ß-Hydroxy-ß-Methylbutyrate (HMB) Supplementation of Resistance Trained Men

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Abstract

A randomised double-blind placebo controlled study design was used to investigate the effects of supplementing 34 resistance trained men (RTM) with 3g/d of ß-hydroxy-ß-methylbutyrate or a cornstarch placebo on strength and body composition over a 9 week supplementation period.

At the beginning of the study period, questionnaires were given to each participant. Prior to and following the period of supplementation; anthropometric measurements were taken, including 8 skinfold sites, height, and body weight; body composition was measured using bioelectrical impedance analysis: strength was assessed using 1 repetition maximum (1RM) strength testing on the leg extension, bench press, and preacher curl apparatus; and food intakes were assessed using 3-day dietary records. During the supplementation period, all participants completed the same resistance training programme and physical activity was assessed using training log book records.

Prior to the supplementation period, a significant difference was found between the two supplementation groups for initial body mass indices (BMI: HMB 26.2 ± 0.8; Placebo 22.8 ± 0.9, P=0.014). There was no significant difference found between the HMB and Placebo supplemented groups for any other baseline anthropometric (P>0.056), or strength measurements (P>0.583).

Over the study duration there was no significant difference found in number of training sessions between the two supplemented groups (P>0.056). Following the supplementation period there was no significant change in anthropometric measurements (P>0.095), nor actual strength (P>0.086) over the study duration. However, percent change in leg

extension strength increased significantly more for the HMB-supplemented group than the placebo group (I.E: HMB 14.7 ± 3.6%; Placebo 4.84 ± 2.8%, P=0.041).

During the supplementation period there was a significant difference found between the dietary intakes of some nutrients between the supplementation groups. The HMB group tended to consume a greater percent of energy from carbohydrates, and had a higher maltose intake. The HMB group had a lower percent of energy from fats in the diet, and consumed lower average cholesterol intake than the placebo group (P<0.047). Several study participants failed to meet the recommended dietary intakes for adult New Zealanders of certain nutrients. The average intakes of energy from carbohydrates, intakes of vitamin A, vitamin E, vitamin B6, potassium, magnesium, calcium, and selenium were low for some participants.

The conclusion of this study was that there was no beneficial effect of HMB supplementation on body composition in resistance trained humans, however there was a significant increase found in leg extension strength with HMB supplementation in response to resistance exercise over the 9 week supplementation period.

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Chapter 1 Introduction

It is well documented that elite strength athletes readily experiment with sports supplements (Sandoval, et. al., 1989; Williams, 1989; Manore, et. al., 1993; Brill & Keane, 1994; Butterfield, 1996; Slater, *et. al.*, 2001). Sports supplement use has also increased in popularity in recent years amongst recreational resistance trained individuals and sports enthusiasts (Rubinstein & Federman, 2000). In fact some studies have shown supplement use to be higher in recreational resistance trained individuals than competitive bodybuilders (Kleiner, *et. al.*, 1994).

Recreational resistance trained individuals are bombarded with numerous products, all claiming to enhance recovery, muscle strength and growth, and/or fat loss. (Philen, *et. al.*, 1992; Burke, *et. al.*, 2000). Muscle building supplements are now available at supermarkets, by mail order, over the internet, in gyms, as well as in health stores, reflecting the increasing popularity of sports supplements (Grunewald & Bailey, 1993; Clarkson & Rawson, 1999). Many of which have little or no evidence supporting their claims (Grunewald & Bailey, 1993; Butterfield, 1996; Clarkson, 1996).

HMB is one of the latest, more expensive (Ahrendt, 2001) ergogenic supplements aimed at resistance trained individuals (Slater, *et. al.*, 2000). Though many studies of HMB supplementation have been done prior to this study, mainly with animals and some studies involving humans, there are some concerns regarding the methodology of those previous studies. The purpose of this study therefore is to validate or dispute claims of increased strength, increased fat free mass, and body fat loss with HMB supplementation of recreational resistance trained men.

Recreational resistance trained men (RTM) differ from other strength athletes and competitive bodybuilders in their level of training (Lamar-Hildebrand, *et. al.*, 1989; Kleiner, *et. al.*, 1994), dietary intakes, and supplement use (Lamar-Hildebrand, *et. al.*, 1989; Sandoval, *et. al.*, 1989; Bamman, *et. al.*, 1993; Withers, *et. al.*, 1997).

There was little data found on the exercise, dietary, and supplementation patterns of recreational resistance trained men (RTM), or data distinguishing RTM from strength athletes and competitive bodybuilders. Therefore the exercise patterns, nutrition factors, and supplement use of resistance trained individuals have been investigated in this study, along with the effects of HMB supplementation.

1.1 Physical Activity Patterns of Resistance Trained Men

The resistance training sessions of individuals exercising for physical fitness and appearance goals are unique compared to athletes training for bodybuilding, power lifting, weightlifting, and sport specific training (Lambert & Flynn, 2002). In addition, physical fitness, training levels, and commitment also vary considerably within recreational resistance trained individuals (Sandoval, *et. al.*, 1989; Kleiner, *et. al.*, 1994).

There is limited data describing the exercise programmes of RTM (Sandoval, et. al., 1989), however some studies comparing non-competitive resistance trained individuals to competitive bodybuilders do indicate more consistent exercise patterns and less seasonal variation in training than competitive bodybuilders (Lamar-Hildebrand *et. al.*, 1989; Sandoval, *et. al.*, 1989; Bamman, *et. al.*, 1993). Recreational resistance trained individuals were generally found to devote less time to resistance exercise, aerobic exercise, and posing routines than competitive bodybuilders (Lamar-Hildebrand *et. al.*, 1989).

1.2 Adaptations Associated with Resistance Exercise

Numerous studies of resistance training techniques have been carried out, varying training frequency, volume (sets, repetitions, loads), and type (free weights or machines; isotonic, isokinetic, or isometric exercise; and concentric or eccentric contractions) (Feigenbaum & Pollick, 1999). Although there is still controversy regarding optimum conditions for maximum muscle adaptation and strength increase, it is generally understood that the degree of strength increase is a function of muscle overload at near maximal intensities (Narici, et. al., 1989).

1.2.1 Neural Adaptations

With initial training, the maximum voluntary contraction produced by an individual increases over a short period of time without a corresponding increase in muscle strength and size (MacDougall, 1986; Narici, *et. al.*, 1989; Jones & Folland, 2001). Adaptations within the nervous system, known as neural adaptations (Sale, 1988) are thought to play an important role in initial maximum voluntary contraction increase with training (MacDougall, 1986). However, the evidence regarding neural adaptations are hard to interpret and controversial (Sale, 1988; Jones, *et. al.*, 1989; Garfinkel & Cafarelli, 1992; Jones & Folland, 2001).

A possible mechanism of neural adaptation includes increased motor unit activation with training (Heyward, 1998; Jones *et. al.*, 1999). Untrained individuals may not be able to fully recruit high threshold motor units in some muscle groups (Fleck & Kraemer, 1997; Jones & Folland, 2001), at different speeds, limb lengths, joint positions (Jones & Round, 1990), or with fatigue (Behm, 1995). Another possible mechanism is increased motor neuron firing frequency (Fleck & Kraemer, 1997; Heyward, 1998; Häkkinen, *et. al.*, 2001; Jones & Folland, 2001), or maximum force production may occur at lower motor neuron firing frequencies (Sale, 1988). Neural adaptation may also occur via increased synchronisation of motor unit firing frequency, or changes in the recruitment pattern of motor units (Fleck & Kraemer, 1997). However research indicates that asynchronous

recruitment of motor units produces greater force than synchronous (Jones, *et. al.*, 1989; Narici, et. al., 1989; Behm, 1995). Increase in synchronisation of motor units with training may only increase the rate of force development (Sale, 1988).

Exercise induced disinhibition is thought to be a neural adaptation to training, increasing the stimulus transmitted to the muscle by reducing neural inhibitory stimulus (Deschenes, *et. al.*, 1994; Häkkinen, *et. al.*, 2001). These latter are thought to be produced in the central nervous system during maximum force and low velocity movements (Fleck & Kraemer, 1997). The neuromuscular junction can only produce excitatory impulses, not inhibitory impulses (Deschenes, *et. al.*, 1994). Experiments indicate reduced disinhibition and increased activation of motor units when an individual experiences anger, fright, or disinhibition, (Klausen, 1990) via drugs, hypnosis, or excitement (Sale, 1988; McArdle, et. al., 1991).

Finally, improved activation and co-ordination of muscles in a specific movement (Rutherford & Jones, 1986; Sale, 1988), is thought to occur through increased activation of agonists and synergists, and decreased co-contraction of antagonist muscle groups (Sale, 1988). Evidence includes differences in strength when training and testing strength in different body positions (Jones, *et, al.*, 1989); strength increases in the untrained limb with unilateral limb training (Jones, *et. al.*, 1989; Klausen, 1990); decreasing co-activation of antagonist muscles with training, measured by electromyography (Häkkinen, *et. al.*, 2001); and little or no increase in strength and size of stabilisation muscles with increasing force development of agonist muscles in a specific movement (Rutherford & Jones, 1986).

1.2.2 Morphological Changes

Long term responses to resistance training include hypertrophy of the muscle group (Narici, *et. al.*, 1989; Garfinkel & Cafarelli, 1992; MacDougall, 1992; Narici, *et. al.*, 1996), hypertrophy of individual muscle fibres (Garfinkel & Cafarelli, 1992; Häkkinen, *et. al.*, 2001), increased myofibril number and size, and increased contractile protein content (Garfinkel & Cafarelli, 1992; MacDougall, 1992).

With hypertrophy, the predominance of Type IIB to Type IIA muscle fibre subtypes is thought to change (Staron & Hikida, 2000); the sarcoplasmic reticulum and T-tubule membranes increase (MacDougall, 1992); connective tissue such as ligaments and tendons increase in size and strength (MacDougall, 1986; Heyward, 1998); and bone strength and density increase (Heyward, 1998). However the proportion of capillaries and mitochondria decrease, due to the increased contractile protein content (MacDougall, 1986; MacDougall, 1992).

1.2.3 Muscle Architecture (Ultra-structural Adaptations)

Training may increase both fibre cross sectional area and angle of pennation (angle of muscle fibre insertion into the tendon). Therefore fibre cross sectional area and corresponding strength is increased to a larger degree than indicated by anatomical cross sectional area. This in itself is not thought to cause significant strength increase (Jones, *et. al.*, 1989).

Training may cause an increase in contractile material packing by closer arrangement of myofilaments and myofibrils, decreased connective tissue between fibres, and decreased intramuscular fat, therefore increasing the amount of force produced per cross sectional area of muscle (Jones, *et. al.*, 1989; Jones & Round, 1990). Indirect evidence of this includes increased density of contractile material, and greater increases in fibre area than anatomical cross sectional area with resistance training (Jones, *et. al.*, 1989). However this evidence is controversial (Jones & Round, 1990).

Other possible changes in muscle architecture include increased attachments from the tendon to intermediate sarcomeres, acting to increase the force produced per cross sectional area (Jones, *et. al.*, 1989; Narici, *et. al.*, 1989). Muscle hypertrophy is known to stimulate increased collagen, and therefore connective tissue synthesis (Jones & Round, 1990); however this theory of attachments to individual sarcomeres would be extremely difficult to prove.

1.2.4 Biochemical Changes

Biochemical factors associated with muscle hypertrophy include a slight increase in ATP and creatine phosphate stores due to increased hypertrophy of Type II fibres (MacDougall, 1986). Creatine phosphokinase, myosin ATPase, myokinase enzyme activity also increase (McArdle, *et. al.*, 1991; Heyward, 1998). Phosphofructosekinase and other glycolytic enzymes remain the same or decrease, and mitochondrial enzymes decrease with the decreasing proportion of mitochondria (MacDougall, 1986).

1.3 Nutritional Factors Affecting Resistance Trained Men

Chronic resistance training over long periods of time is thought to increase protein synthesis and produce increased muscle hypertrophy and strength. One mechanism for this may be by increased basal level of protein synthesis with resistance training, so that the muscle is in positive protein balance more often, and hypertrophy results (Tipton & Wolfe, 2001). Alternatively, the mechanism may be via a series of small increases in net positive muscle protein balance due to each bout of resistance exercise, adding up to hypertrophy over time (Tipton & Wolfe, 2001).

1.3.1 Dietary and Supplementation Patterns

There was little data found on the eating habits of RTM, or data distinguishing RTM from competitive bodybuilders. The diets of RTM are thought to be less rigid and restrictive (Kleiner, *et. al.*, 1994), and include more varied food groups than competitive bodybuilders (Sandoval & Heyward, 1991). Energy intake is thought to be similar to competitive bodybuilders in the competition phase of training (Bazzarre, et. al., 1994; Kleiner, *et. al.*, 1994), but less than off-season competitive bodybuilders and other strength athletes

(Bazzarre, et. al., 1994). Some studies indicate that protein intakes of RTM are similar to that of other amateur athletes (Bazzarre, et. al., 1994) while other studies indicate protein intakes far exceed requirements (Pearce, 1988; Lemon, *et. al.*, 1992; Tarnopolsky, 2000). Protein consumption in this group is often influenced by hearsay, trial and error, and self experimentation, and the belief that excessive intakes of protein may benefit muscle hypertrophy and strength performance (Pearce, 1988). Due to a less restrictive diet, dietary fat intake is higher in RTM than competitive bodybuilders (Lamar-Hildebrand, *et. al.*, 1989; Sandoval, *et. al.*, 1989; Sandoval & Heyward, 1991).

Intake of macronutrients in RTM is similar to competitive bodybuilders for cholesterol, fibre, and zinc. High cholesterol, low fibre and zinc intakes appear to be common for RTM and competitive bodybuilders. In addition the diets of RTM have also been found to be low in copper, and some studies have found low vitamin C intakes compared to the RDA (Bazzarre, et. al., 1994).

Supplement use is thought to be higher in RTM and regional competitive bodybuilders than elite level competitive bodybuilders (Kleiner, *et. al.*, 1994). Studies also indicate a higher prevalence of supplement usage than the general population (Bond & Keane, 1994; Naylor & Garg, 1996; Kleiner, 2000). Supplement use varies with age group, gender, training level and commitment (Kleiner, *et. al.*, 1994). The most popular supplements used are amino acid and protein supplements, and vitamin and mineral supplements (Pearce, 1988; Bond & Keane, 1994; Naylor & Garg, 1996). Supplement intakes seem to be influenced by the belief that vitamin and mineral requirements cannot be met with food intake alone (Naylor & Garg, 1996); and that protein and amino acid supplements are required to increase muscle size and strength gains (Lemon, *et. al.*, 1992; Bond & Keane, 1994; Naylor & Garg, 1996).

1.3.2 Nutritional Concerns

There seems to be numerous misconceptions and much inaccurate nutrition knowledge amongst RTM (Naylor & Garg, 1996). Dietary and supplement intakes are influenced by a

great number of sources including other RTM and competitive bodybuilders, friends and family (Naylor & Garg, 1996), supplement advertisements, and magazine articles (Philen, *et. al.*, 1992; Manore, *et. al.*, 1993; Kleiner, 2000). The main nutritional concerns regarding RTM include; ensuring a balanced diet is consumed, as some nutrients may be consumed in excessive amounts, while others in inadequate amounts (Pearce, 1988; Kleiner, 2000); the energy requirement of resistance training exercise and the amount of muscle protein degradation during exercise; weight control; and excessive supplement usage (Pearce, 1988; Bond & Keane, 1994; Naylor & Garg, 1996).

1.3.3 Carbohydrate Recommendations

The carbohydrate recommendations for RTM are in the range 5-7g/kg/day (Hawley & Burke, 1998; Howe, *et. al.*, 2002). These recommendations are based on the carbohydrate intakes required for muscle glycogen replenishment (Kleiner, 2000), as carbohydrate is the major fuel used during resistance exercise (Lemon, 1998). Carbohydrate ingestion also increases blood glucose, and therefore blood insulin concentrations, which are known to retard muscle protein breakdown (Roy, et. al., 1997; Gibala, 2000), and stimulate amino acid uptake by muscle fibres (Lemon, 1998; Gibala, 2000). In addition, the eccentric component of resistance exercise causes significant damage to the muscle fibre structure, which is thought to result in impaired muscle glycogen storage and insulin resistance, therefore increasing the carbohydrate requirement of RTM (Lambert & Flynn, 2002).

1.3.4 Protein Recommendations

Protein recommendations are in the range 1.2-2.0 g/kg/day (Pearce, 1988; Lemon, *et. al.*, 1992; Tarnopolsky, 2000). These recommendations are based on nitrogen balance studies and protein kinetics studies (using isotopic tracers) of individuals performing resistance exercise, and including a two standard deviation safety margin (Tarnopolsky, *et. al.*, 1988; Lemon, *et. al.*, 1992; Lemon, 1998). Extra protein is required for increased protein synthesis and muscle growth (Lemon, 1998); repair of damaged muscle fibres, and to provide for enzymes with increased activity (Pearce, 1988).

Surplus amino acids may provide for increased protein synthesis and decreased degradation (Lemon, 1991). However, evidence of the effects of high protein intakes on muscle hypertrophy is equivocal, intakes of greater than 2.62g/kg/day have not been shown to increase muscle hypertrophy and/or strength gains to a significant degree (Lemon, *et. al.*, 1992). Protein intakes above 1.5-1.8g/kg/day lead to nutrient overload and additional amino acids are oxidised for energy or stored as body fat rather than contributing to muscle protein accretion (Pearce, 1988; Lemon, *et. al.*, 1992).

Possible adverse health effects of habitual high protein intakes include; increased workload on the kidneys; increased urination and dehydration; increased urinary calcium loss; high fat intakes with high intakes of animal protein; and the potential toxicity of high doses of individual amino acid supplements (Lemon, 1998).

1.3.5 Timing of Nutrient Intake

Muscle protein synthesis increases to a greater degree when a pre exercise meal is consumed compared to post exercise meal. Protein feeding prior to resistance exercise may increase amino acid uptake into muscles during exercise (Tipton, *et. al.*,2001; Tipton & Wolfe, 2001). Carbohydrate feeding prior to resistance exercise produces high initial muscle glycogen levels, and increases glycogen synthesis during rest periods between sets, therefore sparing muscle glycogen (Lambert & Flynn, 2002).

Protein and carbohydrate ingestion following exercise elevates circulating amino acids and insulin concentrations. Insulin is thought to increase amino acid uptake by muscle fibres, therefore stimulating muscle protein synthesis, and retarding breakdown (Roy, *et. al.*, 2000; Tipton & Wolfe, 2001).

1.4 Muscle Metabolism and Resistance Exercise

1.4.1 Energy Storage in Skeletal Muscle

Intramuscular stores of ATP are small (~5mmol/kg) (Hargreaves, 2000; Casey & Greenhaff, 2000). ATP is utilized for muscle contraction and relaxation, ATP dependent ion pumps (Billeter & Hoppeler, 1992; Casey & Greenhaff, 2000; Lambert & Flynn, 2002), and biosynthesis reactions (Wagenmakers, 2000).

Creatine phosphate stores are larger (20mmol/kg) (Hargreaves, 2000), creatine phosphate diffuses readily throughout the muscle fibre protoplasm, and is utilised by the myofilaments, where it is hydrolysed for ATP if required (Billeter & Hoppeler, 1992). Glucose is stored as glycogen granules between myofibrils in muscle fibre and undergoes glycolysis (glycogenolysis) for energy within the cell cytoplasm (Jones & Round, 1990; Billeter & Hoppeler, 1992).

Lipid is stored as droplets within the endomysial space between muscle fibres, and some minute droplets occur within fibres (Jones & Round, 1990; Billeter & Hoppeler, 1992). Intramuscular lipids may be used by muscle during high intensity exercise such as resistance training, but are a very minor fuel source (Watt, *et. al.*, 2002).

1.4.2 Muscle Metabolism during Resistance Exercise

Resistance training consists of high intensity intermittent exercise performed over a prolonged period of time. A resistance training session may consist of up to 15-20 sets of 6-20 repetitions (Lambert & Flynn, 2002).

Unlike other high intensity exercise, resistance exercise is performed at 60-80% of 1 repetition maximum (1RM) and is taken to momentary muscular failure, which differs from maximal effort high intensity exercise such as sprints taken to muscular fatigue. Therefore the metabolic response is smaller for resistance exercise compared to 30 seconds of other

high intensity exercise (Lambert & Flynn, 2002). However, blood flow to the muscle is restricted during resistance exercise contractions due to intra-muscular blood pressure, increasing the amount of creatine phosphate degradation and lactate accumulation compared to other high intensity exercise of the same duration (Lambert & Flynn, 2002, MacDougall, *et. al.*, 1999).

As resistance exercise is not continuous, the duration of work done by a given muscle group is less than the duration of the entire exercise session (Lambert & Flynn, 2002), and rest periods between sets allow some replenishment of muscle fuel stores and normalisation of intramuscular pH (Parcell, *et. al.*, 2002).

Initial energy is supplied by the phosphagen system, muscle ATP is hydrolysed to ADP and organic phosphate (Maughan, *et. al.*, 1997; Gastin, 2001). Muscle ATP stores are small and last only 1-2 seconds (Casey & Greenhaff, 2000). Creatine phosphate is broken down to creatine and phosphate and used to regenerate ATP, this occurs immediately with the initiation of exercise, and lasts approximately 1.3 seconds (Gastin, 2001). The phosphagen system contributes to initial rapid forceful contractions during resistance training, but has a limited capacity to supply muscle energy (Maughan, *et. al.*, 1997; Hawley & Burke, 1998). To prevent a significant decrease in intramuscular ATP other mechanisms are utilised to regenerate ATP (Maughan, *et. al.*, 1997).

The anaerobic glycolytic system becomes the main energy source once initial ATP and creatine phosphate stores are depleted (Haff, *et. al.*, 2000). The anaerobic glycolytic system is also activated with the initiation of exercise, ATP production peaks at about 20 seconds and is maintained for 2-5 minutes (Liebman & Wilkinson, 1994; Maughan, *et. al.*, 1997; MacDougall, *et. al.*, 1999). Anaerobic glycolysis produces ATP via the incomplete breakdown of muscle glycogen (glycogenolysis) to lactic acid (Liebman & Wilkinson, 1994; Maughan, *et. al.*, 1997). Its capacity for producing ATP is much greater than the phosphagen system, but the rate of ATP production is slower (Liebman & Wilkinson, 1994; Maughan, *et. al.*, 1997; Gastin, 2001).

During rest periods between sets oxidative creatine phosphate resynthesis occurs (Rico-Sanz & Mendez Marco, 2000), muscle fibre pH is normalised (Parcell, *et. al.*, 2002), and oxidative glycogen synthesis occurs, sparing muscle glycogen to some degree (Lambert & Flynn, 2002).

Studies of resistance exercise indicate that approximately 1.6% of energy is derived from stored ATP, approximately 16.3% from creatine phosphate hydrolysis to ATP, and approximately 82.1% from anaerobic glycolysis (based on measurements of ATP, creatine phosphate remaining, and muscle lactate production) (MacDougall, *et. al.*, 1999; Lambert & Flynn, 2002).

Fatigue is caused by decreasing muscle pH, increasing ADP concentrations, increasing organic phosphate concentrations, creatine phosphate depletion, and muscle glycogen reduction (Lambert & Flynn, 2002). During a resistance training workout the mechanisms causing muscle fatigue during the first set of 10 repetitions differ to the mechanisms causing fatigue after the twentieth set of 10 repetitions (Maughan, *et. al.*, 1997; Gastin, 2001; Lambert & Flynn, 2002). Fatigue or momentary muscle failure during one set is thought to be mainly due to creatine phosphate depletion. Fatigue after multiple sets is mainly due to muscle acidosis and metabolite accumulation if limited rest periods are taken (1-3 minutes), and low intramuscular glycogen levels if adequate rest periods are taken (2-3 minutes) (Lambert & Flynn, 2002; Parcell, *et. al.*, 2002).

1.4.3 Muscle Metabolism following Resistance Exercise

Following resistance training muscle protein turnover is increased (Tipton & Wolfe, 1998; Tipton & Wolfe, 2001). In the fasted state resistance exercise increases muscle protein breakdown (Biolo, *et. al.*, 1997). In the fed state resistance exercise increases muscle protein synthesis (Phillips, et. al., 1997).

Amino Acids

Resistance exercise and amino acid availability appear to act synergistically to increase muscle protein synthesis (Phillips, *et. al.*, 1999). Several theories have been proposed to explain this.

One possible mechanism involves maintaining intracellular free amino acid pool levels. Following resistance exercise protein synthesis increases, this may initially deplete the intracellular amino acid pool, and therefore protein breakdown is initiated to maintain levels of the intracellular amino acid pool (Tipton & Wolfe, 1998; Tipton & Wolfe, 2001; Wolfe, 2001). When exogenous amino acids are supplied, protein breakdown is retarded (Lemon, 1998; Tipton & Wolfe, 1998; Tipton & Wolfe, 2001; Wolfe, 2001). The intracellular amino acid pool controls muscle protein turnover and stimulates increased intracellular amino acid transport and availability (Tipton & Wolfe, 1998).

Another mechanism is regulation of protein synthesis via amino acid availability (Tipton & Wolfe, 2001). Following resistance exercise amino acid intracellular transport is increased (Biolo, *et. al.*, 1997) which may control protein synthesis, or protein synthesis may stimulate and control the increase in amino acid transport (Biolo, *et. al.*, 1997). There are two explanations for the role of amino acid availability in the stimulation and control of protein synthesis; the amount of amino acids required for translation to proteins may be greater than that available within the muscle cell, therefore any increased amino acid availability will charge tRNA and drive protein synthesis; another explanation is that specific amino acids may be required to signal the initiation of translation (Tipton & Wolfe, 2001). Studies show increased intracellular transport of alanine, leucine, and lysine with exercise (Phillips, *et. al.*, 1999).

Other theories relate to differing pathways of protein breakdown. Resistance exercise may stimulate protein breakdown via a separate pathway to protein breakdown during rest. When exogenous amino acids are supplied, protein breakdown due to exercise is retarded, but basal protein breakdown is not (Wolfe, 2001). For example, resistance exercise may

stimulate the lysosomal protein breakdown pathway, which is sensitive to amino availability (Tipton & Wolfe, 2001). Increased muscle protein degradation may be initiated by resistance exercise due to structural damage, myofibrillar disruption (MacDougall, *et. al.*, 1995), or stimulated by neural proteases (Phillips, *et. al.*, 1997; Biolo, *et. al.*, 1995), and unrelated to protein synthesis.

Carbohydrates and Fats

The influence of these substrates is less important in the regulation of muscle protein synthesis. Any influence is likely to be mediated via hormone action (Tipton & Wolfe, 2001). For instance carbohydrate ingestion increases blood glucose concentrations, and therefore blood insulin concentrations, which are known to decrease muscle protein breakdown (Roy, et. al., 1997).

1.5 The Role of Leucine and other Branched-Chain Amino Acids

1.5.1 Branched-Chain Amino Acids (BCAA)

The branched-chain amino acids are widespread in the average diet, and make up 15-25% of protein foods (Stipanuk & Watford, 2000; Layman, 2003). Branched-chain amino acids are not metabolised in the liver like other amino acids, but in peripheral tissues (Stipanuk & Watford, 2000; Layman, 2003), as branched-chain amino acid transaminases occur mainly in the heart, brain, and skeletal muscle, with small amounts in the kidney and adipose tissue (Linder, 1991).

The muscle intracellular free amino acid pool represents only around 1% of the total amino acids in the body; however it is responsible for providing substrates for protein synthesis, carbon for energy via the tricarboxylic acid cycle (Krebs cycle, citric acid cycle), amino

nitrogen for alanine and glutamine production. Increased levels of plasma branched-chain amino acids stimulate the release of insulin, and thus muscle protein synthesis, and retard protein degradation (Matthews, *et. al.*, 1981; Nair, *et. al.*, 1992; Anthony, *et. al.*, 1999; Blomstrand & Saltin, 2001; Layman, 2002; Layman, 2003; Pitkänen, *et. al.*, 2003).

Of the branched-chain amino acids only leucine stimulates muscle protein synthesis (Mitch & Clark, 1984), and high levels of decarboxylated branched-chain amino acids products are required to decrease muscle protein degradation (Mitch & Clark, 1984).

1.5.2 Leucine

Leucine is metabolically active with a half-life of only around 45 minutes in the body (Mero, 1999). The roles of leucine in muscle include:

- Protein synthesis.
- Energy substrate.
- Regulation of branched-chain amino acids uptake into muscle fibre via the L-carrier system transporters (Odessey & Goldberg, 1972; Hood & Terjung, 1987).
- Source of α-amino nitrogen for gluconeogenic amino acids.
- Metabolic signal to initiate protein synthesis (Matthews, *et. al.*, 1980; Schneible, *et. al.*, 1981; Tessari, *et. al.*, 1985; Anthony, *et. al.*, 1999; Layman, 2002; Layman, 2003).
- Retardation of muscle protein degradation (Hong & Layman, 1984; Mitch & Clark, 1984; Nair, *et. al.*, 1992; Mero, 1999; Blomstrand & Saltin, 2001).

