapts to the stress and the skill becomes easier to perform (Cureton, 1988). Adaptation explains why people who begin training are often sore after starting a new routine, but after doing the same exercise for weeks and months the athlete has little, if any, muscle soreness. This also explains the need to vary the routine and continue to apply the overload principle if continued improvement is desired. Muscles do adapt to the loads they are working against (Tesch, 1992). Initially exercises are tough, but gradually one becomes accustomed and can repeat more repetitions at maximum levels than previously. The rate of adaptation depends on a several factors that include rest periods and correct diets (Hardy, 1986). This may not be the same for each of the muscle groups being trained. The rest periods between sets are of great importance, because they allow for positive adaptations to occur. Not only are the rest periods important but also alternating between light and heavy days (intensity) to allow for the same adaptations and to prevent injury and soreness (Hardy, 1986). Athletes who lift heavy weights will allow 72 hours to pass before training the same muscle group again (Hardy, 1986).

1.3.1.5   Progression

Once the body has adapted to the current training level, exercise stress should be increased as dictated by the overload principle if further increases in strength are

desired (Kraemer, 1989). This principle is the basis of progressive resistance training.

Progression should be done gradually, once it has been approved. Progression can be applied by increasing any of the following: the number of sets, repetitions or the load or by decreasing resting periods in between sessions or sets (Kraemer, 1989). The two variables that are most often changed are the load and number of repetitions. Very important though, is that the individuality once again plays a role and therefore the progression process will need to be applied accordingly (Kraemer, 1989). For example if gains in strength is the objective, then heavier weights should be used, but if the goal aims towards endurance then the same weight should be lifted for more repetitions.

The principle of progression implies that there is an optimal level of overload that should be achieved and an optimal timeframe for this overload to occur (Hardy, 1986; Kraemer, 1989). Overload should not be increased too slowly or improvement is unlikely. Overload that is increased too rapidly will result in injury or muscle damage. Beginners can exercise progressively by starting near threshold levels and gradually increase in frequency, intensity, and time within the target zone. Exercising above the target zone is counterproductive and can be dangerous (Blimkie, 1988). For example, the weekend athlete who exercises vigorously only on weekends does not exercise often enough, and so violates the principle of progression. Many people, who consider them self to be regular exercisers, violate the principle of progression by failing to exercise above threshold levels and in the exercise target zone. Clearly, it is possible to do too little and too much exercise to develop optimal fitness.

The Principle of Progression also makes us realize the need for proper rest and recovery. Continual stress on the body and constant overload will result in exhaustion and injury (Hardy 1986; Blimkie, 1988). One should not (and cannot) train hard all the time. Doing so will lead to overtraining and a great deal of physical and psychological damage will result.

1.3.1.6   Maintenance

Once the desired level of strength or endurance has been achieved it is possible to maintain that level of fitness by reducing the amount of work, but then the intensity should remain at the same constant level (Wallin, 1985).

1.3.1.7   Retrogression/Plateau/Reversibility

Improvements do not occur in a linear manner, even if the coach does have a perfect laid out plan. Even with progressive increasing loads, there will be times that when performance will stay at the same level (plateau) or even show a decrease (retrogression). It may be due to either overtraining or individual differences that exist among the subjects (Ethyre, 1987). If overtraining is thought to be the cause of the plateau or retrogression effect, it would be wise to decrease the level of intensity or to apply the periodisation principle that we discussed earlier.

Reversibility occurs when an individual ceases to train. The rate of detraining (reversibility) depends on the level of strength the individual has attained. Strength is maintained more easily than endurance during the detraining period (Werring, 1999).

1.3.1.8   Warm-Up and Cool Down

This principle is the most important prior to and after training, because a proper warm up session raises the body to the correct temperature and is often recommended to prevent injury and muscle soreness. There is evidence that suggests this theory is true, even if hasn't been proven conclusively. Increased body temperature also increases the speed of the muscle contraction and relaxation as well as the enzymatic reactions (Enoka, 1988).

Warm ups for resistance training athletes can be either general or very specific and are recommended for weight lifting and isokinetic exercise (Perrin, 1993). General warm ups involve the major muscle groups and involve activities such as jogging or skipping rope. Specific warm up activities for resistance training programs include performing the exercises that are going to be in the program, but they should be performed at a level far below the intensity level that is going to be used during the exercise (Alter, 1988; Cornelius, 1988).

The cool down period is just as important as the warm up session. A cool down period, followed by stretching is highly recommended after training sessions. The cool down may aid in a decrease in muscle soreness and stiffness and therefore aid in the increase of flexibility. Cooling down is also important in the prevention of venous blood pooling in the lower extremities of the body (Alter, 1988; Cornelius, 1988).

In the end all these applied principles of resistance training are utilised in order to reach one or more of the following goals:

1. To increase muscle strength.
2. To increase muscle power output.
3. To increase muscle growth (hypertrophy).
4. To lower subcutaneous body fat percentages.
5. To decrease the onset of muscular fatigue during resistance training.

It is important to note that all these goals are not applicable to all athletes who perform resistance training, but rather that they will make use of the principles to achieve one or more of the goals as mentioned earlier (Ormsbee *et al.,* 2007).

1.3.2   Resistance exercise, the cardiovascular system, and muscle blood flow

The cardiovascular system regulates the heart rate as well as effective distribution of blood in the vascular circuit to maintain blood pressure, and thus blood flow, in response to the body's metabolic and physiologic needs (McArdie, 1985; Laughlin, 1999).

1.3.2.1   *Cardiac output*

Cardiac output (CO) can increase markedly to meet the demands we put on our body. Cardiac output can increase from a resting volume of approximately 5 liter to 25 liter in young female marathon runners and even to 35 liter in young male marathon runners (Kirwan, 1988).

Increased sympathetic stimulation increases heart rate and stroke volume by increasing contractility, resulting in more complete ejection of blood from the heart. Parasympathetic activity increases after the sympathetic stimulus has passed,

reducing heart rate, which results in cardiac output returning to normal. Cardiac muscle fibers are stretched by increased venous return, resulting in greater contraction force and an increase in stroke volume (Despopoulos & Sibernagl, 1991; Ober, 1997). A slow heart-beat allows more time for ventricular filling, thereby increasing stroke volume, and an extremely rapid heart rate decreases stroke volume due to a low venous return. Exercise activates the sympathetic nervous system, increasing heart rate, contractility and stroke volume. The higher heart rate and squeezing action of skeletal muscles on veins, increases venous return causing an increase in stroke volume. A sudden drop in blood pressure decreases stroke volume due to low venous return, but sympathetic activity increases heart rate and normal cardiac output is maintained. Rising blood pressure (BP) decreases heart rate, by reducing sympathetic activity, and decreases stroke volume by increasing arterial pressure. A sudden drop in blood volume (e.g. blood loss) decreases the stroke volume (low venous return) and increases the heart rate (sympathetic activity), maintaining cardiac output (Despoulos & Sibernagl, 1991; Ober, 1997).

The sympathetic catecholamines, adrenaline and noradrenaline, accelerate heart frequency and increase myocardial contractility, whereas the parasympathetic neurotransmitter, acetylcholine, acts via the vagus nerve to slow the heart frequency (McArdie, 1985). The increase in heart rate that accompanies exercise is due, in part, to a reduction in vagal tone. Recovery of the heart rate immediately after exercise is a function of vagal reactivation. A delayed fall in the heart rate during the first minute after exercise, which may be a reflection of decreased vagal activity, is a powerful predictor of: overall mortality, independent of workload, the presence or absence of myocardial perfusion defects; and changes in heart rate during exercise (Cole, 1999).

Humoral factors also contribute to modify the heart rate during exercise, enabling it to speed up rapidly in anticipation of exercise and to increase to 200 beats per minute or higher in maximum exercise (McArdie, 1985). These mechanisms can initiate changes in peripheral circulation according to the work performed, e.g. by vasodilatation of peripheral blood vessels and a higher mean arterial pressure.

Two forms of work can be distinguished on the basis of heart rate:

1. Light work (non-fatiguing work)
2. Heavy work (fatiguing work)

A progressively increased training load, and over training, in female endurance athletes did not induce significant changes in intrinsic resting heart rate or cardiac autonomic modulation. Resting heart rate however decreased with heavy endurance training and overtraining, and maximal oxygen uptake was correlated with high cardiac parasympathetic modulation (Uusitalo, 1998).

Forjaz *et al.* (1998) investigated the post-exercise changes in blood pressure, heart rate (HR) and rate pressure (RP) product [RP = HR X systolic blood pressure (BP)] at different exercise intensities. Varying exercise intensities (30%-80% of VO_{2peak}) in young normotensive humans did not influence the magnitude of post-exercise hypotension, but exercise at 30% of VO_{2peak} decreased post-exercise heart rate and rate pressure (Forjaz, 1998). Carmouche *et al.* (1998) investigated the effect of maximum heart rate on oxygen kinetics and exercise performance at low and high workloads. Normal heart rate was linearly related to oxygen consumption during exercise and maximum heart rate of the normal sinus node was approximated by the formula: HRmax = (220 - age) with a variance of approximately 15%. The nominal upper rate for most permanent pacemakers is levelled at the 120-beats/min mark. This value falls well below the maximum predicted heart rate for most patients and results in the possibility that the chronotropic responses of rate adaptive pacemakers during moderate and maximal exercise workloads may be less than optimal. Therefore, by programming the upper rate of rate adaptive pacemakers based on the age of the patient improves exercise performance and exertion symptoms during both low and high exercise workloads as compared with a standard nominal value of 120-beats/min (Carmouche, 1998).

Heart rate monitoring is important for assessing the total level and pattern of energy expenditure and physical activity in medium size and larger scale epidemiological studies (Wareham, 1997). Increasing the precision of measurements of total energy expenditure in population-based epidemiological studies is important for accurately quantifying the relationship between this exposure and disease (Livingstone, 1997; Wareham, 1997). The use of a heart rate monitor as a means of modulating physical

activity will enable the setting of clear, precise, observable limits on physical activity, and self-controlling of an acceptable level of physical activity (Bar-Mor, 1999).

The stroke volume (SV) of the heart raises 20-30% after the onset of work and then remains largely constant (Astrand & Rodahl, 1986). Since cardiac output is the product of heart rate and SV, it seems clear that further increases in cardiac output are due to changes in heart rate. This underlines the significance of heart rate as an indicator of cardiac output as well as the energy expenditure of exercise (Vander, 1985). Figure 1.4 indicates the factors that have an influence on stroke volume that can further impact the cardiac output.

Only when the strain is maximal, does cardiac output decline slightly because the rapid heart rate then limits the filling time and thus the SV of the ventricles (Fritzsche, 1999).

Nobrega *et al.* (1997) had investigated mechanisms for increasing stroke volume during static exercise, at a fixed heart rate, in humans. The increase in SV, during fixed HR at a resting level (73 ± 3 beats/min) utilized a combination of increased contractility and the Frank-Starling mechanism. However, during HR fixed at peak exercise rate (107 ± 4 beats/min) a greater left ventricular contractility mediated the increase in SV (Nobrega *et al.,* 1997).
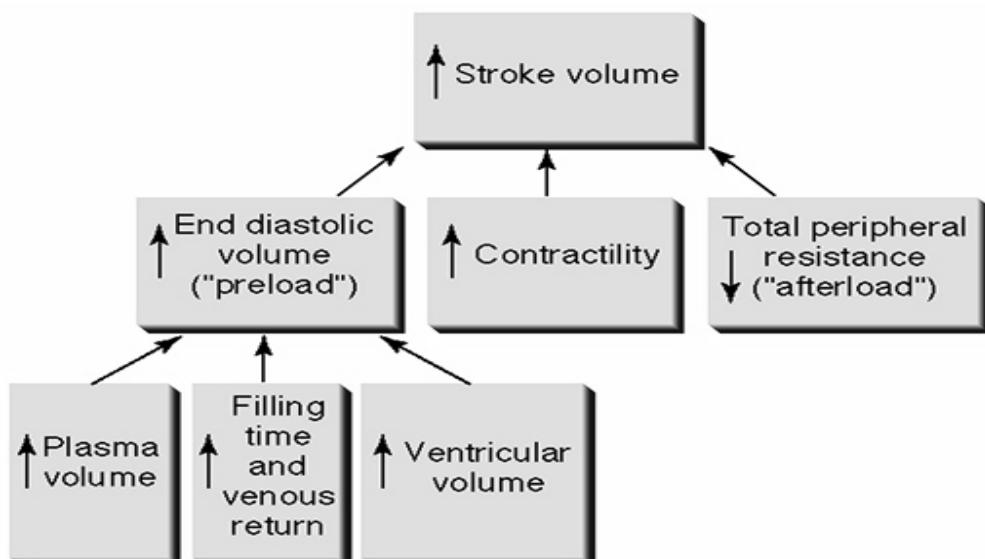

Figure 1.4: Factors increasing stroke volume (Vander, 1994)

1.3.3 Resistance exercise, blood pressure and blood flow

Exercise had proven to be a useful measurement to lower blood pressure in many guidelines for the management of hypertension, and subsequently the intensity of exercise recommended was lowered from moderate to mild (lactic threshold or 50% maximum oxygen uptake) (Arakawa, 1999).

Increasing the level of physical activity and changes to a "healthy" diet (low in total and saturated fat; energy intake balanced with expenditure to maintain or achieve optimal body weight; low in salt; high in fruits, vegetables, legumes, and whole grains) were reported to reduce blood pressure by between two and four mmHg (Arakawa, 1999; Margetts, 1999).

The multi factorial antihypertensive mechanism involves sympatholytic as well as diuretic actions through activation of relevant metabolic pathways, e.g. decrease in endogenous ouabain-like substance, and increase in prostaglandin E, urinary dopamine and kallikrein excretion. Other risk factors, such as sugar and lipid metabolism and insulin resistance, are improved (Arakawa, 1997). A prospective epidemiologic study has suggested that a physically active lifestyle will be important in the prevention of cardiovascular complications and will also have a substantial impact on population mortality patterns (Arakawa, 1997; Margetts 1999).

1.3.4 Resistance exercise and oxygen uptake

At the onset of exercise, the importance of rapid increases in cardiac output, and blood flow to the working fibers has been established by showing that small decreases in O_2 supply at the onset of exercise cause delay in the increase in O_2 utilization (Hughson, 1999). Oxygen uptake increases by a factor depending on performance level and efficiency (Astrand & Rodahl, 1986). Oxygen requirements, of the active muscles, during exercise can be increased virtually instantaneously by as much as twenty-fold. During light work a steady state is reached after 3-5 min, where the rate of oxygen uptake and consumption are equal. Until the steady state is reached, the dependence on the small oxygen reserve of the myoglobin-bound O_2 is greater. A study (Pederson, 1999) examined the influence of hyperoxia on peak oxygen uptake (VO_{2peak}) and peripheral gas exchange during exercise with the

quadriceps femoris muscle. Similar VO_{2peak} values, despite higher O_2 driving pressure (60% O_2), indicates a peripheral limitation for VO_{2peak}. This may relate to saturation of the O_2 turnover rate in the mitochondria during exercise. It can also be caused by tissue diffusion limitation related to lower O_2 conductance. Heavy muscular work causes a continuous rise in oxygen uptake until a maximum is reached.

The maximum capacity for the aerobic re-synthesis of ATP is indicated as the maximum oxygen consumption (VO_{2max}) (Welch, 1973; Martin, 1997). The VO_{2max} is influenced by factors such as exercise history (training), genetics, sex, age, body temperature, respiration and body fat (McArdie, 1985; Hayashi, 1999). Training contributes a large portion (30-50%) to the difference between sedentary and athletic values (Kissouras, 1972). Both active and sedentary people's VO_{2max} values increase when they undertake serious aerobic training, and VO_{2max} is an appropriate indicator of improved aerobic fitness (Martin, 1997). Cohen-Solal also evaluated the ratio of peak oxygen consumption to peak heart rate (peak oxygen pulse) as a predictor of long-term prognosis in chronic heart failure. Results showed that peak oxygen pulse has lower prognostic value than peak oxygen consumption, especially when the latter is indexed to predicted values (Cohen-Solal, 1997).

According to Bouchard (1988), there is also genetic variability in the determinants of endurance performance. The most notable determinant is the ratio of fast twitch (FT) to slow twitch (ST) fibre volume of skeletal muscles (Martin, 1997). In addition some people are more responsive to training at an early age; whereas others respond at a later age. According to Astrand and Rodahl (1986), performance gradually deteriorates and VO_{2max} declines at about 1% per year from age 25 onward.
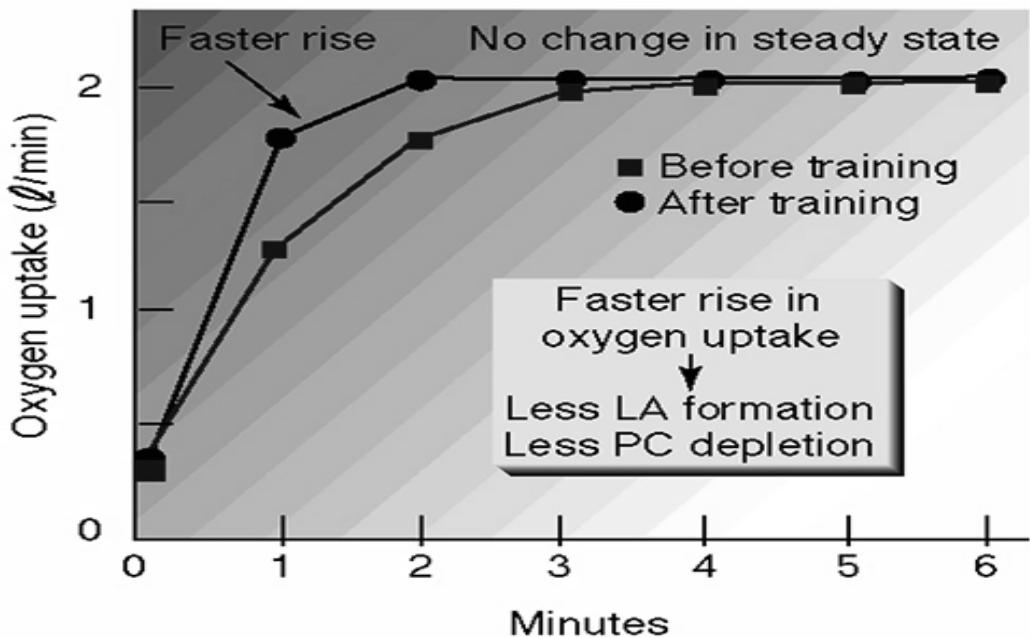
Figure 1.5: Effects of endurance training on O_2 deficit (Proctor & Joyner, 1997)

Heart rate, an important component of VO_{2max}, also declines at a rate of about six beats per decade, resulting in a decline (unless stroke volume increases appropriately) in maximum cardiac output. Muscle cells seem to deteriorate more rapidly after the age of 60. Campbell et al. have suggested that nervous system degeneration during the late years may induce the above mentioned changes. Changes in e.g. muscle oxidative capacity and body fat play an important role in the age-associated decline in maximal O_2 uptake (VO_{2max}) (Proctor & Joyner, 1997). Proctor and Joyner (1997) attempted to clarify these issues by examining the relationship between several indexes of muscle mass (by using dual-energy X-ray absorptiometry) and treadmill VO_{2max}. The results suggested that the reduced VO_{2max} seen in highly trained older men and women is due, in part, to a reduced aerobic capacity per kilogram of active muscle independent of age-associated changes in body composition, i.e. replacement of muscle tissue by fat. The reduced aerobic capacity per kilogram of muscle probably results from age-associated reductions in maximal O_2 delivery (cardiac output and/or muscle blood flow) (Proctor & Joyner, 1997). Figure 2.4 indicates the relationship between oxygen uptake and time and its effect on the O_2 uptake (VO_{2max}).