1.5.3 Leucine Metabolism with Resistance Exercise

Plasma leucine levels decrease by around 30% with resistance exercise (Mero, 1999). The role of leucine during exercise is not well elucidated; with the main role thought to be protein metabolism during recovery following resistance exercise (Blomstrand & Saltin, 2001).

During resistance exercise oxidation of leucine contributes little to energy requirements (Hood & Terjung, 1987), however with low muscle glycogen stores there is an increased use of amino acids for energy, especially leucine (Schneible, *et. al.*, 1981; Mero, 1999; Layman, 2002).

During recovery, following resistance exercise plasma leucine concentrations are significantly decreased (Mero, 1999; Layman, 2002; Pitkänen, *et. al.*, 2003). Following resistance exercise leucine directly stimulates protein synthesis via the insulin signalling cascade. Leucine stimulates Kinase Mammalian Target of Rapamycin, which initiates phosphorylation and activation of Eukaryotic Initiation Factor 4 complex and Ribosomal Protein Serine/Threonine 6 Kinase (70kDa) which in turn initiates translation of muscle mRNA (Anthony, *et. al.*, 1999; Anthony, *et. al.*, 2002; Layman, 2002; Layman, 2003). Leucine indirectly retards protein degradation via its transamination product, α-ketoisocaproate (Hong & Layman, 1984; Mitch & Clark, 1984; Nair, *et. al.*, 1992; Mero, 1999; Blomstrand & Saltin, 2001). This decrease in leucine may also indicate degradation of leucine to provide substrates for the purine nucleotide cycle to replenish muscle ATP stores (Pitkänen, *et. al.*, 2003), and synthesis and release of alanine and glutamine, which stimulate hepatic gluconeogenesis (Hagg, *et. al.*, 1982).

1.6 The Leucine Metabolite ß-hydroxy-ß-methylbutyrate

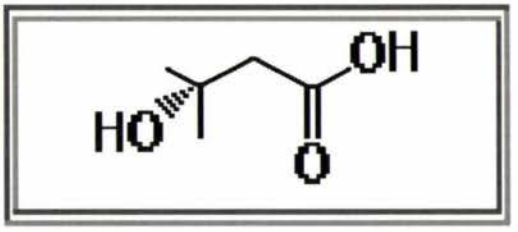
1.6.1 Chemical Description of ß-hydroxy-ß-methylbutyrate

HMB or ß-hydroxy-ß-methylbutyrate is also known as beta-hydroxy-beta-methylbutyrate (beta-hydroxy-beta-methylbutyric acid) (Nissen, S., *et. al.*, 2000) 3-hydroxy-3-methylbutyrate (3-hydroxy-3-methylbutyric acid) (Sigma-Aldrich, 2002), hydroxymethylbutyrate, beta-hydroxyisovalerate (beta-hydroxy-isovaleric acid) (Nissen, *et. al.*, 2000), and 3-hydroxyisovalerate (Hendler, *et. al.*, 2001) (Figure 1.1). HMB may be represented as free acid, salt, ester, or lactone forms (Nissen, *et. al.*, 2000).

HMB does not occur in different structural isomers, or sterioisomers (Nissen, 1991). Structural isomers occur where a compound has the same molecular formula, but the atoms are arranged in different sites. Sterioisomers may be described as two compounds where the atoms are arranged in the same sites, but are orientated differently in space (Brady, 1990). Therefore HMB does not have L- or D- forms, as leucine does (Nissen, 1991).

The Chemical Abstract Registry Number for HMB is 625-08-1. HMB has the molecular formula $C_5H_{10}O_3$, and a molecular weight of 118.1. In its free acid form it is a slightly yellow, clear viscous liquid (Sigma-Aldrich, 2002; Alfa Aesar, 2002). HMB has a boiling point of 128 $^{\circ}$C (7mmHg), flash point >110 $^{\circ}$C, is not an explosive hazard, and its density at 20 $^{\circ}$C is approximately 1.1 g/cm^3. HMB is also fully miscible in water (Alfa Aesar, 2002). In its free acid form, HMB is an irritant to eye, skin, and respiratory system (Alfa Aesar, 2002).

Figure 1.1: ß-hydroxy-ß-methylbutyrate Chemical Structure (Nissen, *et. al.*, 2000)



The preferable form of HMB is its salt form, especially a water soluble salt, or salt that becomes water soluble in human or animal stomach or intestine (Nissen, *et. al.*, 2000). The most preferable salts of HMB include sodium, potassium, magnesium, chromium, and calcium. The most commonly used salt is the calcium salt, but any non-toxic, alkali metal or alkaline earth metal salt may be used.

1.6.2 Food Sources of ß-hydroxy-ß-methylbutyrate

Sources of HMB include both plant and animal foods (Gallagher, *et. al.*, 2000). HMB is found in foods such as catfish, citrus fruits, and trace amounts in alfalfa, corn, silage, soybeans, and animal feedstuffs (Nissen, *et. al.*, 1994; Gordon, 1996). It is also found in breast milk, and is endogenously produced in the body (DiPasquale, 2000).

1.6.3 Leucine Catabolism and ß-hydroxy-ß-methylbutyrate Formation

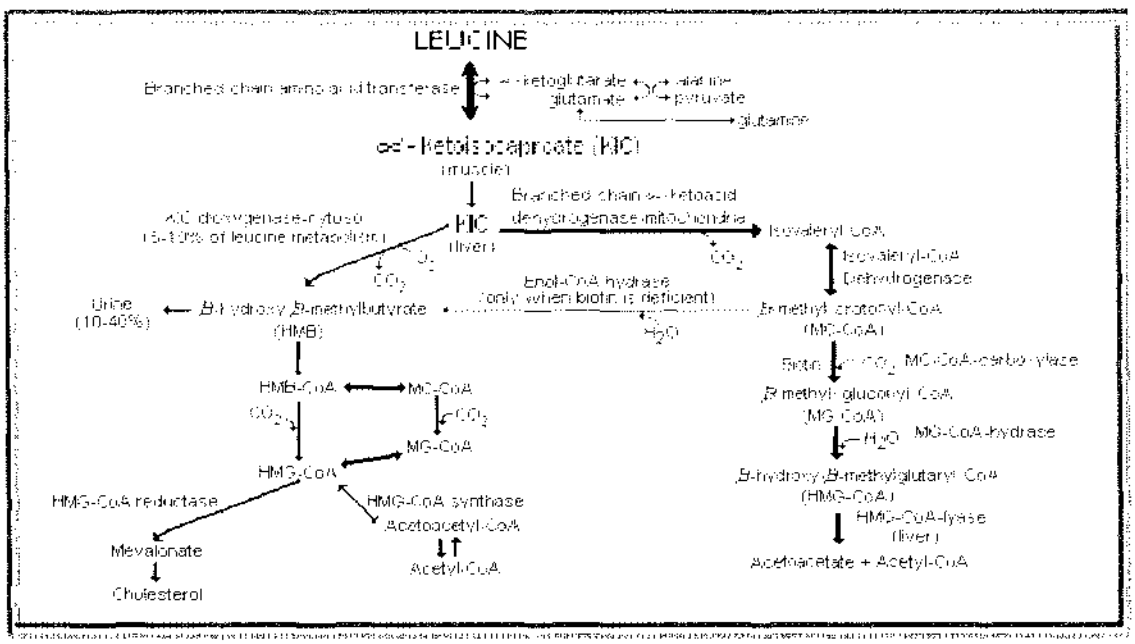
The initial step of leucine catabolism occurs in the muscle fibre sarcoplasm (cytosol) or mitochondria (Nissen & Abumrad, 1997). Leucine undergoes a reversible transamination by an animotransferase enzyme, the α-amino group is transferred to pyruvate to form alanine, or to α-ketoglutarate to form glutamate, also producing a branched-chain α-ketoacid, α-ketoisocaproate (Hutson, *et. al.*, 1980; Matthews, *et. al.*, 1981; Nissen & Haymond, 1981; Hagg, *et. al.*, 1982; Mero, 1999; Stipanuk & Watford, 2000; Layman, 2002). This reaction is reversible, and α-ketoisocaproate may be reaminated to leucine, oxidised in skeletal muscle for energy, or released into the blood stream and transported by the plasma protein albumin to other tissues (Hutson, *et. al.*, 1980; Nissen, *et. al.*, 1981; Hagg, *et. al.*, 1982; Nissen, *et. al.*, 1982; Mero, 1999).

α-ketoisocaproate catabolism occurs mainly in the liver where it is further catabolised by an irreversible oxidative decarboxylation (Hutson, *et. al.*, 1980) via branched-chain α-ketoacid dehydrogenase, to isovaleryl CoA (Hutson, *et. al.*, 1980; Layman, 2002). This

may be further catabolised to β-hydroxy-β-methylglutaryl CoA, and further catabolised to acetoacetate and acetyl-CoA (Di Pasquale, 1997; Stipanuk & Watford, 2000). These reactions are thought to occur within the liver mitochondria (Nissen & Abumrad, 1997).

A minor pathway of α-ketoisocaproate catabolism also takes place in the liver. α-ketoisocaproate is oxidised by the enzyme α-ketoisocaproate dioxygenase to HMB (Sabourin & Bieber, 1981; Van Koevering & Nissen, 1992; Di Pasquale, 1997; Gallagher, *et. al.*, 2000; Vukovich, *et. al.*, 2001) (Figure 1.2, below). Approximately 5% of leucine is metabolised to HMB via this pathway (Vukovich, *et. al.*, 2001). This reaction was thought to occur within the liver cell cytosol. The majority of HMB production occurs in the liver, some in the kidney, and low rates may occur in the muscles and other tissues (Nissen & Abumrad, 1997). HMB may also be produced via hydration of methylcrotonyl-CoA by crotonase enzyme in 3-methylcrotonyl-CoA or biotin deficiency (Sousa, *et. al.*, 1996).

Figure 1.2: Diagram of Leucine and β-hydroxy-β-methylbutyrate Metabolism in the Body (Nissen & Abumrad, 1997)



1.6.4 Chemical Production of ß-hydroxy-ß-methylbutyrate (ß-hydroxy-isovalerate)

The earliest method of chemical synthesis of HMB is described as the manufacture of isovaleric acid. The substrate, methyl isobutyl ketone is oxidised using a solution of alkali metal hypohalite, preferably hypochlorite as catalyst, and simultaneously produces isovaleric acid and chloroform (Bing, *et. al.*, 1950).

This method has been further described by Coffman and colleges (1958) as the preparation of *ß*-hydroxyisovaleric acid. The substrate diacetone alcohol is added to a solution of sodium hypochlorite (sodium hydroxide, chloroform, and water), with stirring and cooling. The product is extracted using methyl ethyl ketone to give *ß*-hydroxyisovaleric acid (Coffman, *et. al.*, 1958). This process has been described in detail in Appendix 13.

In the early 1990s, Nissen and colleagues began experimenting with HMB supplementation of animals. The process used to synthesise HMB for these studies was an adaptation of the chloroform reaction above. *ß*-hydroxy-*ß*-methyl butyric acid was made palatable by conversion to its calcium salt via neutralisation with calcium hydroxide, and crystallisation (Nissen, 1990; Nissen, 1990; Nissen, 1991; Nissen, 1992; Nissen, 1993). The first patent describing the commercial manufacture of HMB was filed in 1996, titled, 'Process for manufacturing 3-hydroxy-3-methylbutanoic acid. The most common synthetic process for the production of HMB for the use of animal or human supplementation appears to be the chloroform method using diacetone alcohol (4-hydroxy-4-methyl-2-pentanone) as the substrate, and various catalysts and large scale reactor designs (Barac, 1998; McCoy *et. al.*, 2000; McCoy *et. al.*, 2001; McCoy *et. al.*, 2002). The large scale manufacture of HMB is described in greater detail in Appendix 13.

1.7 ß-hydroxy-ß-methylbutyrate Metabolism and Proposed Roles in the Body

Animal and human studies suggest that naturally occuring HMB plasma levels are in the range 0.2-0.4 g/day (Nissen, *et. al.*, 1996; Gallagher, *et. al.*, 2000). These levels increase 5-10 times with leucine supplementation (Nissen, *et. al.*, 1996), and 10-20 times with HMB supplementation of 3g/day (Gallagher, *et. al.*, 2000).

1.7.1 ß-hydroxy-ß-methylbutyrate Metabolism

The half life of HMB in the body is only approximately 2.3 hours (Vukovich, *et. al.*, 2001). HMB is utilised by the body in three possible ways. The main fate is thought to be conversion of HMB to ß-hydroxy-ß-methylglutaryl-CoA (Figure 1.5, previous) in several tissues, including the immune system, mammary glands, and muscle fibres (Nissen & Abumrad, 1997), providing a cytosolic source of ß-hydroxy-ß-methylglutaryl-CoA and a precursor and carbon source for cholesterol synthesis (Van Koevering & Nissen, 1992; Nissen & Abumrad, 1997; Vukovich, *et. al.*, 2001). In periods when cholesterol is in demand, such as for cell growth and repair, ß-hydroxy-ß-methylglutaryl-CoA is a rate limiting substrate for cholesterol synthesis (Gallagher, *et. al.*, 2000). The second metabolic fate is a small loss (~29%) of HMB via urine (Di Pasquale, 1997; Vukovich, *et. al.*, 2001). The third metabolic fate is thought to be further use by muscle fibre (Di Pasquale, 1997).

1.7.2 Proposed Actions of ß-hydroxy-ß-methylbutyrate

During conditions such as rapid growth, stress, and physical activity, an increased requirement of HMB may occur, which is not met by endogenous metabolism or dietary intake (Nissen, *et. al.*, 1994).

ß-hydroxy-ß-methylbutyrate Effect on Blood Lipid Profile

The proposed role of HMB in cholesterol synthesis has been well described in several studies (Van Koevering & Nissen, 1992; Nissen & Abumrad, 1997; Peterson, *et. al.*, 1999; Gallagher, *et. al.*, 2000; Vukovich, *et. al.*, 2001). HMB supplementation has also been shown to lower serum cholesterol and LDL levels in animals and humans (Nissen & Sharp, 2003). Evidence of this has been demonstrated in rabbit (Ostaszewski, *et. al.*, 1997), steer (Van Koevering, *et. al.*, 1994), and human supplementation studies (Nissen, *et. al.*, 2000).

There are two theories for this decrease in serum cholesterol. The first theory is competition between HMB and ß-hydroxy-ß-methylglutaryl-CoA for binding to liver ß-hydroxy-ß-methylglutaryl-CoA enzyme, due to the similar structures of these compounds, decreasing liver cholesterol synthesis (Ostaszewski, *et. al.*, 1997). Another may be via tissues other than liver synthesising cholesterol in cell cytosol from the HMB precursor, acting as a negative feedback for liver cholesterol synthesis (Vukovich & Dreifort, 2001).

ß-hydroxy-ß-methylbutyrate and Body Fat Metabolism

Evidence of any HMB influence on fat metabolism is controversial. HMB supplementation in steers was shown to cause a significant increase in the ratio intramuscular to subcutaneous fat (Van Koevering, *et. al.*, 1994).

One proposed mechanism for a role of HMB in fat metabolism is an increase in fat oxidation in muscle cells, stimulated by HMB. Evidence for this comes from unpublished study abstracts, however further studies have not supported this theory (Vukovich & Dreifort, 2001).

Proposed Role of ß-hydroxy-ß-methylbutyrate in the Mammary Gland and Milk Production

Leucine has been shown to increase mammary development, lactational performance, and milk fat secretion in animals. Current opinion is that the leucine metabolite HMB may be responsible for these effects (Nissen, *et. al.*, 1994). HMB has been shown to increase milk fat, milk cholesterol, and milk HMB content in sows (Nissen, *et. al.*, 1994; Nissen & Abumrad, 1997). The role of HMB in mammary gland metabolism is unknown (Nissen, *et. al.*, 1994; Gordon, 1996), however its role in cholesterol synthesis may be related to increased cholesterol synthesis in the mammary gland and increased milk cholesterol content (Nissen, *et. al.*, 1994).

ß-hydroxy-ß-methylbutyrate Effects on the Immune System

Both animal and human supplementation studies indicate no adverse effects of short term acute HMB supplementation (Gallagher, *et. al.*, 2000). In fact HMB appears to act as an immunomodulater. The effect of HMB on the immune system seems to be selective, and appears to stimulate mainly the macrophage (initial immunological defence) and B-lymphocyte (antibody production and secretion) systems (Peterson, *et. al.*, 1999).

In vitro studies of HMB indicate that HMB acts as an immunostimulator in cultures of sheep, cattle, and trout lymphocytes, and chicken macrophages (Nissen & Abumrad, 1997; Siwicki, *et. al.*, 2001). With the addition of HMB lymphocyte immune cell function, lymphocyte blastogenesis, macrophage proliferation, phagocytic potential, and cell membrane receptor number increased (Peterson, *et. al.*, 1999; Siwicki, *et. al.*, 2000). However, studies of isolated human lymphocytes are less clear. Human lymphocytes appear to act in a biphasic manner with HMB, at low concentrations lymphocytes increase blastogenesis, and at high concentrations lymphocytes decrease blastogenesis (Nissen & Abumrad, 1997).

The effects of HMB on the immune system in *in-vivo* studies are less clear. Some studies of HMB supplementation in animals have found decreased morbidity and mortality in stressful situations (Nissen, *et. al.*, 1994; Nissen, *et. al.*, 2000) however other studies have found no stimulatory effects of HMB on the immune system (Gatnau, *et. al.*, 1995).

Evidence for positive effects of HMB on the immune system include; enhanced B-cell and T-cell proliferation and capacity and increased macrophage nitrite levels in broiler chickens (Nissen, et. al., 1994; Peterson, *et. al.*, 1999); increased macrophage phagocytic capacity, leukocyte respiratory activity, and increased number and efficacy of antibody secreting cells in trout (Peterson, *et. al.*, 1999; Siwicki, et. al., 2001); and increased white blood cell volume in horses (Miller, 1998).

However other studies have found no immune response in broiler chickens, lambs, and weanling pigs with HMB supplementation (Gatnau, *et. al.*, 1995). In addition studies of human supplementation indicate no differences in white blood cell volume, polysinophils, lymphocytes, or eosinophils (Gallagher, *et. al.*, 2000).

Theories to explain the HMB mechanism of action in the immune system include, direct action of HMB on macrophages and B-lymphocytes, provision of an energy source for the immune system, or via cholesterol synthesis and utilisation for cell membrane integrity and receptor function within immune system cells (Peterson, *et. al.*, 1999).

ß-hydroxy-ß-methylbutyrate as an Ergogenic Aid

HMB is promoted as an ergogenic aid to athletes; however results from preliminary studies of HMB supplementation with exercise are equivocal (Slater, *et. al.*, 2000). The proposed actions of HMB during exercise include:

- Altered oxidative capacity of muscle cells (Vukovich & Dreifort, 2001).

- Retardation of muscle damage and proteolysis in endurance or resistance exercise

(Nissen & Abumrad, 1997; Gallagher, *et. al.*, 2000; Knitter, *et. al.*, 2000; Slater, *et. al.*, 2001).

- Promotion of muscle protein synthesis (Ostaszewski, et. al., 1997).

- Enhanced strength and muscle accretion with resistance training (Nissen & Abumrad, 1997; Gallagher, *et. al.*, 2000).

1.7.3 Proposed Mechanisms of Action

HMB may affect the oxidative capacity, and decrease lactate production of muscle cells, therefore enhancing exercise performance. If HMB acts via a protein sparing effect, then cytosol and mitochondrial proteins may be increased therefore increasing the oxidative capacity of muscle cells. Also, an increase in protein content of cells is known to increase their buffering capacity (Vukovich & Dreifort, 2001). Proteolysis may be reduced (Nissen, *et. al.*, 1996; Clark, *et. al.*, 2000; Nissen, *et. al.*, 2000; Vukovich, *et. al.*, 2001) by modulating enzymes that cause muscle protein catabolism (Slater & Jenkins, 2000). HMB may act as an ergogenic aid by reducing exercise induced proteolysis and allowing continued training and decreasing over-training (Kreider, et. al., 1999; Paddon-Jones, *et. al.*, 2001; Vukovich & Dreifort, 2001).

Another theory is that HMB provides an *β*-hydroxy-*β*-methylglutaryl-CoA source for cholesterol synthesis, therefore increasing membrane structural integrity and repair of damaged membrane in muscle cells in times of stress such as resistance exercise (Knitter, *et. al.*, 2000; Nissen, *et. al.*, 2000; Panton, *et. al.*, 2000; Slater & Jenkins, 2000; Jówko, *et. al.*, 2001; Slater, *et. al.*, 2001; Nissen & Sharp, 2003). Damaged and stressed cells may not be able to supply adequate *β*-hydroxy-*β*-methylglutaryl-CoA to keep up with the cholesterol synthesis required for cell and plasma membrane functions without HMB supplementation (Nissen, *et. al.*, 2000).

Finally HMB is possibly utilised as a structural component of cell membranes (Slater & Jenkins, 2000), as HMB or as a polymer or co-polymer of HMB. β-hydroxybutyrate a chemically similar compound is utilised as its polymer, poly-β-hydroxybutyrate, as a component of the Ca-channel of cell membranes. HMB and β-hydroxybutyrate are chemically similar and HMB has been found covalently bound in tissues (Nissen, *et. al.*, 1996).

1.7.4 Evidence

Evidence of altered oxidative capacity comes from both animal and human *in-vivo* studies. Studies of horses indicate HMB may increase endurance via an increase in aerobic capacity. Results suggest an increase in red blood cells, globulin, and white blood cells (Miller, 1998). A study of endurance cyclists indicate HMB supplementation increases VO_2 max relative to blood lactate level, but not the VO_2 peak itself. Possibly via decreased lactate production with exercise, or increased clearance of lactate following exercise (Vukovich & Dreifort, 2001).

Evidence of retardation of muscle damage and proteolysis are numerous. Initially *in-vitro* studies of rat and chicken muscle cells produced evidence that HMB retards proteolysis and stimulates synthesis (Nissen & Abumrad, 1997; Peterson, *et. al.*, 1999; Vukovich, *et. al.*, 2001). In addition, HMB supplementation of animals has been shown to decrease biochemical markers of muscle protein damage and proteolysis. HMB supplementation of rats indicates decreased levels of cathepsins (a lysosomal protease) and calpain following intense exercise (Jank, *et. al.*, 2000). HMB supplementation in horses has also been shown to lower creatine kinase levels following exercise (Miller, 1998). Human HMB supplementation studies also indicate decreased muscle proteolysis assessed via decreased levels of biological markers 3-methylhistidine (3MH), creatine kinase, and lactate dehydrogenase with endurance exercise (Nissen & Abumrad, 1997; Knitter, *et. al.*, 2000; Panton, *et. al.*, 2000; Jówko, *et. al.*, 2001; Slater, *et. al.*, 2001; Vukovich, *et. al.*, 2001).

The evidence for increased muscle protein synthesis and fat free mass accretion is more controversial. The protein concentration of rabbit carcasses, fed an HMB supplemented diet (0.04g, 0.18g, and 0.88g ingested HMB) increased significantly compared to controls (Ostaszewski, *et. al.*, 1997). The body weight and carcass yields of broiler chickens was increased in some studies of HMB supplementation, however no significant difference was found in other studies (Nissen *et. al.*, 1994). Body weight of 21 day old piglets was increased in some studies of HMB supplementation of lactating sows, however not in others (Nissen, *et. al.*, 1994; Gatnau, *et. al.*, 1995). Studies of HMB supplementation of feedlot steers was shown to alter the ratio of intramuscular to subcutaneous fat; however it had no effect on live weight (Van Koevering, *et. al.*, 1994). Studies of HMB supplementation of growing lambs produced an increase in plasma free amino acids, but failed to stimulate skeletal muscle protein synthesis (Papet, *et. al.*, 1997).

Studies of HMB supplementation with resistance training are reviewed separately following.

1.8 β-hydroxy-β-methylbutyrate Supplementation with Resistance Exercise

Despite equivocal results of HMB supplementation studies on muscle protein turnover in animals and no clear mechanism of HMB action within muscle fibres, several studies have investigated HMB supplementation in humans with resistance exercise. Discussion has been limited to human supplementation studies published in peer reviewed journals, studies published in abstract form only were excluded. A summary of studies reviewed is presented in Table 1.1.

Nissen and colleagues (1996) were the first to study the effects of HMB supplementation in humans. Two separate studies were undertaken. The first examined strength, body composition, markers of muscle damage, HMB blood and urine levels with resistance exercise, and HMB supplementation at two levels of protein intake. Nissen and colleagues

(1996) concluded that exercise induced muscle proteolysis was significantly decreased with HMB supplementation, as measured by urinary 3-methyl histidine and creatine kinase. In addition strength was significantly increased in the HMB compared to placebo groups. This study has been previously criticised for its use of a protein nutrient supplement, supplying additional calories and vitamins to the protein groups, therefore any effect on increased muscle strength or muscle mass may not be attributed solely to HMB supplementation (Slater & Jenkins, 2000). In addition, the use of six treatment groups decreased the statistical power of the study due to low sample sizes in each group. The training status of subjects was ill defined, subjects had not resistance trained for 3 months prior to the study, but previous experience was unknown. Study subjects were initially randomised into treatment groups; however one subject was later switched to a different treatment group after baseline assessment of body weight, possibly introducing bias.

In the second study Nissen and colleagues (1996) examined strength, and body composition during seven weeks of HMB supplementation with resistance exercise. They concluded that with HMB treatment, fat free mass was significantly increased compared to the placebo group. This study has been previously criticised for its use of the same HMB and protein nutrient supplement as used in the first study, providing greater protein and some vitamin content compared to the control in an orange drink mix (Slater & Jenkins, 2000). The training status of subjects was ill defined, and the HMB group had lower initial bench press strength, possibly indicating a greater potential for strength gains if representative of less training experience (Slater & Jenkins, 2000).

Kreider and co-workers (1999) further investigated the role of HMB supplementation on strength, body composition, and markers of muscle catabolism with resistance exercise. They concluded that HMB supplementation significantly increased serum and urinary concentrations of HMB, however no ergogenic effect was observed (Kreider, *et. al.*, 1999). This study could be criticised for limited study duration compared to other HMB supplementation studies. Lack of supervision of subject training may have resulted in workouts not intense enough to produce a training induced catabolic response. In addition, it is questioned whether blood testing for 3-methyl histidine requiring dietary restrictions

and an 8 hour fast prior to testing, and 1RM strength testing during the same day was wise.

Gallegher, *et. al.*, (2000) continued investigation on the effects of HMB supplementation on strength and body composition with resistance training. The researchers concluded that daily supplementation of HMB with resistance training significantly increased fat free mass, and decreased plasma creatine kinase activity following intense exercise. However strength gains during the study were less obvious. In addition the researchers concluded that higher doses of HMB (76mg/kg/day) did not promote additional strength or fat free mass gains compared to the recommended dose of 3g/day. This study may be criticised for lack of definition of training status of subjects.

Panton and colleagues (2000) investigated the effects of gender and training status on the action of HMB on strength, body composition, and muscle catabolism with resistance training. The researchers' concluded that gender and training status had no effect on the action or effectiveness of HMB. In addition, supplementation with HMB significantly suppressed exercise induced plasma creatine kinase, upper body strength increased significantly, and fat free mass increased but not significantly, compared to the placebo group. This study has previously been criticised because the study period was small (4 weeks), sample sizes were small therefore reducing the statistical power of the study, and originally no significant differences were found until trained and untrained subjects results were pooled. In addition, subjects training status was not clearly indicated. Untrained subjects who had not trained the previous 6 months may differ from individuals who have never trained. In fact baseline data suggests trained subjects were weaker than untrained. This study may be further criticised for comparing data between the genders, when two important differences in training occurred. During the study, changes were made in the women's resistance training programme due to complaints of fatigue and soreness (from the men), and lower body 1RM testing was performed on the leg extension machine, while men performed on the leg press equipment.

In an attempt to further elucidate the mechanism of action of HMB, Slater and co-workers investigated the effects of HMB supplementation on urinary testosterone: epitestosterone

ratio in males. This study examined urine samples at baseline, day 7, and 14. No other parameters were investigated. The authors concluded that HMB supplementation had no influence on the urinary testosterone: epitestosterone ratio, therefore any anabolic effects occurred via an alternative mechanism and required the training stimulus of resistance exercise.

Paddon-Jones and colleagues (2001) concentrated their study on one aspect of the proposed actions of HMB, the reduction of exercise induced muscle damage. The conclusions reached from this study were that HMB supplementation had no beneficial effect on symptoms of muscle damage. This study may be criticised for only assessing muscle damage via delayed onset muscle soreness, swelling, and recovery from exercise. Further measures of muscle damage, such as levels of 3-methyl histidine, creatine kinase, and lactate dehydrogenase were not carried out during this study.

Jówko and colleagues (2001) examined the effects of creatine and HMB supplementation on strength and body composition with resistance exercise, in an effort to further elucidate the mechanism of action of HMB. Jówko and colleagues (2001) concluded that creatine and HMB supplementation both increase fat free mass and strength and the effects are additive, therefore occurring via differing mechanisms. However, the increase in fat free mass from HMB alone was not significant (P=0.08). This study may be criticised for small sample numbers, and unclear indication of subject training status. Subjects were considered untrained if they had not trained during the previous 6 months (this may differ from individuals who have never trained).

Slater, *et. al.*, (2001) studied the effects of HMB supplementation on strength, body composition, and markers of muscle damage, in highly trained subjects. Subjects, were involved in sports at national level, and had a minimum of two years training experience. The researchers concluded that HMB supplementation of trained subjects had no influence on strength, body composition, or biochemical markers of muscle damage. This study may be criticised for its limited study duration, and small sample numbers within each treatment group. In addition this study suffered from the same limitations as the study by Kreider and

co-workers (1999), blood testing for 3-methyl histidine and 1RM strength testing occurred on the same day.

The final study reviewed, was conducted by Vukovich and co-workers (2001), and investigated HMB supplementation on elderly subjects undergoing resistance training. Potential changes in strength and body composition were assessed. The researchers concluded that the HMB supplemented group significantly increased fat free mass and decreased fat mass; however there were no significant differences in strength increase. The results of this study may not be comparable to the present study due to the age of subjects involved, and possible decreased training intensity due to subject training status, age, and frailty.

1.9 Study Justification and Objectives

1.9.1 Study Justification

Several studies of HMB supplementation with resistance trained individuals have been carried out previous to this study as described above. However results have been indeterminate, and there have been concerns regarding their methodology. The purpose of this study is to validate or dispute claims of increased strength, increased fat free mass, and decreased fat mass with HMB supplementation during a period of resistance training, in trained men.

The methodological problems of previous studies hope to be avoided by increasing the study duration, increasing sample sizes, standardising and assessing subject dietary intakes, using trained subjects with a minimum of one year experience, and randomly allocating subjects to HMB or placebo supplementation groups in a double blind manner.

1.9.2 Study Objectives

The objectives of this study are as follows:

- To assess and compare muscular strength of subjects in the HMB and Placebo groups prior to, and following a 9-week resistance training programme.

- To assess and compare body composition of subjects in the HMB and Placebo supplemented groups prior to, and following a 9-week resistance training programme.

- To assess and compare body fat distribution of subjects in the HMB and Placebo supplemented groups prior to, and following a 9-week resistance training programme.

- To assess the effectiveness of standardisation of dietary intakes of subjects.

- To determine the effects, if any, of HMB supplementation with resistance exercise on trained men.

Note: ***Abbreviations used in Table 1.1 on the following page include:***

BW = **Body Weight**
Carb = **Carbohydrate**
Prep = **Prepared Meals**
1RM = **One Repetition Maximum**
TOBEC = **Total Body Electrical Conductivity**
DEXA = **Dual Energy X-ray Absorptiometer**
3MH = **3-Methyl Histidine**
CK = **Creatine Kinase**
LDH = **Lactate Dehydrogenase**
ALT = **Alanine Aminotransferase**
AST = **Aspartate Aminotransferase**
N = **Nitrogen**
FFM = **Fat Free Mass**
BIA = **Bioelectrical Impedance Analyser**
CT = **Computerised Tomography**
DOMS = **Delayed Onset Muscle Soreness**
CRTN = **Creatinine**
%BF = **Percent Body Fat**

Table1.1: Summaries of the Previous HMB Studies Reviewed

AUTHORS	Nissen, *et. al.*,	Nissen, *et. al.*,	Kreider, *et. al.*,	Gallagher, *et. al.*,	Panton, *et. al.*,
YEAR	1996	1996	1999	2000	2000
DURATION	3 wks	7 wks	28 days	8 wks	4 wks
SUBJECT NO.	41	32	40	37	84
GENDER	Male	Male	Male	Male	Male & Female
AGE	19-29yrs	19-22yrs	Mean 25.1 yrs	18-29	20-40yrs
TRAINING STATUS	No resistance exercise (3 mnth prior)	Some exercise	Trained (>1yr)	No resistance exercise (3 mnth prior)	Trained & Untrained
SUPPLEMENT CONTROL PRIOR TO STUDY	No	No	No creatine, HMB, β agonists (8wk prior)	No supplements (3 mnth prior)	No
GROUPED BY	Random, blind study	Random	Double blind match BW	Double blind match BW	Random, double blind
PLACEBO	No	Orange drink	Carb, protein supplement	?	Rice flour
SUPPLEMENT	HMB, protein, orange juice	HMB + nutrient shake	Carb, protein, HMB	HMB in foil pkt	Ca-HMB + KH_2PO_4
HMB (g)	1.5 or 3g/day	3g/day	3 or 6g/day	3G or 76mg/kg/d	3g/day
SUPPLEMENT COMPLIANCE	Urine test	?	Empty supplement packets, blood/urine	Empty supplement packets, blood	Log
DIET	3 prep meals/day	No	Diary/log	3day food recall	No
TRAINING COMPLIANCE	Supervised	?	Training Log	Supervised	Supervised
STRENGTH	1RM	1RM	1RM	Dynamometer, isokinetic	1RM
BODY COMPOSITION	TOBEC	TOBEC	DEXA	Skinfold	Underwater weighing
MUSCLE DAMAGE	3MH, CK	No	CK, LDH, ALT, AST, uric acid, N, creatinine	CK	CK
RESULTS	Decrease 3MH, & CK; increase strength.	Increase FFM; increase bench press 1RM.	No beneficial effect.	Decrease plasma CK; increase FFM.	Decrease plasma CK; increase upper body 1RM.

AUTHORS	Slater, *et. al.,*	Paddon-Jones, *et. al.,*	Jówoko, *et. al.,*	Slater, *et. al.,*	Vukovich, *et. al.,*
YEAR	2000	2001	2001	2001	2001
DURATION	14 days	6 days	3 wks	6 wks	8 wks
SUBJECT NO.	6	17	40	27	32
GENDER	Male	Male	Male	Male	Male & Female
AGE	30-34yrs	Mean 21.4yrs	19-23yrs	Mean 24.7yrs	Mean 70yrs
TRAINING STATUS	Recreationally active	Untrained	Not resistance trained	Highly trained	Untrained
SUPPLEMENT CONTROL PRIOR TO STUDY	No	No	No HMB, creatine (3 mnth prior)	No creatine, HMB, or supplements, (8wk prior)	No
GROUPED BY	No	Double-blind match BW	Random, double-blind	Double blind parallel	Random double-blind
PLACEBO	No	40mg/kg $CaCO_4$	Rice flour, glucose	Rice flour	Rice flour
SUPPLEMENT	HMB capsules	40mg/kg Ca-HMB	Ca-HMB, Creatine	HMB, time release HMB	Ca-HMB + KH_2PO_4
HMB (g)	3g/day	~3.4g/day	3g/day	3g/day	3g/day
SUPPLEMENT COMPLIANCE	?	No	No	Empty bottles returned	Questionnaire, blood test
DIET	No	No	Prep meals, 24hr recall	3-day food records	No
TRAINING COMPLIANCE	No	Supervised	Supervised	Supervised	Supervised
STRENGTH	No	Elbow flexor torque	1RM	3RM	1RM
BODY COMPOSITION	No	Upper arm girth	BIA	DEXA	Skinfold, CT, DEXA
MUSCLE DAMAGE	No	Report DOMS, post-ex torque	CRTN, CK, urea N	CK, LDH, CRTN, urea, cortisol, testosterone	No
RESULTS	No influence on testosterone.	No beneficial effect.	Increase strength; decrease serum CK; synergistic to creatine.	No beneficial effect.	Increase FFM; decrease % BF.

Chapter 2 Methods

This project was reviewed and approved by the Massey University Regional Human Ethics Committee, Albany Campus, MUAHEC 02/063. A copy of the human ethics application may be found in Appendix 1. In accordance with the human ethics application, each participant signed a consent form prior to participation, see Appendix 3.

2.1 Subject Recruitment

Advertisement for study participation was via pamphlets and notices in gyms and health centres throughout the Auckland region. Due to the advertisement in gyms, home exercisers were excluded from the study; it is possible that the exercise patterns of home, and commercial gym exercisers differs slightly. Study participants were resistance trained men, aged 20-30, attending gyms, living in the Auckland region, and who volunteered for this study. A copy of the flyer and brochure may be found in Appendix 2.

Information sheets including a brief description of the ß-hydroxy-ß-methylbutyrate (HMB) supplements and proposed action, study outline and description of procedures involved, confidentiality procedures and rights of volunteers, consent forms and self addressed envelopes were mailed out to all men interested in participating in the study. Study information sheets may be found in Appendix 3.

2.1.1 Selection Criteria

Participants were chosen for the study from those respondents who met the study selection criteria:

- **Gender:** Males were studied to avoid any hormone response and sensitivity variations with the menstrual cycle in women potentially affecting metabolism (Heyward, 1998) and strength (Jones & Folland, 2001).

- **Age:** Participants aged 20 to 30 years were selected to avoid any physiological and metabolic differences associated with adolescent or older individuals from potentially affecting results.

- **Resistance Training Experience:** A minimum of one year of previous resistance training experience was required. This decision was made for three reasons: Firstly, to avoid any influence of neural adaptation weakening the significance of the strength results. Secondly, strength trained individuals were thought to differ from untrained individuals in muscle substrate stores, muscle enzyme activities (MacDougall, 1986), hormonal sensitivities (Biolo, *et. al.*, 1995), and protein turnover in response to resistance exercise (Tarnopolsky, *et. al.*, 1988; Lemon, 1998; Roy *et. al.*, 2000; Tipton & Wolfe, 2001). Therefore resistance trained individuals may not respond to HMB supplementation in the same manner as untrained individuals (Nissen, *et. al.*, 1996; Clark, *et. al.*, 2000; Nissen, *et. al.*, 2000; Slater & Jenkins, 2000; Slater, *et. al.*, 2001; Vukovich, *et. al.*, 2001). Finally, trained individuals have less potential for change with resistance exercise, and may therefore more clearly indicate changes associated with supplementation (Slater & Jenkins, 2000; Slater *et. al.*, 2001).

- **Medical History:** All participants were healthy, with no known medical conditions that would contraindicate resistance exercise. There was considered to be no risk of harm to healthy participants if exercises were conducted in a safe environment and according to instructions given.

- **Intake of Other Sports Supplements:** Participants were asked to discontinue use of other sports supplements prior to beginning the study period, including other HMB products, protein powders, protein shakes, protein bars, and products containing creatine, to reduce the possibility of a synergistic effect on HMB from other sports supplements.

2.1.2 Sample Size

Thirty four resistance trained males (RTM) participated in the treatment (n = 19) and control groups (n = 15) of the study.

2.2 Study Design

Experimental Design

The experimental design used in this study was a double blind randomised design. An outside party assigned subjects to the treatment or control group so that neither the subjects nor the researcher were aware which subjects were taking the HMB or the placebo (Burke, *et. al.*, 2000).

Justification of Experimental Design

A control group was used to remove any psychological effect of being supplemented and included in the study, otherwise known as a 'placebo effect' (Burke, *et. al.*, 2000). Subjects were randomly assigned to the treatment and control groups to remove any bias or initial differences between the groups (Vincent, 1995). In addition, this study was double blind to remove any effect from inadvertent encouragement by the researcher to improve the performance of the treatment group, also known as the 'halo effect' (Burke, *et. al.*, 2000).

Study Length

The study was conducted for 11 weeks in total, including 10 weeks of resistance training, and 9 weeks of supplementation. The resistance training programme began on week 2 and continued until week 11, for a total of 10 weeks; the supplementation period began on week 2 and continued until week 10, for a total of 9 weeks.

Justification of Study Length

Thus far results from HMB supplementation on resistance trained individuals have been variable; therefore this aspect of HMB supplementation was thought to be worth further investigation over a longer study period than has previously been undertaken with resistance trained individuals.

2.2.1 Supplementation

The HMB was sourced from Musashi Ltd (Notting Hill, Victoria, Australia). The batch number was 20011118. The specifics of this batch were as follows;

Appearance:	white crystalline powder
Ca content:	13.70%
HMB content:	84.69%
Loss on Drying:	1.52%
Pb:	<10ppm max.
As:	<2ppm max.

The placebo was made up of maize starch, sourced from National Starch and Chemical NZ Ltd (Auckland, New Zealand) with the product number NOVATION 2700 and BDH (Palmerston North, New Zealand) with the product number 30261. This placebo was chosen because it closely resembled the HMB powder.

The HMB and placebo were packaged in the same type of gelatine capsule (size 00) provided by Pharmaceutical Compounding (Auckland, New Zealand). Each capsule contained 0.5g of HMB weighed in batches of 10, or approximately 0.5-1.0g of placebo. All subjects were required to consume 6 capsules per day, divided into 3 doses of 2 capsules throughout the day. A third party coded and allocated packets of capsules for collection by study subjects.

The men received three grams of HMB supplemented during this study, as a dose-responsive increase in muscle strength and lean muscle size is thought to occur up to 3g per day (Slater *et. al.*, 2001; Slater & Jenkins, 2000; Gallagher, *et. al.*, 2000). Capsules were taken three times per day to maintain high circulating plasma HMB levels throughout the day as the plasma HMB half life is approximately 2.3 hours (Vukovich, *et. al.*, 2001).

2.2.2 Training Programme

The training log booklets, including all exercise descriptions (Westcott, 1996; Mann, 1997; Moran & McGlynn, 1997; Bompa & Cornacchia, 1998) and sketches of each exercise were designed by the researcher. The exercise descriptions were approved, and the training programme designed by a qualified Personal Trainer (Network Certificate in Personal Training), Mark Woodgate. The training programme design was a three day split; each major muscle group was trained 1-2 times per week, for 5-7 sets of 5-15 repetitions, and 3-4 exercises per muscle group. For an example of the complete training log book see Appendix 5.

Justification

To ensure adherence, the resistance training programme was designed to maximise muscle mass and strength increase, without being too time intensive or monotonous. The amount of spare time of the individual and the variety in an exercise programme both influence the drop-out rate from exercise programmes (Heyward, 1998).

The same resistance training programme was given to participants in both the HMB and placebo groups to ensure the total training volume and mode of resistance training performed by subjects was the same. Muscle groups were trained and tested on the same type of apparatus and range of motion to avoid a training response specificity influencing strength over contraction velocity, muscle length, and range of motion (Rutherford & Jones, 1986; Sale, 1988; Jones, *et, al.,* 1989; McArdle, *et. al.,* 1991; Fleck & Kraemer, 1997).

2.3 Data Collection Methods

2.3.1 Study Questionnaire

Questionnaire Development and Pre-testing

A study questionnaire was prepared and administered to four resistance trained individuals from the general public prior to beginning the study. Several changes were made from the original draft of the study questionnaire. The original phrasings of some questions were changed to make them easier to understand. In addition, a question on supplement usage and beliefs was added after the initial trial questionnaire indicated high supplement usage in resistance trained men.

The questionnaire was divided into three sections. The first section was composed of questions assessing the demographic details of the study group, including age, ethnicity, education, and occupation. Questions regarding occupation were based on NZSCO occupational coding (NZSCO, 2002). The second section consisted of a semi-quantitative food frequency questionnaire to give an estimate of food group consumption frequency (Mullen, *et. al.,* 1984) of resistance trained men in this age group. Questions were based on the New Zealand National Nutrition Survey (1999) questionnaire and suggested food portion sizes. The third and final section of the questionnaire consisted of questions assessing the previous physical activity and training habits of recreationally resistance

trained men. An example of the study questionnaire has been included in Appendix 6.

Justification

Recent studies indicate some significant differences in the dietary habits and supplementation patterns of resistance trained men compared to both the general population and competitive bodybuilders (Lamar-Hildebrand, *et. al.*, 1989; Sandoval, *et. al.*, 1989; Sandoval & Heyward, 1991; Bamman, *et. al.*, 1993; Bazzarre, et. al., 1994; Naylor & Garg, 1996; Withers, *et. al.*, 1997). A food frequency questionnaire has the following advantages; it is quick to perform; inexpensive; may be used to analyse large populations (Mullen, *et. al.*, 1984); can be used to measure consumption over a day, week, month, or year (Lee & Nieman, 1996); gives a good measure of individual intake compared to household food account data; and is reasonably accurate for frequently eaten foods (Mullen, *et. al.*, 1984). Disadvantages include; food intake is measured using standard portion sizes so is not as accurate as measured food records; it does not give a precise estimate of energy and nutrient intake (Lee & Nieman, 1996); and it is not accurate for infrequently eaten foods (Mullen, *et. al.*, 1984).

Several studies have looked at the energy and nutrient requirements of competitive body builders; however it is known that exercise and training programs of competitive bodybuilders and resistance trained men differ in training level, intensities, and frequencies (Lamar-Hildebrand, *et. al.*, 1989; Kleiner, *et. al.*, 1994). Therefore when evaluating the nutritional requirements of resistance trained men, a closer assessment of physical activity levels would prove advantageous.

2.3.2 Dietary Assessment via Diet Records

Prior to, and at the completion of the study, subjects were asked to record all food and drink consumed over three days, including two weekdays and one weekend day. Portion size or food volume consumed and any leftovers were estimated. Subjects were also asked to record type and brand of foods consumed, cooking methods, and homemade recipes.

Prior to starting the study, dietary recommendations were given to each subject, containing advice on appropriate nutrition to maximise muscle size and strength increases with resistance training, in an effort to standardise participant intakes. In addition to this, all subjects were reminded not to consume other sports supplements that may influence the action of HMB. An example of the dietary record sheet and dietary recommendations given to participants, have been included in Appendices 9 and 10.

Justification

Estimated food records were used to assess subject's usual dietary intake of energy and nutrients prior to, and during the study. This method of dietary intake measurement was chosen because it was considered to be a less expensive and less time intensive method when analysing the intakes of a relatively large group. The food records were then analysed for changes over the study duration and differences between the HMB and Placebo groups that may have influenced strength increase and body fat loss. The study dietary recommendations were used in an attempt to standardise subject's dietary status during the study, as adequate nutrition status will provide optimum conditions to maximise the action of HMB.

Advantages of using estimated food records include; several days may be analysed giving a more representative estimate of subject's usual dietary intake (Lee, & Nieman, 1996); recording weekdays and weekend days reduces day to day variations in dietary intake (Tarasuk, & Beaton, 1992); do not require memory recall; subjects record their own dietary intakes in relative privacy and therefore are less likely to omit foods due to embarrassment in front of an interviewer (Lee, & Nieman, 1996; Todd, *et. al.*, 1983).

Disadvantages include; all subjects must be literate and motivated to keep records (Todd, *et. al.*, 1983); estimated food records are less accurate than weighed records; limited record periods don't include seasonal variations in food intake (Beaton, 1994); the act of recording food intake may alter subject's food intakes and underestimate usual intakes (Lee, & Nieman, 1996).

Use of food composition tables and databases introduce errors when foods chosen from the database differ in growing, storing, processing, and/or cooking methods; bioavailability of individual nutrients due to season or region; substitution of similar food for food item or recipe not contained in the database; and incorrect identification of food item in the database (Lee, & Nieman, 1996).

2.3.3 Anthropometric Measurements

Skinfold Measurements

The standard specifications of ISAK (International Society for the Advancement of Kianthropometry) were used when locating and measuring skinfolds (Marfell-Jones, 1999; Mellow, 1999).

Initially eight sites were identified along the right hand side of the body using bony landmarks, and marked with a water based felt-tip pen (Mellow, 1999; Heyward, 1998; Heyward & Stolarczyk, 1993). The eight sites were measured in sequence two to three times. A copy of the data record sheets for the anthropometric profile, BIA results, and strength data has been included in Appendix 7.

A Slim Guide skinfold calliper was used which has a precision of 1 mm (Heyward & Stolarczyk, 1993). A Luftkin retractable measuring tape, model W606PM, was used to aid identification of anatomical landmarks, and was read to the nearest millimetre.

Justification

Skinfold measurement's were taken from each participant to measure the initial pattern of fat distribution, and the change in fat distribution and fat free mass during the study period due to HMB and placebo supplementation.

Advantages of using the skinfold method include the wide use and validation of this method in numerous studies. As well this method allows a measure of fat distribution over the body (Yoke, 1995). Disadvantages include the assumptions that subcutaneous and internal fat distribution is similar in all individuals, and a constant relationship between subcutaneous and internal fat distribution. Error may be introduced into this method from measurer skill, calliper type, and subject individual variability. It would have been preferable to use Harpenden skinfold callipers with a constant jaw pressure of $10g/cm^2$, rather than the Slim Guide callipers that were used. The Harpenden callipers have greater accuracy and precision of measurement. Individual variability may be a factor when studying resistance trained men, as skinfolds are more difficult to take in heavily muscled subjects due to the difficulty in separating the subcutaneous fat layer from muscle tissue (Heyward & Stolarczyk, 1993).

Body Fat Estimation

The equation used to estimate percent body fat was the Durnin & Womersley four-site method (Durnin & Womersley, 1974; Lee & Neeman, 1996).

Justification

This equation was used to give an estimate of percent body fat from the skinfold sites that were measured. The advantages of using this skinfold equation were that this was a well known and well validated equation; it was one of the more popular body fat equations currently in use on New Zealand populations; it was developed on a fairly similar population to that used in this study; and it uses more than one skinfold to predict body fat. The more sites used in the equation the greater the accuracy (Mellow, 1999). However, the validity of this equation decreases due to the differences between the population used to develop this body fat equation and the study population. There were a large proportion of sedentary individuals in the Durnin and Womersley (1974) study, and all individuals in this study were recreational bodybuilders; The Durnin and Womersley (1974) study used data from a Scottish population (Mellow, 1999), and there was a fair proportion of Asian

individuals (12.5%) in this study population. The Durnin and Womersley (1974) body fat equation was validated against a level II method, densitometry (under water weighing). Densitometry is not a direct measure of body fat, though it is thought to be the gold standard in body fat measurement. Densitometry also uses a complex regression equation to calculate body fat levels, and this potentially further reduces the accuracy of using the Durnin and Womersley (1974) equation (Mellow, 1999).

Other methods of measuring body fat have been discussed in the bioelectrical impedance analysis section below.

Height Measurement

Subjects were asked to remove footwear, and stand on a level surface with feet together, heels, buttocks, shoulders, and head pressed back against a wall (Wilson, *et .al.*, 1993). A plate was lowered onto the individuals head, using a level to ensure a horizontal intersect with the tape measure. The individual was asked to take a deep breath and stand straight, while the measurement was taken. Height was measured to the nearest millimetre (Wilson, *et .al.*, 1993).

Justification

Height was measured to calculate body mass index as an indication of long-term health risks for this population, and to calculate total body water for use in blood alcohol concentration calculations.

Weight Measurement

Each subject was measured in light clothing with shoes removed. Subjects were asked to stand on the middle of the scales, and look straight ahead (Mellow, 1999).

Tanita Digital Personal Scales were used with a maximum capacity of 150kg, with graduation of 100-200g increments. Weight was measured in kilograms to 1 decimal place.

Justification

Body weight was measured prior to the start of supplementation during visit 2, and after nine weeks of supplementation during visit 4. Body weight was used to calculate percent change in weight for the study duration, body mass index, and total body water for use in blood alcohol concentration calculations.

2.3.4 Bioelectrical Impedance Analysis

The bioelectrical impedance analyser instrument passes a low electrical current (50 kHz) through the body and measures the impedance or restriction of flow of the electrical current received back at the bioelectrical impedance analyser. This initially measures total body water, which is than converted to percent fat mass and percent fat free mass using a pre-programmed algorithm (Mellow, 1999; Heyward, 1998; Heyward & Stolarczyk, 1993). The procedures followed for use of the BIA instrument were those provided by the manufacturer (Impedimed Pty Ltd, Australia).

A single frequency bioelectrical impedance analyser instrument was used in tetra-polar mode at 50 kHz, 200 μA, model BIM 4, supplied by Impedimed Pty Ltd (Queensland, Australia).

Justification

This measurement was taken as an indication of fat free mass change over the duration of the study.

Advantages of using bioelectrical impedance analysis are that it was a quick and easy method to use; was a non-invasive technique; and required minimal training of the

researcher (Yoke, 1995). The disadvantages of this method include; errors introduced by the individual's hydration level and incorrect placement of electrodes (Yoke, 1995); instrument variation of up to 36 Ω between brands (Heyward, 1998; Heyward & Stolarczyk, 1993); and decreased reliability since the regression equation was unknown.

Other methods for measuring body fat levels include; Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Dual Energy X-ray Absorptiometry (DEXA), densiometry (under water weighing), plethysmograph (BODPOD) (Mellow, 1999), required the use of expensive equipment, were not portable, and therefore were considered impractical for this study.

2.3.5 Strength Testing Using the One Repetition Maximum Method

The one repetition maximum (1RM) estimates strength via measurement of the heaviest weight an individual is able to lift through the full range of motion, for one repetition of an exercise (McMurray, 1999).

Prior to the strength test date, subjects were asked not to train within 12 hours of the test, and were advised to bring their usual training clothes and gear. Immediately prior to strength testing, each subject was asked to warm-up for five minutes on aerobic equipment (Dowson, 1999). The exercise apparatus was adjusted to suit the size of the individual, and the seat and bench height positions noted for later retesting. A starting weight close to 40-60% of the individual's perceived 1RM was chosen for 5-10 repetitions as a warm-up. The subject was given a one minute rest period and allowed to stretch if required. A weight close to 60-80% of the subject's perceived 1RM was chosen for 3-5 repetitions. The subject was again given a one minute rest period. As the weight lifted came closer to the perceived 1RM, conservative increases of 1-5 kg increments were chosen. For each attempt where the individual was able to perform more than one lift, the weight was increased after a one minute rest period until failure to complete the lift (Kraemer & Fry, 1995). The researcher observed subject positioning and technique, ensuring the full range

of movement was completed for each exercise attempt, as well as giving encouragement to each subject. The strength testing procedures have been described in more detail in Appendix 8.

Leg Extension Apparatus

The Leg Extension apparatus had both an adjustable seat, and adjustable pin loading up to a maximum weight of 96kg, from FitnessWorks (Auckland, New Zealand).

Bench Press Apparatus

A Pro Smith press with counter balance, Olympic barbell, Olympic cast plates, and Pro Super adjustable incline bench, from FitnessWorks (Auckland, New Zealand) and ProGym brands were used to test 1RM bench press strength.

Bicep Preacher Curl Apparatus

FitnessWorks (Auckland, New Zealand) and ProGym Pro preacher curl adjustable bench, and assorted dumbbells (cast plates on chrome handles) were used to measure 1RM bicep preacher curl strength.

Justification

Isotonic strength testing was done at the beginning and end of the study period. The 1RM method was used as an estimate of individual strength increases during the study period, and to compare the strength gains between the HMB and placebo groups. Strength tests were performed twice at the beginning and twice at the end of the study, to give an indication of the measurement reliability. Measurements were taken from three different muscle groups, arms (biceps curl), trunk (bench press), and lower body (leg extension), to increase the correlation between individual strength measurements and total body strength (Haskell & Kiernan, 2000).

The 1RM method was used because it was useful to predict performance in other dynamic activities (Wilson, 1994); the protocol has good reliability when used in experienced subjects (Wilson, 1994); and the use of resistance exercise machines reduced the need for a spotter. To avoid risk of diurnal variations in strength, each subject was tested at approximately the same time of day when possible.

Disadvantages of the method included; the use of free weights since the 1RM protocols were designed for machines, the learning effect may be increased if subjects were not familiar with the exercise due to the co-ordination involved when using free weights; the resistance exercise machines may reduce the range of motion of the movement; and test reliability may be affected by the measurer's knowledge of lifting and spotting techniques. Other methods for measuring strength include; isometric strength testing which measures maximum force applied against an immovable resistance measured using a tensiometer, load cell, or strain gauge over a 3-4 second period; and isokinetic strength testing which involves testing maximum strength throughout a range of motion at set velocities using specialised isokinetic equipment. Both methods require specialised instruments and equipment which were not available for this study (Wilson, 1994).