1.3.5   Resistance exercise, fatigue and recovery

Evidence states that fatigue is likely if the average intensity of efforts requires more than 40% of the individual's maximum oxygen uptake over long periods (Egger, 1992). Long-term exercise and the consequent depletion of energy stores and accumulation of lactic acid (the "fatigue substance") will reduce performance capacity (Astrand & Rodahl, 1986). Other physiological factors that may contribute are glycogen and/or mineral depletion, an increase in morning heart rate, progressive weight loss, decreased sleep effectiveness, loss of appetite and dehydration (Martin, 1997). Reductions in muscle blood flow with dehydration during moderately intense prolonged exercise in the heat, do not impair the delivery of glucose and free fatty acids (FFA), or the removal of lactate, but elevates carbohydrate oxidation and lactate production. Therefore the results indicate that hyperthermia, rather than altered metabolism, is the main factor underlying the early fatigue during prolonged exercise in the heat, with dehydration (Gonzalez-Alonso, 1999). During the recovery phase following physical work, the energy stores are replenished and the lactic acid is eliminated. During the replenishment stage of recovery the CrP stores are also replenished (Meyer *et al.,* 2002). Below the endurance limit the work movements allow sufficient muscular relaxation time for nutrients to be replenished and the metabolic end products to be transported away (Martin, 1997), so-called non-fatiguing work. Takahashi *et al.* (1998) examined the influence of light exercise on cardiac responses during recovery from exercise by using heart rate (HR), stroke volume (SV), and cardiac output (CO). It was concluded from the results that light post-exercise physical activity plays an important role in facilitating venous return from the muscles and in restoring the elevated HR to the pre-exercise resting level (Takahashi, 1998). Trained individuals have a faster regulation of post exercise metabolism (faster recovery heart rate) than untrained individuals when exercising at either the same relative or same absolute work rate (Takahashi, 1998). In both dynamic and static work there is an expenditure of chemical energy, which in static work, is completely transformed into the heat of maintenance at a rate proportional to the muscle tension times the duration of static work (Astrand & Rodahl, 1986). During dynamic work (above the endurance limit) the relaxation times amount to less than that required for rapid recovery. Replenishment of the nutrients and removal of the lactic acid are incomplete and fatigue builds up (Astrand & Rodahl, 1986). If the replenishment rate of nutrients

could be increased, it could be hypothesized that fatigue would take longer to build up and a trainer could therefore train for extended periods of time and in the end increase the specific parameters they might focus on. Koike *et al.* (1998) investigated the kinetics of oxygen uptake during recovery after exercise in cardiac patients. The recovery of oxygen uptake and heart rate was significantly delayed in patients with severely impaired left ventricular function (Koike, 1998).

1.4 DELAYED ONSET MUSCLE SORENESS (DOMS)

Regular exercise does have beneficial properties, but many people don't continue with their training due to pain and discomfort they experience when they start training or haven't trained before in their lifetimes. Although exercise physiologists have studied fatigue for quite some time, it is still to be precisely defined and many questions remain regarding the basic causes of fatigue (Plowman *et al.,* 1991).

Fatigue is generally defined the as transient loss of work capacity resulting from proceeding work. This physiological phenomenon presents one of the most fundamental problems for both research and practical applications. Fatigue limits performance in normal conditions and even more so in disease. It produces a general feeling of discomfort and frustration and interferes with well-being (Simonson, 1971).

There are two types of soreness that are related to training and exercise. Injuries and sprains are not included in this category. The first type is acute soreness which occurs during or directly after exercise and the second type is Delayed Onset Muscle Soreness (DOMS), which develops 12 hours after the conclusion of training (Cheung, 2000).

Acute soreness: Muscle soreness during and immediately after exercise usually reflects simple fatigue, caused by a build-up of chemical waste products of exercise (Cheung, 2000). If so, the discomfort will often subside after a minute or two of rest. Once the soreness goes away, a person can usually continue exercising without any residual effects. If discomfort persists despite a rest period, a person should stop his /her activity and rest the part of the body that is involved. A person should not

proceed with his / her workout until they are able to exercise the affected area without pain.

Delayed Onset Muscle Soreness is common after a workout; particularly if a person is not used to the exercise or if it is a new type of exercises that has not been performed before (Cheung, 2000). For example you haven't performed a 10 km run in over 6 months and then come back to try and run at the same pace you did 6 months ago. The next day you'll experience soreness, but the muscles will also feel weak and stiff. This is a normal response to any unusual exertion and is part of the muscles adaptation process that leads to greater strength once the muscles have recovered. The soreness is usually the most intense the first 2 days within the training session and subsides thereafter in the following days (De Vries, 1994).

1.4.1 Causes of DOMS

There is no clear explanation to what causes DOMS, but there are a few theories to what may be the cause of DOMS (Armstrong, 1986). The most common and acceptable theory to the cause of DOMS is that it might be the result of microscopic tearing of the muscle fibres (Armstrong, 1986). The amount of tearing depends on how intense; for what time duration the exercise is performed and on the type of exercise that is performed. Exercises that perform eccentric contractions (the muscle is forcefully lengthened while still contracting) seem to cause the highest amount of tearing within the effected muscle (Armstrong, 1986). The eccentric contractions provide a braking action and they occur in activities such as descending stairs, running downhill, lowering weights, and performing the downward movements of squats and push-ups.

In addition to microscopic tearing, swelling may take place in and around a muscle (De Vries, 1994), which can also contribute to delayed soreness. Such swelling increases pressure on the neighbouring structures, resulting in greater muscle pain and stiffness (De Vries, 1994). Eccentric muscle contractions tend to cause both micro tearing and swelling.

1.4.2 Preventing DOMS

There are a few principles that can be applied to minimize or prevent DOMS or any other soreness altogether. The most important of these principles is to warm up properly before any exercise and to cool down and stretch just as thoroughly afterwards (De Vries, 1994). Research has shown that muscular (dynamic) stretching is more effective than static stretching and that static stretching failed to demonstrate the same reduction in pain (McGlynn, 1979).

The next applicable principle is to allow the muscles that you are being trained to adapt to the exercise (Armstrong, 1986). So rather start at a lower level of intensity than try to perform the exercise at the same intensity it was previously subsided. Kelly (1982) suggested that the lower intensity of training causes the relief in pain due to an increased release in endorphins as a result of the exercise (Kelly, 1982).

These principles are applicable to all activities, especially resistance or weight training (Kelly, 1982). The best advice would be to refrain from any sudden changes in the intensity or type of exercises.

1.4.3   Dealing with DOMS

The best efforts may be applied to prevent DOMS from occurring, but there will be times that they do fail. Most fitness experts will concur that the soreness will fade within 3 to 7 days without any special treatment (De Vries, 1994). However, there are a few things that can be done that might reduce the soreness quicker and decrease the recovery time.

If a person does have any soreness the best they can do would be to avoid any vigorous exercise that could further increase the discomfort and to rather spend time and train at a lower intensity level the areas of the body that haven't been affected (Kelly, 1982). The logic behind this is that exercising affected areas (by performing low intensity aerobic exercises) increases the blood flow to the affected areas which could aid in the "rehabilitation" or recovery process.

Other measures include applying ice, gently stretching, and massaging the affected muscles, which may be helpful for some people and poses little risk if done sensibly (Kelly, 1982; Sellwood, 2007).

Also, the use of non-steroidal anti-inflammatory medications like aspirin and ibuprofen may reduce the soreness temporarily, but they won't actually speed up the recovery time (Hasson, 1990; Arendt-Nielsen, 2007). They will decrease the discomfort. These medications are characterized as over the counter (OTC) products and don't need a subscription to obtain the products. There is also evidence that Vitamin C can decrease soreness.

The last thing to remember is to abstain from any vigorous training and fully recover from any soreness and stiffness before participation in any intensive activities.

Throughout this chapter it becomes clear that the body is in need of additional supplementation to overcome the effects of intense exercise (increased cardiac output, increased oxygen uptake, occurrence of fatigue and DOMS) that utilize plenty of the body's energy substrates.

The possibility exists that if the protein breakdown within the body could be limited during resistance training, it could hypothetically shorten recovery time of athletes for training and increase the gains in muscle power output, while improving fat utilization (decrease in body fat percentage) (Baty *et al.,* 2007).

1.5   HMB (beta-hydroxy-beta-methylbutyrate)

Only two supplements can justifiably and consistently claim that they are powerful enough to improve lean body mass and strength. One is creatine, with its relatively simple mechanisms and volumes of successful research. In HMB, we have a supplement with similarly compelling research, but quite complex.

HMB is a metabolite of the amino acid leucine that is found in all dietary protein (Nissen & Van Koevering, 1996). Leucine is also one of the three branched chain amino acids (the other two are isoleucine and valine), a trio of compounds that together possess unique performance-enhancing effects. However, what separates leucine from the other two, and from all other amino acids for that matter, is its role in

regulating protein synthesis and protein breakdown. Scientists speculated that there was a "mystery" compound behind all these protein-sparing effects. Extensive studies suggested that HMB may be the bioactive component of leucine metabolism that plays a regulatory role in protein metabolism, or it was the key that unlocked the complex door of protein synthesis and anti-catabolism (prevents protein breakdown) (Nissen & Sharp, 1996). Research that was done in animals, suggests HMB plays some role in protein metabolism, especially in stressful situations. Kornasio *et al.* (2009) hypothesized that HMB would directly regulate muscle-cell proliferation and differentiation and would attenuate apoptosis during muscle degradation and atrophy.

1.5.1   Problem statement

What is the effect of HMB supplementation on the Lean Body Mass (LBM) and muscle power output of males who perform resistance exercise programs (gym) for recreational purposes, after a combination of resistance weight training, eating a balanced set diet and supplementation with HMB for 8 weeks.

1.5.2   Objectives

The objectives of the proposed study are to:

1. Determine whether HMB supplementation will increase Lean Body Mass (LBM)

2. Determine whether HMB supplementation will increase muscle power output

3. Determine whether HMB supplementation will have an effect on creatine kinase activity and body fat percentage.

1.5.3   Hypothesis

Three hypotheses are identified for the proposed study based on each of the objectives:

1. HMB supplementation increased Lean Body Mass (LBM)

2. HMB supplementation increased muscle power output

3. Creatine kinase activity and fat percentage decreased with HMB supplementation.

CHAPTER 2

BETA-HYDROXY-BETA-METHYLBUTYRATE (HMB): A COMPLETE LITERATURE REVIEW

2.1 INTRODUCTION

In the competitive world of professional sport, sportsmen and women have looked increasingly to scientific nutritional strategies to increase muscle and lean body mass (LBM) to better their performances (Garrow & Summerbell, 1995). This has created a desire to develop a variety of nutritional supplements for these purposes (Kreider, 2000). Consequently there has been experimented with a variety of substances to satisfy this demand. A substance which has also shown increasing popularity in the USA is the leucine metabolite HMB (Kornasio, 20009; Thomson, 2009). It is commercially available as calcium HMB monohydrate under U.S. patents: 5,348,979 (a method for improving nitrogen retention), 5,360,631 (a method decreasing low-density and total cholesterol), 6,103,764 (a method for increasing aerobic capacity of muscle), 4,992,470 (method of enhancing immune response), and 6,031,000 (composition comprising β-hydroxy-β-methylbutyrytic acid and at least one amino acid and methods of use). It has been hypothesised that the HMB decreases proteolysis in muscle cells after resistance exercise thus increasing lean body mass and strength, but the exact mechanism has not been experimentally proven (Nissen, 1990; Nissen, 1996; Mero, 1999; Knitter, 2000; Kreider, 2000; Nissen et al., 2000; Panton, 2000; Slater, 2000; Jówko, 2001; Flakoll, 2004). It is also hypothesised that it decreases muscle damage by being converted to β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) which serves as a carbon source for synthesis of cholesterol which is incorporated into cellular membranes (Nissen, 1990; Nissen, 1996; Knitter, 2000; Nissen et al., 2000; Panton, 2000 & Slater *et al.,* 2000). It has also been shown that HMB is safe to use by humans as a nutritional supplement and may even decrease cardiovascular risk factors (Nissen et al., 2000)

2.1.1 Leucine, α-Ketoisocaproate (KIC) and HMB Metabolism

Previous research has demonstrated that ingestion of essential amino acids and their metabolites induce anabolic effects with the potential to increase and promote

gains in LBM and strength after resistance exercise training (Kraemer, 2009). Branched Chain Amino Acids (BCAA) have been used as a supplement in various situations and have shown to be of considerable use (Sax, 1989). Results found that only creatine (18 previous studies) and HMB (9 studies) had sufficient data supporting their ability to enhance LBM and various indexes of performance (Gabriel *et al.,* 2008).

The amino acid leucine is transaminated in the cytosol and mitochondria of muscle cells to form α-ketoisocaproate (KIC). KIC is then transported to the liver where it is oxidized (Frexes-Steed, 1960; Nissen, 1996; Mero, 1999; Slater *et al.,* 2000). KIC can be oxidized to yield acetoacetate and acetyl-CoA. This takes place through 5 steps (Figure 2.1). It was suggested in 1981 by Sabourin that KIC can also be oxidized to HMB via another separate metabolic pathway (Nissen, 1996). Through this pathway KIC is oxidized by the enzyme KIC dioxygenase to yield HMB, this reaction requires oxygen and takes place in the cytosol of the liver and is unique from other enzymes in the leucine catabolic pathway (Mero, 1999; Nissen, 1996; Sapir, 1983). According to Nissen and Abumrad (1996), KIC dioxygenase is very similar to an enzyme active in tyrosine catabolism, *p*-phenylpyruvate dioxygenase. Nissen and Abumrad (1996) also reported significant increases in plasma HMB concentrations if pigs were fed food enriched with leucine, KIC and isovaleric acid illustrating that they are important precursors for HMB. The most important metabolic fate of HMB seems to be the synthesis of HMG-CoA. HMG-CoA serves as substrate for the enzyme HMG-CoA reductase, in the anabolism of cholesterol in humans. There is no doubt about the incorporation of HMB carbon into cholesterol, but the percentage of HMB actually present in the total cholesterol is unclear (Nissen & Abumrad, 1996; Gallagher *et al.,* 2000a). Another metabolic fate of HMB like other metabolites is excretion in urine.
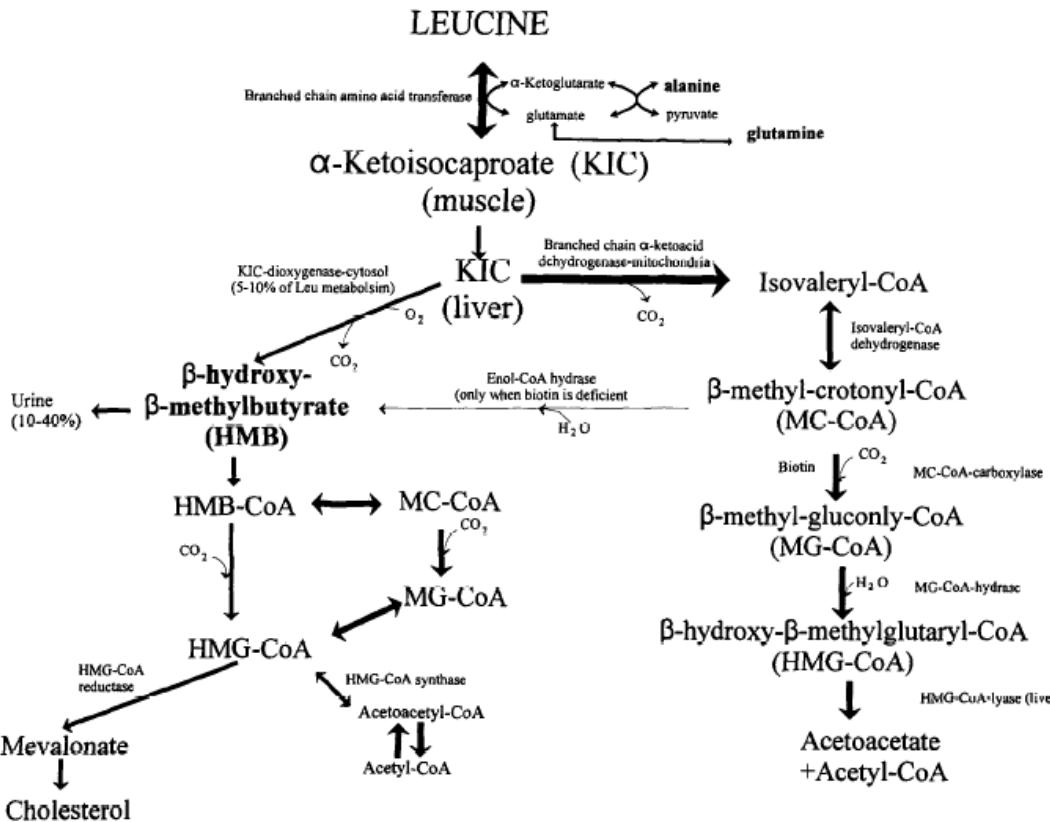
Figure 2.1: Overview of leucine, α-ketoisocaproate (KIC) and β-Hydroxy β-Methylbutyrate in mammals. The enzymes and major co-factors are listed with each reaction. The metabolism of HMB is based upon isotopic data which suggested that HMB is converted to β-Hydroxy β-Methylglutyrate-CoA (HMG-CoA) in the cytosol and ultimately to cholesterol (Sabourin, 1981).
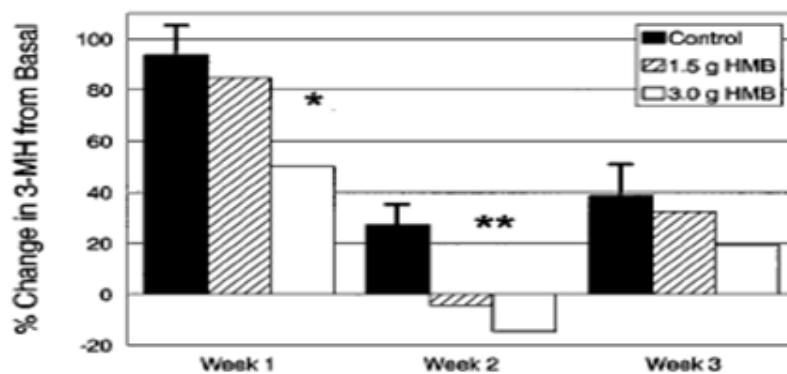
Figure 2.2: Changes in urinary 3-methylhisitdine (3-MH) in subjects undergoing exercise-resistance training and supplemented Ca-HMB. *$P < 0.04$: **$P < 0.001$ (Nissen, 1996)

There are various dependant measures that are used to study the effects of HMB supplementation. These measures include different measures of strength (isokinetic, isometric and dynamic), and also functionality exercises in the elderly (Gabriel et al, 2008). Another parameter that is measured regularly includes markers of muscle damage, specifically plasma concentrations of CK, LDH and 3-MH (Janssen. 1989; Nuviala, 1992; Knitter, 2000).