2.3.6 Physical Activity Records

Training Log Books

A red coloured training log book was provided to each participant for the first five weeks of the study, and a separate blue coloured training log book for the final five weeks. Subjects were asked to maintain their current participation in other physical activities in conjunction with the study resistance training programme. The training log books included space to record resistance training compliance; other physical activities participated in; as well as room for notes of any effects or side-effects of the supplementation experienced by participants.

Justification

The training log books allowed a record of subject compliance in the study resistance training programme as well as participation in other physical activities. An advantage of using the log books was that they allowed recording of activities immediately following participation or at the end of each day, therefore recall was more convenient for participants. However the disadvantage in the use of log books was that they were time demanding and may influence a subject's behaviour (Haskell & Kiernan, 2000).

Other methods of collecting this information include self reported surveys (see study questionnaire above); diaries which generate a complete record over a limited time period such as a few days; and physiological monitoring. Both diaries and physiological monitoring would have generated vast amounts of unnecessary data and were considered to be too involved for a study of this size.

2.4 Data Collection Programme

Data was gathered during four visits at weeks 1, 2, 10 and 11. Data collection consisted of a study questionnaire, anthropometric measurement, BIA body composition analysis, 3-day food records, strength testing, resistance training compliance records, supplementation compliance records, and additional physical activity records.

Visit One (Approximately 30 minutes)

During visit 1, three-day dietary record sheets, study questionnaires, and training log books for weeks 1-5 were handed out to each participant to take home, with instruction on how to complete them. Muscular strength was measured during a series of One Repetition Maximum (1RM) exercise trials for leg extension, Smith machine bench press, and preacher curl apparatus. Additional instruction on how to perform unfamiliar exercises in

the study resistance training programme was given to participants where required.

Visit Two (Approximately 60 minutes)

The study questionnaire was collected from participants during visit two. The questionnaire was used to assess demographic details, and previous eating, exercise, and supplement habits. The three-day dietary records were also collected during visit two. Dietary aims for the study period were discussed, and the avoidance of other sports supplements for the duration of the study period was emphasised to subjects. Anthropometric measurements were taken; eight skinfold sites were measured using skinfold callipers; height and body weight measured; and body composition measured using a bioelectrical impedance analyser. The strength tests from visit one were repeated. The first week supply of supplement capsules were handed out, and each week thereafter to all participants.

In Between Visits

At a half-way point during the study period the completed training log books were collected, and log books for weeks 6-10 were supplied to each participant.

Visit Three (Approximately 30 minutes)

The final three-day dietary record sheets were handed out to each study participant. Muscular strength was measured using the same procedure as visit 1. Prior to the final assessment participants should have finished their supply of supplement capsules. All participants were advised to record the number of any capsules remaining, and to refrain from consuming further capsules.

Visit Four (Approximately 60 minutes)

The final 3-day dietary records were collected once completed. Final Anthropometric

measurements were taken as above in visit two. The strength tests from visit three were repeated. Participants were asked to return the final training log books as soon as possible following visit 4.

2.5 Data Processing and Computer Entry

2.5.1 Questionnaire

Each question was coded. Questions that allowed an open-ended answer were further examined for relevance, and then coded. Some answers required immediate action from the researcher. For example, subjects confirming steroid use were removed from the study; subjects on medications possibly affected by the HMB supplement (or calcium in the HMB supplement) were contacted; subjects on medications that may have influenced the action of the HMB supplement were removed from the study.

An updated Widmark Equation (Watson, *et. al.*, 1981) was used to calculate subjects total body water, and therefore blood alcohol concentrations based on data gathered from the questionnaire and anthropometric records (subject's age, height, and weight, usual type of alcohol and volume consumed). The usual blood alcohol concentrations during a drinking session were calculated for 20 subjects (Watson, *et. al.*, 1981).

2.5.2 Supplementation

During the final visit, each subject reported the number of capsules remaining, so compliance could be evaluated.

2.5.3 Dietary Records

Food intakes were entered into the FoodWorks database (described below), and mean intakes were averaged over three days. For any foods not present in the database, the

closest food type and cooking method was chosen, entire recipes entered, and/or the manufacturer of the food item contacted regarding nutrient content.

2.5.4 Anthropometric Records

Median skinfold (SF) measurements were used for each site, and then percent change in each skinfold site over the study duration was calculated.

Sum of six skinfolds was calculated from the following formula;
Σ = (Triceps + Subscapular + Supraspinale + Abdominal + Front Thigh + Medial Calf)
(Wilson, *et .al.*, 1993).

The regression equation used to estimate body fat from the skinfold sites measured was the Durnin and Womersley (1974) equation. Initial and final body fat percentages, fat mass and fat free mass were then compared over the duration of the study. BIA and skinfold methods of measuring body composition were then compared.

Body density and body fat percentages were calculated from the following formulae;
Body Density = 1.1610 – 0.0632 logΣ (Iliac Crest + Subscapular + Triceps + Biceps)
%Body fat = [(4.95/Body Density) - 4.5] * 100
(Durnin & Womersley, 1974; Lee & Neeman, 1996).

BMI was calculated from height and weight measurements using the following formula;
BMI = Weight in kg / (Height in cm) ^{2}
(Deakin, 2000).

2.5.5 Bioelectrical Impedance Analysis

The BIA was used to measure fat mass (kg), percent body fat, fat free mass (kg), and percent fat free mass. The percent change in each of these measurements was then calculated over the study duration.

2.5.6 Strength Measurements

Strength measurements were averaged over the initial two visits, and the final two visits. Strength measurements were also averaged over right and left hand side of the body. Actual change in strength, and percent change in strength were calculated over the duration of the study for each strength measurement (leg extension, bench press, and bicep preacher curl).

2.5.7 Resistance Training Programme and Activity Records

The resistance training programme log book was used to assess subject compliance. The activity records were grouped into; high intensity exercise (sports at competition level, track events, training sprints, etc); medium intensity exercise (non-competitive running and jogging, indoor cardiovascular exercise, non-competitive cycling, sports training, roller-blading, etc); and low intensity exercise (less intense activities such as golf, walking, gentle hiking, table tennis, gardening, and sports with short bursts of activity and long periods of rest such as recreational surfing and water-skiing).

2.6 Data Checking

During data entry, each data point was checked as it was typed in to Excel and each data value on screen was checked against the original on paper, to ensure there were no typing errors. Once all of the data had been entered into the computer it was checked for outliers, and any outliers found, were compared to the original data to confirm actual results.

Nutrient intake data was checked in the FoodWorks software by checking nutrient levels for outliers. For example one subject had very high sodium levels, and upon checking outliers it was found that 44 pieces of bread had been entered rather than 4 pieces.

Data from FoodWorks was copied and pasted to Excel, the first and last data points from each column were checked against the original FoodWorks data to ensure data was pasted into the correct columns and rows.

2.7 Data Analysis and Statistics

2.7.1 Software

The FoodWorks Nutrition Database (Version 2) from Xyris Software Australia was used to analyse the dietary intakes of the study subjects at the beginning and end of the study. The addition of 3g HMB or placebo (corn starch) was not analysed in the diets as participant supplementation compliance was not 100%, and Version 2 of the FoodWorks software did not provide the facility to analyse amino acid content of foods.

Two spreadsheet programmes were used to analyse study data, Microsoft Excel (2002), and MINITAB for Windows (Version 13.32, 2002).

2.7.2 Statistics

The differences between the HMB and placebo groups in strength, anthropometric, and nutrient results were tested using the Two Sample-T test if the results were normally distributed or the non-parametric Kruskal-Wallis test if results were not normally distributed.

Regressions were carried out to determine any relationship between age, ethnicity, anthropometric measurements, energy intake, nutrient intake, habitual alcohol intake, and incidence of alcohol binging on nutrient intakes and the various strength and anthropometric measurements performed during the study.

The results of this study are generally represented in the text as Mean ± Standard Error of the Mean (SEM) or Median and Upper and Lower quartiles. Where the difference between the HMB and placebo groups has been calculated, results are represented as Mean ± SEM and probability value (P-value) for a confidence interval of 95%.

Bar graphs and line graphs were used to graphically represent results. Results are represented as Mean ± SEM, or ranked from smallest to greatest.

2.8 Subject Feedback

At the completion of the study all participants were sent a summary of their individual results in relation to the study. Where relevant, information regarding nutrient food sources was included if the participant was at risk of low nutrient intakes or nutrient deficiencies. All participants were encouraged to contact the researcher to discuss their results and to answer any questions regarding their results; especially those at risk of low nutrient intakes (see Appendix 12).

Chapter 3 Results

Results accumulated during this study have been separated into the following sections; sample size; demographics of participants; anthropometry; physical activity; strength; diet; supplementation; and health and lifestyle.

3.1 Sample Size

Participant Retention

Thirty four subjects participated in this study. Complete data was obtained from 20 participants, and limited data was obtained from 12. Four participants withdrew from the study due to unspecified reasons, 2 moved out of the area, 1 withdrew due to an unrelated illness, and 1 participant withdrew due to time constraints and was unable to continue participation in the study. The data from two participants was excluded from the study as participants failed to comply with study criteria.

There was no significant difference found between numbers of participants who withdrew from either treatment group.

3.2 Demographics of Study Participants

3.2.1 Age

Participants were all aged between 20 to 30 years. Mean age in the HMB supplemented group was 24.0 ± 0.9; and in the control group was 24.9 ± 1.2.

3.2.2 Ethnicity

The majority (78.1%) of participants in the study were of European ethnicity. Table 3.1 below, shows the distribution of ethnicity in the study group.

Table 3.1: Participant Ethnicity

ETHNICITY	SUBJECT NO.	%
European	25	78.1
Maori	1	3.1
Asian	4	12.5
Indian	2	6.3

3.2.3 Education and Occupation

Half (50%) of the participants gave 7th form as the highest level of education completed (Table 3.2). Around half (56.3%) of study participants were currently studying at tertiary level (Table 3.3).

Table 3.2: Participant Highest Level of Education

HIGHEST LEVEL OF EDUCATION	SUBJECT NO.	%
5th form	1	3.1
6th form	2	6.3
7th form	16	50.0
Certificate	3	9.4
Diploma	6	18.8
Degree	4	12.5

Of those participants in full time work, the majority were involved in the service and sales industry (18.8%). Participant occupations were classified using NZSCO occupational coding (NZSCO, 2002) and are illustrated in Table 3.3, following.

Table 3.3: Participant Occupation.

OCCUPATION	SUBJECT NO.	%
Legislation, Administration, Management	3	9.4
Professional	1	3.1
Associate Professional, Technician	2	6.3
Clerk	1	3.1
Service, Sales	6	18.8
Trade	1	3.1
Student	18	56.3

3.3 Anthropometry

3.3.1 General Anthropometric Characteristics of the Study Group

Eighteen (56.2%) study participants had remained at their current body weight for six

months or longer prior to the study. Body weight and other anthropometric characteristics of the HMB and Placebo groups have been presented in the following table (Table 3.4).

Table 3.4: Anthropometric Measurements of the Study Group (Mean ± SEM)

ANTHROPOMETRIC MEASUREMENTS	HMB (n=12)	PLACEBO (n=9)	P-value
Initial Height (cm)	179.7 ± 1.6	181.0 ± 1.6	0.582
Final Height (cm)	179.7 ± 1.6	181.0 ± 1.6	0.582
Initial Weight (kg)	84.9 ± 3.6	74.7 ± 3.6	0.063
Final Weight (kg)	85.0 ± 3.7	75.3 ± 3.4	0.069
Initial BMI (kg/cm^2)	26.2 ± 0.8	22.8 ± 0.9	0.014
Final BMI (kg/cm^2)	26.2 ± 0.8	22.9 ± 0.9	0.015
[1]Initial Sum 6 Skinfolds (mm)	72.5 ± 10.0	64.2 ± 11.0	0.594
[1]Final Sum 6 Skinfolds (mm)	69.7 ± 9.0	65.9 ± 11.0	0.790
Initial SF Fat Mass (kg)	15.3 ± 1.9	12.0 ± 1.7	0.209
Final SF Fat Mass (kg)	15.2 ± 1.9	12.4 ± 1.5	0.253
Initial SF Fat Free Mass (kg)	69.6 ± 2.4	62.8 ± 2.4	0.056
Final SF Fat Free Mass (kg)	69.8 ± 2.4	62.8 ± 2.4	0.055
Initial SF %Body fat	17.5 ± 1.6	15.6 ± 1.6	0.399
Final SF %Body fat	17.4 ± 1.5	16.2 ± 1.4	0.568
[2]Initial BIA Fat Mass (kg)	17.7 ± 2.0	13.2 ± 2.0	0.126
[2]Final BIA Fat Mass (kg)	17.1 ± 2.4	13.3 ± 1.9	0.414
Initial BIA Fat Free Mass (kg)	67.2 ± 2.4	61.4 ± 2.0	0.084
Final BIA Fat Free Mass (kg)	67.9 ± 2.4	61.8 ± 1.8	0.060
Initial BIA %Body fat	20.4 ± 1.6	17.1 ± 1.8	0.197
Final BIA %Body fat	19.5 ± 2.0	17.19 ± 1.7	0.406
Initial TBW Fat Free Mass (kg)	65.8 ± 1.8	61.3 ± 1.7	0.088
Final TBW Fat Free Mass (kg)	65.9 ± 1.9	61.5 ± 1.6	0.095
Initial TBW %Body fat	21.9 ± 1.3	17.4 ± 1.8	0.054
Final TBW %Body fat	21.9 ± 1.3	17.8 ± 1.6	0.056

Note: SF fat mass, fat free mass and percent body fat levels were calculated using the Durnin & Womersley equation (1974). Total body water was calculated using the method of Watson, *et. al.*, (1981). [1]HMB group n=11. [2] Kruskal-Wallis non-parametric test used. Abbreviations; BMI = body mass index, SF = skinfolds, BIA = bioelectrical impedance analysis, and TBW = total body water.

There was a significant difference found between the mean body mass index of the two groups, the body mass index of the HMB group were; initial 26.2 ± 0.8; final 26.2 ± 0.8, and the placebo group; initial 22.8 ± 0.9; final 22.9 ± 0.9. There was also a significant difference found between the initial body fat estimated from total body water of the two groups, the initial body fat estimation of the HMB group was; 21.9 ± 1.3; and the placebo group; 17.4 ± 1.8.

There was no significant difference found in the initial or final skinfold measurements of the HMB and Placebo supplemented groups (P>0.177) (see Table 3.5).

Table 3.5: Skinfold Measurements of the Study Group (Mean ± SEM)

SKINFOLD MEASUREMENTS	HMB (n=12)	PLACEBO (n=12)	P-value
[1]Initial Triceps (mm)	8.3 ± 1.1	8.2 ± 1.3	0.943
Final Triceps (mm)	8.3 ± 0.9	8.7 ± 1.4	0.819
[1]Initial Sub scapular (mm)	13.8 ± 2.0	10.6 ± 1.2	0.356
[1]Final Sub scapular (mm)	14.0 ± 2.2	10.8 ± 1.2	0.594
[1]Initial Biceps (mm)	4.5 ± 0.9	3.3 ± 0.5	0.253
[1]Final Biceps (mm)	3.9 ± 0.6	3.3 ± 0.5	0.453
[1]Initial Iliac Crest (mm)	18.9 ± 2.7	15.8 ± 3.0	0.337
[1]Final Iliac Crest (mm)	18.5 ± 2.6	16.4 ± 2.2	0.570
[1]Initial Supraspinale (mm)	14.0 ± 2.7	10.3 ± 2.0	0.240
[1]Final Supraspinale (mm)	13.3 ± 2.8	10.8 ± 1.7	0.749
Initial Abdominal (mm)	20.4 ± 3.5	15.6 ± 3.2	0.327
Final Abdominal (mm)	19.6 ± 3.3	15.6 ± 3.0	0.387
[1,3]Initial Thigh (mm)	15.9 ± 3.0	14.0 ± 2.8	0.512
[3]Final Thigh (mm)	14.6 ± 2.3	13.0 ± 2.7	0.522
[3]Initial Calf (mm)	9.8 ± 1.9	7.0 ± 1.1	0.177
[2,3]Final Calf (mm)	7.8 ± 1.1	7.0 ± 1.2	0.381

Note: [1] Placebo group n=8. [2] HMB group n=11. [3] Kruskal-Wallis non-parametric test.

3.3.2 Anthropometric Changes over the Study Period

There was no significant difference found between the HMB and Placebo supplemented groups for any of the eight skinfolds measured over the study duration (P>0.135) (see Table 3.6, below).

Table 3.6: Change in Skinfold Thickness over Study Duration (Mean ± SEM)

CHANGE IN SKINFOLDS	HMB (n=12)	PLACEBO (n=9)	P-value
[3]Triceps (mm)	-0.02 ± 0.4	-0.5 ± 0.4	0.803
Sub scapular (mm)	-0.2 ± 0.5	-0.2 ± 0.3	0.975
[3]Biceps (mm)	0.6 ± 0.4	-0.09 ± 0.2	0.135
Iliac Crest (mm)	0.4 ± 0.4	-0.5 ± 1.3	0.502
Supraspinale (mm)	0.6 ± 0.6	-0.5 ± 0.7	0.214
Abdominal (mm)	0.8 ± 0.3	0.03 ± 0.7	0.316
[1,3]Thigh (mm)	1.4 ± 1.0	-0.6 ± 1.3	0.669
[2,3]Calf (mm)	0.6 ± 0.3	0.07 ± 0.3	0.517

Note: [1] Placebo group n=8. [2] HMB group n=11. [3] Kruskal-Wallis non-parametric test.

There was no significant difference found between the HMB and Placebo supplemented groups for change in weight, sum of six skinfolds, fat mass, fat free mass, or percent body fat (P>0.095) over the study duration (Table 3.7).

Table 3.7: Change in Anthropometric Measurements over Study Duration (Mean ± SEM)

CHANGE IN MEASUREMENTS	HMB (n=12)	PLACEBO (n=9)	P-value
Weight (kg)	0.08 ± 0.40	0.52 ± 0.44	0.471
BMI (kg/cm^2)	-0.02 ± 0.1	-0.2 ± 0.1	0.438
[1]Sum of 6 Skinfolds (mm)	-2.75 ± 1.50	1.70 ± 2.00	0.095
SF Fat Mass (kg)	-0.08 ± 0.24	0.46 ± 0.35	0.222
[2]SF Fat Free Mass (kg)	0.17 ± 0.32	0.06 ± 0.25	0.915

CHANGE IN MEASUREMENTS	HMB (n=12)	PLACEBO (n=9)	P-value
SF %Body fat	-0.13 ± 0.23	0.67 ± 0.47	0.161
BIA Fat Mass (kg)	-0.60 ± 0.61	0.11 ± 0.56	0.402
BIA Fat Free Mass (kg)	0.68 ± 0.57	0.41 ± 0.53	0.731
BIA %Body fat	-0.93 ± 0.75	0.04 ± 0.73	0.368
TBW Fat Free Mass (kg)	-0.03 ± 0.1	-0.2 ± 0.2	0.452
TBW %Body fat	-0.01 ± 0.1	-0.4 ± 0.3	0.454

Note: SF fat mass, fat free mass, and percent body fat levels were calculated using the Durnin & Womersley equation (1974). Total body water was calculated using the Watson, *et. al.*, method (1981). [1] HMB group n=11. [2] Kruskal-Wallis non-parametric test. Abbreviations; BMI = body mass index, SF = skinfolds, BIA = bioelectrical impedance analysis, and TBW = total body water.

3.4 General Physical Activity Habits of the Study Group

In addition to resistance training, study participants were regularly involved in a range of other physical activities. The most popular physical activity other than resistance training was running, both prior to the study (questionnaire recall indicates 34% participation), and during the study period (training log book records indicates 43% participation).

Other physical activities performed by study subjects during the study period have been grouped into the following categories; high intensity exercise, medium intensity exercise, and low intensity exercise. There was no significant difference between the HMB and placebo supplemented groups in duration or intensity over the study period (Table 3.8).

Prior to the study other physical activities were performed on average 2.8 ± 0.3 times per week for an average of 62.6 ± 4.9 minutes per session. During the study other physical activities were performed on average 2.9 ± 0.2 times per week for an average of 54.6 ± 1.8 minutes per session.

Table 3.8: Recorded Physical Activity Intensity and Duration during the Study Period (Mean ± SEM)

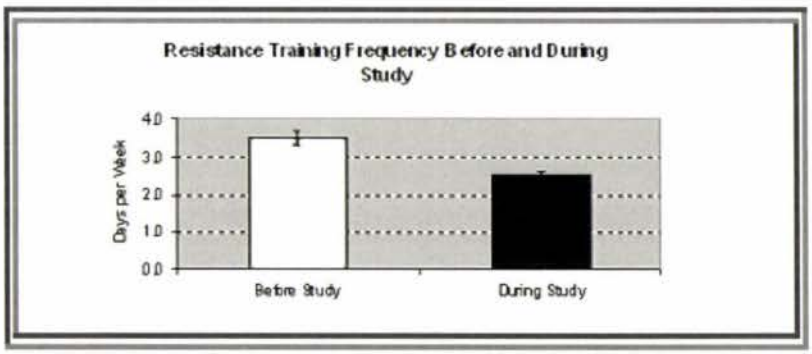
ACTIVITY DURATION (MIN)	HMB	PLACEBO	P-value
High Intensity Exercise	2.4 ± 1.6	0.0 ± 0.00	NA
Medium Intensity Exercise	49.9 ± 13.3	46.8 ± 12.2	0.865
Low Intensity Exercise	33.8 ± 11.6	36.8 ± 18.7	0.893
Total Exercise (excl. Resistance Training)	86.0 ± 16.4	83.2 ± 19.2	0.913

Note: Excludes Resistance Training. Physical Activity Records Weeks 1 through 5 HMB group n=17, Placebo n=10; Weeks 6 through 10 HMB group n=10, Placebo n=9.

3.5 Strength

The average length of resistance training experience was 4.3 ± 0.6 years. The maximum recalled resistance training experience was 14.5 years. Prior to the study, resistance training was performed on average 3.5 times per week, for an average of 66.9 ± 3.2 minutes per session. During the study, resistance training was performed on average 2.5 times per week, as illustrated in Figure 3.1 below. There was no significant difference between the HMB and placebo supplemented groups in average number of resistance training sessions over the study period (P>0.056).

Figure 3.1: Resistance Training Frequency Before and During Study (Mean ± SEM)



3.5.1 Baseline Strength Levels of the Study Group

There were no significant differences (P>0.298) in post-trial and pre-trail strength measurements between the HMB and Placebo groups (Table 3.9).

Table 3.9: Pre-trial and Post-trial Strength Measurements (Mean ± SEM)

STRENGTH	HMB (n=13)	PLACEBO (n=9)	P-value
Initial LE Strength (kg)	37.1 ± 2.1	37.2 ± 2.9	0.993
Final LE Strength (kg)	42.0 ± 1.8	38.6 ± 2.6	0.298
[1]Initial BP Strength (kg)	53.9 ± 5.0	52.2 ± 6.3	0.839
[1]Final BP Strength (kg)	62.1± 5.4	60.8 ± 6.6	0.888
[2]Initial PC Strength (kg)	17.7 ± 0.8	16.9 ± 1.3	0.583
[2]Final PC Strength (kg)	19.6 ± 0.9	18.8± 1.3	0.654

Note: [1] HMB group n=11. [2] HMB group n=12. Abbreviations; LE = leg extension, BP = bench press, and PC = bicep preacher curl.

3.5.2 Strength Changes Over the Study Period

There were no significant differences in actual change in strength during the study (P>0.086), however percent change in leg extension strength did increase significantly for the HMB supplemented group over the study duration (Tables 3.10 and 3.11).

Table 3.10: Strength Change over Study Duration (Mean ± SEM)

STRENGTH		INITIAL	FINAL	P-value
HMB (n=13)	LE Strength (kg)	37.1 ± 2.1	42.0 ± 1.8	0.086
	[1]BP Strength (kg)	53.9 ± 5.0	62.1 ± 5.4	0.140
	[2]PC Strength (kg)	17.7 ± 0.8	19.6 ± 0.9	0.361
PLACEBO (n=9)	LE Strength (kg)	37.2 ± 2.9	38.6 ± 2.6	0.276
	BP Strength (kg)	52.2 ± 6.3	60.8 ± 6.6	0.723
	PC Strength (kg)	16.9 ± 1.3	18.8 ± 1.3	0.307

Note: [1] HMB group n=11. [2] HMB group n=12. Abbreviations; LE = leg extension, BP = bench press, and PC = bicep preacher curl.

Leg Extension strength increased significantly for the HMB supplemented group during the study period, compared to the placebo group; HMB 14.7 ± 3.6%; Placebo 4.84 ± 2.8%, as indicated in Table 3.11 and Figures 3.2 and 3.3.

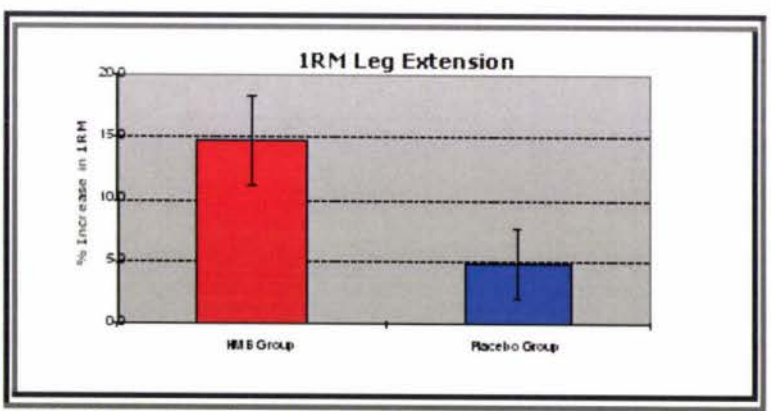
Table 3.11: Percent Change in Strength Measurements for HMB and Placebo Groups (Mean ± SEM)

%CHANGE	HMB (n=13)	PLACEBO (n=9)	P-value
%Change LE	14.7 ± 3.6	4.8 ± 2.8	0.041
[1]%Change BP	16.5 ± 4.1	18.9 ± 4.7	0.710
[2]%Change PC	10.5 ± 2.1	12.3 ± 2.1	0.563

Note: [1] HMB group n=11. [2] HMB group n=12. Abbreviations; LE = leg extension, BP = bench press, and PC = bicep preacher curl.

The percent increase in leg extension represented as a red bar graph is clearly greater than that for the placebo group represented as a blue bar graph, below.

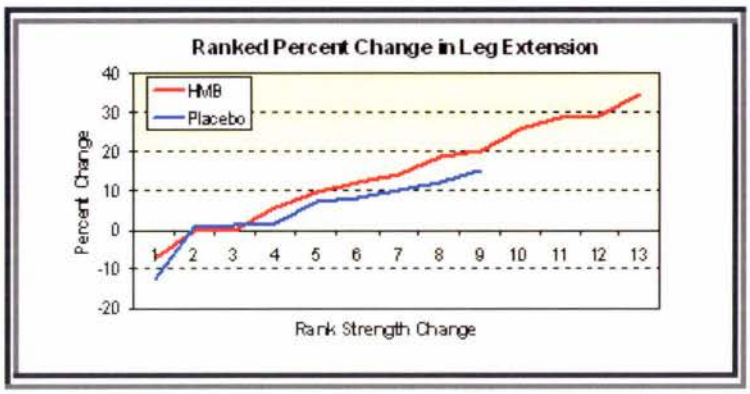
Figure 3.2: Percent Change in Leg Extension Strength (Mean ± SEM)



Note: (Leg extension; HMB 14.7 ± 3.6%; 4.8 ± 2.8% Placebo).

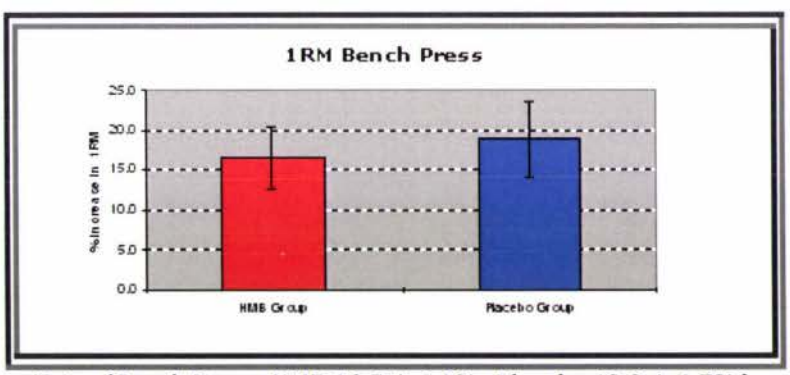
A line graph representing ranked percent change in leg extension strength for the HMB and placebo groups is given in Figure 3.3, over page. Representing the data in this way gives a clear picture of how strength changed amongst the two supplemented groups.