2.2 THE EFFECT OF HMB ON MUSCLE DAMAGE

During the participation in intense resistance or endurance exercise, delayed-onset muscle membrane damage occurs (Armstrong, 1986). This is due to the damage caused to structural and functional protein in muscle cells as well as damage caused to muscle membranes (Knitter, 2000; Slater *et al.,* 2000). When muscle membranes are damaged enzymes like creatine kinase (CK) leaks from the cell and the increases plasma CK levels which serves as an indication of the extent of muscle damage (Jówko, 2001). It could therefore be reasoned that lower plasma CK levels could be an indication of less muscle damage within exercising muscles. Therefore it could also be hypothesized that HMB might also be beneficial in minimizing the effects of DOMS.

Two other substances used to quantify muscle damage after exercise is the enzyme lactate dehydrogenase (LDH) and 3-methylhistidine (3-MH) (Nissen, 1996; Knitter, 2000; Panton 2000; Slater *et al.*, 2000). 3-MH levels is directly proportional to muscle protein turnover, thus increasing levels of 3-MH indicates increased proteolysis, and vice versa (Figure 2.2) (Nissen, 1996). In experiments done by Slater *et al.* (2000) with people using HMB and performing resistance exercise, a decrease in these three parameters have been found (Nissen, 1996; Panton, 2000; Slater *et al.,* 2000). Also experiments done by Knitter et al. (2000) on people after a prolonged run also using HMB, the same metabolic results were found: a decrease in the plasma levels of CK and LDH (Figure3.3) (Knitter *et al.,* 2000).

This clearly illustrates that HMB helps to reduce muscle damage that occurs during intense exercise (Gallagher *et al.,* 2000a of b; Van Someren, 2005).
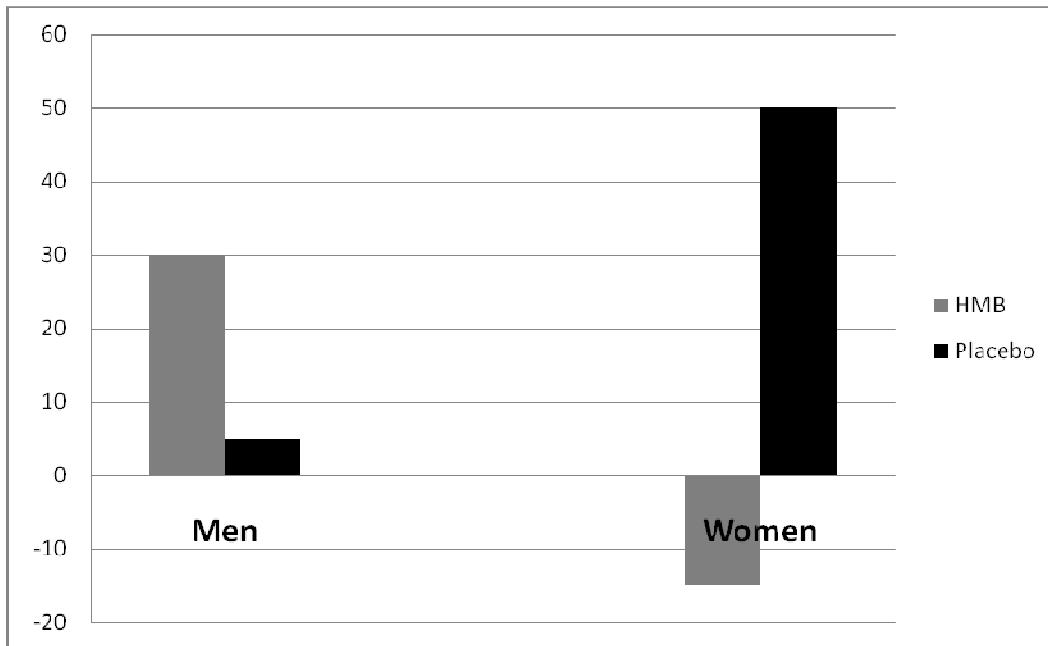
Figure 2.3: Change in creatine phosphokinase (CK) activity following 4 weeks of HMB supplementation combined with resistance training in men and women cohorts. (Panton, 2000)

A hypothesis for this phenomenon refers to the conversion of HMB to HMG-CoA. HMG-CoA serves as a carbon donor for the synthesis of cholesterol (Knitter 2000; Nissen 1996; Slater *et al.,* 2000). The increased cholesterol synthesised is then incorporated into membranes of the cell, increasing its structural integrity and speeding up recovery of the damaged membranes. This will cause less CK, LDH and 3-MH to leak out of the cell, into the bloodstream and will lead to decreased plasma concentrations of these three compounds (Nissen, 1996; Knitter 2000).

A study was performed to indicate the beneficial use of HMB to counteract muscle wasting in elderly women. The author presented evidence that indicated an increase in muscle functionality that was also reflected by an increase in limb circumferences and grip strength of the hands (Flakoll, 2004)

In a medical trial HMB was used to investigate the efficacy of a mixture HMB, glutamine, and arginine as nutritional treatment for rheumatoid cachexia. The amino acid mixture increased the fat free mass of affected individuals and the experimental group reported fewer gastrointestinal complaints compared to the placebo group. (Marcora, 2005).

The most recent studies (Kraemer, 2009; Kornasio, 2009; Rowlands, 2009) show that HMB is an effective anti catabolic. Kraemer (2009) indicated the beneficial changes in hormonal responses and markers of muscle damage in response to a 12 week resistance exercise training schedule. Biochemical research has presented evidence that the direct effects of HMB such as the increase in internal growth factor (IGF-1) suggest its positive influence in preventing muscle wasting (Kornasio, 2009). Baier (2009) used HMB in a protein cocktail during a year-long study to indicate its effects on protein metabolism within elderly women. The study showed that consumption of the amino acid cocktail (consisting primarily of HMB) increased protein turnover and lean tissue in the elderly individuals.

2.3   THE EFFECT OF HMB ON MUSCLE STRENGTH AND GROWTH

It has been hypothesized that a daily intake of 3 g HMB, combined with resistance training, will increase lean body mass and total body strength. It was found that lean tissue increased in a dose responsive manner. A gain of 0.4, 0.8 and 1.2 kg of lean mass for 0, 1.5 and 3 g-HMB respectively during a 3 week trial period. In the strength department they recorded a total net increase of 337.8, 529.4 and 707.1 kg for 0g, 1.5 g and 3 g-HMB respectively (Nissen *et al.,* 2000). The same type of results were also found in a study done by Panton *et al.* (2000) where men and women supplemented with 3 g Ca-HMB per day did resistance exercise under supervision of trainers. They showed increases in lean body mass and a decrease in body fat percentage. The men gained 1.2 kg of lean mass and women gained 1.1 kg, calculated with the seven skinfold method. The supplemented men group also increased their upper body strength by 9.9 kg versus the placebo group who increased their upper body strength by 7.0 kg after the 4 week experimental period. The women supplemented with HMB increased their upper body strength with 5.1 kg and versus the placebo group who increased theirs by only 3.2 kg (Panton *et al.,* 2000). Another study found that lean body mass increased by 1.24 kg for the HMB supplemented group and only 0.84 for the placebo group. The strength of the HMB group accumulatively increased by 58.87 kg, where the placebo group increased by 19.72 kg (Jówko, 2001).

The effect that HMB has on people with different training status has been questioned. In a study completed by Kreider *et al.* (2000). The researchers found (Figure 2.5) that there is a difference in the response of untrained and trained athletes (Knitter, 2000; Kreider, 2000; Wilson, 2000). The experiments concluded that untrained athletes showed a larger response (thus a greater increase in lean body mass and strength) to HMB supplementation than trained athletes (Panton *et al.,* 2000). However Panton (2000) found that training status had no effect on the effects of HMB in supplemented subjects. In the same study gender also did not have an effect on the increase of lean body mass during the observation period. In a study done by Nissen and Abumrad (1996) subjects were also divided into trained and untrained groups. Untrained subjects didn't train at least for four months prior to the study. Both groups experienced increases in lean body mass and strength during the 4 week experiment (Figure 2.4) with no significant differences in lean mass gains. The subjects received 3 g HMB per daily (Nissen, 1996). Slater *et al.* (2000) suggest that more tightly controlled and larger studies need to be done to investigate this and the total effect of HMB on people thoroughly (Slater *et al.,* 2000).
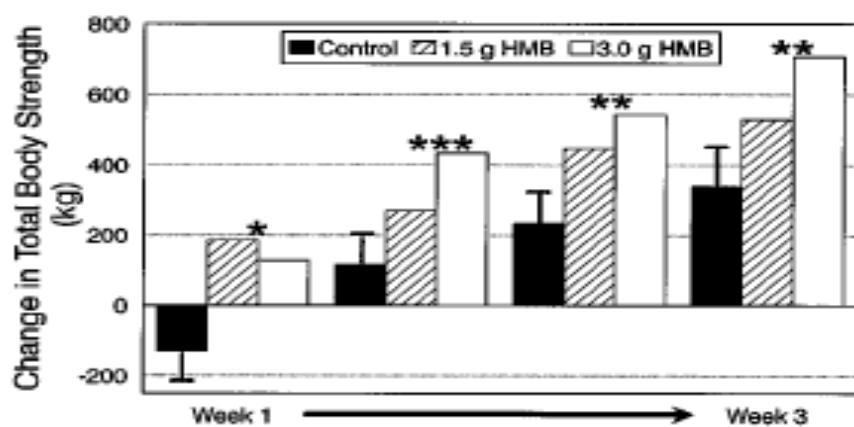

Figure 2.4: Change in muscle strength (total of upper and lower body exercises) from week 1 to week 3 in subjects supplemented with Ca-HMB. Each set of bars represents one complete set of upper and lower body workouts. ***$P<0.01$; **$P<0.02$; *$P<0.03$ (significant linear effect of HMB supplementation). (Slater *et al.*, 2000)
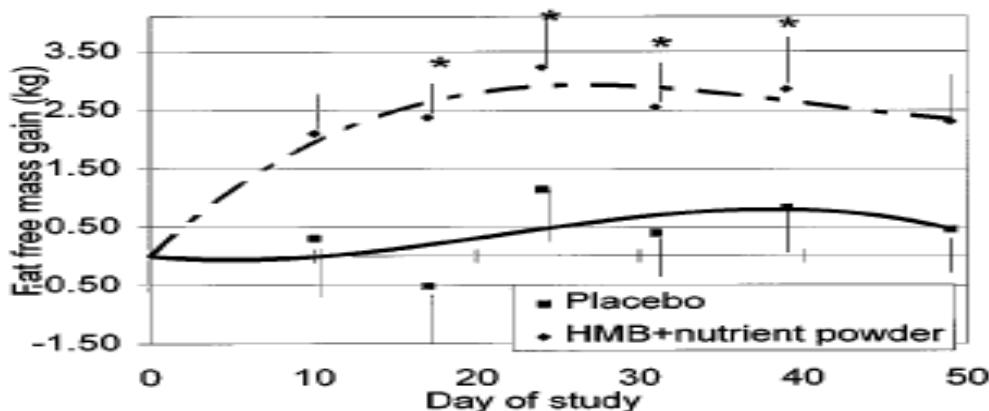
Figure 2.5: Change in fat-free mass during study 2 as measured by total body electrical conductivity. Values are means ± SE for control group (n = 13) that received a carbohydrate drink (Placebo) and HMB group (n = 15) that received 3 g Ca-HMB/day mixed in a nutrient powder (HMB + nutrient powder). Lines, best fit of data to cubic equation. *Significant difference between control and HMB groups at a given time, *P*<0.05. (Slater *et al.,* 2000)

More studies up until 2009 support previous research. These studies show the beneficial effects of HMB with regard to increases in muscle strength and growth. Between 2003 and 2009 there have been at least 5 studies with published results. The muscular effects of HMB weren't as effective in trained athletes (Ransone, 2003; O'Connor, 2007), but more effective in untrained athletes who supplemented or initiated resistance training for the first time (Rowlands, 2009).

One study researched the aerobic-performance of HMB. The study concluded that HMB supplementation positively affected selected components of aerobic performance in active college students (Lamboley, 2007).

2.4 THE SAFETY OF HMB SUPPLEMENTATION

As with any new consumable product its safety to the public needs to be tested and proven. Any side effects can be costly to the consumer and the manufacturer. The safety of HMB has been thoroughly tested (Nissen *et al.,* 2000; Baxter *et al.,* 2005). The study specifically tested the emotional profile, any adverse effects and finally blood chemistry (Very Low Density Lipoproteins, Low Density Lipoprotiens), blood pressure and haematology.

The emotional tests showed no significant effects due to HMB (dosage of 3 g HMB per day was used). The only significant change was that there was a decrease in the number of subjects feeling "dull, tired, drowsy, sluggish, bored and droopy". There were also no significant adverse effects. No changes were found either in the pre-treatment or treatment periods in categories for example stomach-ache, dizziness, rash, numbness etc.

When cholesterol levels were measured at the beginning of the study, the participants were divided into two groups, firstly subjects with total cholesterol below 5.17mmol/L and secondly subjects with total cholesterol above 5.17 mmol/L. Significant decreases in total cholesterol were found in both groups. The low cholesterol group decreased by 2.5% and the high cholesterol decreased by 5.8%. A huge part of the decrease is due to the decrease in low density lipoproteins (LDL) in both groups (4.2% for low and 7.3% for high cholesterol). On the other hand high density lipoprotein (HDL) concentrations showed no decline. Systolic blood pressure showed a decline in all subjects. When they were divided into two groups, low and high risk, the high risk group showed an increased decline of the systolic pressure (high risk being above 130 mmHg). Diastolic pressure showed no significant change. No significant differences were found for liver function indicators between the control group and the placebo group. The indicators used were bilirubin, lactate dehydrogenase, alkaline phosphatase, serum glutamate pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), Gamma glutamyl transpeptidase (GGT), and iron. These results were also found in a study done by Nissen and Abumrad (1996), where no changes were found for the enzymes SGOT, SGPT, alkaline phosphotase and lactate dehydrogenase (Nissen et al., 2000). They also reported a decrease in the LDL concentrations of the HMB supplemented group. No significant differences were found for the majority of the general blood chemistry categories (Nissen *et al.,* 2000). However there was a 1.9% decrease in potassium concentrations, a 5% increase in the albumin/globulin ratio and a significant 0.48% decrease in the hematocrit of the HMB supplemented group.

Table 2.1: Effect of 3 g/d supplemental ß-Hydroxy-ß-Methylbutyrate (HMB) on blood lipid and blood pressure (BP) in a summary in humans in 9 clinical studies (Nissen *et al.,* 2000)

	All subjects					Lower risk subjects					Higher risk subjects				
	Screen	Delta	Change %	HMB effect[2]	Exp × HMB[2]	Screen	Delta	Change %	HMB effect[2]	Exp × HMB[2]	Screen	Delta	Change %	HMB effect[2]	Exp × HMB[2]
						Subjects with cholesterol < 5.17 mmol/L					Subjects with cholesterol ≥ 5.17 mmol/L				
Total cholesterol, mmol/L															
Control	4.68	−0.034	−0.7			4.24	0.057	1.3			5.72	−0.036	−0.6		
HMB	4.76	−0.176	−3.7	0.03	0.92	4.29	−0.106	−2.5	0.04	0.46	5.72	−0.334	−5.8	0.03	0.21
HDL cholesterol, mmol/L															
Control	1.14	0.049	4.3			1.09	0.052	4.7			1.26	0.028	2.3		
HMB	1.17	0.008	0.7	0.04	0.57	1.13	0.003	0.2	0.06	0.77	1.30	0.003	0.2	0.53	0.59
VLDL cholesterol, mmol/L															
Control	0.53	−0.009	−1.7			0.50	0.011	2.3			0.64	−0.070	−10.9		
HMB	0.54	−0.016	−3.0	0.79	0.36	0.51	0.009	1.7	0.93	0.30	0.58	−0.054	−9.4	0.78	0.09
LDL cholesterol, mmol/L															
Control	2.97	−0.062	−2.1			2.66	−0.008	−0.3			3.83	0.034	0.9		
HMB	3.05	−0.173	−5.7	0.05	1.00	2.69	−0.114	−4.2	0.09	0.60	3.90	−0.284	−7.3	0.01	0.33
Total/HDL cholesterol															
Control	4.31	−0.16	−3.7			4.09	−0.12	−2.9			4.96	−0.12	−2.4		
HMB	4.24	−0.17	−4.0	0.95	0.49	3.96	−0.07	−1.8	0.63	0.32	4.64	−0.31	−6.7	0.22	0.42
Triglycerides, mmol/L															
Control	1.22	−0.019	−1.6			1.13	0.034	3.0			1.64	−0.207	−12.6		
HMB	1.23	−0.066	−5.3	0.46	0.08	1.16	−0.028	−2.4	0.46	0.19	1.29	−0.114	−8.8	0.41	0.10
						Subjects with systolic BP < 130					Subjects with systolic BP ≥ 130				
Systolic BP, mm Hg															
Control	133	−0.8	−0.6			121	2.0	1.7			143	−4.4	−3.1		
HMB	134	−4.4	−3.3	0.05	0.16	121	0.9	0.7	0.76	0.18	144	−9.3	−6.5	0.05	0.39
Diastolic BP, mm Hg															
Control	73.0	−3.6	−4.9			68.7	−2.5	−3.6			77.7	−5.4	−6.9		
HMB	73.7	−3.7	−5.0	0.96	0.52	67.9	−3.3	−4.9	0.70	0.42	78.3	−5.0	−6.4	0.83	0.30

1. Results presented are least-square means from the combined analysis of the nine studies (*n* = 134 placebo and *n* = 128 HMB for blood lipids and *n* = 112 placebo and *n* = 110 HMB for blood pressure). Screen values are the initial values of the participating subjects and delta values are the change over the study. Blood pressures were not measured in Studies 5 and 9.

2. *P*-values for HMB effect and Experiment by HMB interaction as determined by ANOVA.