Figure 3.3: Ranked Percent Change in Leg Extension Strength



No significant difference in percent strength increase in bench press was found. However, as can be seen in Figures 3.4 and 3.5, the mean percent change in bench press strength for the placebo group (18.9 ± 4.7%) was slightly greater than that of the HMB group (16.5 ± 4.1%), but not significantly so.

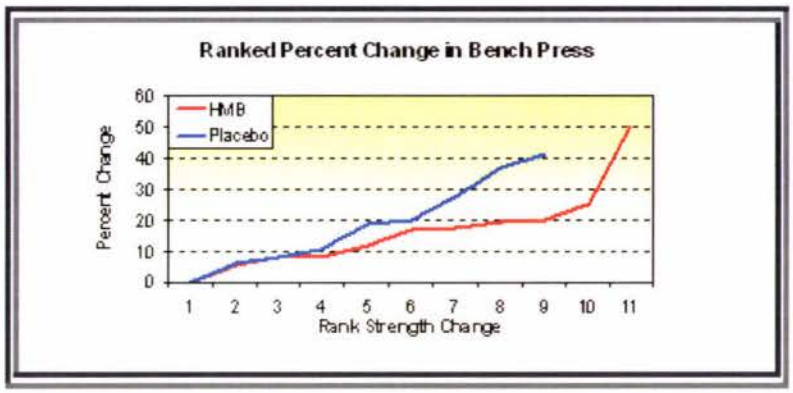
Figure 3.4: Percent Change in Bench Press Strength (Mean ± SEM)



Note: (Bench Press; HMB 16.5 ± 4.1%; Placebo 18.9 ± 4.7%).

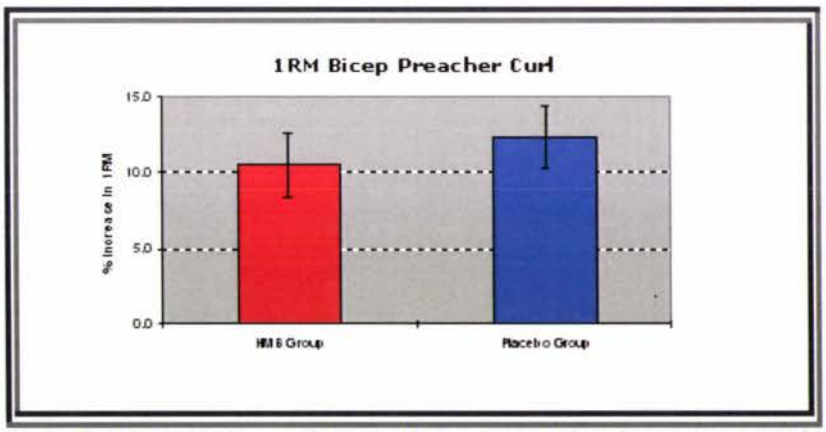
A line graph representing ranked percent change in bench press strength for the HMB and placebo groups is given in Figure 3.5, over page.

Figure 3.5: Ranked Percent Change in Bench Press Strength



No significant difference in percent strength increase in bicep preacher curl was found. As can be seen in Figures 3.6 and 3.7, the mean percent change in bicep preacher curl strength for the placebo group (12.3 ± 2.1%) was slightly greater than that of the HMB group (10.5 ± 2.1%), but again, not significantly so.

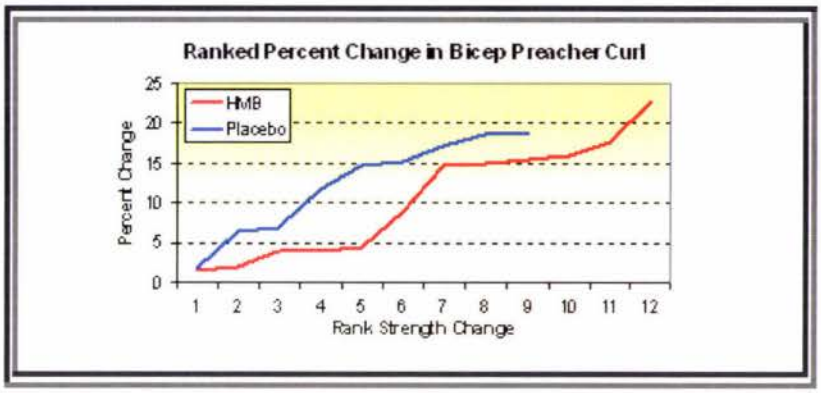
Figure 3.6: Percent Change in Bicep Preacher Curl Strength (Mean ± SEM)



Note: (Bicep preacher curl; HMB 10.5 ± 2.1%; Placebo 12.3 ± 2.1%).

A line graph representing ranked percent change in bicep preacher curl strength for the HMB and placebo groups is given in Figure 3.7, over page.

Figure 3.7: Ranked Percent Change in Bicep Preacher Curl Strength



3.6 Diet

3.6.1 Description of Dietary Habits Prior to Study

The majority (87.5%) of participants described their eating habits as 'eating a variety of all foods'. A description of study participants eating habits is given below in Table 3.12.

Table 3.12: Description of Eating Habits

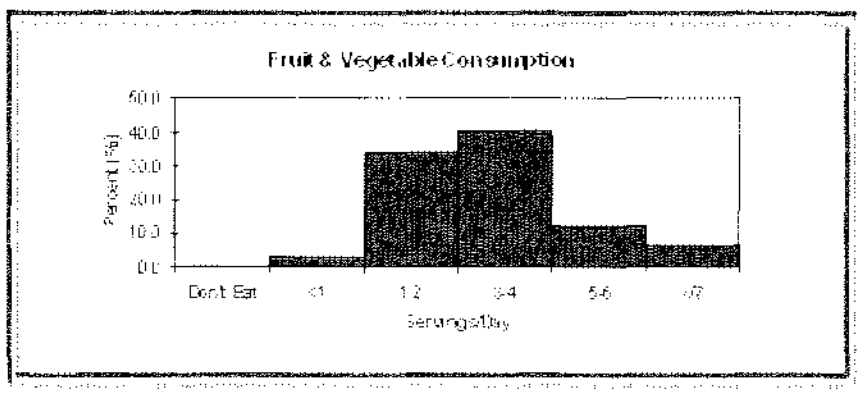
DESCRIPTION OF EATING HABITS	SUBJECT NO.	%
Eat variety of all foods	28	87.5
Eat eggs, dairy, fish, chicken, but no other meat	2	6.3
Eat eggs, dairy, meat, but avoid chicken, fish	1	3.1
Eat chicken, beef, dairy, but no eggs, fish	1	3.1

Only three participants described aversions or allergies. Allergies were to seafood, and aversions were to eggs, beer, and 'hard meats' (steak, lamb, and pork fillets). No explanation was given for these food aversions.

Foods were classified as one of the six following categories; fruit and vegetables, cereal, breakfast cereal, breads and bakery products, dairy products, and meats. The following table represents participant consumption of these food groups (Table 3.13).

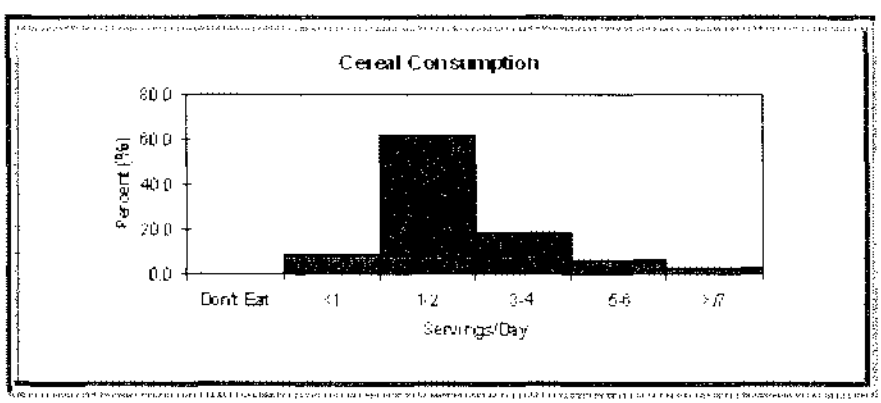
Only 4 (12.5%) participants consumed five or more servings of fruit and vegetables, and one participant (3.1%) ate less than one serving per day (Figure 3.8).

Figure 3.8: Recalled Fruit and Vegetable Consumption



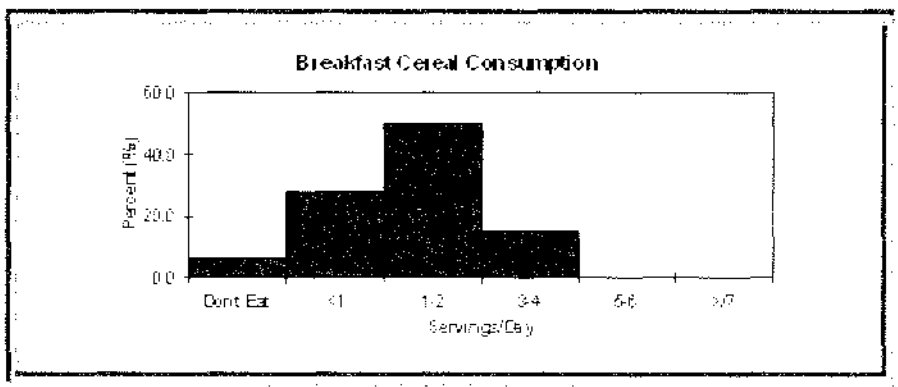
The majority (62.5%) of participants consumed one to two servings of cereals per day, and one (3.1%) participant consumed seven or more servings of cereals (excluding breakfast cereals) per day (Figure 3.9).

Figure 3.9: Recalled Cereal Consumption



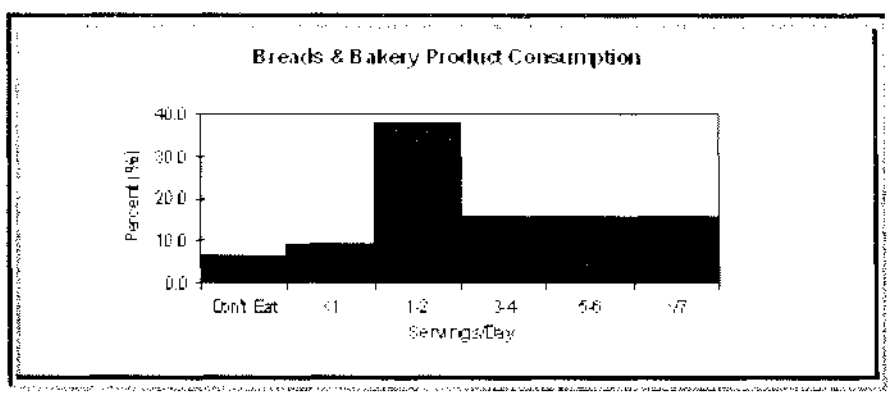
Two (6.3%) participants did not eat breakfast cereals, however, half (50.0%) of the participants consumed one or two servings of breakfast cereals per day (Figure 3.10).

Figure 3.10: Recalled Breakfast Cereal Consumption



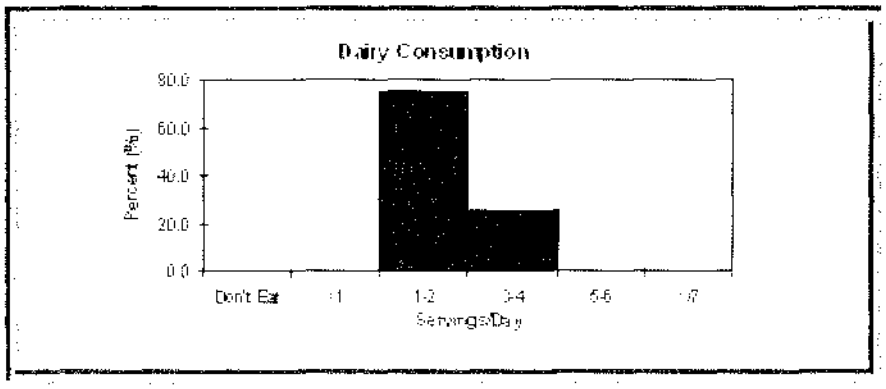
Two (6.3%) participants did not eat breads or bakery products at all. Of those participants that did consume breads and bakery products, most (37.5%) consumed one to two servings of breads or bakery products per day (equivalent to one sandwich) (Figure 3.11).

Figure 3.11: Recalled Breads and Bakery Product Consumption



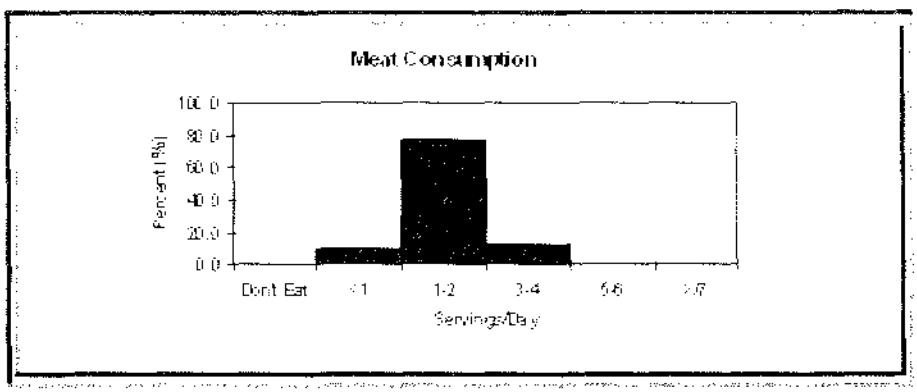
The majority (75.0%) of study participants consumed one to two servings of dairy products daily, and the remaining (25.0%) participants consumed three to four servings each day (Figure 3.12).

Figure 3.12: Recalled Dairy Consumption



The majority (78.1%) of participants consumed one to two servings of meat per day, as indicated in Figure 3.13.

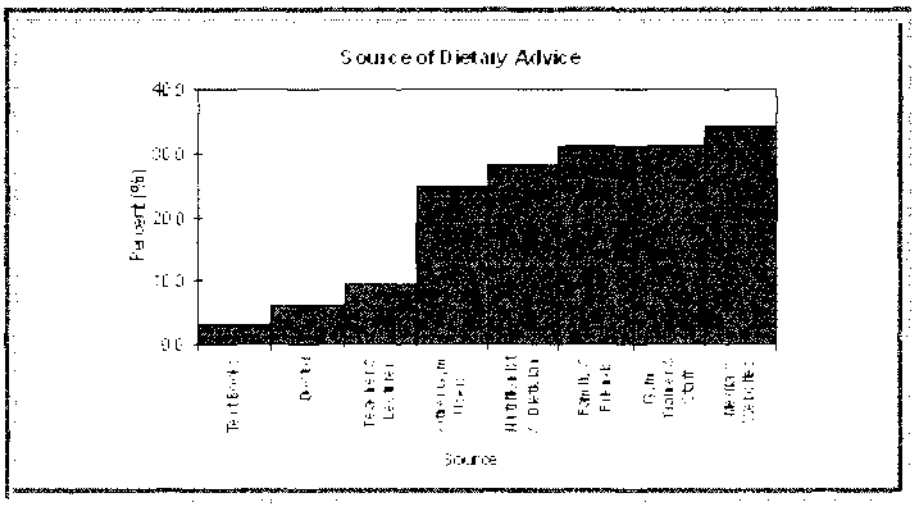
Figure 3.13: Recalled Meat Consumption



3.6.2 Influences on Dietary Habits Prior to Study

The major sources of dietary advice were the popular media and websites (34.4%), family and friends (31.3%), gym trainers and staff (31.3%), nutritionists and dieticians (28.1%), and other gym users (25.0%), as illustrated in Figure 3.14.

Figure 3.14: Source of Dietary Advice



Eight (25.0%) participants had stable eating habits for less than six months. Three participants had practised some form of restrictive dieting in the past. The most common was a high protein and low carbohydrate diet.

3.6.3 General Dietary Intakes during the Study

Differences in Nutrient Intake during the Study

No significant differences in nutrient intakes were found between the initial and final dietary records from study participants (Table 3.13), therefore initial and final three day dietary records were averaged to give a better description of usual nutrient intake of the group.

Table 3.13: Differences in Nutrient Intake between Initial and Final Three Day Dietary Records (Mean ± SEM)

NUTRIENT INTAKE	INITIAL (n=28)	FINAL (n=21)	P-value
Food Weight (g)	2705 ± 215	2471 ± 241	0.472
Energy (KJ)	10997 ± 729	10586 ± 563	0.657
Energy (cal)	2627 ± 174	2529 ± 134	0.657
[2] Protein (g)	143.6 ± 11	125.5 ± 9.2	0.312
[1] Protein (g/kg)	1.8 ± 0.2	1.6 ± 0.1	0.305
Protein (%)	22.4 ± 1.0	20.5 ± 1.2	0.252
Carbohydrate (g)	294.0 ± 21	281.2 ± 18	0.644
Carbohydrate (%)	42.9 ± 1.4	42.2 ± 1.4	0.763
[1] Carbohydrate (g/kg)	3.7 ± 0.3	3.5 ± 0.2	0.597
[1] Fibre (g)	24.7 ± 1.9	24.3 ± 2.4	0.558
Total Sugars (g)	128.1 ± 12	116.9 ± 9.2	0.473
Starch (g)	165.0 ± 13	163.5 ± 12	0.929
[2] Glucose (g)	25.0 ± 3.3	20.8 ± 2.1	0.554
Fructose (g)	21.2 ± 2.1	21.1 ± 2.5	0.958
Sucrose (g)	49.2 ± 4.7	49.8 ± 5.7	0.936
[2] Lactose (g)	28.3 ± 7.8	20.7 ± 2.8	0.936
[2] Maltose (g)	4.2 ± 0.7	4.2 ± 0.7	0.777
[2] Total Fat (g)	92.4 ± 8.5	97.1 ± 8.2	0.473
Total Fat (%)	31.3 ± 1.6	33.8 ± 2.0	0.317
[2] Saturated Fat (g)	39.8 ± 4.4	40.9 ± 4.2	0.544
Mono Fat (g)	31.9 ± 3.0	33.7 ± 2.7	0.661
[2] Poly Fat (g)	12.0 ± 1.2	12.1 ± 1.2	0.762
[2] Cholesterol (mg)	428 ± 34	401 ± 33	0.621
[2] Alcohol (g)	9.1 ± 3.3	6.2 ± 2.8	0.175
[2] Water (g)	2168 ± 228	1905 ± 230	0.419
Vitamin A (µg) (Retinol Equivalents)	943 ± 95	882 ± 66	0.601
[2] Vitamin C (mg)	154 ± 42	91.0 ± 14	0.170
[2] Vitamin E (mg)	10.7 ± 1.0	9.6 ± 1.1	0.312
[2] Vitamin D (µg)	3.1 ± 0.6	3.3 ± 0.6	0.708
[2] Thiamin (Vitamin B1) (mg)	3.1 ± 0.7	2.3 ± 0.3	1.0
[2] Riboflavin (Vitamin B2) (mg)	3.7 ± 0.9	2.7 ± 0.2	0.911

NUTRIENT INTAKE	INITIAL (n=28)	FINAL (n=21)	P-value
[2] Niacin (Equivalents) (Vitamin B3) (mg)	57.7 ± 4.4	52.3 ± 3.8	0.385
[2] Pyridoxine (Vitamin B6) (mg)	3.3 ± 0.6	2.5 ± 0.3	0.518
[2] Folate (Vitamin B9) (µg)	338 ± 31	321 ± 33	0.731
[2] Cyanocobalamin (Vitamin B12) (µg)	8.2 ± 1.2	8.0 ± 0.8	0.538
Sodium (mg)	4002 ± 324	3957 ± 280	0.349
Potassium (mg)	4383 ± 403	3805 ± 271	0.240
Magnesium (mg)	405 ± 33	383 ± 29	0.625
[2] Calcium (mg)	1216 ± 215	995 ± 92	0.920
[2] Phosphorus (mg)	2118 ± 198	1878 ± 149	0.572
Iron (mg)	17.7 ± 1.3	17.3 ± 1.2	0.843
[2] Zinc (mg)	17.7 ± 1.3	15.4 ± 1.1	0.303
[2] Manganese (µg)	4954 ± 361	4224 ± 513	0.098
[2] Copper (mg)	2.0 ± 0.2	2.7 ± 0.7	0.903
[2] Selenium (µg)	95.0 ± 13	89.5 ± 13	0.952

Note: [1] Initial group n= 26. [2] Kruskal-Wallis non-parametric test.

Macronutrient Intake Calculated from 6-Day Dietary Records

The mean protein, carbohydrate, and fat intakes were 139.3 ± 9.7g, 287.7 ± 17.6g, and 93.5 ± 7.4g respectively. Mean alcohol intake was 7.5 ± 2.3g (equivalent to 0.75 of a serving of beer wine, or spirits), and the maximum intake was 42.9g (equivalent to 4.3 servings of beer, wine, or spirits). Energy and macronutrient intakes of the study group are displayed below in Table 3.14.

Table 3.14: Energy Intake from Macronutrients

ENERGY INTAKE	MEAN ± SEM	MEDIAN	LOWER QUARTILE	UPPER QUARTILE	MIN	MAX
Energy (kJ)	10823.1 ± 591.3	10880.4	8272.6	12381.8	5559.8	17662.2
Energy (cal)	2585.5 ± 141.3	2599.2	1976.3	2957.9	1328.2	4219.4
Protein (g)	139.3 ± 9.7	126.7	98.3	173.4	56.2	259.1
Protein (g/kg)	1.7 ± 0.1	1.5	1.3	2.1	0.7	3.7
Carbohydrate (g)	287.7 ± 17.6	275.9	219.7	367.0	145.7	453.8

ENERGY INTAKE	MEAN ± SEM	MEDIAN	LOWER QUARTILE	UPPER QUARTILE	MIN	MAX
Carbohydrate (g/kg)	3.6 ± 0.2	3.6	2.6	4.3	1.5	6.5
Total Fat (g)	93.5 ± 7.4	84.9	60.2	112.4	43.4	190.0
Alcohol (g)	7.5 ± 2.3	0.3	0.0	12.9	0.0	42.9
% Protein	22.0 ± 1.0	21.1	18.4	25.8	13.7	32.6
% Carbohydrate	42.4 ± 1.3	43.1	39.0	46.1	27.8	56.5
% Total Fat	32.2 ± 1.6	32.8	18.4	25.8	13.8	46.8
% Alcohol	1.8 ± 0.5	0.1	0.0	3.3	0.0	7.6

Carbohydrate Intake

The mean intake of fibre was 24.9 ± 1.8g. Mean energy intake derived from total sugars was 19.0 ± 1.1%, sucrose 7.4 ± 0.6%, and starch 25.3 ± 1.0%, as indicated in Table 3.15, following.

Table 3.15: Carbohydrate Intake

ENERGY INTAKE AS CARBOHYDRATES	MEAN ± SEM	MEDIAN	LOWER QUARTILE	UPPER QUARTILE	MIN	MAX
Fibre (g)	24.9 ± 1.8	24.9	17.9	28.2	13.0	51.0
Total Sugars (g)	122.4 ± 9.2	120.2	83.6	150.4	43.2	243.7
Sucrose (g)	47.7 ± 4.1	48.0	30.7	61.3	9.8	92.7
Starch (g)	164.5 ± 11.5	147.6	118.3	198.9	64.0	285.2
Fibre %	3.9 ± 0.2	3.9	3.1	4.3	2.5	7.1
Total Sugars %	19.0 ± 1.1	18.4	15	22.4	8.7	35.1
Sucrose %	7.4 ± 0.6	7.3	5.8	9.4	2	14.9
Starch %	25.3 ± 1.0	24.5	22	28.9	15.8	36.6

Lipid Intake

Mean saturated fat and cholesterol intake was 40.2 ± 4.1g, and 424.0 ± 30.8 mg respectively. The maximum intake of saturated fat was 97.9g, and cholesterol 857.7mg. Energy intake from lipid sources are displayed in Table 3.16 following.

Table 3.16: Lipid Intake

ENERGY INTAKE AS LIPIDS	MEAN ± SEM	MEDIAN	LOWER QUARTILE	UPPER QUARTILE	MIN	MAX
Saturated Fat (g)	40.2 ± 4.1	36.9	24.1	48.2	11.5	97.9
Monounsaturated Fat (g)	32.5 ± 2.5	29.6	22.6	39.1	14.9	66.9
Polyunsaturated Fat (g)	12.1± 0.9	12.0	8.5	15.6	4.5	19.5
Cholesterol (mg)	424.0 ± 30.8	366.3	324.4	524.7	195.5	857.7
Saturated Fat %	13.9 ± 1.1	13.4	10.7	16.9	3.7	31.9
Monounsaturated Fat %	11.4 ± 0.6	12	9.1	13.2	4.8	17.3
Polyunsaturated Fat %	4.3 ± 0.2	3.9	3.4	4.8	2.3	7.4

Differences in Nutrient Intake between Supplementation Groups

There were some significant differences found in percentage of energy from carbohydrate (%), percentage of energy from total fat (%), intake of maltose (g), and cholesterol (mg) between the HMB and placebo groups (Table 3.17).

Table 3.17: Differences in Nutrient Intake between HMB and Placebo Groups (Mean ± SEM)

NUTRIENT INTAKE	HMB (n=17)	PLACEBO (n=11)	P-value
Food weight (g)	2805 ± 272	2413 ± 210	0.265
Energy (KJ)	10744 ± 828	10945 ± 841	0.866
Energy (cal)	2567 ± 198	2615 ± 201	0.866
[2] Protein (g)	138.5 ± 14	140.6 ± 14	0.689
[1]Protein (g/kg)	1.6 ± 0.2	1.8 ± 0.2	0.523
Protein (%)	21.9 ± 1.2	22.3 ± 1.8	0.868
Carbohydrate (g)	305 ± 25	261.3 ± 23	0.210
[1]Carbohydrate (g/kg)	3.7 ± 0.4	3.4 ± 0.3	0.494
Carbohydrate (%)	45.1 ± 1.4	38.3 ± 1.8	0.007
Fibre (g)	25.9 ± 2.8	23.3 ± 1.5	0.418

NUTRIENT INTAKE	HMB (n=17)	PLACEBO (n=11)	P-value
Total Sugars (g)	134.8 ± 13	103.2 ± 11	0.072
Starch (g)	169.4 ± 16	157.0 ± 17	0.958
[2] Glucose (g)	27.7 ± 4.6	18.7 ± 1.8	0.086
Fructose (g)	23.4 ± 2.3	17.9 ± 2.1	0.084
Sucrose (g)	50.0 ± 4.7	44.1 ± 7.8	0.522
[2] Lactose (g)	28.5 ± 7.3	19.8 ± 3.3	0.760
Maltose (g)	5.0 ± 0.7	2.5 ± 0.7	0.019
Total Fat (g)	85.1 ± 9.5	106.7 ± 11	0.152
Total Fat (%)	29.7 ± 2.1	35.9 ± 2.0	0.043
[2] Saturated Fat (g)	35.1 ± 4.7	48.0 ± 7.4	0.151
Mono Fat (g)	29.3 ± 3.2	37.5 ± 3.8	0.108
Poly Fat (g)	10.9 ± 1.2	14.0 ± 1.0	0.065
[2] Cholesterol (mg)	361.6 ± 23	520 ± 60	0.046
[2] Alcohol (g)	6.3 ± 3.1	9.3 ± 3.7	0.442
Water (g)	2267 ± 271	1836 ± 181	0.198
Vitamin A (µg) (Retinol Equivalents)	846.0 ± 92	1006 ± 93	0.230
[2] Vitamin C (mg)	139.0 ± 35	108.8 ± 15	0.655
Vitamin E (mg)	9.6 ± 1.6	12.0 ± 1.6	0.167
[2] Vitamin D (µg)	3.1 ± 0.7	3.0 ± 0.5	0.588
[2] Thiamin (Vitamin B1) (mg)	3.0 ± 0.6	2.3 ± 0.4	0.655
[2] Riboflavin (Vitamin B2) (mg)	3.6 ± 0.7	2.9 ± 0.3	0.525
Niacin (Equivalents) (Vitamin B3) (mg)	56.9 ± 6.7	59.0 ± 4.7	0.819
[2] Pyridoxine (Vitamin B6) (mg)	2.9 ± 0.5	2.9 ± 0.3	0.572
[2] Folate (Vitamin B9) (µg)	325.0 ± 45	334.2 ± 17	0.230
[2] Cyanocobalamin (Vitamin B12) (µg)	7.9 ± 1.1	7.8 ± 1.0	0.888
Sodium (mg)	4028 ± 364	3514 ± 403	0.354
Potassium (mg)	4094 ± 480	4295 ± 326	0.732
Magnesium (mg)	397.0 ± 40	393.6 ± 30	0.947
[2] Calcium (mg)	1272 ± 224	919 ± 103	0.525
Phosphorus (mg)	2087 ± 228	1940 ± 157	0.600
Iron (mg)	17.7 ± 1.5	17.5 ± 1.5	0.909
Zinc (mg)	17.1 ± 1.5	16.2 ± 1.2	0.661
Manganese (µg)	4961 ± 555	4323 ± 355	0.342
[2] Copper (mg)	2.6 ± 0.5	1.9 ± 0.2	0.465
[2] Selenium (µg)	99.1 ± 18	87.2 ± 11	0.724

Note: [1] HMB group n=15. [2] Kruskal-Wallis non-parametric test.