All of the studies investigated in this review had very similar findings to the effect of HMB supplementation. HMB seems to decrease the amount of muscle damage experienced by subjects undergoing intense resistance exercise. The most likely explanation for this is that HMB may play a role in cholesterol synthesis (Nissen, 1996; Slater *et al.,* 2000; Knitter, 2000; Nissen et al., 2000; Panton, 2000). HMB which is then converted to HMG-CoA serves as a carbon donor for cholesterol synthesis. The cholesterol is then incorporated into cellular membranes of muscle cells. This incorporation increases membrane integrity and speeds up recovery after exercise. This then helps the body to faster increase lean body mass and strength. There also seem to be negative feedback inhibition on the synthesis of LDL, because of increased cholesterol synthesis. Some studies showed a decrease in LDL (Nissen, 1996; Nissen *et al.,* 2000). It is also hypothesized that HMB decreases

proteolysis in muscles which increases the lean body mass and strength of subjects. The exact mechanism with which this takes place is not yet known but being investigated. It is however supported by varies studies which found a decrease in the plasma levels of CK, LDH and 3-MH in HMB supplemented subjects (Nissen, 1996; Slater *et al.,* 2000; Knitter, 2000; Panton, 2000). It has also been proven that HMB is safe to use by athletes to increase lean body mass and strength, because it has no influence on blood chemistry and haematology (Nissen *et al.,* 2000). Also, HMB decreases cardiovascular risk factors like total cholesterol by decreasing in particular the plasma levels of LDL (Nissen *et al.,* 2000).

The above mentioned data and results were researched and verified in a meta-analysis by Gabriel *et al.* (2008). All the performed safety tests indicated that HMB is a safe product to use when used properly over a period of a few months (2 to 3 months).

CHAPTER 3

EFFECT OF BETA-HYDROXY-BETA-METHYLBUTYRATE (HMB) SUPPLEMENTATION ON THE BODY-COMPOSITION AND MUSCLE POWER OUTPUT OF NON COMPETITIVE SPORTING MALES BETWEEN 19 AND 24 YEARS WHO PERFORM RESISTANCE WEIGHT TRAINING THREE TIMES A WEEK FOR 8 WEEKS.

3.1 INTRODUCTION

Obesity is a major problem facing our society and is the main cause of many diseases (Sorof & Daniels 2002). With weight maintenance programmes, these diseased conditions can be managed within narrow limits, thus improving an individual's quality of life. An increase in energy intake leads to an increase in body weight. Energy expenditure needs to increase to eventually match the higher energy intake. Body weight would then stabilize at a new and higher value (Hill & Commerford, 1996). A biological set point for body weight, much like the set points for any negative biological feedback, helps return the body to a certain weight (Calle, Thun, Petrelli, Rodriguez & Heath, 1999).

Nutrient balance exists for both proteins and carbohydrates. Excess nutrients are oxidised and not converted to fat, whereas excess fat is stored in adipose tissue. Weight control is achieved by maintaining the fat balance. Diets with a high fat-to-carbohydrate ratio are linked to obesity. A reduction in caloric intake by dieting or fasting can decrease the basal metabolic rate (BMR), while physical activity is important in maintaining it (Mole, 1990). The thermic effect of food represents a small part of total energy expenditure and is not predictive of obesity. Moderate exercise promotes the expenditure of large amounts of fat and calories, achieving weight-loss. Other advantages of exercise include increased cardio respiratory fitness, and HDL cholesterol (Hirsch, 1995).

A subject's response to endurance exercise is determined by the resultant response of several interrelated control systems. This response (performance) is finally determined by a tightly attached partnership (ratio) between oxygen transfer to and carbon dioxide removal from the exercising cells and secondly (not less important) the capacity of the

cells to produce ATP aerobically (Weber, 1984b; Brown, Wiener & Brown, 1985; Sue & Wasserman, 1989). The accurate assessment of body composition in particular fat mass is of considerable importance to practitioners of sports medicine. Physical activity is an important component of life, contributing to quality and function, that reflects a multi factorial, closely regulated integration of the functions of several organ systems (Van de Wolle, Peres & Monod, 1987; Sue, 1994).

The body is put under stress during resistance exercise (gym), because various forces (such as lifting a weight against gravity) are applied to the different muscles and joints (Tiidus & Ianuzzo, 1983). Intense exercise (weight lifting) can cause disruption of and/or degradation of structural proteins in the muscle fibres as well as in the connective tissue (Tiidus & Ianuzzo, 1983). More intense training has an even greater effect on the body. Excessive or an unaccustomed exercise to the body is the most common cause for DOMS (Knitter *et al.,* 2000). Downhill running and resistance training (weight training) are forms of exercise that involve plenty of eccentric contractions (contractions of the muscle while being stretched) that have a more severe effect on the muscle fibres, because eccentric contractions cause micro tears in muscle fibres (Knitter *et al.,* 2000). Trying to lessen the amount of protein breakdown during high-intensity exercise could be beneficial to training people (Armstrong, 1986; Evans, & Cannon, 1991; Hough, 1992). The body can physiologically adapt to exercise up to a point, but needs some aid in diminishing the amount of protein breakdown taking place (Knitter *et al.,* 2000). It seems as if additional nutrients may be needed to aid in muscle and strength gains and recovery from resistance exercise which disrupts muscles (Florini, 1985). Both professional and amateur sport competitors do make use of additional nutrients to aid in either maintaining or gaining strength and/or stamina depending upon the sport type's needs (Steven, Nissen, & Sharp, 2003).

The anti catabolic effects of the branched chain amino acid, leucine and certain of its metabolites for example α-ketoisocaproate (KIC) has been known for over 30 years (Nissen, Sharp, Ray, Ratmacher, Rice, Fuller, Connelly, & Abumrad, 1996). Animal studies showed that leucine and KIC were nitrogen sparing (Van Koevering, & Nissen, 1992). HMB is a metabolite of the branched chain amino acid leucine and is produced from KIC (Nair, Schwartz, & Wells, 1992; Kreider, Ferreira, Greenwood, &

Wilson, 2000). Both leucine and KIC are proposed to decrease nitrogen and protein loss by inhibiting protein breakdown (Van Koevering, & Nissen, 1992; Nair, Schwartz & Wells, 1992; Nissen, & Sharp, 1996; Steven *et al.,* 2003). Animal studies with leucine supplementation showed an increase in muscle-cell and immune-cell production of animals whose muscles suffered from severe stress or trauma (starvation of muscle tissue during which proteolysis was greatly elevated) (Kreider, 2000). Plasma concentrations of HMB range between 1 to 4 µM but can increase up to 10-fold after leucine has been added to a diet (Nair, 1992). Data suggests that a 60-gram dose of leucine or 20-gram dose of KIC produces these anabolic effects (Zhang, Talleyrand, Ratmacher, Nissen & Van Koevering, 1993).

HMB, a normal product of human metabolism, is naturally present in mother's milk and is found (in small quantities) in the foods we eat for example grapefruit and catfish (Nissen *et al.,* 2000). HMB is a water-soluble compound and is excreted in the urine in proportion to dietary intake (Zhang *et al.,* 1993). Therefore, based on the chemistry of HMB, it would be predicted that HMB is a safe compound. HMB is classified as a dietary supplement (Nissen & Sharp, 1996). HMB is derived from KIC when KIC is oxidized in the liver by the enzyme KIC-dioxygenase (Van Koevering, & Nissen, 1992).

Due to the concerns that the public may have about the unhealthy manner in which young adults try to attain healthy looking bodies and the unhealthy products they use to achieve this aim (Nissen, Sharp, Panton, Vukovich, Trappe & Fuller 2000), researchers have studied HMB to determine its safety. Every human study included an extensive safety profile which screened for adverse reactions and organ function (Nissen, & Sharp, 1996). Psychological profiles and physical exams were performed in each study. There were no indications in any of the tests that HMB presented any danger to the subjects (Baxter, Carlos, Thurmond, Rehani, Bultman & Frost, 2005). The only change in blood chemistry related to a decrease in a total and low-density-lipoprotein (LDL) cholesterol levels.

The mechanism of HMB is not exactly known, but the latest research indicates that HMB might supply a precursor to muscle and to the immune system that supports maximal cellular repair (Steven *et al.,* 2003). This hypothesis reasons that the

muscle membrane is more rapidly repaired after exercise induced muscle damage. Muscle growth is supported by having an adequate supply of this substrate in the muscle for membrane expansion. Another hypothesis is that HMB supports a decrease in protein turnover or muscle damage (Van Koevering, & Nissen, 1992). This decrease in muscle breakdown could in turn result in more rapid recruitment of neural motor units by muscle fibres and may act as a catalyst to faster strength increases.

HMB works in the laboratory, but does it work in the real world (Nissen, & Sharp, 1996)? It seems that the role of HMB is to shift the balance of protein metabolism in favour of new muscle growth, and it also appears to minimize the breakdown of muscle tissue (Steven *et al.,* 2003). HMB has been shown to reduce the effects of DOMS twenty four hours after exercise and reduce the signs and symptoms of exercise-induced muscle damage in non-resistance trained males following a single bout of eccentrically biased resistance training (Sabourin & Bieber, 1983). This decrease in muscle breakdown could result in more rapid neural recruitment by the muscle fibres and may act as a catalyst to faster strength increases (Nissen, & Sharp, 1996). In doing so, HMB can help support a consistent increase in muscle-tissue growth and it appears that HMB supplementation may enhance the muscle-building and fat-burning effects of exercise (Nissen, & Sharp, 1996; Gallagher, Carrithers, Godard, Schulze & Trappe, 2000; Steven *et al.,* 2003).

HMB is a natural by product of the body. HMB is a metabolite of the amino acid leucine and is produced from KIC. If there is damage to the muscle it can be reasoned that muscle damage could lead to a decrease in physical performance. A hypothesis was proposed that, when combined with exercise, HMB might be able to up-regulate the body's endurance (tempo of exercise) and accelerate the rate at which to gain muscle and burn fat, that will allow the subject to enhance his lean body mass (LBM) & increase his power output.

3.2 MATERIALS AND METHODS

3.2.1 Research design

The study was a double blind clinical trial. A total number of 40 subjects (male, aged 19 – 24 years) participated. Each participant had to complete a series of pre- and post-tests which consisted of the following.

1. Isokinetic testing of the hamstring (Bicepsa Femoris) and quadriceps muscle (Cybex resistance trainer).
2. Anthropometric measurements (Heath-Carter Method).
3. Strength test to determine weight lifting capability of the subject on incline leg press, lat pull down and bench press.

Before the pre evaluation, the subjects were not allowed to eat any food for 4 hours, to exercise for 12 hours or consume alcohol for 24 hours. The subjects participated in a supervised exercise program for 1 hour, three times a week. This program was followed for 8 weeks. In addition, each subject received a dosage of 3 g of HMB each day for 6 days of each week. During the 8 weeks they also followed a set diet and meal plan (APPENDIX C) to lessen the chance of any external factors that could influenced the study. After the 8 weeks have passed, the subjects were re-evaluated. Results were compared between pre- and post test values.

3.2.2 Materials

- HMB – supplied by EAS, a company providing nutritional sport supplements. They were formerly known as NSA (Natural Supplement Association), but changed the name to EAS.
- Placebo – supplied by EAS. The active ingredients are listed in milligrams for each capsule:

  1. Talc purified BP   161 mg
  2. Avicel ph102   64 mg
  3. Potassium Phosphate Monobasic   112 mg
  4. Arocil 200 (Silicon Dioxide)   8 mg
  5. Calcium Phosphate Tribasic   32 mg
  6. Magnesium Stearate   3 mg

- HMB capsules – supplied by EAS. The HMB capsules contain all the same active ingredients as the placebo. There is 250 mg of HMB in each capsule.

- Resistance weight training program (8 weeks) which was specifically developed for the study (developed by Mr. Anton Zackey employed by Institute of Sport Research (ISR) of the University of Pretoria (UP) and registered with the Health Professions Council of South Africa (HPCSA))
- Isokinetic testing apparatus (Cybex Machine) supplied by Institute of Sports Research (ISR) at University of Pretoria (UP)
- Gym equipment supplied by the ISR at UP

3.2.3 Sample

Male students who live in the residential housing of the University of Pretoria were the focus for volunteers to determine a study population. This was done to control external factors from affecting the study population.

All volunteers were screened before they could participate. The process involved filling in a questionnaire to establish physical background information and a physical evaluation. The evaluation consisted of body-composition measurements, initial evaluation of strength (weight lifting capabilities test) and blood creatine kinase quantification. Potential subjects were excluded if they showed any evidence or history of diabetes mellitus, cardiac, renal, liver or pulmonary disease. They were also excluded if they had any joint, muscle or bone injury within the last three months of the start of the study. No subjects were excepted if they either used another anabolic product or have participated in any other training programs within the three months prior to the trial.

3.2.4 Methods

1. After completing the pre-tests the 40 subjects were divided into two groups in such a manner that the two groups were as homogenous as possible (Figure 3.1 to 3.4 and Table 3.1 to 3.6). The tables provide a descriptive analysis of the two study groups. Figures 3.1 to 3.4 support the descriptive analysis values of Tables 3.1 to 3.6 of the two study groups and Figures 3.5 to 3.9 provide visual evidence of the various parameters that had a statistical significant change from a baseline value. Stratification rules were applied on body composition and initial strength capability to insure the two groups are

homogenous. By randomization the experimental and control groups were then decided.

2. Each participant had undergone a series of pre-tests to evaluate their muscle power output and total body composition.

    - Isokinetic (Cybex) test to evaluate quadriceps and hamstring strength, 1 repetition maximum test on all the major muscle groups (Latissimus Dorsi, Quadriceps, Hamstrings/Bicepsa Femoris, Trapeziums, Pectoralis Major) (Li, Wu, Maffulli, Chan & Chan, 1996). This method of testing was chosen due to the fact that isokinetic testing with the Cybex neutralizes the effect of resistance training familiarity among the different subjects. Therefore it equalized both groups.

    - Body composition measurements (anthropometric measurements including: height, weight of participant and skin folds of Triceps, Biceps, Sub scapula, Iliac Crest, Front Thigh and Abdomen using the Heath-Carter method to determine the percentage body fat) (Carter, 2002) (APPENDIX A).

    - Maximal weight lifts (1 repetition maximum) 1RM of the incline leg press, bench press and lat pull downs.

3. Blood sample collections were taken only after the cybex test, before and after the 8 weeks of the trial, to evaluate the creatine kinase activity in the body. CPK isoenzyme testing can help pinpoint the exact source of the damaged tissue. Only 1ml of blood from each subject was necessary for testing. The sample was cooled down to between 4-8 degrees Celsius. It was then centrifuged and 200 µl of serum was then used to determine the creatine kinase value. Creatine kinase is an in indicator of metabolism (Van Deursen, Ruitenbeek, Heerschap, Jap & Ter Laak, 1994). It is the muscle enzyme that catalyzes the reaction during which creatine phosphate is hydrolysed to donate a single phosphate from ADP to ATP. This reaction takes place during the contraction of skeletal muscles. The isoenzyme testing was performed by Drs. du Buisson, Bruinette, Kramer Inc.

4. Three capsules (HMB/Placebo) were taken with 200 ml of water three times a

day (during main meals), with no less than 4 hours in between following dosages. The prescribed dosages added up to 3 g/day of HMB. This protocol was followed for six days in a week and then suspended on the seventh day. The dosage prescription has been studied and proven. (Nissen, & Van Koevering 1990, Nissen, & Sharp 1996, Nissen, & Sharp 2000). Gallagher *et al.,* (2000a) found that 6 grams of HMB did not improve LBM or strength gains over 3 grams per day. Vukovich *et al.* (2001) investigated the digestion patterns of HMB and his results confirmed that 1 gram of HMB should be taken 3 times per day to equal the 3 g/d optimal dosage.

5.   Three 1-hour training session were scheduled for each week. Each session concentrated on a different part of the body to insure no overtraining or straining of a muscle group.   Session 1, focused on Pectorals (Chest), Triceps and Calve muscles. Session 2, the muscles of the upper legs (Quadriceps, Hamstrings) and shoulders (Deltoids). Session 3, then focused on the back (Trapeziums and Trapezoids), Biceps and calves. Both groups followed the prescribed weight training program for 8 weeks (APPENDIX B). As the weeks passed, each participant had to increase the resistance accordingly to ensure that the level of exertion was maintained. A 2-by-2 rule was applied to determine the increase in load. If the subject could do 2 more repetitions than recommended in two consecutive sessions, the weight was increased in the following session. Each exercise and load of all the subjects was recorded.

6.   For the duration of the experimental period (8 weeks) the subjects followed a prescribed diet that was provided to them by the food services of the University of Pretoria's Residential Housing (APPENDIX C). Three meals were taken each day. The distribution of the meals was recorded by a staff member of the residential housing of the University of Pretoria. Record keeping of between meal snacking was also done by the subjects. This procedure monitored the external factors during training sessions and provided evidence for exclusion of further participation if necessary. After the 8 weeks passed, subjects were re-evaluated to compare the results with the pre-test values.

3.3 RESULTS

3.3.1 Considerations

The experimental group (HMB users) was compared with a control group (no supplementation) with respect to the following outcomes:

- Maximal weight lifting capabilities (bench press, leg press & lat pull down, 1 repetition maximum, and isokinetic testing).
- Anthropometric measurements (skin folds used to calculate subcutaneous fat percentage, circumferences of the biceps, triceps and calves).
- Blood creatine kinase activity (as markers of catabolism).

The sample size calculations were based on maximal weight lifting capability (bench press) which was of primary interest in this study (Figure 3.3 and Table 3.4). Three major muscle groups are involved and it was reasoned that the effect on these groups would follow similar trends and hence bench press was considered. Of interest was to compare groups with respect to improvement from a baseline outcome (used resistance weight/load as a ratio of body mass).

An improvement of 10% (body weight x 0.1) was regarded as clinically relevant and a sample of 15 subjects per group did have 10% power to detect such a difference at the 0.05 level of significance, assuming a standard deviation of 0.08 (general performance was expected to be between 0.8 and 1.1 times body weight and crude estimate of sd = ([1.1 − 0.8]/3.92). To provide for a 20% (4 subjects) expected dropout rate 16 subjects were included into each group.

3.3.2 Data analysis

Descriptive statistics included mean, standard deviation and range. The primary analysis addressing the outcome variables of interest were employed and an analysis of covariance comparing groups (Mann-Whitney test) with respect to change from baseline and using the baseline as a covariate. Within groups changes from baseline were tested using student's Wilcoxon Signed Ranks Test. Testing was done at the 0.05 level of significance and should data not confirm to normality, data analysis would resort to nonparametric procedures.

Since the sample was relatively small and consisted of only 16 subjects per group, non-parametric statistics was used to analyse the data. Non-parametric tests, also known as distribution-free tests are a class of test that does not rely on a parameter estimation and/or distribution assumptions (Howell, 1992). The major advantage attributed to these tests is that they do not rely on any seriously restrictive assumptions concerning the shape of the sampled populations and thus accommodates small samples as in the case of this study.

The following statistical data analysis procedures were used:

a. *Descriptive statistics*. Descriptive statistics are primarily aimed at describing the data. The mean, standard deviation, minimum and maximum scores for each measurement per group were determined for reference purposes.

b. *Inferential statistics*: Test hypotheses about differences in populations on the basis of measurements made on samples of subjects (Tabachnick & Fidell, 1996).

(1) *The Mann-Whitney Test:* The Mann-Whitney test is used for testing differences between means when there are two conditions and different subjects have been used in each condition. This test is a distribution-free alternative to the independent samples t-test. Like the t-test, Mann-Whitney tests the null hypothesis that two independent samples (groups) come from the same population (not just populations with the same mean). Rather than being based on parameters of a normal distribution like mean and variance, Mann-Whitney statistics are based on ranks. The Mann-Whitney statistic is obtained by counting the number of times an observation from the group with the smaller sample size precedes an observation from the larger group. It is especially sensitive to population differences in central tendency (Howell, 1992). The rejection of the null hypothesis is generally interpreted to mean that the two distributions had different central tendencies, in other words, that there is a significant difference between the two groups on a specific variable measured.

This test was used to determine significant differences between the experimental and control groups on all variables measured.

(2) *The Wilcoxon Signed Ranks Test*: The Wilcoxon test is used in situations in which there are two sets of scores to compare, but these scores come from the same subjects. This test is the distribution-free analogue of the t-test for related samples. According to Howell (1992) it tests the null hypothesis that two related (matched) samples were drawn either from identical populations or from symmetric populations with the same mean. This test was used to determine whether statistically significant differences existed between the pre- and post-tests obtained for various variables measured within the same group.

The data was captured onto Microsoft excel and converted to SPSS (Statistical Package for Social Sciences) in order to do the analysis. The data analysis had the following aims:

- To determine whether significant differences existed between the two groups on all variables measured after the study had been completed.
- To determine whether significant differences existed between the pre- and post-intervention measurements within the same group.

The collected data therefore had to either confirm or deny the following hypotheses (parameters) of the experimental study.

1. HMB supplementation increased Lean Body Mass (LBM).

2. HMB supplementation increased muscle power output.

3. Creatine-kinase activity and body fat percentage decreased with HMB supplementation.

Baseline values for the two study groups were collected prior to the intervention. The data provides evidence that the two groups were statistically homogenous for the measured parameters at the onset of the study. Data was collected for body composition (LBM, body fat percentage, length, and mass), muscle power output

(bench press, leg press, and lat pull down lifting capabilities, and isokinetic testing) and markers of muscle damage (blood creatine kinase activity).

The results are presented in the following order:

a.   Descriptive statistics for the two groups on all measurements in table form (Table 3.1 to 3.6).

b.   Graphic representation of the comparison of the two groups at the 0.05 level of significance for the various measurements during the pre test and post test (Figure 3.1 to 3.9).

3.3.3   Results

As indicated previously, Mann-Whitney U-tests were used to determine whether statistically significant differences existed between the two groups on various variables measured. Since this statistical technique is based on mean rank, the mean rank scores will be shown in all figures. For actual mean scores please refer to the descriptive statistics tables above. Only statistically significant differences on the 5% level of significance will be graphically presented. It is important to note that statistically significant differences in scores do not necessarily reflect clinical differences.

The Wilcoxon Signed Ranks test was used to determine whether statistically significant changes took place between measurements taken before (pre-test) and after (post-test) the intervention, within the same group regarding the various variables.

The descriptive statistical results (as defined in 3.3.2) are included simply as frame of reference for the reader to see how the two groups performed on all the measurements. The results are presented in Tables 3.1 to 3.6:

The values for LBM and body fat were both given as a percentage of total body weight and also in kilograms (kg) of total body weight (Table 3.1 and Figure 3.1). The pre test values show that the two groups were homogenous with regard to LBM and body fat at the onset of the study. This is also true for the two groups with reference

to the various muscle circumference measurements (biceps, triceps, and calves) in Table 3.2 and body shape of all the test subjects in Figure 3.2. These values were statistically similar with regards to mean and standard deviation for LBM, body fat percentage calculations, muscle circumferences and body shape measurements for the two groups during the pre test.

Table 3.1 : Descriptive statistics for the experimental and control group for body composition.

Group		N	Minimum	Maximum	Mean	Std. Deviation
Experimental Group	PRE TEST					
	Fat %	14	5.4	19.8	14.414	4.5221
	Fat (kg)	14	4	21	12.93	5.274
	LBM %	14	80.2	94.6	85.586	4.5221
	LBM (kg)	14	63.7	86.1	74.093	7.3240
	POST TEST					
	Fat %	14	5.5	20.5	15.079	4.7957
	Fat (kg)	14	4.1	21.9	13.693	5.6651
	LBM %	14	79.5	94.5	85.036	4.7472
	LBM (kg)	14	62.5	85.1	73.907	6.7678
	Valid N (listwise)	14				
Control Group	PRE TEST					
	Fat %	15	7.8	24.4	14.160	4.1930
	Fat (kg)	15	6	25	12.13	4.839
	LBM %	15	75.6	92.2	85.807	4.2377
	LBM (kg)	15	60.3	79.2	71.640	5.9267
	POST TEST					
	Fat %	15	8.1	22.7	15.200	4.2334
	Fat (kg)	15	6.9	22.8	13.213	4.8981
	LBM %	15	77.3	91.9	84.833	4.1972
	LBM (kg)	15	61.2	80.8	72.280	6.5895
	Valid N (listwise)	15				

Table 3.2 : Descriptive statistics for the experimental and control group for anthropometry (bicep, tricep and calve circumferences).

Group		N	Minimum	Maximum	Mean	Std. Deviation
Experimental Group	**PRE TEST**					
	Biceps	14	32	44	36.29	2.942
	Calve	14	20.2	42.8	37.621	5.5476
	Triceps	14	5.2	25.2	14.357	6.0147
	POST TEST					
	Biceps	14	32.2	44.6	37.271	3.1463
	Calve	14	20.0	43.5	38.121	5.6206
	Triceps	14	6.8	25.4	14.943	5.8787
	Valid N (listwise)	14				
Control Group	**PRE TEST**					
	Biceps	15	31	41	35.56	3.314
	Calve	15	16.4	40.9	36.560	5.9829
	Triceps	15	2.5	17.0	6.120	3.8638
	POST TEST					
	Biceps	15	32.6	41.8	36.880	3.0115
	Calve	15	35.1	41.8	38.500	2.0160
	Triceps	15	2.5	15.1	5.920	3.6985
	Valid N (listwise)	15				

Table 3.3 shows the various skin fold measurements (used to calculate subcutaneous body fat percentage) for the experimental and control groups. The collective data shows no significant statistical difference between the two groups for skin folds during the pre test of the study.

The skin fold values provide additional information to support that the two study groups were statistically homogenous during the pre test.

Table 3.3 : Descriptive statistics for the experimental and control group for anthropometry (Skin folds)

Group		N	Minimum	Maximum	Mean	Std. Deviation
Experimental Group	PRE TEST					
	Supra-Ilium	14	7.2	56.1	28.250	15.2956
	Sub-Scapula	14	7.6	32.2	15.407	6.9575
	Para-Umbilicus	14	10.0	40.3	25.469	11.0265
	Thigh	14	10.2	32.2	18.029	6.9525
	Calve	14	4.0	20.2	12.493	5.5814
	POST TEST					
	Supra-Ilium	14	7.6	45.2	25.743	12.6285
	Sub-Scapula	14	7.8	26.4	13.807	5.6243
	Para-Umbilicus	14	9.0	41.8	24.671	11.1368
	Thigh	14	7.6	30.4	18.629	7.4653
	Calve	14	4.8	22.0	12.864	5.8190
	Valid N (listwise)	14				
Control Group	PRE TEST					
	Supra-Ilium	15	9.0	75.1	28.800	19.2953
	Sub-Scapula	15	6.9	30.9	14.413	6.9141
	Para-Umbilicus	15	11.2	59.8	24.964	12.9858
	Thigh	15	8.1	32.6	19.573	7.2982
	Calve	15	5.1	21.2	13.073	4.9701
	POST TEST					
	Supra-Ilium	15	9.1	62.3	25.360	15.4445
	Sub-Scapula	15	6.7	30.8	13.540	6.0861
	Para-Umbilicus	15	10.9	47.5	23.133	10.8857
	Thigh	15	7.6	35.0	18.847	7.0847
	Calve	15	4.3	19.9	11.807	4.4035
	Valid N (listwise)	15				

Table 3.4 : Descriptive statistics for the experimental and control group for power output (lifting capabilities)

Group		N	Minimum	Maximum	Mean	Std. Deviation
Experimental Group	**PRE TEST**					
	Leg press (kg)	14	170	380	270.00	60.383
	Bench press (kg)	14	80	150	104.29	17.415
	Lat pull down (lbs)	14	150	256	191.86	29.231
	POST TEST					
	Leg press (kg)	14	210	395	330.00	55.490
	Bench press (kg)	14	90	160	110.00	19.612
	Lat pull down (lbs)	14	160	293	210.64	33.214
	Valid N (listwise)	14				
Control Group	**PRE TEST**					
	Leg press (kg)	15	150	330	248.67	50.266
	Bench press (kg)	15	60	120	94.33	16.783
	Lat pull down (lbs)	15	130	200	162.67	20.517
	POST TEST					
	Leg press (kg)	15	210	400	308.33	56.241
	Bench press (kg)	15	70	140	98.80	17.913
	Lat pull down (lbs)	15	140	220	178.00	22.741
	Valid N (listwise)	15				

Tables 3.4 and 3.5 describe the two groups with regard to their muscle power output abilities.

Table 3.4 shows the similar strength (power output) values for the two groups (lifting capabilities), especially with regard to bench press outputs.

Isokinetic measurements with the Cybex scientifically and quantitatively provided data that indicates the two groups were homogenous with regard to isokinetic power output during the pre test (Table 3.5 & Figure 3.4).

Table 3.5 : Descriptive statistics for the experimental and control group for power output (Cybex [isokinetic] measurements)

Group		N	Minimum	Maximum	Mean	Std. Deviation
Experimental Group	**PRE TEST**					
	Right Quadricep (Nm)	16	220	334	271.25	30.576
	Left Quadricep (Nm)	16	222	328	260.25	29.388
	Right Hamstring (Nm)	16	119	209	157.75	26.625
	Left Hamstring (Nm)	16	103	217	147.50	28.439
	POST TEST					
	Right Quadricep (Nm)	16	228	380	278.69	42.797
	Left Quadricep (Nm)	16	220	395	274.06	43.545
	Right Hamstring (Nm)	15	123	206	166.13	22.769
	Left Hamstring (Nm)	16	15	199	151.94	43.478
	Valid N (listwise)	15				
Control Group	**PRE TEST**					
	Right Quadricep (Nm)	16	203	342	267.00	42.280
	Left Quadricep (Nm)	16	183	320	260.94	39.768
	Right Hamstring (Nm)	16	94	171	141.06	23.985
	Left Hamstring (Nm)	16	99	255	145.75	34.887
	POST TEST					
	Right Quadricep (Nm)	16	0	359	259.63	81.156
	Left Quadricep (Nm)	16	197	361	272.88	42.256
	Right Hamstring (Nm)	15	0	190	150.13	47.979
	Left Hamstring (Nm)	16	40	180	147.44	33.570
	Valid N (listwise)	16				

Table 3.6 : Descriptive statistics for the experimental and control group for blood tests (CK activity)

Group		N	Minimum	Maximum	Mean	Std. Deviation
Experimental Group	Pre-test Blood Creatine Kinase Activity (c/ul)	11	108	945	348.45	283.274
	Post-test Blood Creatine Kinase Activity (c/ul)	9	93	696	207.89	188.504
	Valid N (listwise)	9				
Control Group	Pre-test Blood Creatine Kinase Activity (c/ul)	14	67	781	371.79	243.077
	Post-test Blood Creatine Kinase Activity (c/ul)	14	76	735	250.71	188.837
	Valid N (listwise)	14				

Table 3.6 contains data that describes the two study groups' creatine kinase activity levels during the pre test. This information provides the baseline values for creatine kinase activity (as a marker of muscle catabolism) and it was used to evaluate the last of the three hypotheses of the experimental study (creatine kinase activity and fat percentage decrease with HMB supplementation).

In summary, all the descriptive statistics indicate the similar starting values for measured parameters between the two groups and provide the baseline data that serves as the foundation for the interpretation of the results of the post test values.

The post test results were calculated as the change (difference) from the baseline values that were collected during the pre test.

Statistically, no significant differences were found on the 5% level of significance between the two groups for any of the other pre- and post-test variables measured. Thus, at the onset of the experiment the two groups did not differ significantly with regard to these measured variables.

The Mann-Whitney test could not find any statistical differences during the pre test at the 5% level of significance with regard to the tested parameters (mass, body length, body fat percentage) that were used to calculate LBM. These values are summarized in Figure 3.1 and 3.2. LBM was expressed in kilograms of total body mass and also as a percentage of the total body weight.
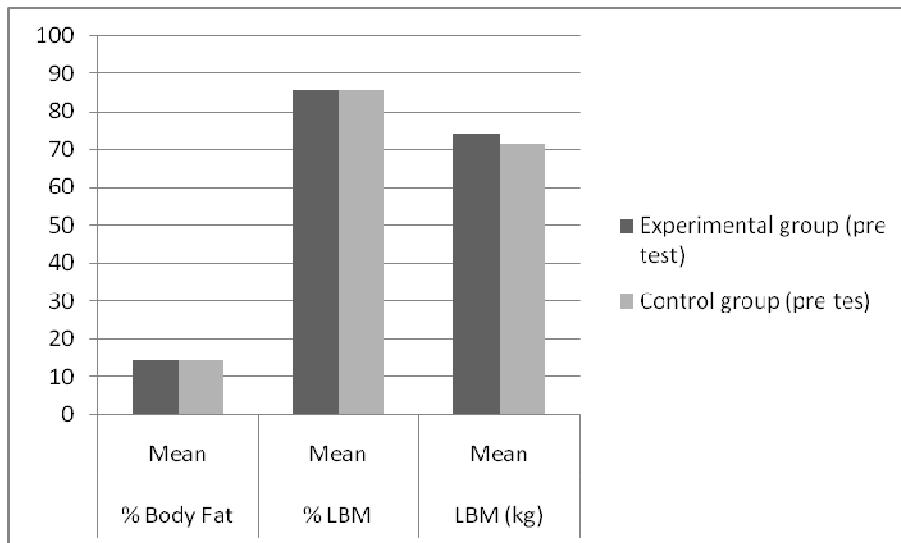

**Figure 3.1 : Body composition of the two study groups prior to the study**
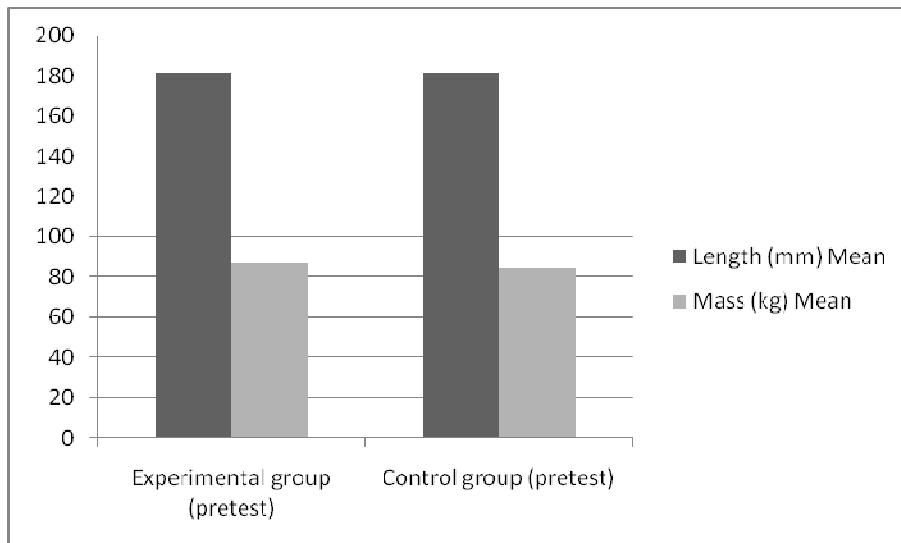
Figure 3.2 : Body shape & size of the two study groups prior to the study

The Mann-Whitney statistically indicated significant differences at the 5% level of significance between the two groups for pre-test lat pull down, but not for leg press

and bench press values during the pre test (Figure 3.3). This alteration might be attributed to the subjects' familiarity to the exercise and therefore bench press and leg press was chosen as an equalizer to measure power output (lifting capabilities).
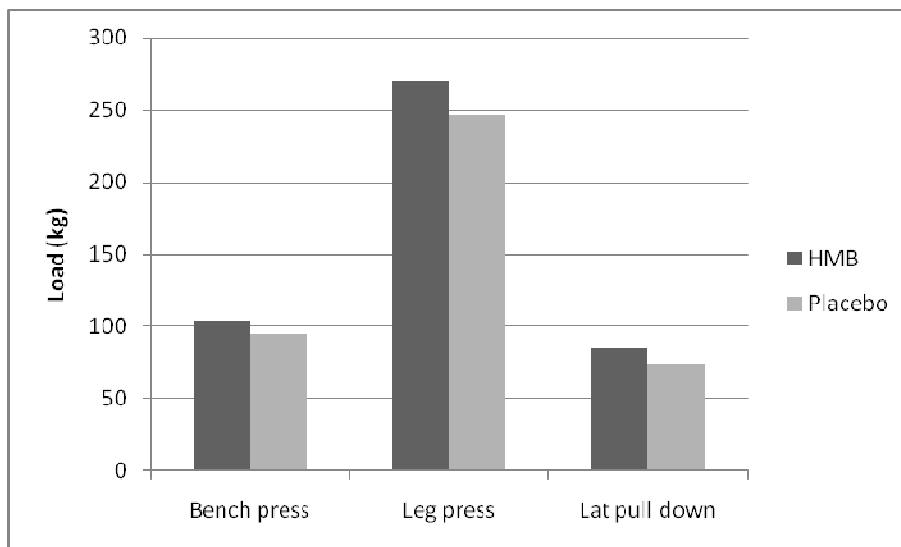

**Figure 3.3 : Initial lifting capabilities of the two study groups prior to the study**
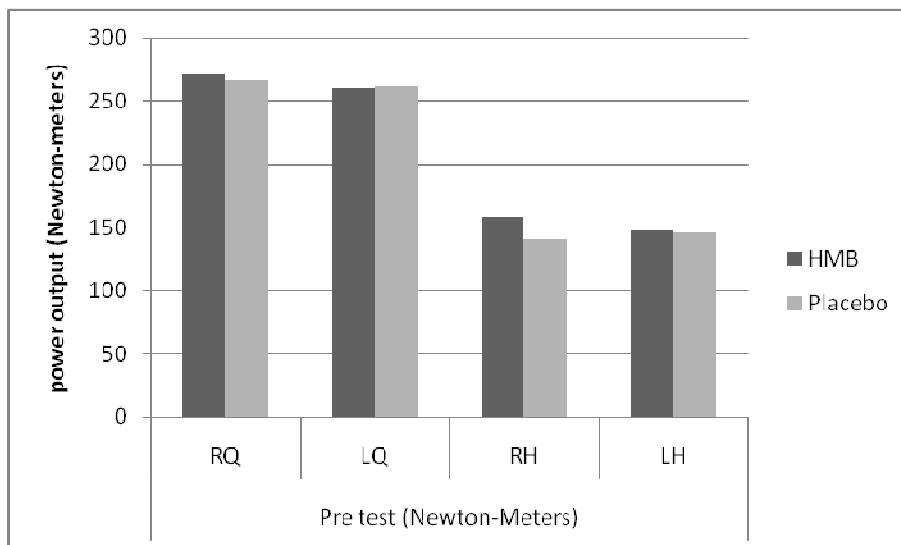
Figure 3.4 : Cybex results during the pre test for both study groups

RQ = Right Quadricep; LQ = Left quadriceps; RH = Right hamstring; LH = Left hamstring

The best quantitative values for power output were collected during the isokinetic testing (Figure 3.4) and the Mann-Whitney test couldn't find any significant statistical

difference at the 5% level of significance between the two groups for isokinetic power output during the pre test.