3.7 Supplementation

3.7.1 Supplementation Patterns Prior to Study

The types of supplements taken by study participants prior to the beginning of the study period, were categorised into five groups: sports supplements, weight-loss supplements, vitamin/mineral supplements, herbal supplements, and pharmaceuticals (not a supplement, but added for completeness of records). One participant admitted to steroid use in the past, and was excluded from the study. The same participant was the only competitive bodybuilder from the study group. Eighteen (58.1%) study subjects were taking supplements regularly before the study period. Sports supplements were the most popular; and of these, protein powders were the most used, followed by creatine (Table 3.18).

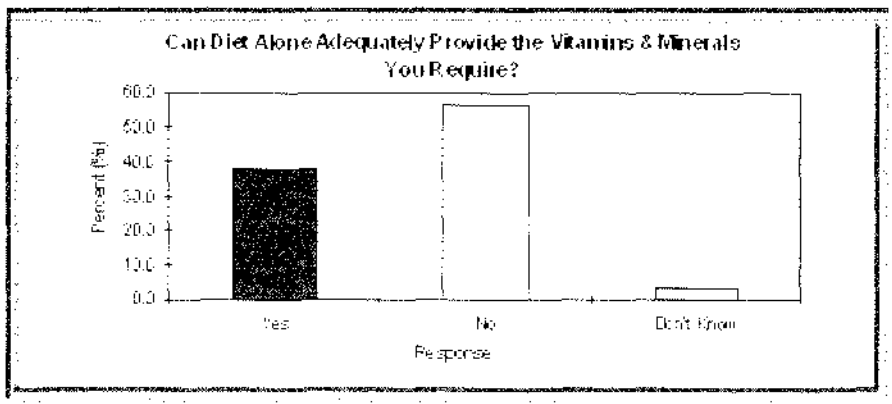
Table 3.18: Supplement Popularity

SUPPLEMENT TYPE	SUPPLEMENT NAME	SUBJECT NO.	%	SERVINGS/WEEK
Sports	Protein Powder	12	38.7	12.4
	Protein Shake (RTD)	1	3.2	3
	Protein Bar	1	3.2	7
	Creatine	4	12.9	11.2
	Branched chain Amino Acids	1	3.2	7
	L-glutamine	2	6.5	10.5
Weight-loss	Fat Burner	2	6.5	17.5
Vitamin/Mineral	Multi- Vitamin/Mineral	2	6.5	7
	Antioxidant	2	6.5	5.3
	Berocca B vitamins	1	3.2	3.5
	Vitamin C	2	6.5	7
Herbal	Garlic, Vitamin C & Horse	1	3.2	21
	Fenugreek & Marshmallow	1	3.2	14
	Tribulus	1	3.2	7
Pharmaceutical	Steroid	1	3.2	Unknown

Eighteen (56.3%) subjects believed that diet alone could not provide an adequate vitamin

and mineral intake. Figure 3.15 gives a graphical representation of this below.

Figure 3.15: Participant Beliefs on Vitamin and Mineral Supplementation

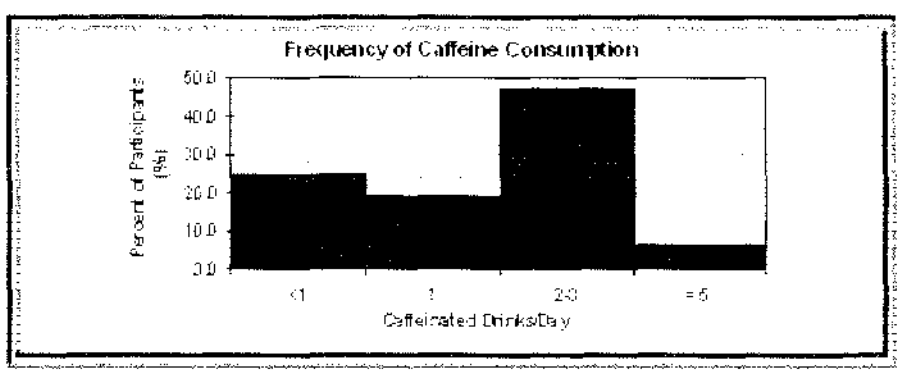


3.8 Health and Lifestyle

3.8.1 Caffeine Consumption

Only one participant did not consume caffeinated beverages, over half of the participants (54.8%) consumed two or more caffeinated beverages, and two (6.3%) participants consumed five or more caffeinated beverages per day. Figure 3.16 below represents caffeine consumption by study participants who drank caffeinated beverages.

Figure 3.16: Frequency of Caffeine Consumption



3.8.2 Smoking Habits

Eight (25.0%) participants have smoked in the past and four (12.9%) participants were currently smokers. Of the smokers in the study, the majority smoked less than five cigarettes per day.

3.8.3 Alcohol Consumption

Twenty five (78.1%) subjects from the study regularly consumed alcohol. When drinking alcohol, beer (25.0%), or beer and wine (21.9%) were the most popular beverages chosen (Table 3.19).

Table 3.19: Type of Alcoholic Beverage Consumed

ALCOHOL TYPE	SUBJECT NO.	%
Beer	8	25.0
Wine	2	6.3
Spirits	3	9.4
Beer & Wine	7	21.9
Beer & Spirits	4	12.5
Wine & Spirits	1	3.1
Don't Drink Alcohol	7	21.9

Almost half (43.8%) of the study subjects consumed alcohol one to three times per week, two (6.3%) subjects drank alcohol daily. Frequency of alcohol consumption appears in Table 3.20 over the page.

Table 3.20: Frequency of Alcoholic Beverage Consumption

FREQUENCY OF ALCOHOL CONSUMPTION		
Frequency	SUBJECT NO.	%
< 1 time/month	1	3.1
1-2 times/month	6	18.8
1 time/week	7	21.9
2-3 times/week	7	21.9
daily	2	6.3

The Alcohol Advisory Council of New Zealand advises men to consume no more than 6 standard drinks per drinking session (Matheson, 2002). Eight (25%) subjects usually consumed more than 6 standard drinks per drinking session. In addition, blood alcohol concentrations were able to be calculated for 20 subjects based on reported consumption during a usual drinking session. Of the subjects who drank alcohol, seven (58%) usually drank until over the blood alcohol limit for operating a motor vehicle.

Table 3.21: Calculated Blood Alcohol Concentrations for a Usual Drinking Session

USUAL BLOOD ALCOHOL CONCENTRATION DURING A DRINKING SESSION		
BAC	SUBJECT NO.	%
< 0.08%	5	42
> 0.08%	7	58

Note: Abbreviations used BAC = blood alcohol concentration

Chapter 4 Discussion

This was the first HMB supplementation study done in New Zealand. It is important to note that there were several differences between this study and previous studies investigating HMB supplementation in humans. This study did not incorporate techniques such as; analysis of HMB levels in blood and urine (Nissen, *et. al.*, 1996; Kreider, *et. al.*, 1999; Gallagher *et. al.*, 2000; Vukovich, *et. al.*, 2001); preparation of participant meals (Nissen, *et. al.*, 1996; Jówko, *et. al.*, 2001); supervision of participant resistance exercise sessions (Nissen, *et. al.*, 1996; Gallagher *et. al.*, 2000; Panton, *et. al.*, 2001; Paddon-Jones, *et. al.*, 2001; Jówko, *et. al.*, 2001; Slater, *et. al.*, 2001; Vukovich, *et. al.*, 2001); measurement of biochemical markers of muscle damage (Nissen, *et. al.*, 1996; Kreider, *et. al.*, 1999; Gallagher *et. al.*, 2000; Panton, *et. al.*, 2001; Paddon-Jones, *et. al.*, 2001; Jówko, *et. al.*, 2001; Slater, *et. al.*, 2001), as previous studies have done.

This study investigated the effects of HMB supplementation on resistance trained men (RTM) in response to resistance exercise. Due to the lack of current research on this group, particular attention was paid to the exercise, dietary, and supplementation patterns of RTM.

4.1 Demographics of Study Participants

4.1.1 Participants

Gyms close to the Massey University Albany campus were chosen including two gyms on University campuses, and therefore there were a high proportion of students participating in this study. Student eating and exercising behaviours may not reflect the RTM population as a whole.

4.1.2 Age

There are likely to be age related effects on exercise habits, eating habits, and supplement usage, therefore these results can only be generalised for RTM between the ages of 20 and 30 years. There were no significant differences in age between the two treatment groups. In addition, there were no significant correlations between age and gain in muscle strength, or relationships between age, muscle strength change and treatment group.

4.1.3 Ethnicity

There was no significant difference in distribution of European and Non-European ethnicities between the HMB and Placebo groups (P=0.965). Ethnicity had no effect on physical activity patterns, or strength change during the study (P>0.152), as indicated in Tables 4.1 and 4.2. However, baseline bicep preacher curl strength was significantly greater for Europeans compared with non-Europeans, the mean load successfully lifted in the bicep preacher curl for Europeans was 18.3 ± 0.7; and non-Europeans, 13.5 ± 0.2. This difference did not seem to have any apparent effect on percent change in bicep preacher curl strength over the study duration.

Table 4.1: Effect of Ethnicity on Physical Activity Patterns

ACTIVITY DURATION (MIN)	EUROPEAN	NON-EUROPEAN	P-value
[1] High Intensity Exercise	2.0 ± 1.0	0.0 ± 0.0	0.274
[1] Medium Intensity Exercise	50.7 ± 11	43.7 ± 22	0.505
[1] Low Intensity Exercise	43.3 ± 13	11.9 ± 7.6	0.298
Total Exercise (excl. Resistance Training)	95.9 ± 14	55.6 ± 22	0.152

Note: [1] Used to indicate use of the Kruskal-Wallis non-parametric test.
Physical Activity Records Weeks 1 through 5 European group n=20, Non-European group n=7; Weeks 6 through 10 European group n=14, Non-European group n=5.

Table 4.2: Effect of Ethnicity on Strength

STRENGTH	EUROPEAN (n=17)	NON-EUROPEAN (n=5)	P-value
Initial LE Strength	37.9 ± 1.4	34.5 ± 5.9	0.603
[1]Initial BP Strength	57.1 ± 3.7	37.2 ± 9.4	0.142
[2]Initial PC Strength	18.3 ± 0.7	13.5 ± 1.2	0.016
%Change in LE Strength	9.8 ± 2.9	13.8 ± 5.9	0.562
[1,3]%Change in BP Strength	10.7 ± 1.6	13.7 ± 3.8	0.370
[2]%Change in PC Strength	14.5 ± 2.5	29.8 ± 9.6	0.222

Note: [1] European group n=16, Non-European n=4. [2] Non-European group n=4.
[1] Kruskal-Wallis non-parametric test.

When comparing European participants to non-European, there was a significant difference between intake of total sugars, glucose, total fat, saturated fat, monounsaturated fat, sodium, and calcium. European participants consumed significantly greater amounts of sugars, glucose, total fat, saturated fat, monounsaturated fat, sodium, and calcium than non-Europeans (Table 4.3).

Table 4.3: Effect of Ethnicity on Nutrient Intake

NUTRIENT INTAKE	EUROPEAN (n=22)	NON-EUROPEAN (n=6)	P-value
Food weight (g)	2844 ± 910	1943 ± 397	0.081
Energy (KJ)	11374 ± 627	9172 ± 1084	0.112
Energy (cal)	2717 ± 160	2191 ± 259	0.112
[1] Protein (g)	143.8 ± 10	125.7 ± 24	0.689
Protein (%)	21.9 ± 1.3	22.5 ± 1	0.755
Carbohydrate (g)	296.0 ± 20	262.7 ± 37	0.449
Carbohydrate (%)	41.5 ± 1.5	45.4 ± 2.3	0.182
Fibre (g)	25.4 ± 1.8	23.3 ± 4.9	0.695
Total Sugars (g)	132.1 ± 11	93.4 ± 14	0.047
Starch (g)	163.0 ± 13	168.9 ± 28	0.853
[1] Glucose (g)	26.8 ± 3.8	16.11 ± 1.8	0.026
Fructose (g)	22.7 ± 2.0	16.9 ± 2.6	0.101

NUTRIENT INTAKE	EUROPEAN (n=22)	NON-EUROPEAN (n=6)	P-value
Sucrose (g)	50.4 ± 4.8	39.4 ± 7.8	0.257
[1] Lactose (g)	27.6 ± 5.9	17.6 ± 5.1	0.194
Maltose (g)	4.3 ± 0.7	3.3 ± 0.8	0.337
Total Fat (g)	101.3 ± 8.9	70.4 ± 7.6	0.016
Total Fat (%)	33.0 ± 1.8	29.7 ± 3.1	0.385
[1] Saturated Fat (g)	44.5 ± 5.1	27.1 ± 2.7	0.036
Mono Fat (g)	35.1 ± 3.0	24.8 ± 2.9	0.024
Poly Fat (g)	12.7 ± 1.0	10.4 ± 1.8	0.287
[1] Cholesterol (mg)	445 ± 35	361 ± 64	0.194
[1] Alcohol (g)	9.1 ± 3.0	2.7 ± 2.3	0.675
Water (g)	2267 ± 209	1588 ± 306	0.092
Vitamin A (µg) (Retinol Equivalents)	961 ± 67	751 ± 177	0.303
[1] Vitamin C (mg)	144 ± 27	77.3 ± 23	0.095
Vitamin E (mg)	11.3 ± 1.0	8.1 ± 1.2	0.067
[1] Vitamin D (µg)	3.0 ± 0.6	3.1 ± 0.8	0.559
[1] Thiamin (Vitamin B1) (mg)	2.8 ± 0.5	2.5 ± 0.6	1.000
[1] Riboflavin (Vitamin B2) (mg)	3.5 ± 0.6	2.2 ± 0.4	0.099
Niacin (Equivalents) (Vitamin B3)	58.5 ± 3.6	44.1 ± 8.2	0.153
[1] Pyridoxine (Vitamin B6) (mg)	3.1 ± 0.4	2.5 ± 0.7	0.457
[1] Folate (Vitamin B9) (µg)	352 ± 33	258 ± 44	0.095
[1] Cyanocobalamin (Vitamin B12)	8.5 ± 0.9	5.8 ± 1.0	0.144
Sodium (mg)	4158 ± 311	2831 ± 369	0.015
Potassium (mg)	4493 ± 349	3213 ± 597	0.094
Magnesium (mg)	421 ± 27	319 ± 62	0.165
[1] Calcium (mg)	1248 ± 178	790 ± 168	0.047
Phosphorus (mg)	2140 ± 171	1697 ± 298	0.226
Iron (mg)	18.0 ± 1.2	16.5 ± 2.7	0.633
Zinc (mg)	17.4 ± 1.2	14.8 ± 1.8	0.262
Manganese (µg)	4645 ± 327	4906 ± 1145	0.833
[1] Copper (mg)	2.5 ± 0.4	1.8 ± 0.4	0.111
[1] Selenium (µg)	98.5 ± 15	82.1 ± 18	0.614

Note: [1] Kruskal-Wallis non-parametric test.

The equation used to estimate percent body fat and fat free mass from skinfolds was the Durnin & Womersley four-site method (Hawes & Martin, 2001; Durnin & Womersley,

1974). This is a popular body fat equation used on New Zealand populations and was developed on a fairly similar population to that used in this study, however because it was developed on a Scottish population, and our study included four Asian subjects (12.5%) and two Indian subjects (6.3%), the validity of using this equation may be decreased in this instance. Body fat and fat free mass were also estimated using the bioelectrical impedance analyser; this also has decreased reliability for use on this population since the regression equation used was unknown.

There were no significant differences between the European and non-European groups for initial or final skinfold measurements. However percent change in subscapular skinfolds was significantly different between Europeans and non-Europeans; the mean percent change in subscapular skinfolds for Europeans was -1.4 ± 1.4%; and non-Europeans 10.9 ± 3.6%.

4.1.4 Education & Occupation

Around half of the study participants were students, and the exercise, dietary, and supplementation behaviours of students may not reflect the RTM population as a whole.

4.2 Anthropometry

There was a significant difference found between the mean body mass indices of the two groups. The body mass index provides reference standards of healthy body weight range for a given height (Deakin, 2000). The body mass index is not thought to be appropriate for athletes with large muscle mass, who are inappropriately classified as overweight. The body mass index is also not a good representation of body composition (Deakin, 2000). However, in this instance there was quite a good correlation between body mass index and fat free mass (R-Sq > 69.6%). A line graph representing ranked baseline and final body mass indices for the HMB and placebo groups are shown in Figures 4.1 and 4.2, over the page.

Figure 4.1: Comparison of Ranked Initial Body Mass Indices for HMB and Placebo Groups

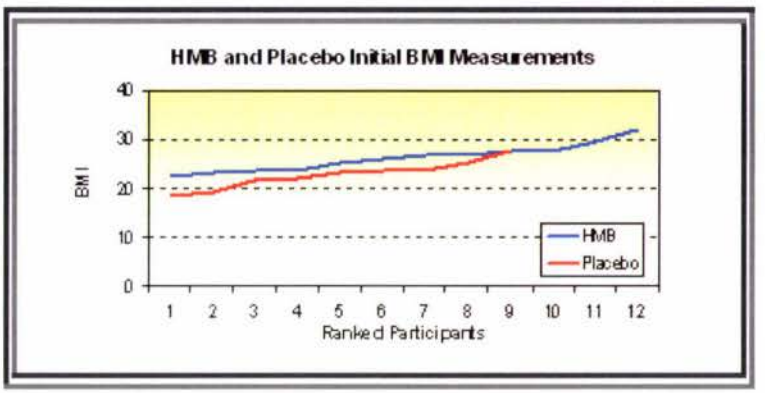
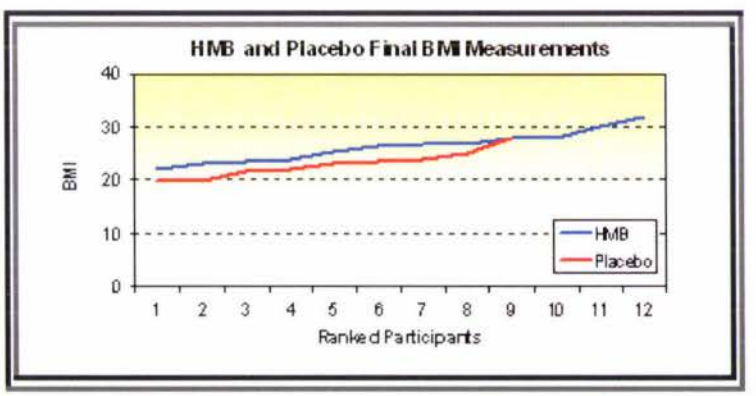


Figure 4.2: Comparison of Ranked Final Body Mass Indices for HMB and Placebo Groups



Although the HMB group had greater initial body mass indices, this did not equate to greater baseline strength, the baseline strength was not significantly different between the two supplemented groups. Regression statistics indicate that although there was a relationship between body mass index and strength, body mass index did not have a large influence on strength.

4.2.1 Comparison of Methods used to Estimate Body Composition

Three procedures were used to estimate body composition change during this study. The methods included the ISAK eight site skinfold method plus Durnin and Womersley four-site equation (1974); single frequency bioelectrical impedance analyser; and Watson and colleagues total body water estimate of density (1981) plus estimation of percent body fat from body density (Durnin and Womersley, 1974).

The estimation of body fat percent based on the Watson and colleagues (1981) total body water estimation of density and percent body fat, differed significantly to the estimate based on skinfolds and the Durnin and Womersley (1974) equation. The bioelectrical impedance analyser method and the skinfold and Durnin and Womersley (1974) estimation of percent body fat did not significantly differ, nor did the bioelectrical impedance analyser method and the Watson and colleagues (1981) total body water estimation of density and percent body fat differ significantly. The bioelectrical impedance analyser and Watson and colleagues (1981) total body water methods appeared to give closer estimations of percent body fat. The skinfold and Durnin and Womersley (1974) method estimated consistently lower levels of body fat than the other two methods used in the study, as indicated in Table 4.4, below.

Table 4.4: Comparison of Methods used to Estimate Body Composition

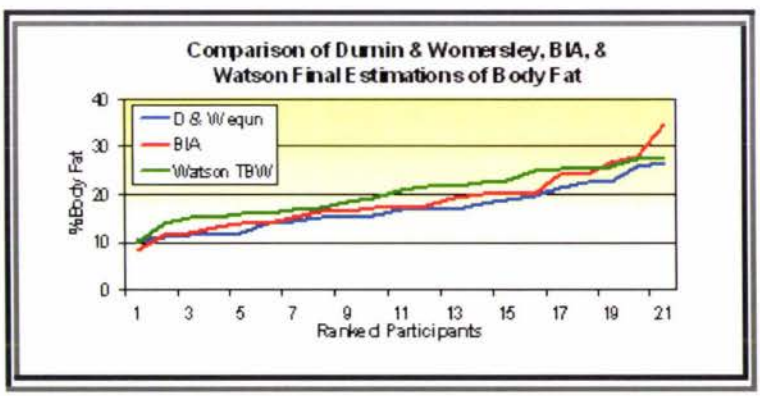
BODY FAT ESTIMATES	DURNIN & WOMERSLEY	BIA	WATSON'S TBW EQUN	P-value
Initial (n=21)	16.7 ± 1.1	19.0 ± 1.2	.	0.175
	16.7 ± 1.1	.	19.9 ± 1.1	0.049
	.	19.0 ± 1.2	19.9 ± 1.1	0.570
Final (n=21)	16.9 ± 1.0	18.5 ± 1.4	.	0.366
	16.9 ± 1.0	.	20.1 ± 1.1	0.039
	.	18.5 ± 1.4	20.1 ± 1.1	0.355

Note: Abbreviations used EQUN = equation, BIA = bioelectrical impedance analyser, TBW = total body water.

Line graphs representing ranked percent body fat estimations using the three methods are shown below (Figures 4.3, and 4.4). The Durnin & Womersley (1974) equation appears to underestimate percent body fat compared to the other two methods.

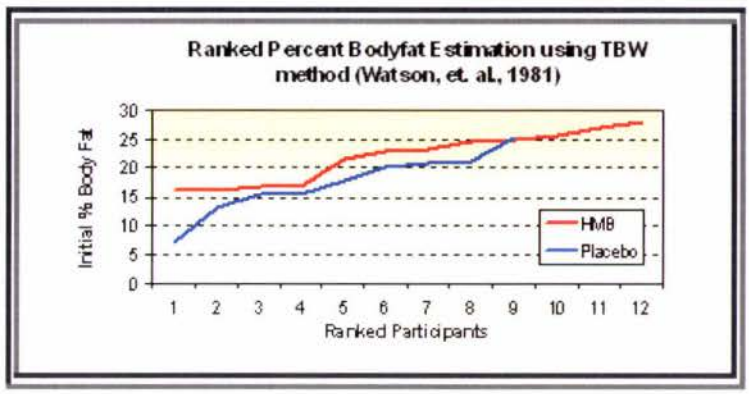
Figure 4.3: Comparison of Methods used to Estimate Initial Body Fat Percentages

Figure 4.4: Comparison of Methods used to Estimate Final Body Fat Percentages



The HMB group had significantly greater initial percent body fat as estimated by the Watson and colleagues (1981), total body water method (Figure 4.5). This difference in percent body fat had no influence on change in body fat over the duration of the study (P=0.296).

Figure 4.5: Comparison of Ranked Baseline Estimates of Percent Body Fat using the Total Body Water Equation



4.2.2 General Anthropometric Characteristics of the Study Group

In comparison to skinfolds of 19-24 year old males taken during the National Nutrition Survey in 1997 (triceps 12.2 ± 0.5mm and subscapular 14.1 ± 0.7mm (Mean ± SEM) (Russell *et. al.*, 1999), skinfold thickness of study participants (triceps 8.4 ± 0.6mm and subscapular 12.6 ± 0.9 (Mean ± SEM) were less. This study indicates that RTM are leaner than the general New Zealand population.

However, in comparison to the body fat levels of competitive bodybuilders, which range from 9.1% off-season to 4.1-7.2% during competition (Sandoval, *et. al.*, 1989; Bamman, *et. al.*, 1993; Withers, *et. al.*, 1997), the mean body fat levels of study participants, 16.7 ± 1.1% calculated by skinfolds and the Durnin & Womersley equation (1974); 19.0 ± 1.2% measured by bioelectrical impedance analyser; and 20.03 ± 0.8% estimated by Watson and colleagues (1981) total body water equation, were much greater. The large differences in mean body fat levels clearly indicates that RTM anthropometry differs to that of competitive bodybuilders, therefore RTM anthropometric profiles need to be studied separately from competitive bodybuilders.

4.2.3 Anthropometric Changes over the Study Period

There was no significant difference found between the HMB and Placebo supplemented groups; change in weight; individual skinfolds; sum of six skinfolds; fat mass; fat free mass; or percent body fat during this supplementation study. These results agree with the majority of previous studies of HMB supplementation in humans reviewed, which found no significant effect on body composition (Nissen, *et. al.*, 1996; Panton, *et. al.*, 2000; Kreider, *et. al.*, 1999; Gallagher, *et. al.*, 2000; Jówko, *et. al.*, 2001; Slater, *et. al.*, 2001).

The proposed mechanism of HMB effect on body composition is via increased fat oxidation in muscle cells. The majority of the evidence comes from study abstracts (not reviewed). The literature discussion in the introduction was limited to human studies published in peer reviewed journals; studies published in abstract form only, were ignored. It seems that adequate scientific support for any effect of HMB on fat oxidation in adipose or muscle tissue is lacking.

4.3 Strength

4.3.1 Resistance Training Compliance

Resistance training compliance was monitored using training log books, and no significant difference was found in the number of training sessions between treatment groups. This method of regulating subject compliance may introduce bias, as self recording by subjects is likely to be higher than actual results, and may reflect what the subjects believe the researcher is after rather than actual results (Panton, *et. al.*, 2000). In addition not all training log books were completed and returned by study subjects (75% compliance by subjects who completed the study). Subject compliance of all study requirements, has been represented in Table 6.1 in Appendix 11.

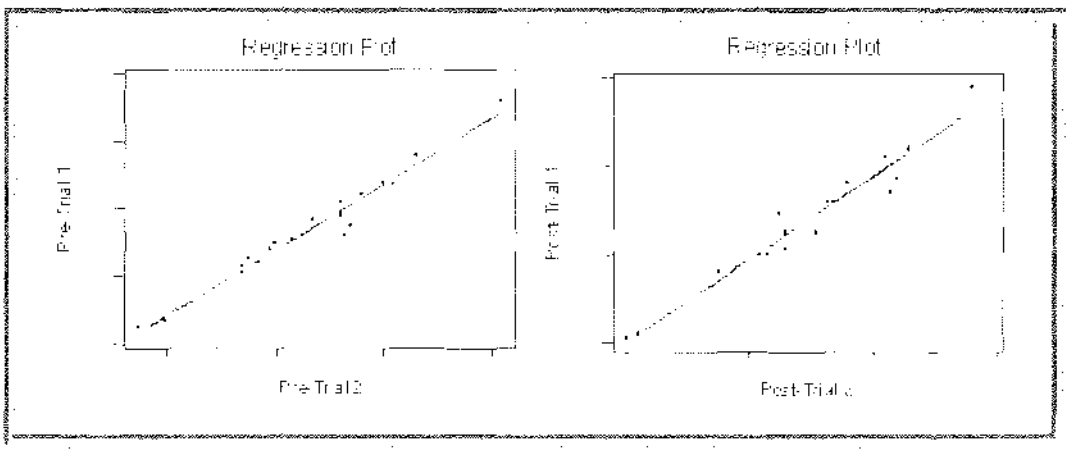
It was unusual that neither treatment group significantly gained strength over the study duration. This might be due to inadequate training intensity for muscle physiological adaptation, during the unsupervised training sessions, and agreed with a previous study that indicated less intense training and smaller strength increases in unsupervised compared to supervised subjects undergoing resistance training (Mazzetti, *et. al.*, 2000).

During the study the average duration of resistance training actually decreased once participants followed the study resistance training programme rather than their own individual programmes. The study resistance training programme advised training three times per week. However frequency decreased to an average of 2.5 ± 0.1 times per week during the study, from 3.5 ± 0.2 times per week prior to the study. Reasons for this difference are unclear. Compliance may have dropped due to lack of interest in the study resistance training programme; the number of student participants declined as the study duration coincided with the university exam period; errors in recall of previous training frequency in the questionnaire; and decreased compliance recording training sessions in the log books.

4.3.2 Strength Changes Over the Study Period

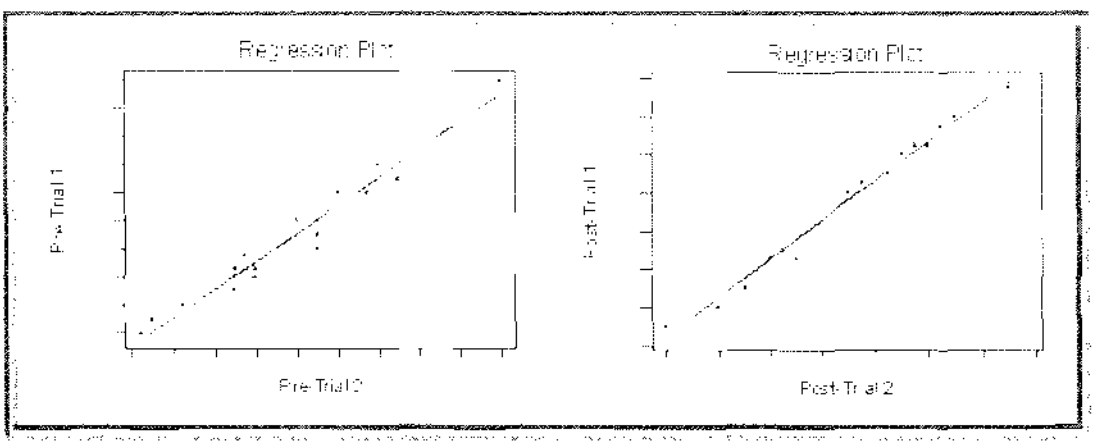
The pre-trial 1RM strength measurements taken during visit 1 and 2 were closely correlated; as were the post-trial strength measurements taken during visit 3 and 4 ($R^2 >$ 90.2%), indicating good precision with the 1RM method used. The accuracy of this method may have been reduced due to large increase in weight increments used in the bicep preacher curl strength test, where the range of weighted plates and dumbbells was limited. The regression statistics are shown below in Figures 4.6 to 4.8.

Figure 4.6: Leg Extension Pre-trial and Post-trial Strength Measurements



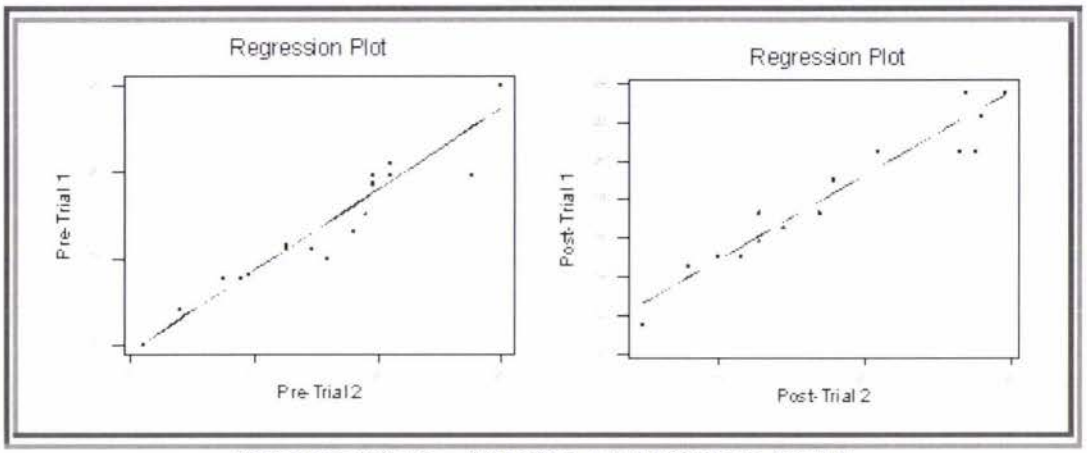
Note: Pre-Trial 1 = -0.284678 + 0.975016 Pre-Trial 2
S = 1.45847 R-Sq = 96.7% R-Sq(adj) = 96.5%
Post-Trial 1 = 1.63827 + 0.936705 Post-Trial 2
S = 1.67689 R-Sq = 94.9% R-Sq(adj) = 94.6%

Figure 4.7: Bench Press Pre-trial and Post-trial Strength Measurements



Note: Pre-Trial 1 = -4.27099 + 0.995648 Pre-Trial 2
S = 4.77150 R-Sq = 95.1% R-Sq(adj) = 94.9%
Post-Trial 1 = -3.61556 + 1.02778 Post-Trial 2
S = 2.33919 R-Sq = 98.8% R-Sq(adj) = 98.7%

Figure 4.8: Bicep Preacher Curl Pre-trial and Post-trial Strength Measurements



Note: Pre-Trial 1 = 0.213947 + 0.939938 Pre-Trial 2
S = 1.11180 R-Sq = 90.6% R-Sq(adj) = 90.2%
Post-Trial 1 = 1.65540 + 0.879550 Post-Trial 2
S = 1.01021 R-Sq = 92.3% R-Sq(adj) = 91.8%

There were no significant differences in actual change in strength for any of the exercises tested for either supplemented group during the study (P>0.086). However, percent change in leg extension strength did increase significantly for the HMB supplemented group compared to the placebo group over the study duration. Previous studies have found both an increase in strength (Nissen, *et. al.*, 1996; Panton, *et. al.*, 2000; Vukovich, *et. al.*, 2000; Jówko, *et. al.*, 2001) and no significant difference in strength (Kreider, *et. al.*, 1999; Slater, *et. al.*, 2001; Vukovich *et. al.*, 2001) with HMB supplementation in humans undergoing resistance training

It is unclear why the strength increase found in this study showed a significant difference for only the lower body. Previous studies (Nissen, *et. al.*, 1996; Panton, *et. al.*, 2000; Vukovich, *et. al.*, 2000) have also found HMB supplementation to increase strength in selective muscle groups. Nissen and colleagues (1996) and Vukovich and colleagues (2000) both found HMB supplementation to increase strength to a greater degree in lower body compared to upper body, as did the results of this study. However Panton, and colleagues (2000) found the opposite effect, HMB supplementation increased strength significantly in upper body muscle groups and not in lower body, in sharp contrast to the

results of this study. It seems implausible that HMB acts selectively on certain muscle groups therefore the apparent selectively of HMB found in these studies may indicate a difference in training intensity or frequency of certain body parts, as HMB requires the stimulus of stress such as exercise induced muscle damage and proteolysis to exert its effect.

4.4 Diet

There was very little reported research found on the dietary habits of recreational resistance trained individuals, and no detailed studies found on the dietary habits of New Zealand resistance trained individuals.

4.4.1 Description of Dietary Habits of Study Participants

Significant differences in the intakes of some nutrients were found between the two supplemented groups. The percentage of energy intake from carbohydrates was significantly higher in subjects from the HMB group compared to the placebo group; however actual carbohydrate intake in grams was not significantly different between the two groups. The carbohydrate intake of both treatment groups was lower than optimum for resistance exercising individuals. The carbohydrate recommendations for RTM will be discussed in more detail later in this section.

In addition the intake of maltose was significantly greater in the HMB group compared to placebo. In general only small amounts of maltose are consumed in the diet. Maltose naturally occurs in sprouted grain plants such as wheat and barley, and in honey, and is used for brewing beer and producing malted foods (Belitz & Grosch, 1999; Lewis, 2000). There was no significant difference in beer consumption between the supplementation groups to explain the difference in maltose intake.

The percentage of energy intake from fats, and intake of cholesterol were significantly lower in subjects from the HMB group compared to placebo. Lipids may be used by muscle during high intensity exercise such as resistance training, but are a very minor fuel source (Watt, *et. al.*, 2002); therefore should not affect training and strength performance.

4.4.2 The Dietary Habits of Study Participants in Comparison with the Recommended Dietary Allowances

The latest available U.S. and Canadian Dietary Reference Intakes (DRI) were used to assess participant's intakes of nutrients, along with the Lower Reference Nutrient Intakes (LRNI) from the UK Dietary Reference Values (DRV).

Several study participants failed to meet the Recommended Dietary Allowances (RDA), some failed to meet the Lower Reference Nutrient Intakes (LRNI), and a few exceeded the Tolerable Upper Intake Level (UL) of certain nutrients. The Recommended Dietary Allowances represent the average dietary intakes sufficient to provide most of the population (97%) with their daily nutrient levels (IOM, 2000). The Adequate Intakes (AI) were used where Recommended Dietary Allowances were not available, and represent intake levels based on observed intakes of groups of healthy individuals. The Lower Reference Nutrient Intakes represent nutrient levels adequate for only a small number (3%) of the population, and most of the population habitually consuming intakes at these levels risk nutrient deficiencies (Salmon, 1991). The Tolerable Upper Intake Levels represent the maximum daily intake of nutrients posing no risk of adverse health effects to most of the population (IOM, 2000; Stark, 2000). Nutrient intakes of the study group have been represented in Table 4.5, over the page.

For the macronutrients, the median percent energy from protein of study subjects (21.1%) was within the Recommended Dietary Allowances (10-35%). Median intake of dietary protein in g/kg bodyweight per day (1.5g/kg/d) however was greater than Recommended Dietary Allowances (0.8g/kg/d). Only one study participant consumed less than the Recommended Dietary Allowances for protein. The median percent energy intake from

carbohydrate of the study subjects (43.1%) was lower than Recommended Dietary Allowances (45-65%).

Several vitamin and mineral intakes were below both two thirds of the Recommended Dietary Allowances and the Lower Reference Nutrient Intakes. Vitamin intakes below two thirds of the Recommended Dietary Allowances included; vitamin A (25.0% of participants below ⅔RDA and 7.1% below LRNI); vitamin C (21.4% participants below ⅔RDA); vitamin E (39.3% participants below ⅔RDA); vitamin B6 (3.6% participants below ⅔RDA and 3.6% below LRNI); and folate (21.4% participants below ⅔RDA). Mineral intakes below the two thirds of the Recommended Dietary Allowances included; potassium (21.4% participants below ⅔RDA and 3.6% participants below LRNI); magnesium (14.3% participants below ⅔RDA and 3.6% below LRNI); calcium (17.9% participants below ⅔RDA and 3.6% below the LRNI); and selenium (3.6% participants below ⅔RDA and 3.6% below LRNI).

Table 4.5: Comparison of Median Participant Nutrient Intakes with Recommended Dietary Allowances and Lower Reference Nutrient Intakes

NUTRIENT (n=28)	MEDIAN	UQ	LQ	% <RDA	% <⅔RDA	% <LRNI
Protein (g)	126.7	173.4	98.3	0	0	NA
[1]Protein (g/kg)	1.5	2.1	1.3	3.8	0	NA
[1,3]Protein (g/kg) for RTM	1.5	2.1	1.3	3.8	0	NA
Protein (%)	21.1	25.8	18.4	0	0	NA
Carbohydrate (g)	275.9	367.0	219.7	0	0	NA
[1,3]Carbohydrate (g/kg) for RTM	3.6	4.3	2.6	24.0	9.0	NA
Carbohydrate (%)	43.1	46.1	39.0	74.1	0	NA
Fibre (g)	24.9	28.2	17.9	89.3	39.3	NA
[4]Total Sugars (%)	18.4	22.4	15.0	17.9	NA	NA
[5]Sucrose (%)	7.3	9.4	5.8	21.4	NA	NA
Total Fat (%)	32.8	37.9	25.7	10.7	0	NA
Saturated Fat (%)	13.4	16.9	10.7	21.4	10.7	NA
Mono Fat (%)	12.0	13.2	9.1	NA	NA	NA
Poly Fat (%)	3.9	15.6	8.5	96.4	57.1	NA

NUTRIENT (n=28)	MEDIAN	UQ	LQ	% <RDA	% <½RDA	% <LRNI
[6]Alcohol (g)	0.3	12.9	0.0	100	NA	NA
Vitamin A (µg) (Retinol Equivalents)	952.6	1074.2	601.5	42.9	25.0	7.1
Vitamin C (mg)	115.2	170.4	68.3	46.4	21.4	0
Vitamin E (mg)	9.9	12.7	7.1	85.7	39.3	NA
Thiamin (Vitamin B1) (mg)	2.1	3.1	1.4	14.3	0	0
Riboflavin (Vitamin B2) (mg)	2.8	3.6	1.8	10.7	0	0
Niacin (Equivalents) (Vitamin B3)	55.9	68.3	40.4	0	0	0
Pyridoxine (Vitamin B6) (mg)	2.8	3.1	1.9	17.9	3.6	3.6
Folate (Vitamin B9) (µg)	301.0	388.7	245.5	82.1	21.4	0
Cyanocobalamin (Vitamin B12) (µg)	6.9	9.3	4.4	0	0	0
[2]Sodium (mg)	3781.0	4588.1	2484.6	3.6	0	0
[2]Potassium (mg)	3983.0	5277.9	2950.0	67.9	21.4	3.6
Magnesium (mg)	374.0	487.0	288.1	60.7	14.3	3.6
[2]Calcium (mg)	927.0	1269.0	683.7	57.1	17.9	3.6
Phosphorus (mg)	1918.5	2289.8	1429.4	0	0	0
Iron (mg)	17.3	21.7	13.3	3.6	0	0
Zinc (mg)	16.1	18.7	13.3	7.1	0	0
Selenium (µg)	74.1	107.6	52.0	32.1	3.6	3.6

Note: Abbreviations UQ = upper quartile, LQ = lower quartile, RDA = Recommended Dietary Allowances, and LRNI = Lower Reference Nutrient Intake. [1]Sample number n=26. [2] Adequate Intakes (AI). [3]Study Recommendations. [4]Total Sugar intakes compared with Added Sugar Recommendations. [5]Sucrose intakes compared with New Zealand Nutrition Task Force Recommendations for Sucrose and other Free Sugars. [6]Alcohol Advisory Council of New Zealand guidelines. (Salmon, 1991: IOM 1997; IOM 1998; IOM 2000; IOM 2001; IOM 2002; Matheson, 2002).

The protein intakes of New Zealanders are generally high (Matheson, 2002). Most study participants (96.2%) consumed greater than the Recommended Dietary Allowance for protein (0.8g/kg/d), and more than half of the study subjects (60.7%) exceeded the study recommendations for protein (1-1.4g/kg/d), represented in Figures 4.9 and 4.10. Two participants were found to consume protein in amounts that far exceeded requirements (four times the Recommended Dietary Allowances, or greater than 2.8g/kg/d), although this was not as common an occurrence in this group as previously thought (Pearce, 1988; Lemon, *et. al.*, 1992; Tarnopolsky, 2000). However one study subject consumed less than the

Recommended Dietary Allowances for protein, and three (11.5%) participants were below the study recommendations for individuals regularly performing resistance training.

Proteins, peptides, and amino acids are required for structural purposes such as tissue structure, growth and repair; also regulatory purposes such as hormones, transport systems, metabolism enzymes, and immune system function; as well as a variety of other processes (McNurlan & Garlick, 2000; Tarnopolsky, 2000; Matheson, 2002). Additional dietary protein is required by RTM for repair of damaged muscle fibres, to provide for increased enzyme activity, and for muscle protein accretion and muscle hypertrophy (Pearce, 1988; Lemon, 1998; Tarnopolsky, 2000)

Intakes of dietary protein above study recommendations (1-1.4g/kg/d) were not thought to provide additional benefit to muscle gain, but up to 2g/kg/d should not lead to any adverse health effects (Tarnopolsky, *et. al.*, 1988; Carroll, 2000; Tarnopolsky, 2000). Intakes of dietary proteins above requirements, increases the oxidation of protein for energy and storage as body fat (Pearce, 1988; Lemon, *et. al.*, 1992; Tarnopolsky, 2000). In addition, protein intake above requirements may lead to reduced carbohydrate intake and therefore affect resistance exercise performance (Tarnopolsky, *et. al.*, 1988; Carroll, 2000; Tarnopolsky, 2000). Excessive protein intakes (greater than 2.8g/kg/d) increase urination and may lead to dehydration, increase calcium excretion, and may aggravate any existing renal disease due to increased strain on the kidneys (Lemon, 1998; Tarnopolsky, 2000).

Figure 4.9: Comparison of Participant Protein Intakes with Recommended Dietary Allowances

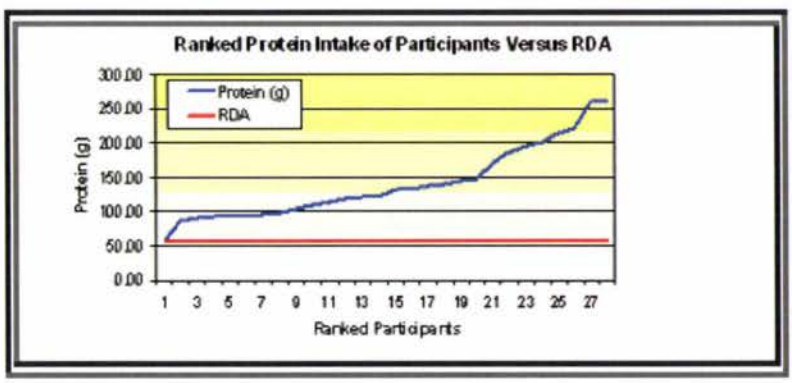
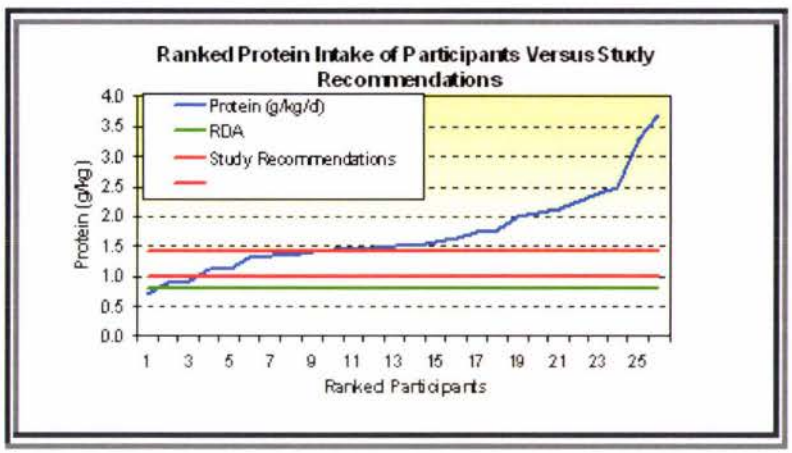


Figure 4.10: Comparison of Participant Protein Intakes with Study Recommendations for RTM



The Acceptable Macronutrient Distribution Ranges (AMDR) for carbohydrate are 45-60% of total energy intake (IOM, 2002). Seventy one percent of study participants failed to consume within these ranges (Figure 4.11). All participants consumed greater carbohydrate intake than the Recommended Dietary Allowances (130g), however the majority (92.3%) were below the study recommendations (5-7g/kg/d) for RTM (Figure 4.12).

The main function of dietary carbohydrate is energy supply. Carbohydrate is digested and absorbed into the body mainly as glucose, the major fuel of the body and its tissues (McGrane, 2000). In addition to this role, dietary carbohydrate is also required as a source of beneficial vitamins, minerals, and non-nutrients inherently contained within carbohydrate-rich foods such as fruits, vegetables, and whole grains (Linder, 1991). Carbohydrate intake is important for RTM as the preferred fuel for resistance training exercise (Liebman & Wilkinson, 1994; Maughan, *et. al.*, 1997; Hawley & Burke, 1998; Lemon, 1998; Gastin, 2001; Howe, *et. al.*, 2002). The study recommendations were based on the carbohydrate requirements for muscle glycogen replenishment (Kleiner, 2000) retardation of muscle protein breakdown (Roy, et. al., 1997; Gibala, 2000), and stimulation of amino acid uptake by muscle fibres (Lemon, 1998; Gibala, 2000).

Low intakes of carbohydrate lead to fatigue and often inadequate and unbalanced nutrient intakes (O'Connor, *et. al.*, 2000). The low carbohydrate intakes of some RTM may lead to

decreased levels of muscle glycogen and therefore utilisation of muscle protein for gluconeogenesis to maintain blood glucose concentrations while exercising, and an associated reduction in resistance exercise performance (O'Connor, *et. al.*, 2000). Extremely low carbohydrate intakes (around 50-100g/day) lead to ketosis (Driskell, 2000; Matheson, 2002), nausea, headaches, fatigue, and bad breath (O'Connor, *et. al.*, 2000). None of the study participants were found to follow extremely low carbohydrate diets, although two participants had admitted to having done so in the past.

Figure 4.11: Comparison of Participant Carbohydrate Intakes with Recommended Dietary Allowances

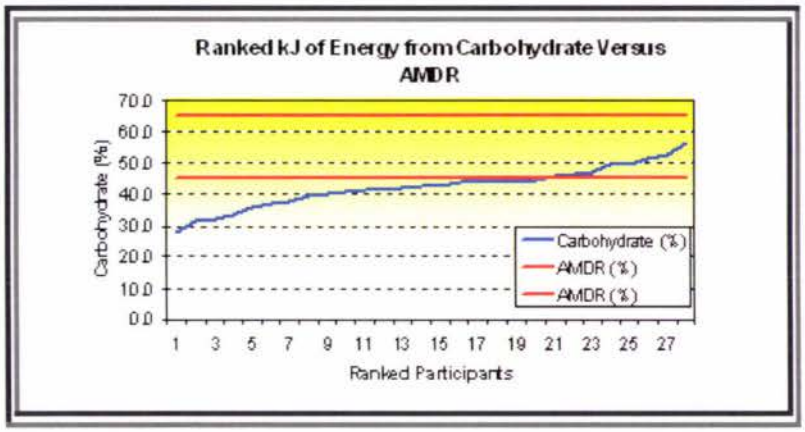
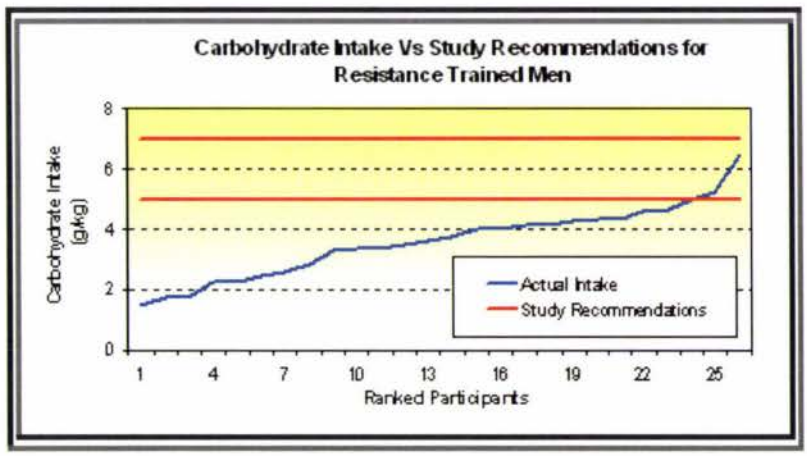


Figure 4.12: Comparison of Participant Carbohydrate Intakes with Study Recommendations for RTM

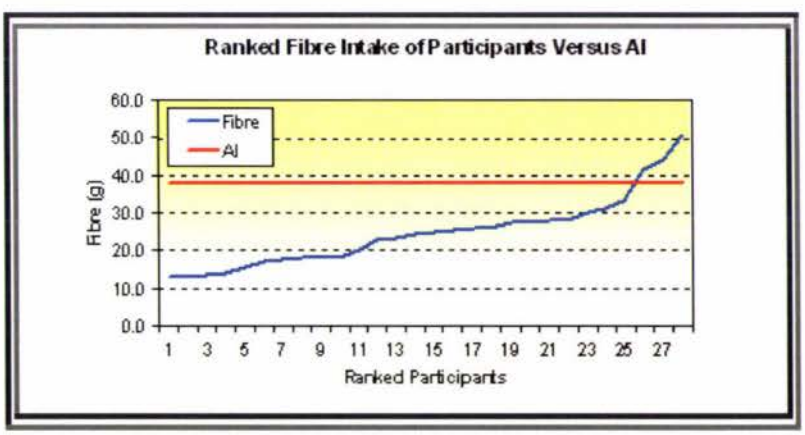


An Adequate Intake for individuals of total dietary fibre from foods is 38g/d for males aged 19-50 years (IOM, 2002). The majority (89.3%) of study subjects failed to consume the Recommended Intakes for Individuals of dietary fibre; this is not surprising considering their low carbohydrate intakes, as indicated previously.

The health benefits of fibre include; maintaining gastrointestinal integrity and function (Linder, 1991); increasing stomach distension and producing a feeling of fullness; slowing the absorption of food and therefore providing glycaemic control; binding to and causing the excretion of bile acids, new bile acids are produced from cholesterol thus lowering cholesterol; decreasing production of micelles from bile acids, thus slightly decreasing fat and cholesterol absorption; diluting, and possibly binding to and removing, toxic and cancer causing compounds; altering colon pH, and therefore reducing bacterial reactions producing cancer-causing compounds; and the prevention of constipation (Baghurst *et. al.*, 1996).

If the low fibre intakes found in some of the study participants (Figure 4.13) continue into later life, these individuals risk increased incidence of atherosclerosis, some cancers, diabetes, and possibly some gallbladder disease (Baghurst *et. al.*, 1996).

Figure 4.13: Comparison of Participant Fibre Intakes with Adequate Intakes



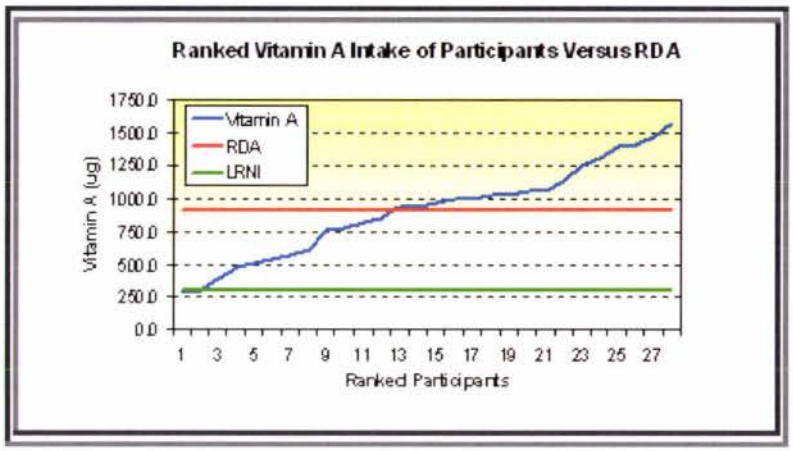
There is no Tolerable Upper Intake Level for cholesterol, as any incremental increase in dietary cholesterol above zero is thought to increase risk of coronary heart disease (IOM, 2002). However two study participants were consuming excessive levels of dietary cholesterol.

Moderate intakes of dietary cholesterol, have only a small effect on blood cholesterol levels, and the main effect is via down regulation of LDL receptors involved in LDL breakdown and internalisation. Excessive intakes of dietary cholesterol (around 800mg), have an appreciable effect on blood cholesterol (Mann, 2000), and the high intakes of study participants are of serious concern as this habitual level of cholesterol intake may lead to the development of atherosclerosis and heart disease or stroke later in life (Mann, 2000). In addition individuals with Familial Combined Hyperlipidemia (occurring in 10% of individuals with documented heart disease and around 0.5% of population), a condition associated with increased low density lipoprotein (LDL) and very low density lipoprotein (VLDL) plasma cholesterol (Voors-Pette & de Bruin, 2001), show a heightened sensitivity to the effects dietary cholesterol and increased risk of coronary artery disease (Mann, 2000).

It used to be rare to find vitamin A deficiency in developed countries (Noy, 2000); and the 1997 National Nutrition Survey estimated that inadequate vitamin A intake occurred in 1.9% of the population, with a higher prevalence of inadequate intake in males 19-24 years of age (Matheson, 2002). However the findings of this study indicated that two study participants (7.1%) were habitually consuming less than the Lower Reference Nutrient Intakes for vitamin A (Figure 4.14). If this situation continues for a significant period of time body stores of vitamin A will be depleted and symptoms of deficiency may arise (Matheson, 2002).

Vitamin A has important roles in vision, reproduction, immune function, cell signalling, and cell differentiation and proliferation (Noy, 2000). Deficient vitamin A intake is associated with impaired immune system defence, impaired lymphocyte function, eye and vision problems, and are thought to lead to several types of cancer later in life (Noy, 2000).