The various changes from a baseline value are presented in Figures 3.5 to 3.8.
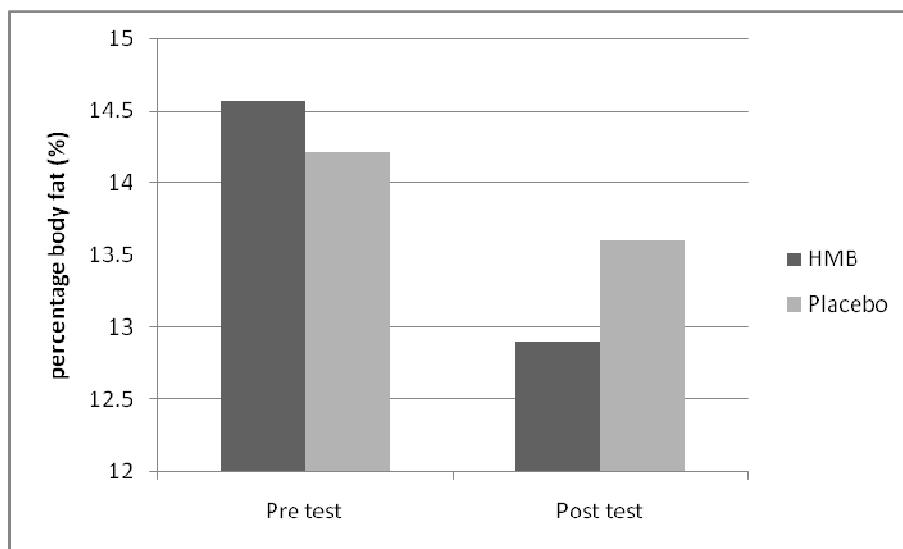
Figure 3.5 : Results for changes in total body fat percentages

Both study groups showed decreases for body fat percentage, but the body fat percentage decrease was higher in conjunction with HMB supplementation than in the control group as can be seen in Figure 3.5. When the data in Figure 3.5 is viewed together with blood creatine kinase activity levels in Figure 3.9 it could be reasoned that less protein is used as a fuel source for metabolism.
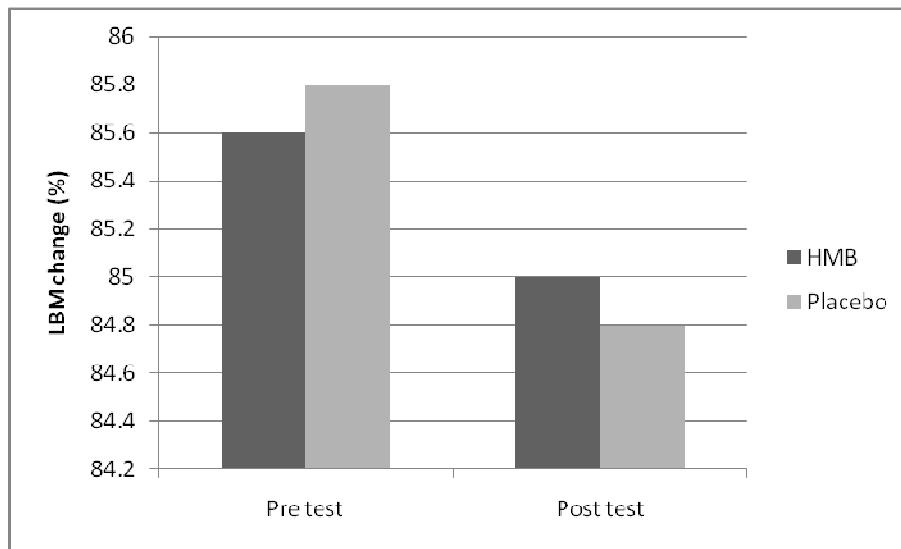
Fig 3.6 : Change in LBM values from a baseline values.

In Figure 3.6 the data indicates that the experimental group's decrease in post test values for LBM was smaller than within the control group. Post test indications confirm that supplementation with HMB has a statistical greater effect on improving LBM values of resistance trainers from a baseline.
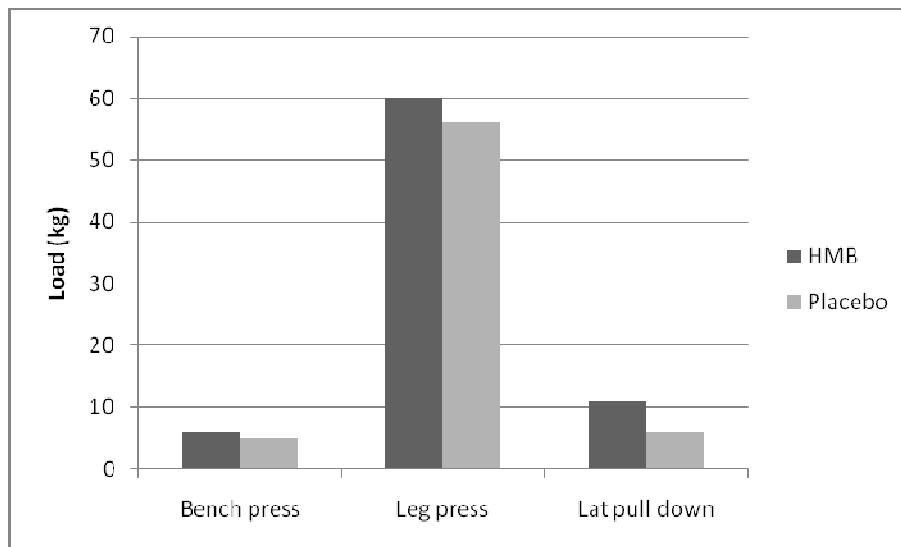

Figure 3.7 : 1 Repetition Maximum results for leg press, bench press and lat pull downs

HMB supplementation has resulted in a higher increase of weight lifting capability from a baseline value in the experimental group than in the control group (Figure

3.7). This gives an indication that the muscle power output of the experimental group has increased by a greater number with relation to the control group. The application of the Mann-Whitney Test showed statistical increase for both groups, but the increases within the experimental group showed a greater change from a baseline. The differences between the groups were greater than 5%.

Isokinetic post test indications (Figure 3.8) show that an improvement from a baseline in the experimental group was greater than gains within the control group, but it is unclear why the right hamstring (RH) tested less efficient in the experimental group than the control group.

Both lifting capabilities and isokinetic testing results confirm that HMB supplementation increased muscle power output with regard to lifting capability and isokinetic testing.
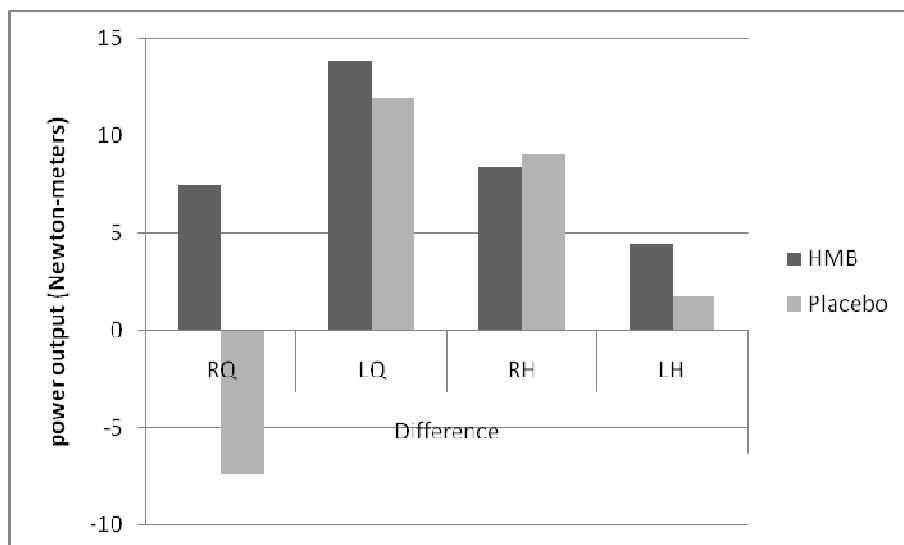

Figure 3.8 : Cybex improvements from a baseline value (isokinetic improvements).
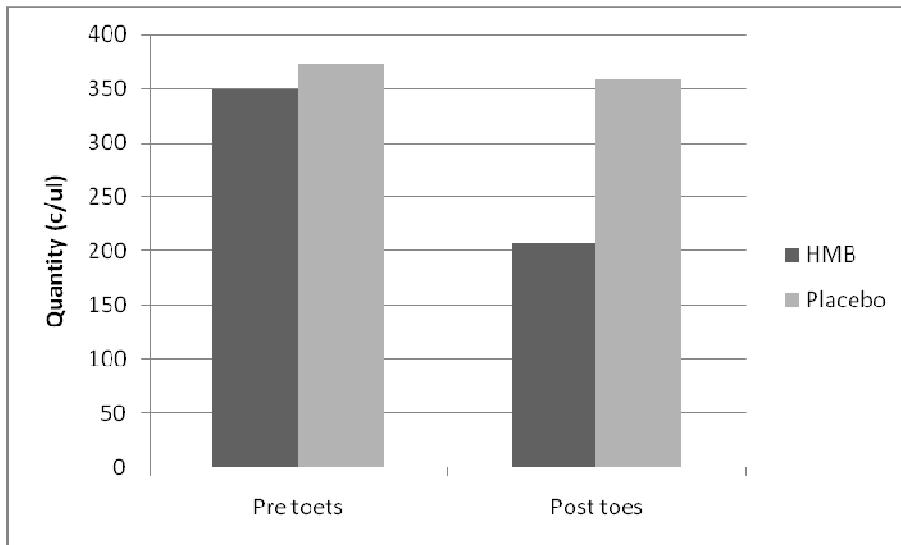
Figure 3.9 : Creatine kinase activity results

As mentioned earlier, HMB supplementation decreased body fat percentages and increased LBM. The values in Figure 3.5 and 3.6 confirm that supplementation with HMB decrease body fat percentages. The creatine kinase activity levels decreased markedly within the experimental group from a baseline value in Figure 3.9 when viewing the creatine kinase activity levels for the post test.

With HMB supplementation the activity of creatine kinase has lowered by a greater margin than without the supplementation. This indicates that less protein breakdown occurs with HMB supplementation. When protein breakdown decreases, the amount of fat in the body is also lowered (due to an increase in free fatty acid metabolism).

This data confirms that supplementation with HMB decreases creatine kinase activity levels within individuals.

3.4   DISCUSSION

Exercise is generally considered to be good for health and its effects on body composition have been widely studied (Aulin, 1995; Fielding, 1995; Lukaski, Bolonchuk, Siders & Milne, 1996; Terbizan & Seljevold, 1996; Wallberg-Henriksson, Rincon & Zierath, 1998). Regular moderate exercise should be considered as a viable means of treating depression, anxiety and improving mental well-being, physical self perceptions and in some cases global self-esteem in the general public

(Fox, 1999). Total energy expenditure varies as a result of differences in duration, frequency and intensity of physical activities (Van Baak, 1999).

During moderate (below lactate threshold) exercise and also with rapidly incremental work, pulmonary and muscle oxygen uptake (VO_2) increases as a linear function of work rate (Poole & Richardson, 1997). The muscles, in heavy muscular work, have to be supplied with up to 500 times more oxygen (O_2) than when the body is in a resting state and at the same time, larger quantities of carbon dioxide (CO_2) and lactate, have to be removed (Despopoulos & Sibernagl, 1991).

Exercise as part of a weight loss program has been shown to help preserve, or even increase, fat free mass (Blair, 1993, Garrow & Summerbell, 1995, Saris, 1995, Kraemer, Volek, Clark, Gordon, Incledon & Puhl, 1997, Pérusse, Collier, Gagnon, Leon, Rao & Skinner, 1997), of which muscle is an important component. This increased ratio of fat free mass to fat mass has important health consequences. Muscles are metabolically active and an increase in muscle mass results in an increase in the resting metabolic rate (Saris, 1995) and the $VO_{2\,max}$ (Proctor & Joyner, 1997). Diseases known to be associated with obesity are decreased in subjects with less fat and an increased fat free mass, making fat loss more important than weight loss (Bryner, Toffle, Ullrich & Yeater, 1997).

An exercise program can therefore contribute to good health by reducing the risk of some chronic diseases, for instance, coronary heart disease, non-insulin dependent diabetes and hypertension (Bjorntorp, 1995). It can also help keep older people active (Evans, 1995) and independent for longer. These beneficial results are not only a result of a decrease in body fat, but also an increase in the muscle component of the body which is of critical importance.

Garrow & Summerbell showed that a person's level of physical activity can have an impact on body composition, and that specific aerobic exercises in conjunction with a restricted diet, resulted in significant differences in body composition (decrease in body fat) (Garrow & Summerbell, 1995). The associated increase in LBM and the muscle component affords added health benefits.

Supplementation should be considered only by people who are serious about performing in competitive sport. This study has indicated that both groups have gained muscle strength over the course of the 8 weeks (data analysis), but the gains were greater in the experimental group.

It is important to notice that in practically any investigation the possibility of obtaining contradictory results is high, based on the inherent noise found across human participants (Schmidt, 1999). The primary target of the study was to investigate the physiological and performance effects of HMB, but during such research the participant's social milieu, motivations, self-confidence, and current emotive states also play a role (Weinberg, 2003; Jones, 2003; Wilson, 2006). The most effective way to filter out this problem is by obtaining an adequate sample sizes to include in the research.

By evaluating the creatine kinase values and the weight lifting values of the two different groups, it can be concluded that HMB supplementation does lower protein breakdown and the fat percentage in subjects in conjunction with resistance training. There are greater increases in muscle power output with HMB supplementation & also a higher measured LBM value.

Effects of beta-hydroxy-beta-methylbutyrate (HMB) supplementation on the body-composition and muscle power output of non competitive sporting males between 19 and 24 years who perform resistance weight training three times a week for 8 weeks were measured. Physically active men and women may be less likely than their inactive peers to become overweight. Exercise has a favourable effect on body fat distribution, with a reduction in waist-to-hip ratio with increased exercise. Exercise is especially important in maintaining weight loss in overweight persons. Physical activity can directly affect both total energy intake and total energy expenditure. Physical activity can also affect fat balance, and it is becoming clear that imbalances in total energy are largely due to imbalances in fat. Exercise also has additional, beneficial effects on most of the metabolic risk factors for cardiovascular disease and non-insulin dependant diabetes mellitus. Therefore, exercise testing provides a basis for the design of training programs and allows for monitoring progress throughout the training program. Used properly, testing and monitoring is useful to both trainers and subjects.

Therefore, exercise in conjunction with an appropriate diet is of benefit to overweight persons and provided that feasible methods and motivation are available, we recommend exercise as an important part of a weight control program. The effects of ß-Hydroxy-ß-methylbutyrate were tested in a clinical trial. Two homogenous groups of 20 males were evaluated for initial strength capabilities and body composition. For 8 weeks the subjects lifted weights three times a week and followed a balanced diet. Creatine kinase activity decreased with HMB supplementation. Gains in muscle power output were greater in the experimental group. Fat percentage decreases were recorded with HMB supplementation.

It is important to mention that no supplementation should be considered without consulting either a registered medical doctor or dietician.

It can be suggested that further studies should concentrate on the pathway of KIC metabolism as well as the biochemical mechanism of action of HMB to fully comprehend its involvement in establishing hypertrophic effects and shortening recovery time (Tischler, 1982). HMB's mechanisms of action are generally considered to operate through its capacity to stabilze the sarcolemma (Nissen, 1997) and/or attenuate proteolytic pathways (Smith, 2004; Baxter, 2005). The various actions that might play a role in stabilizing the sarcolemma include: the Cholesterol Synthesis Hypothesis (CSH) (Nissen *et al.,* 2000); the antagonistic effects of HMB on proteolytic pathways through the ubiqituin-proteasome proteolysis dependant pathway (Ub-pathway) (Smith, 2004).

Other possible suggestions for further studies should include adjustments to future methodologies. As mentioned in the literature review, the dosage for HMB supplementation has been limited to 3 g/d that are spread into 3 equal dosages for each day of supplementation. There are not plenty of studies that focus on the optimal dosage and frequency of HMB supplementation. Research should focus on optimal HMB administration concerning nutrient timing and the effects of acute HMB administration.

Another suggestion that can be proposed about future studies should include research conflicting results. These conflicting results may be partly attributed to

variability in humans, inadequate sample sizes, and methodological issues including the specificity of testing conditions, cases of overtraining, elicitation of an inadequate training stimulus in experienced participants, limited dependent variables and short duration experiments (Gabriel, 2008).

3.5   CONCLUSION

After completion of the data analysis, the study proved that HMB is an effective anti-catabolic with reference to the parameters of power output, LBM, body fat percentage and creatine kinase activity.

The results of the lifting and isokinetic tests provide statistical evidence that conclude HMB supplementation can increase power output of individuals and supports the theory and results of Panton (2000) and Jówko (2001).

The greater decrease in total fat percentage indicates an increase in LBM within the experimental group in the same fashion as Nissen's previous study showed (2000). It can therefore be concluded that HMB supplementation does increase LBM and decrease body fat percentage.

HMB supplementation lowers creatine kinase activity within individuals and therefore has an anticatabolic effect that supports the theories and results of Gallagher *et al.,* (2000b) and Van Someren (2005).

From the data we can conclude that after a period of 8 weeks, supplementation with HMB in conjunction with a balanced diet and an organized resistance training program can improve an individual's muscle power output, lean body mass and decrease the creatine kinase activity and total percentage body fat within an individual. These results further allow us to conclude that HMB supplementation decreases proteolysis, which allows for faster recovery from exercise and therefore allows for quicker succession of resistance training sessions.

After evaluating the collected data of the study, the following conclusions can be made:

1. HMB supplementation increased Lean Body Mass (LBM)

2. HMB supplementation increased muscle power output

3. Creatine kinase activity and fat percentage decreased with HMB supplementation.

REFERENCES

Alter, M.J. (1988). *The Science of Stretching.* Champaign, IL. Human Kinetics Publishers.

Arakawa, K. (1999). *Exercise, a measure to lower blood pressure and reduce other risks.* Clinical Experiments on Hypertension, 21(5-6):797-803.

Arendt-Nielsen, L. Weidner, M. Bartholin, D. & Rosetzsky, A. (2007). *A double-blind randomized placebo controlled parallel group study evaluating the effects of ibuprofen and glucoseamine sulfate on exercise induced muscle soreness.* Journal of Musculoskeletal Pain, 15(1):21-28.

Armstrong, R.B. (1986). *Muscle damage and endurance events.* Sports Medicine, 3:80-93.

Arts, F.J.P. & Kuipers, H. (1994). *The relation between power output, oxygen uptake and heart rate in male athletes.* International Journal of Sports Medicine, 15:228-31.

Astrand, P. & Rodahl, K. (1986). *Textbook of Work Physiology.* London: McGraw-Hill.

Aulin, K.P. (1995). *Gender-specific issues.* Journal of Sports Sciences, 13:535-S39.

Baier, S. Johannsen, D. Abumrad, N. Rathmacher, J.A. Nissen, S. & Flakoll, P. (2009). *Year-long changes in protein metabolism in elderly men and women supplemented with a nutirion cocktail of beta-hydroxy-beta-methylbutyrate (HMB), L-arginine, and L-lysine.* Journal of Parental & Enteral Nutrition, 33(1):71-82.

Baldwin, J. Snow, R.J. Carey, M.F. & Febbraio, M.A. (1999). *Muscle IMP accumulation during fatiguing submaximal exercise in endurance trained and untrained men.* American Journal of Physiology, 277(1 Pt 2):R295-300.

Baty, J.J. Hwang, H. Ding, Z. Bernard, J.R. Wang, B. Kwon, B. & Ivy, J.L. (2007). *The Effect of A Carbohydrate and Protein Supplement on Resistance Exercise Performance, Hormonal Response, and Muscle Damage.* The Journal of Strength and Conditioning Research, 21(2):306-648.

Bar-Mor, G. Zeevi, B. Yaaron, M. & Falk, B. (1999). *Use of the heart rate monitor to modulate physical activity in adolescents with congenital aortic stenosis: an innovative approach.* Journal of Pediatric Nursing, 14(4):273-7.

Baxter, J.H. Carlos, J.L. Thurmond, J. Rehani, R.N. Bultman, J. & Frost, D. (2005). *Dietary toxicity of calcium beta-hydroxy-beta-methylbutyrate (CaHMB).* Food and Chemical Toxicology, 43(12):1731-41.

Berne, R.M. & Levy, M.N. (1993). *Physiology.* London: Mosby Year Book, p.833-850.

Bjorntorp, P. (1995). *Evolution of the understanding of the role of exercise in obesity and its complications.* International Journal of Obesity Relating to Metabolic Disorders, 19:S1-4.

Blair, S.N. (1993). *Evidence for success of exercise in weight loss and control.* Annual Internal Medicine, 119:702-706.

Bless, C. & Higson-Smith, C. (1995). *Fundamental of social research methods: An African perspective (2nd edition).* Zambia: Juta and Co, Ltd.

Blimkie, C.J.R. (1988). *Resistance training during preadolescence: Issues and controversies.* Sports Medicine, 15(6):389-407.

Bouchard, C. & Boulay, M.R. (1988). *Heredity and trainability of aerobic and anaerobic performances.* Sports Medicine, 5:69-73.

Brand, K. (1997). *Aerobic glycolysis by proliferating cells: protection against oxidative stress at the expense of energy yield.* Journal of Bioenergy and Biomembranes, 29(4):355-64.

Brown, S.E. Wiener, S. & Brown, R.A. (1985). *Exercise performance following a carbohydrate load in chronic airflow obstruction.* Journal of Applied Physiology, 58:1340-1346.

Bryner, R.W. Toffle, R.C. Ullrich, I.H. & Yeater, R.A. (1997). *The effects of exercise intensity on body composition, weight loss, and dietary composition in women.* Journal of the American College of Nutrition, 16(1):68-73.

Cabrera, M.E. Saidel, G.M. & Kalhan, S.C. (1999). *Lactate metabolism during exercise: analysis by an integrative systems model.* Journal of Applied Physiology, 277(5 Pt 2):R1522-36.

Cahill, B.R. Misner, J.E. & Boileau, R.A. (1997). *The clinical importance of the anaerobic energy system and its assessment in human performance.* American Journal of Sports Medicine, 25(6):863-72.

Calle, E.E. Thun, M.I. Petrelli, I.M. Rodriguez, C. & Heath, C.W. (1999). *Body-mass index and mor-tality in a prospective cohort of U.S. adults.* New England Journal of Medicine, 341:1097-1105.

Campbell, M.J. McComas, A.J. & Petito, F. (1973). *Physiological changes in aging muscles.* Journal of Neurology, Neurosurgery and Neuropsychiatry, 36:174-82.

Carmouche, D.G. Bubien, R.S. & Kay, G.N. (1998). *The effect of maximum heart rate on oxygen kinetics and exercise performance at low and high workloads.* Pacing Clinical Electrophysiology, 21(4 Pt 1):679-86.62.

Carter, J.E.L. (2002). *The Heath-Carter Anthropometric Somatotype Instruction Manual.* Surrey, Canada: Rosscraft.

Cheung, C.C. Clifton, D.K. & Steiner, R.A. (2000). *Ur E (ed): Neuro endocrinology of Leptin.* Frontal Hormonal Research. Basel, Karger, 26:87–105.

Chicharro, J.L. Perez, M. Vaquero, A.F. Lucia, A. & Legido, J.C. (1997). *Lactic threshold vs ventilatory threshold during a ramp test on a cycle-ergometer.* Journal of Sports Medicine and Physical Fitness, 37(2):117-21.

Cohen-Solal, A. Barnier, P. Pessione, F. Seknadji, P. Logeart, D. Laperche, T. & Gourgon, R. (1997). *Comparison of the long-term prognostic value of peak exercise oxygen pulse and peak oxygen uptake in patients with chronic heart failure.* Heart, 78(6):572-576.

Cole, C.R. Blackstone, E.H. Pashkow, F.J. Snader, C.E. & Lauer, M.S. (1999). *Heart-rate recovery immediately after exercise as a predictor of mortality.* New England Journal of Medicine, 341(18):1351-1357.

Cornelius, W.L. Hagemann, R.W. & Jackson, A.W. (1988). *A study an placement of stretching within a workout.* Journal of Sports Medicine and Physical Fitness, 28:234-236.

Cureton, K.J. Collins, M.A. Hill, D.W. & Mcelhannon, F.M. (1988). *Muscle hypertrophy in men and women.* Medicine and Science in Sports and Exercise, 20(4):338-344.

Das, A.M. Jolly, R.D. & Kohlschutter, A. (1999). *Anomalies of mitochondrial ATP synthase regulation in four different types of neuronal ceroid lipofuscinosis.* Molecular Genetics & Metabolism, 66(4):349-355.

Davies, C.T. & Barnes, C. (1972). *Negative (eccentric) work. Physiological responses to walking uphill and downhill on motor-driven treadmill.* Ergonomics, 15:121-131.

De Vries, H.A. & Housh, T.J. (1994). *Physiology of Exercise: For Physical Education, Athletics and Exercise Science.* Madison, WI: Brown & Benchmark.

Demant, T.W. & Rhodes, E.C. (1999). *Effects of creatine supplementation on exercise performance.* Sports Medicine, 28(1):49-60.

Despopoulos, A. & Silbernagl, S. (1991). *Color Atlas of Physiology.* New York: Thieme Medical Publishers, p. 46-50.

Dohm, G.L. & Newsholme, E.A. (1983). *Metabolic control of hepatic gluconeogenesis during exercise.* Biochemical Journal, 212:633-39.

Dontas, A.S. Moschandreas, J. & Kafatos, A. (1999). *Physical activity and nutrition in older adults.* Public Health & Nutrition, 2(3A):429-36.

Egger, G. (1992). *Prescribing Exercise Part I: Physical Fitness and Health.* Modern Medicine in SA, P69-78.

Enoka, R.M. (1988). *Neuromuscular basis of Kinesiology.* Campaign, IL; Human Kinetic Publishers, 31-64.

Essen, B. Pernow, B. Gollruck, P.D. & Saltin, B. (1975). *Muscle glycogen content and lactate uptake in exercising muscles. In: Howard H, Poortmans JR. Metabolic adaptations to prolonged physical exercise.* Basel: Dirkhauser, p.130-34.

Ethyre, B.R. & Lee, E.J. (1987). *Dialogue: Comments on proprioceptive neuromuscular facilitation stretching techniques.* Research Quarterly for Exercise and Sport, 58:184-18.

Evans, W.J. & Cannon, J.G. (1991). *The metabolic effects of exercise-induced muscle damage.* Exercise and Sport Science Review, 19:99-125.

Evans, W.J. (1995). *Effects of exercise on body composition and functional capacity of the elderly.* Journals of Gerontology Series A, 50A (Special Issue): 147-150.

Fielding, R.A. (1995). *The role of progressive resistance training and nutrition in the preservation of lean body mass in the elderly.* Journal of the American College of Nutrition, 14(6):587-594.

Flakoll, P. Sharp, R. Baier, S. Levenhagen, D. Carr, C. & Nissen, S. (2004). *Effect of β-hydroxy-β-methylbutyrate, arginine and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women.* Nutrition, 20(5):445-451.

Florini, J.R. (1985). *Hormonal control of muscle cell growth.* Journal of Animal Science, 61:21-37.

Forjaz, C.L. Matsudaira, Y. Rodrigues, F.B. Nunes, N. & Negrao, C.E. (1998). *Post-exercise changes in blood pressure, heart rate and rate pressure product at different exercise intensities in normotensive humans.* Brazilian Journal of Medical Biological Research, 31(10):1247-55.61.

Fox, S. (1989). *Human Physiology.* Dubuque, Brown.

Fox, K.R. (1999). *The influence of physical activity on mental well-being.* Public Health & Nutrition, 2(3A):411-418.

Frexes-Steed, M. Lacy, D.B. Collins, J. & Abumrad, N. (1960). *Role of leucine and other amino acids in regulating protein metabolism in vivo.* American Journal of Physiology, 262(25):E925-E935.

Fritzsche, R.G. Switzer, T.W. Hodgkinson, B.J. & Coyle, E.F. (1999). *Stroke volume decline during prolonged exercise is influenced by the increase in heart rate.* Journal of Applied Physiology, 86(3):799-805.

Gabriel, J.W. Jacob, M.W. & Anssi, H.M. (2008). *Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex and training experience: A review.* Nutrition & Metabolism, 5(10):1186-1211.

Gallagher, P.M. Carrithers, J.A. Godard, M.P. Schulze, K.E. & Trappe, S.W. (2000). *β- hydroxy-β-methylbyturate ingestion. Part I: effects on strength and fat free mass.* Medical Science of Sports Exercise, 32:2109-2115.

Gallagher, P.M. Carrithers, J.A. Godard, M.P. Schulze, K.E. & Trappe, S.W. (2000). *β-hydroxy-β-methylbyturate ingestion. Part II: effects on haematology, hepatic and renal function.* Medical Science of Sports Exercise, 32:2116-2119.

Garrow, J.S. & Summerbell, C.D. (1995). *Meta-analysis: effects of exercise, with or without dieting, on the body composition of overweight subjects.* European Journal of Clinical Nutrition, 49:1-10.

Gomez, T.G. Beach, G. Cooke, C. Hurdey, W. & Goyert, P. (1991). *Normative database for trunk range of motion, strength, velocity, and endurance with the isostation B-200 lumbar dynamometer.* Spine, 16:15-21.

Gonzalez-Alonso, J. Calbet, J.A. & Nielsen, B. (1999). *Metabolic and thermodynamic responses to dehydration-induced reductions in muscle blood flow in exercising humans.* Journal of Physiology, 520(Pt 2):577-589.

Graham, T.E. & Adamo, K.B. (1999). *Dietary carbohydrate and its effects on metabolism and substrate stores in sedentary and active individuals.* Canadian Journal of Applied Physiology, 24(5):393-415.

Hardman, A.E. (1999). *Interaction of physical activity and diet: implications for lipoprotein metabolism.* Public Health & Nutrition, 2(3A):369-76.

Hardy, L. & Jones, D. (1986). *Dynamic flexibility and proprioceptive neuromuscular facilitation.* Research Quarterly for Exercise and Sport, 57:150-153.

Harrison, T.R. & Pilcher, C. (1930). *Studies in congestive heart failure II. The respiratory exhange during and after exercise.* Journal of Clinical Investigations, 8:291.

Hasson, S.M. Daniels, J.G. Divine, J.G. Niebuhr, B.R. Richmond, S. Stein, P.G. & Williams, J. (1990). *Effect of ibuprofen use on muscle soreness, damage, and performance: A preliminary investigation.* Medicine and Science in Sports and Exercise, 25(1):9-17.

Hayashi, N. Ishihara, M. Tanaka, A. & Yoshida, T. (1999). *Impeding O_2 unloading in muscle delays oxygen uptake response to exercise onset in humans.* Journal of Applied Physiology, 277(5 Pt 2):R1274-81.

Heigenhauser, G.J.F. Sutton, J.R. & Jones, N.L. (1983). *Effect of glycogen depletion on the ventilation response to exercise.* Journal of Applied Physiology, 54(2):470-74.

Heigenhauser, G.J. & Parolin, M.L. (1999). *Role of pyruvate dehydrogenase in lactate production in exercising human skeletal muscle.* Advanced Experimental Medicine and Biology, 474:205-18.29.

Hill, J.O. & Commerford, R. (1996). *Physical activity, fat balance and energy balance.* International Journal of Sports Nutrition, 6:80-92.

Hirsch, I. (1995). *Role and benefits of carbohydrate in the diet: Key issues for future dietary guide-lines.* American Journal of Clinical Nutrition, (Suppl.61):996S-l000S.

Hough, T. (1992). *Ergographic studies in muscular soreness.* American Journal of Physiology, 7:76-92.

Howell, D.C. (1992). *Statistical methods for psychology (3rd ed.).* Belmont: Duxbury Press.

Hubley-Koze, C.L. (1991). Testing flexibility. In JD MacDougall. Weuger HA, Green HJ (eds.). *Physiological Testing of the High-Performance Athlete,* Campaign, IL: Human Kinetics Publishers.

Hughson, R.L. & Tschakovsky, M.E. (1999). *Cardiovascular dynamics at the onset of exercise. Medical Science and Sports Exercise,* 31(7):1005-1072.

Hutton, R.S. (1992). *Neuromuscular basis of stretching exercise.* Strenght and Power in Sport. London: Blackwell Scientific.

Ianuzzo, C.D. (1983). *Effects of intensity and duration of muscular exercise on delayed onset muscle soreness and serum enzymes activities.* Medical Science & Sports Exercise, 15:461-465.

Ibrahimi, A. Bonen, A. Blinn, W.D. Hajri, T. Li, X. Zhong, K. Cameron, R. & Abumrad, N.A. (1999). *Muscle-specific Overexpression of FAT/CD36 Enhances Fatty Acid Oxidation by Contracting Muscle, Reduces Plasma Triglycerides and Fatty Acids, and Increases Plasma Glucose and Insulin.* Journal of Biological Chemistry, 274(38):26761-26766.

Iellamo, F. Pizzinelli, P. Massaro, M. Raimondi, G. Peruzzi, G. & Legramante, J.M. (1999). *Muscle metaboreflex contribution to sinus node regulation during static exercise: insights from spectral analysis of heart rate variability.* Circulation, 100(1):27-32.

Janssen, G.M.E. Kuipers, H. Willems, G.M. Does, R.J.M.M. Janssen, M.P.E. & Guerten, P. (1989). *Plasma activity of muscle enzymes: quantification of skeletal muscle damage and relationship with metabolic variables.* International Journal of Sports Medicine, 10(3):S160-S168.

Jones, M.V. (2003). *Controlling emotions in Sport.* The Sport Psychologist, 17:471-486.

Jówko, E. (2001). *Creatine and β-hydroxy-β-methylbutyrate (HMB) additively increase lean body mass and muscle strength during a weight-training program.* Nutrition, 17(7):558-566.

Kavanagh, N.I. Ainscow, E.K. & Brand, M.D. (2000). *Calcium regulation of oxidative phosphorylation in rat skeletal muscle mitochondria.* Biochimica et Biophysica Acta, 1457(1-2):57-70.

Kelly, D.D. (1982). *The role of endorphins in stress-induced analgesia.* Annals of the New York Academy of Sciences, 398:260-270.

Kirwan, J.P. & Costil, D.L. (1988). *Carbohydrate balance in competitive runners during successive days of intense training.* Journal of Applied Physiology, 65(6):2601-6.

Kissouras, V. (1972). *Genetic limit of functional adaptability.* Internationale Zeitschrift fur Angewandte Physiologic, 30:85-94.

Knitter, A.E. Panton, L. Rathmacher, J.A. Petersen, A. & Sharp, R. (2000). *Effect of β-hydroxy-β-methylbyturate on muscle damage after a prolonged run.* Journal of Applied Physiology, 89:1340-1344.

Koike, A. Hiroe, M. & Marumo, F. (1998). *Delayed kinetics of oxygen uptake during recovery after exercise in cardiac patients.* Medical Science & Sports Exercise, 30(2):185-9.

Kornasio, R. Riederer, I. Butler-Browne, G. Mouly, V. Uni, Z. & Halevy, O. (2009). *Beta-hydroxy-beta-methylbutyrate (HMB) stimulates myogenic cell proliferation, differentiation and survival via the MAPK/ERK and PI3K/Akt pathways.* Biochemica et Biophysica Acta, 1793(5):755-63.

Korzeniewski, B. (1998). *Regulation of ATP supply during muscle contraction: theoretical studies.* Biochemistry Journal, 330(Pt 3):1189-95.

Kowalchuk, J.M. Heigenhauser, G.J.F. & Jones, N.L. (1984). *Effect of pH on metabolic and cardiorespiratory responses during progressive exercise.* Journal of Applied Physiology, 57(5):1558-63.

Kraemer, W.J. Fry, A.D. & Frykman, P.N. (1989). *Resistance training and youth.* Pediatric Exercise and Science, 1:336-350.

Kraemer, W.J. Volek, J.S. Clark, K.L. Gordon, S.E. Incledon, T. & Puhl, S.M. (1997). *Physiological adaptations to a weight-loss dietary regimen and exercise programs in women.* Journal of Applied Physiology, 83(1):270-279.

Kraemer, W.J. Hatfield, D.L. Volek, J.S. Fragala, M.S. Vingren, J.L. Anderson, J.M. Spiering, B.A. Thomas, G.A. Ho, J.Y. Quann, E.E. Izquierdo, M. Hakkinen, K. & Maresh, C.M. (2009). *Effects of amino acids supplement on physiological*

adaptations to resistance training. Medicine & Science in Sports & Exercise, 41(5):1111-1121.

Kreider, R.B. Ferreira, M. Greenwood, M. & Wilson, M. (2000). *Effects of Calcium β-HMB Supplementation During Training on Markers of Catabolism, Body Composition, Strength and Sprint Performance.* Journal of Exercise Physiology, 3(4):48-57.

Lamboley, C.R. Royer, D. & Dionne, I.J. (2007). *Effects of beta-hydroxy-beta-methylbutyrate on aerobic-performance components and body composition in college students.* International Journal of Sport Nutrition & Exercise Metabolism, 17(1):56-69.

Laughlin, M.H. (1999). *Cardiovascular response to exercise.* American Journal of Physiology, 277(6 Pt 2):S244-59.

Li, R.C. Wu, Y. Maffulli, N. Chan, K.M. & Chan, J.L. (1996). *Eccentric and concentric knee flexion and extension: a reliability study using the Cybex 6000 dynamometer.* British Journal of Sports Medicine, 30:156-160.

Livingstone, M.B. (1997). *Heart-rate monitoring: the answer for assessing energy expenditure and physical activity in population studies.* British Journal of Nutrition, 78(6):869-71.

Longhurst, J.C. & Stebbins, C.L. (1997). *The power athlete.* Cardiology Clinic, 15(3):413-29.

Lukaski, H.C. Bolonchuk, W.W. Siders, W.A. & Milne, D.B. (1996). *Chromium supplementation and resistance training: effects on body composition, strength, and trace element status of men.* Journal of Clinical Nutrition, 63(6):954-965.

Malina, R.M. & Bouchard, C. (1991). *Growth, Maturation, and Physical Activity.* Champaign, IL. Human Kinetics Publishers.

Margetts, B.M. Little, P. & Warm, D. (1999). *Interaction between physical activity and diet: implications for blood pressure management in primary care.* Public Health & Nutrition, 2(3A):377-82.

Marshall, J.L. Johnson, N. Wickiewicz, T.L. Tischler, H.M. Koslin, B.L. Zeno, S. & Meyers, M. (1980). *Joint Looseness: a function of the person and joint.* Medicine and Science in Sports, 12(3):189-194.

Marcora, S. Lemmey, A. & Maddison, P. (2005). *Dietary treatment of rheumatoid cachexia with beta-hydroxy-beta-methylbutyrate, glutamine and arginine: a randomised controlled trial.* Clinical Nutrition, 24(3):442-454.

Martin, D.E. & Coe, P.N. (1997). *Better Training for distance runners.* United States: Human Kinetics, p.33-81.

Massey, B.H. & Chaudet, N.L. (1956). *Effects of heavy resistance exercise on range of joint movement in young male adults.* Research Quarterly, 27:41-51.

Maughan, R.J. (1999). *Nutritional ergogenic aids and exercise performance.* Nutritional Research Review, 12:255-280.

McArdie, W.D. Katch, F.I. & Katch, V.L. (1985). *Exercise Physiology.* Philadelphia: Lea + Febiger, p.246-268.

McGlynn, G.H. Laughlin, N.T. & Rowe, V. (1979). *Effect of Electromyographic feedback and static stretching on artificially induced muscle soreness.* American Journal of Physical Medicine, 58:139-148.

Mercier, J. & Desplan, J. (1997). *Notion of metabolic adaptation to exercise.* Revue de Pneumologie Clinique, 53(5):231-7.

Mero, A. (1999). *Leucine Supplementation and Intensive Training.* Sports Medicine, 27(6):347-358.

Meyer, B.J. Van Papendorp, D.H. Meij, H.S. & Viljoen, M. (2002). *Human Physiology 3rd Edition.* Pretoria, Juta and Co, Ltd.

Mole, P.A. (1990). *Impact of energy intake and exer-cise on resting metabolic rate.* Sports Medicine, 10:72-87.

Nair, K.S. Schwartz, R.G. & Wells, S. (1992). *Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans.* American Journal of Physiology, 263:928-934.

Nissen, S. Van Koevering, M. & Webb, D. (1990). *Analysis of β-Hydroxy-β-methyl Butyrate in plasma by gas chromatography and mass spectrometry.* Analytical Biochemistry, 188:17-19.

Nissen, S.L. & Abumrad, N.N. (1996). *Nutritional role of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB).* The Journal of Nutritional Biochemistry, 8(6):300-311.

Nissen, S. Sharp, R. Ray, M. Ratmacher, J.A. Rice, D. Fuller, J.C. Connelly, A.S. & Abumrad, N. (1996). *Effect of leucine metabolite β-hydroxy-β-methyulbutyrate on muscle metabolism during resistance-exercise training.* Journal of Applied Physiology, 2095-2104.

Nissen, S. Sharp, R.L. Panton, L. Vukovich, M. Trappe, S. & Fuller, J.C. (2000). *Beta-Hydroxy-beta-methylbutyrate (HMB) Supplementation in Humans Is Safe and May Decrease Cardiovascular Risk Factors.* Human Nutrition and Metabolism, 1937-1945.

Nobrega, A.C.L. Williamson, J.W. Garcia, J.A. & Mitchell, J.H. (1997). *Mechanisms for increasing stroke volume during static exercise with fixed heartrate in humans.* Journal of Applied Physiology, 83(3):712-7.

Nuviala, R.J. Roda, L. Lapieza, M.G. Boned, B. & Giner, A. (1992). *Serum enzyme activities at rest and after a marathon race.* Journal of Sports Medicine & Physical Fitness, 32:180-186.

O'Connor, D.M. & Crowe, M.J. (2007). *Effects of six weeks of beta-hydroxy-beta-methylbutyrate (HMB) and HMB/creatine supplementation on strength, power, and anthropometry of highly trained athletes.* Journal of Strength & Conditioning Research, 21(2):419-423.

Ober, W.C. Garrison, C.W. Welch, K. & Hutchings, R.T. (1997). *Essentials of Anatomy + Physiology.* London: Prentice - Hall International. p. 349-51,414.

Ormsbee, M.J. Thyfault, J.P. Johnson, E.A. Kraus, R.M. Choi, M.D. & Hickner, R.C. (2007). *Fat metabolism and acute resistance exercise in trained men.* Journal of Applied Physiology, 102(5):1767-72v

Paasuke, M. Ereline, J. & Gapeyeva, H. (1999). *Neuromuscular fatigue during repeated exhaustive submaximal static contractions of knee extensor muscles in endurance-trained, power-trained and untrained men.* Acta Physiology Scandinavia, 166(4):319-26.

Panton, L. (2000). *Nutritional supplementation of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB) during resistance training.* Nutrition, 16(9):734-739.

Pederson, P.K. Kiens, B. & Saltin, B. (1999). *Hyperoxia does not increase peak muscle oxygen uptake in small muscle group exercise.* Acta Physiology Scandinavia, 166(4):309-18.73.

Perrin, D.H. (1993). *Isokinetic Exercise and Assessment.* Champaign, IL. Human Kinetic Publishers.

Pérusse, L. Collier, G. Cagnon, J. Leon, A.S. Rao, D.C. Skinner, J.S. Wilmore, J.H. Nadeau, A. Zimmet, P.Z. & Bouchard, C. (1997). *Acute and chronic effects of exercise on leptin levels in humans.* Journal of Applied Physiology, 83:5-10.

Plowman, S.A. Liu, N.Y. & Wells, C.L. (1991). *Body Composition and sexual maturation in premenarcheal athletes and non athletes.* Medicine and Science in Sports and Exercise, 23(1):23-29.

Poole, D.C. & Richardson, R.S. (1997). *Determinants of oxygen uptake. Implications for exercise testing.* Sports Medicine, 24(5):308-20.

Proctor, D.N. & Joyner, M.J. (1997). *Skeletal muscle mass and the reduction of VO_{2max} in trained older subjects.* Journal of Applied Physiology, 82(5):1411-1415.

Ransone, J. Neighbors, K. Lefavi, R. & Chromiak, J. (2003). *The effect of beta-hydroxy-beta-methylbutyrate on muscular strength and body composition in collegiate football players.* Journal of Strength & Conditioning Research, 17(1):34-39.

Rauramaa, R. & Vaisanen, S.B. (1999). *Interaction of physical activity and diet: implications for haemostatic factors.* Public Health & Nutrition, 2(3A): 383-90.46.

Rico-Sanz, J. (1998). *Oxidative metabolism and anaerobic glycolysis during repeated exercise.* Journal of Physiology and Biochemistry, 54(4):217-25.

Robertson, P.W. & Hart, B.B. (1999). *Assessment of tissue oxygenation.* Respiratory Care Clinics of North America, 5(2):221-63.

Rowlands, D.S. & Thomson, J.S. (2009). *Effects of beta-hydroxy-beat-methylbutyrate supplementation during resistance training on strength, body composition, and muscle damage in trained and untrained young men: a meta-analysis.* Journal of Strength & Conditioning Research, 23(3):836-846.

Sabourin, P.J. & Bieber, L.L. (1983). *Formation of β-hydroxyisovalerate by an α-sketoisocaproate oxygenase in human liver.* Metabolism, 32:160-164.

Sapir, D.G. Walser, M. Moyer, E. RosenSchein, N.B. Stewart, P.M. Moreadith, C. Imbembo, A.L. & Munoz, S. (1983). *Effects of α-ketoisocaproate and of leucine on nitrogen metabolism in postoperative patients.* Lancet, 1:1010-1024.

Saris, W.H.M. (1995). *Exercise with or without dietary restriction and obesity treatment.* International Journal of Obesity, 19(Supplement 4):S113-S116.

Sax, H.C. Talamini, M.A. Fischer, J.E. (1989). *Clinical uses of branched-chain amino acids in liver disease, sepsis, trauma and burns.* Archives of Surgery, 121:359-366.

Schurr, A. & Rigor, B.M. (1998). *Brain anaerobic lactate production: a suicide note or a survival kit?* Developing Neuroscience, 20(4-5):348-57.44.

Schmidt, R.A. & Lee, T.L. (1999). *Motor control & learning. 3rd edition.* Champaign: Human Kinetics.

Sellwood, K.L. Brukner, P. Williams, D. Nicol, A. & Hinman, R. (2007). *Ice-water immersion and delayed-onset muscle soreness: a randomised controlled trial.* British Journal of Sports Medicine, 41:392-397.

Shephard, R.L. Berridge, M. & Montelpare, W. (1990). *On the generality of the "sit and reach" test: An analysis of flexibility data for an aging population.* Research Quarterly for Exercise and Sport, 61:326-330.

Simonson, E. (1971). *Physiology of Work capacity and Fatigue.* Springfield, IL:

Slater, G. Jenkins, D. Logan, P. Lee, H. Vukovich, M. Rathmacher, J.A. & Hahn, A.G. (2000). *Beta-hydroxy-beta-methylbutyrate (HMB) supplementation does not affect changes in strength or body composition during resistance training in trained men.* International Journal of Sport Nutrition, Exercise and Metabolism, 11(3): 384-96.

Smith, H.J. Wyke, S.M. & Tisdale, M.J. (2004). *Mechanism of the attenuation of proteolysis-inducing factor stimulated protein degradation in muscle by β-hydroxy-β-methylbutyrate.*
Cancer Research, 64:8731-5.

Sorof, J. & Daniels, S. (2002). *Obesity hypertension in children: a problem of epidemic proportions.* Hypertension, 40(4):441-7.

Steven, L. Nissen, S. & Sharp, R. (2003)*. Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis.* Journal of Applied Physiology, 94: 651-659.

Sue, D.Y. Chung, M.M. Grosvenor, M. & Wasserman, K. (1989). *Effect of altering the proportion of dietary fat and carbohydrate on exercise gas exchange in normal subjects.* American Review of Respiratory Disease, 139:1430-1434.

Sue, D.Y. (1994). *Integrative cardiopulmonary exercise testing: Basis and Application.* Medical Exercise Nutrition & Health, 3:32-55.

Tabachnick, B.G. & Fidell, L.S. (1996). *Using multivariate statistics* (3rd ed.) Northridge: Harper Collins College Publishers.

Takahashi, T. & Miyamoto, Y. (1998). *Influence of light physical activity on cardiac responses during recovery from exercise in humans.* European Journal of Applied Physiology, 77(4):305-11.82.

Terbizan, D.J. & Seljevold, P.J. (1996). *Physiological profile of age-group wrestlers.* Journal of Sports Medicine & Physical Fitness, 36(3):178-185.

Tesch, P.A. (1992). *Training for body building.* PV Komi (ed), Strength and Power in Sport. London: Blackwell Scientific, 357-369.

Thomas, C.L. (1989). *Taber's Cyclopedia Medical Dictionary*, Philadelphia: Davis.

Thomson, J.S. Watson, P.E. & Rowlands, D.S. (2009). *Effects pf mome weels pf neta-hydroxy-beta-methylbutyrate supplementation on strength and body composition in resistance trained men*. Journal of Strength & Conditioning Research, 23(3):827-835.

Tiidus, P.M. & Ianuzzo, C.D. (1983). *Effects of intensity and duration of muscular exerciseon delayed onset muscle soreness and serum enzymes activities.* Medical Science & Sports Exercise, 15:461-465.

Tischler, M.E. Desautels, M. & Goldberg, A.L. (1982*). Does leucine, leucyle-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle?* Journal of Biological Chemistry, 257:1613-1612.

Turcotte, L.P. Richter, E.A. & Kiens, B. (1992). *Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs untrained humans.* American Journal of Physiology, 25:E791-E799.

Uusitalo, A.L. Uusitalo, A.J. & Rusko, H.K. (1998). *Exhaustive endurance training for 6-9 weeks did not induce changes in intrinsic heart rate and cardiac autonomic modulation in female athletes*. International Journal of Sports Medicine, 19(8):532-540.

Van Baak, M.A. (1999). *Physical activity and energy balance.* Public Health & Nutrition, 2(3A):335-9.

Van de Graaff, K.M. & Fox, S.I. (1989). *Concepts of Human Anatomy and Physiology (2nd edition).* Dubuque, IA: Brown, 291-355.

Van de Wolle, H. Peres, G. & Monod, H. (1987*). Standard anaerobic exercise tests*. Sports Medicine, 4:268-89.

Van Deursen, J. Ruitenbeek, W. Heerschap, A. Jap, P. Ter Laak, H. & Wieringa, B. (1994). *Creatine kinase (CK) in skeletal muscle energy metabolism: A study of mouse mutants with graded reduction in muscle CK expression*. Proceedings of the National Academy of Science of the United States if America, 91:9091-9095.

Van Koevering, M. & Nissen, S. (1992). *Oxidation of leucine and α-ketoisocaproate to β-hydroxy-β-methylbyturate in vivo.* American Journal of Physiology, 262(25): E27-E31.

Van Someren, K.A. Edwards, A.J. & Howatson, G. (2005). *Supplementation with beta-hydroxy-beta-methylbutyrate (HMB) and alpha-ketoisocaproic acid (KIC) reduces signs and symptoms of exercise-induced muscle damage in man.* International Journal of Sport, Nutrition, Exercise & Metabolism, 15(4):413-24.

Vander, A.J. Sherman, J.H. & Luciano, D.S. (1985). *Human Physiology: The Mechanisms of Body Function (4th edition).* New York: McGraw-Hill.

Vander, A.J. Sherman, J.H. & Luciano, D.S. (1994). *Human Physiology.* London: McGraw-Hill; p. 315-38.

Vukovich. M.D. Stubbs, N.B. & Bohlken, R.M. (2201). *Body Composition in 70-Year-Old Adults Responds to Dietary β-Hydroxy-β-Methylbutyrate Similarly to That of Young Adults.*
Journal of Nutrition, 131:2049-2052.

Wahren, J. Hagenfeld, L. & Felig, P. (1975). *Glucose and free fatty acid utilization in exercise: studies in normal and diabetic man.* Israelian Journal of Medical Science, 11:551-59.

Wallberg-Henriksson, H.M. Rincon, J. & Zierath, J.R. (1998). *Exercise in the management of non-insulin-dependent diabetes mellitus.* Sports Medicine, 25(1): 25-35.

Wallin, D. Ekblom, B. Grahn, R. & Nordenborg, T. (1985). *Improvement of muscle flexibility: A comparison between two techniques.* American Journal of Sport Medicine, 13:263-268.

Walter, G. Vandenborne, K. Elliott, M. & Leigh, J.S. (1999). *In vivo ATP synthesis rates in single human muscles during high intensity exercise.* Journal of Physiology, 519(Pt 3):901-10.

Wareham, N.J. Hennings, S.J. Prentice, A.M. & Day, N.E. (1997). *Feasibility of heart-rate monitoring to estimate total level and pattern of energy expenditure in a*

population-based epidemiological study: the Ely Young Cohort Feasibility Study 1994-5. British Journal of Nutrition, 78(6):889-900.

Wasserman, K. & Mcllroy, M.B. (1997). *Detecting the threshold of anaerobic metabolism in cardiac patients during exercise.* American Journal of Cardiology, 14: 844-52.

Weber, K.T. Janicki, J.S. & Likoff, M.J. (1984)b. *Exercise Testing in the Evaluation of Cardiopulmonary Disease.* Clinical Chest Medicine, 5(1):173-80.

Weinberg, R. & Gould, D. (2003). *Foundations of Sport and Exercise Psychology*: Human Kinetics.

Welch, H.G. (1973). *Substrate utilization in muscle-adaptations to physical effort.* In: Naughton JP, Hellerstein HK. Exercise testing and exercise training in Coronary heart disease. New York: Academic Press, p.193-97.

Werring, D.J. Clark, C.A. Parker, G.J. Miller, D.H. Thompson, A.J. & Barker, G.J. (1999*). A direct demonstration of both structure and function in the visual system: combining diffusion tensor imaging with functional magnetic resonance imaging.* Neuro Image, 9(3):352-61.

Williams, M.H. & Branch, J.D. (1998)*. Creatine supplementation and exercise performance: an update*. Journal of American College Nutrition, 17(3):216-34.

Wilmore, J.H. (1974). *Alterations in strength, body composition, and anthropometric measurements consequent to a 10 week weight training program*. Medicine and Science in Sports, 6:133-138.

Wilson, G.J. (2006). *The Effects of External Rewards on Intrinsic Motivation*. The Journal of Hyperplasia Research, 6:

Wright, R.L. Swain, D.P. & Branch, J.D. (1999). *Blood pressure responses to acute static and dynamic exercise in three racial groups*. Medical Science Sports Exercise, 31(12):1793-8.

Yoshida, T. (1986). *Arterial lactate and acid-base status during incremental exercise with normoxic and hypoxic conditions in female distance runners.* Medical Science Sports Exercise, 18:560-61.

Yudlen, J. & Cohen, R.D. (1975). *The contribution of the kidney to the removal of a lactic acid load under normal and acidatic conditions in the conscious rat.* Clinical Science, 48:121-31.

Zhang, Z. Talleyrand, V. Ratmacher, J. & Nissen, S. (1990). *Change in plasma beta-hydroxy-beta-methylbutyrate (HMB) by feeding leucine alpha-ketoisocaproate (KIC) and isovaleric acid (IVA) to pigs (abstract).* FASEB J. 7:A392.