Figure 4.14: Comparison of Participant Vitamin A Intakes with Recommended Dietary Allowances



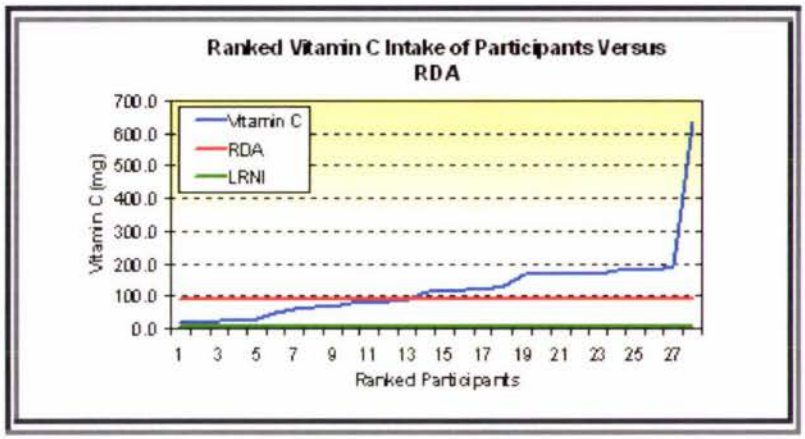
Almost half (46.4%) of study participants were below the Recommended Dietary Allowances, and six (21.4%) subjects below two thirds of the Recommended Dietary Allowances of Vitamin C (Figure 4.15).

Vitamin C acts as an electron donor in enzymes required in collagen synthesis, neurotransmitter synthesis, and pituitary peptide production (Levine, *et. al.*, 2000; Mathews, 2002). Vitamin C has additional roles in connective tissue components, aids iron absorption, scavenges reactive oxygen species, and regenerates α-tocopherol and glutathione antioxidants (IOM 2000; Thurnham, et. al., 2000).

Decreased physical performance has been shown with a vitamin C deficient diet (Fogelholm, 2000).

At an intake of below 10mg/day of vitamin C in healthy subjects, deficiency symptoms would occur at around 40 days. Symptoms include bruising on the skin, haemorrhages occurring around hair follicles, and bleeding gums. Further deficiency would lead to joint pain and accumulation of fluid, slow wound healing, and even bone fractures (Thurnham, *et. al.*, 2000).

Figure 4.15: Comparison of Participant Vitamin C Intakes with Recommended Dietary Allowances

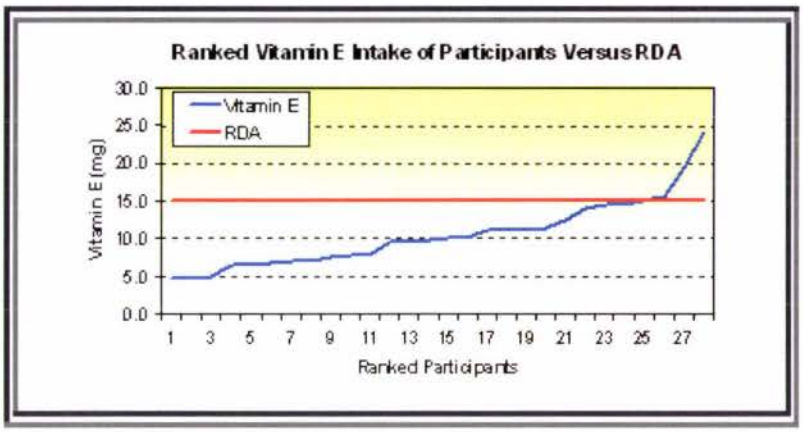


Eleven (39.3%) study participants failed to meet two thirds of the Recommended Dietary Allowances of Vitamin E.

Vitamin E is required for membrane structure and protection, modulation of enzyme activity, cell mediated immune response (Chow, 2000), nerve and muscle function, and male fertility (Linder, 1991). Vitamin E is important in RTM as there may be an increased requirement for vitamin E in active individuals such as the participants of this study. With intense exercise there is an increase in free radical production by the body's normal energy producing reactions. High free radical levels are associated with cell and mitochondrial membrane damage (Fogelholm, 2000). Vitamin E offers some protection against the increased oxidative stress of physical activity (Hawley & Burke, 1998), and may aid recovery from muscle fatigue associated with intense exercise (Driskell, 2000).

Low or deficient vitamin E intakes are associated with short red cell lifespan. There is an association with low vitamin E status and cell and mitochondria membrane damage, weakening immune defence system, aging, cancer, and coronary heart disease (Chow, 2000; Fogelholm, 2000). Participant intakes of vitamin E have been represented in Figure 4.16, over the page.

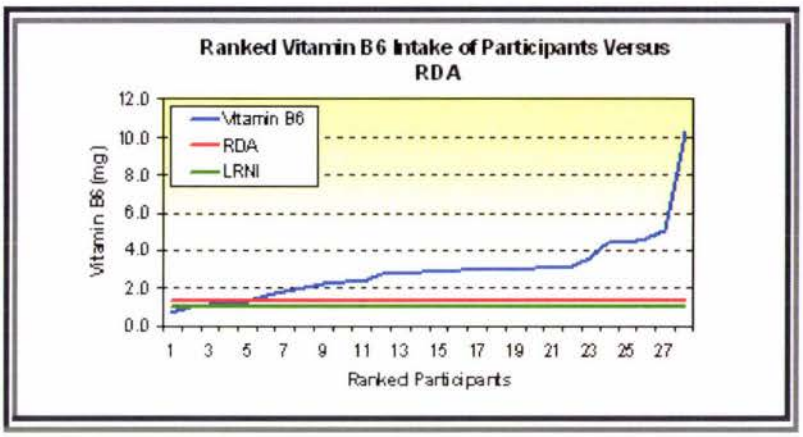
Figure 4.16: Comparison of Participant Vitamin E Intakes with Recommended Dietary Allowances



One subject (3.6%) failed to consume two thirds of the Recommended Dietary Allowances and consumed less than the Lower Reference Nutrient Intakes of vitamin B6 daily. Vitamin B6 deficiencies are rare, as this vitamin is readily found in many foods, such as meat, fish, poultry, yeast, some seeds, and bran (Clarkson, 1998), however deficiency indicators occur more rapidly in high protein diets (Thurnham, *et. al.*, 2000), such as with this group of subjects.

Vitamin B6 is involved in several metabolic pathways in fuel oxidation during exercise, for instance; amino acid oxidation; alanine formation in skeletal muscle and use in liver for gluconeogenesis; glycogen phosphorylase enzymes; and β-oxidation of fatty acids for fuel. Therefore low vitamin B6 status may adversely affect exercise performance and recovery (Sampson, 1997; Manore & Thompson, 2000). Participant intakes of vitamin B6 have been represented in Figure 4.17, over the page.

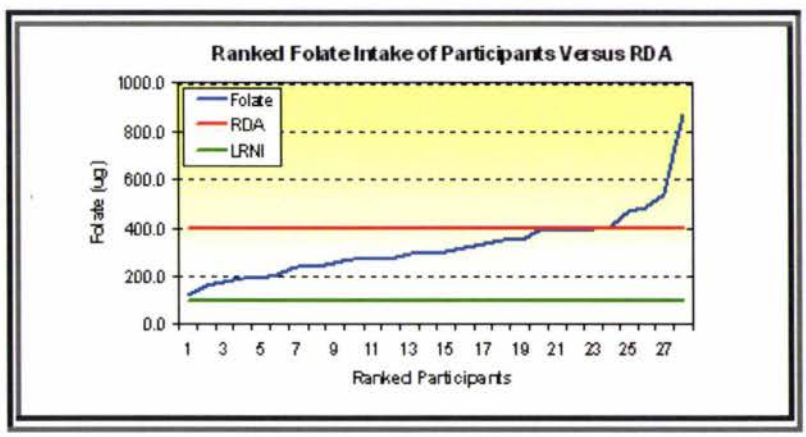
Figure 4.17: Comparison of Participant Vitamin B6 Intakes with Recommended Dietary Allowances



The median intake of folate from foods in study participants (301µg/d) was slightly higher than the median intakes of New Zealand males (278µg/d) (Matheson, 2002). However, six participants (21.4%) were below two thirds of the Recommended Dietary Allowances (Figure 4.18).

Folate has roles as coenzymes in numerous reactions involved in amino acid and nucleotide synthesis (Shane, 2000). Deficient intakes lead to a condition known as macrocytic anaemia. Adequate intakes of folate are lower blood homocysteine concentrations, and therefore risk of cardiovascular disease (Matheson, 2002).

Figure 4.18: Comparison of Participant Folate Intakes with Recommended Dietary Allowances

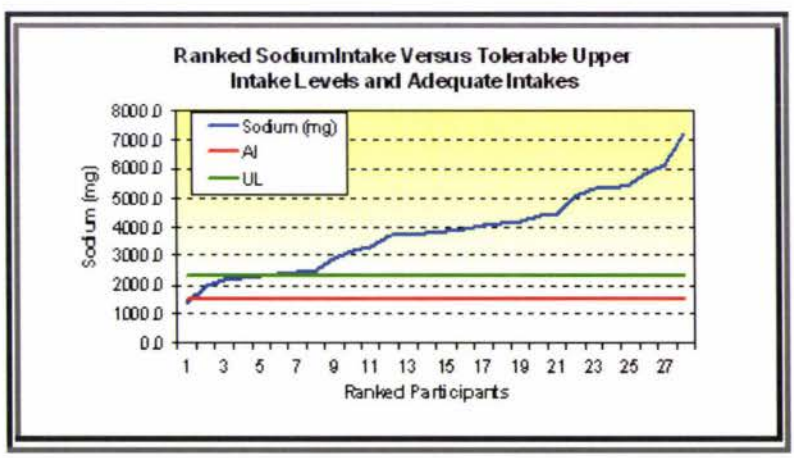


The mean sodium intake of study participants was 3826 mg; calculated from FoodWorks using the two three-day dietary records from the study. Twenty three (82.1%) study participants exceeded the Tolerable Upper Intake Levels for sodium intake, 2300 mg (IOM, 2004). Participant sodium intakes are displayed in Figure 4.19, below.

Sodium is required for the regulation of volume, osmolality, and pH of cells, and active transport across cell membranes (Matheson, 2002).

A previous study indicates high sodium intakes (20,000mg) had no detrimental effects on muscular exercise ability (Fukuba, *et. al.*, 1998), however there is concern regarding the effect of high sodium combined with intense exercise increasing the risk of hypernatremia leading to dehydration, oedema, high blood pressure, and at worst, brain stem injury (Driskell, 2000). High salt intake long term is associated with the development of hypertension, heart disease, stroke (Matheson, 2002), gastric mucosal damage, and gastric cancer (Sheng, 2000). There is also some evidence of increased calcium urinary excretion and negative calcium balance with high salt intake over the long term (Matheson, 2002).

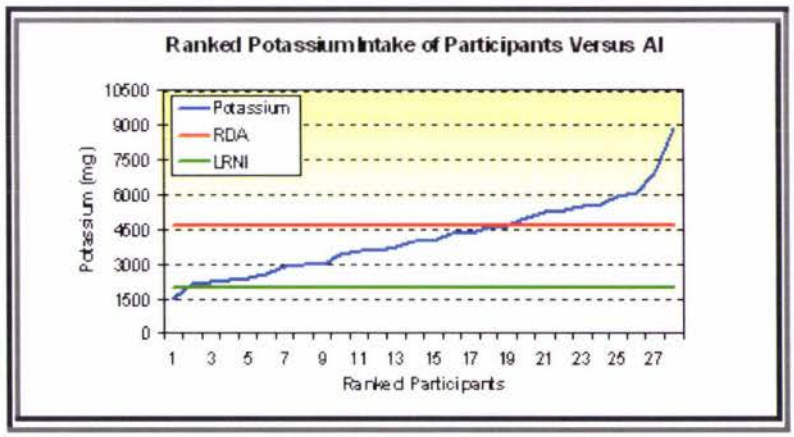
Figure 4.19: Comparison of Participant Sodium Intakes with Tolerable Upper Intake Levels



One subject (3.6%) failed to consume the Lower Reference Nutrient Intakes of potassium (Figure 4.20). Potassium deficiency is rare, as it is commonly found in many foodstuffs (Wrong, 2000). Individuals found deficient in potassium, in most cases have very low total food intake, as is the case with this study, the study participant found with low potassium intake has low total energy intake.

Potassium has important roles in cell fluid movement, and in nerve and muscle function. Adequate potassium aids the removal of excess sodium, important in this group (Salmon, 1991). A deficiency in potassium would lead to fatigue, lethargy, muscle weakness, constipation (Wrong, 2000), and would adversely affect health and exercise performance.

Figure 4.20: Comparison of Participant Potassium Intakes with Adequate Intakes



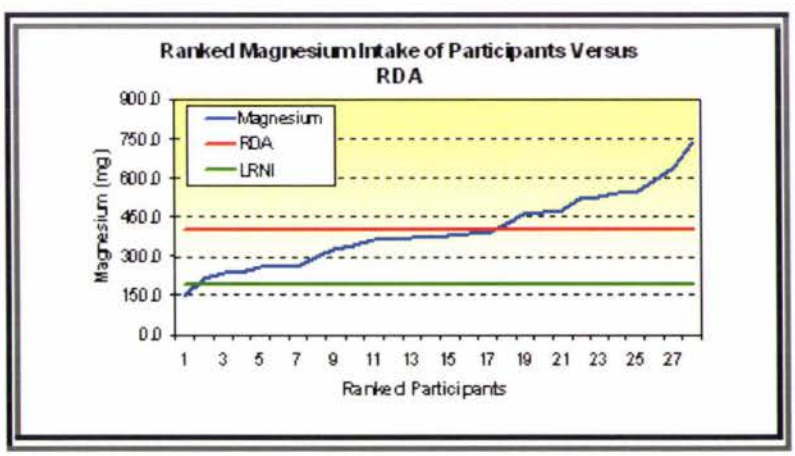
Four (14.3%) participants consumed less than two thirds of the Recommended Dietary Allowances, and one participant (3.6%) failed to consume the Lower Recommended Nutrient Intakes of magnesium.

The roles of magnesium in the body include binding to compounds and neutralising the anion charge; binding to enzymes and other proteins and stabilising the structure; roles in nucleic acid and protein synthesis; mitochondrial ATP synthesis; second messenger systems; and protein, carbohydrate and lipid metabolism pathways (Rude, 2000).

As energy requirements increase with increased exercise and physical activity, there may also be a concurrent increase in magnesium requirements (Deakin, 2000; Fogelholm, 2000). Magnesium has several important roles during physical activity; magnesium is involved in energy production reactions, such as modulating rate limiting enzymes; in muscle contraction-excitation cycles, Mg^{2+} is required to block Ca^{2+} release from calcium channels and therefore lower intracellular Ca^{2+} concentrations; in muscle and nerve excitation, by maintaining membrane electrical potential; and finally magnesium is a co-factor in an enzyme required in carbohydrate metabolism (Haymes & Clarkson, 1998). In addition to the above roles, during intense exercise there is an increased loss of magnesium, in sweat, urine and faeces (Deakin, 2000; Fogelholm, 2000).

Long term low or deficient magnesium intakes may lead to hypertension, atherosclerotic vascular disease, and osteoporosis (Rude, 2000). Low or deficient magnesium intakes in active individuals such as the study participants may result in increased incidence of muscle cramps (Rude, 2000). Participant magnesium intakes compared to the Recommended Dietary Allowances, are displayed in Figure 4.21 below.

Figure 4.21: Comparison of Participant Magnesium Intakes with Recommended Dietary Allowances

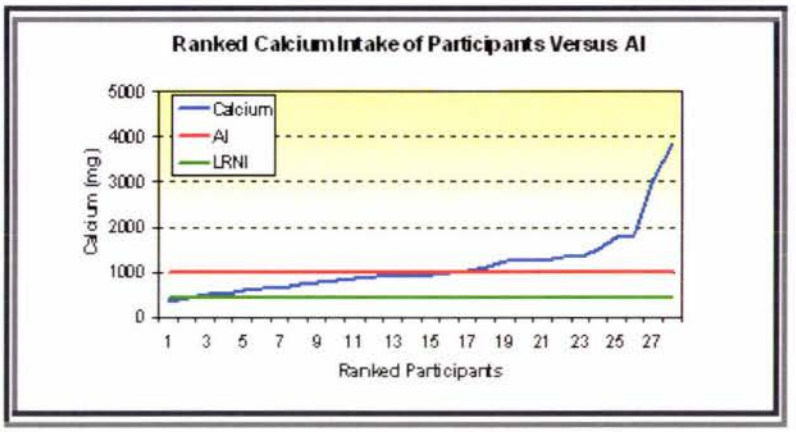


There are no available Recommended Dietary Allowances for Calcium, so Adequate Intake levels are used as goals for individual intake instead (IOM, 1997). Five (17.9%) of the study participants were below two thirds of the Adequate Intakes for calcium, and one (3.6%) subject consumed less than the Lower Reference Nutrient Intakes.

Calcium has a role as a second messenger, for example excitation-contraction of muscle fibres and nerve impulses (Linder, 1991; Chow, 2000); in protein activation, for example in blood clotting enzymes (Chow, 2000); and is an important component of mineralised tissue, such as bone and teeth, around 99% of the body's calcium is found in the skeleton (Chow, 2000; Matheson, 2002). Calcium is an important nutrient for RTM as Ca^{2+} ions have an important role in muscle fibres, where it initiates muscle contraction (Billeter & Hoppeler, 1992; Bowers & Fox, 1988; Gordon, *et. al.*, 2000), and in motor neurons for nerve signalling during physical activity (Haymes & Clarkson, 1998).

An individual in negative calcium balance long term will lose calcium from bone mineral stores, leading to decreased bone mineral density and development of osteoporosis later in life (Chow, 2000). This study also found high sodium and protein intakes in study participants, both of these nutrients are known to increase calcium excretion from the body; combined with low calcium intake this may increase the risk of developing osteoporosis. The low calcium intake of some study participants may lead to deleterious effects on bone density and muscle contraction, which in turn would affect exercise performance (Driskell, 2000). Participant intakes of dietary calcium have been represented in Figure 4.22, over the page.

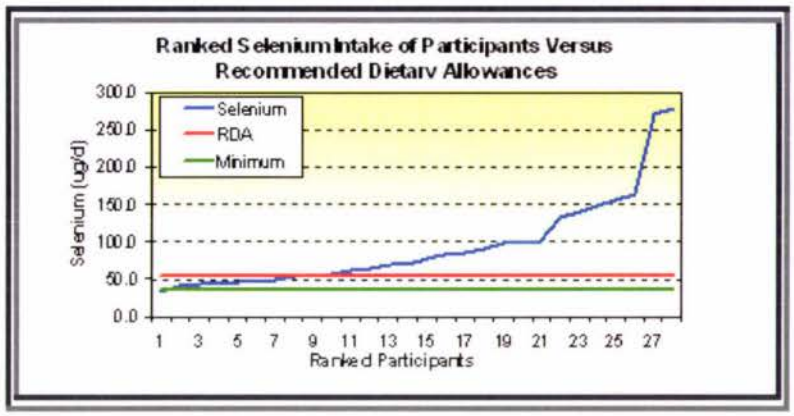
Figure 4.22: Comparison of Participant Calcium Intakes with Adequate Intakes



New Zealand has low soil selenium levels, low levels of selenium in plant and animal foods grown on New Zealand soils, and therefore intakes of New Zealanders (20-60μg) are lower than many other countries (Thomson, 2004). The Dietary Reference Intake for males 19-30 years is 55μg/d (IOM, 2000). A realistic goal for New Zealanders is thought to be a minimum intake of 35μg/d, the level required to reach two thirds maximum glutathione peroxidase activity in the blood (Matheson, 2002). Only one study participant failed to reach the minimum intake goal of 35μg/d selenium (Figure 4.23).

Selenium as a constituent of the many seleno-proteins has a number of important roles in the body, such as thyroid hormone, reproduction, and immune system functions (Matheson, 2002). With intense and strenuous exercise there is an increase in free radical production by the body's normal energy producing reactions (Deakin, 2000), therefore possibly increasing the requirements of antioxidants such as the seleno-protein glutathione peroxidase (Linder, 1991). In addition energy requirements are increased, so there may also be a concurrent increase in selenium requirement in energy production reactions (Deakin, 2000).

Figure 4.23: Comparison of Participant Selenium Intakes with Recommended Dietary Allowances



4.4.3 The Dietary Habits of Study Participants In Comparison with Previous Studies

Prior to starting the study, dietary recommendations were discussed individually with participants, and an information sheet given to each subject containing advice on appropriate nutrition to maximise muscle size and strength increase with resistance training, in an effort to standardize participant intakes and provide optimum conditions to maximise the action of HMB. However this effort at dietary standardisation had no effect on participants eating habits, and dietary intakes as assessed by three day diet records at the beginning and end of the study showed no significant change over the duration of the study.

Very few studies on the eating habits and dietary intakes of RTM were found. Diets of RTM were thought to be less rigid and restrictive (Kleiner, *et. al.,* 1994), and more varied than competitive bodybuilders (Sandoval & Heyward, 1991). Some studies have indicated that protein intakes of RTM were similar to that of other amateur athletes (Bazzarre, et. al., 1994) while others indicated protein intake far exceeded requirements (Pearce, 1988; Lemon, *et. al.,* 1992; Tarnopolsky, 2000). A previous study of 12 recreational resistance trained individuals indicated intakes of cholesterol were high, while intakes of fibre, zinc, and copper were generally low compared to RDA levels (Bazzarre, *et. al.,* 1994).

The fibre intake of study participants (24.9 ± 1.8g) agrees with previous study findings indicating low intakes (Bazzarre, *et. al.*, 1994). The median fibre intake found during the current study was below the recommended dietary allowance.

This study agreed with previous findings of high cholesterol intake in recreational resistance trained individuals (Bazzarre, *et. al.*, 1994); the average cholesterol intake of study participants was 424.0 ± 30.8mg.

The zinc intake of study participants (16.7 ± 1.0mg) differs to previous study findings indicating low intakes (Bazzarre, *et. al.*, 1994). The zinc intakes of present study participants were well within the recommended dietary intake range.

This study agrees with previous studies indicating many misconceptions and inaccurate nutrition knowledge amongst RTM (Philen, *et. al.*, 1992; Manore, *et. al.*, 1993; Naylor & Garg, 1996; Kleiner, 2000). The RTM in this study indicated popular media and websites (34.4%), family and friends (31.3%), and gym trainers and staff (31.3%) were the largest influences on diet and supplement use.

4.5 Supplementation

4.5.1 Supplementation Patterns Prior to the Study

The use of supplements and supplementation patterns of RTM were of specific interest as few previous studies have evaluated supplementation patterns of RTM or distinguished RTM from off-season competitive bodybuilders. The supplementation patterns cannot be compared to competitive bodybuilders, as the few studies that have been done indicate supplement use may in fact be greater in RTM than bodybuilders (Kleiner, *et. al.*, 1994)

Over half of the RTM studied were regularly taking dietary and sports supplements prior to the study. Several participants were taking more than one type of supplement, and one participant admitted to taking 7 different supplements. Protein powders were the most frequently used supplement. This finding was supported by previous studies (Pearce, 1988; Bond & Keane, 1994; Naylor & Garg, 1996). This study found creatine to be the next most popular supplement, which differs to previous studies which have found vitamin and mineral supplements to be more popular. The difference in popularity of creatine use may reflect the increased advertisement and hence popularity of creatine in recent years. Bias may have been introduced to the data on amount and frequency of supplement usage, due to the men who volunteered themselves for this supplementation study. RTM with a higher use of supplements may have been more motivated to take part in a study trialling supplements, than RTM who do not usually take supplements.

Over half of the study subjects believed that diet alone could not provide an adequate vitamin and mineral intake. It has been previously found that two of the major reasons for such supplementation patterns are the belief that vitamin and mineral requirements can not be met with food intake alone (Naylor & Garg, 1996); and that protein and amino acid supplements are required to increase muscle size and strength gains (Lemon, *et. al.*, 1992; Bond & Keane, 1994; Naylor & Garg, 1996). However, this study did not evaluate beliefs regarding the high intake and supplementation of protein found to occur in RTM.

The National Nutrition Survey showed that the most popular dietary supplements used by New Zealanders were multi-vitamin and mineral supplements, used by 19% of the population, compared to use by only 6.5% of study participants.

4.5.2 Supplementation during Study

Product Acceptability

The HMB supplement could be purchased in health stores and from supplement companies in New Zealand as Ca-HMB powder, Ca-HMB in gelatine capsules, and as a flavoured

drink mix. The taste and smell of Ca-HMB powder was found to be very strong, distinct, and unpleasant. Therefore for this study the Ca-HMB was measured into capsules to improve subject compliance. The placebo was a similar looking powder (corn starch) in identical gelatine capsules so the two supplements would be indistinguishable to subjects. Several previous studies have added the HMB supplement to capsules as in this study. However other studies have added HMB to a protein and carbohydrate shake or other flavoured drink mix (Nissen *et. al.*, 1996; Kreider, *et. al.*, 1999; Slater & Jenkins, 2000), and in some cases this altered the energy and protein content of the HMB supplemented group, causing a possible bias in results (Nissen *et. al.*, 1996; Slater & Jenkins, 2000).

Supplement Compliance

Supplementation compliance was monitored by subject recall of remaining supplement capsules during the final visit, and no significant difference was found in the number of capsules remaining between treatment groups (Table 6.1 in Appendix 11). Serum and/or urinary HMB levels were not monitored for supplement compliance, as in previous studies due to time and resource constraints (Nissen *et. al.*, 1996; Kreider *et. al.*, 1999; Gallegher, *et. al.*, 1996; Vukovich *et. al.*, 2001).

Subject supplementation compliance may have been affected by the large capsule size used (size 00), and the number of capsules required to be consumed (6 capsules per day, divided into 3 doses of 2 capsules throughout the day). In addition, compliance may have decreased due to difficulty collecting the supplements once each week, as each subject was required to pick up packets of supplements from one of three locations; the Massey University Recreation Centre, the AIT Recreation Centre, or the New Market Olympic Pools and Fitness Centre. Each participant was aware of the days and times the supplement capsules were delivered to these venues, and were encouraged to meet with the researcher. The subjects often failed to do this, and collected their capsules later.

Participants were asked to discontinue use of other sports supplements prior to beginning the study period, including other HMB products, protein powders, protein shakes, protein bars, and products containing creatine to reduce the possibility of a synergistic effect on HMB from other sports supplements. This was explained to subjects both verbally and was included in the written information sheets all subjects received. However whilst analysing the dietary food records, it was discovered that seven subjects had continued to use protein powders and bars, and one subject had consumed a sports drink containing creatine and leucine. The subject consuming leucine and creatine was subsequently removed from the study. The use of protein supplements by a few of the subjects may introduce a bias when analysing food records and habitual protein intakes of RTM during this study. Protein supplementation should not influence the effect of HMB on strength or body composition during this study, as two previous studies found no synergistic effect of protein and HMB supplementation (Nissen, *et. al.*, 1996; Kreider, *et. al.*, 1999).

4.6 Health and Lifestyle

One participant admitted to steroid use in the past, and was excluded from the study. The same participant was the only competitive bodybuilder in the study group. The desire to compete may have influenced his decision to take steroids. Two subjects were taking medications, which might have been affected by the HMB supplementation, and were contacted regarding this possibility.

4.6.1 Caffeine Consumption

Tea, coffee, and carbonated beverages are the most popular drinks consumed in NZ (Mattheson, 2002). More than half of the study participants (54.8%) consumed two or more caffeinated drinks per day, and two (6.3%) participants consumed five or more.

Caffeine levels greater than 5mg/kg of body mass are thought to stimulate fat metabolism during the initial 15 minutes of exercise (Hawley & Burke, 1998; Burke, *et. al.*, 2000).,

stimulate the central nervous system (Armstrong, 2002) possibly decreasing perception of fatigue, and may have a direct effect on muscle contraction (Hawley & Burke, 1998; Burke, *et. al.*, 2000).

There is a risk of large doses of caffeine and abuse of diuretics in competitive bodybuilders, who utilise caffeine containing products to stimulate diuresis and enhance muscle definition prior to competition (Armstrong, 2002). This study suggests that high caffeine consumption may also be cause for concern in these RTM when high caffeinated beverage consumption was combined with use of a fat burner supplements containing high amounts of caffeine. Two subjects (17.5%) regularly used "fat burner supplements" prior to the study, one subject usually consumed 2-4 caffeinated beverages per day in addition to this, and the other subject consumed five or more caffeinated beverages. The caffeine content of beverages such as coffee, tea, and carbonated drinks ranges from 30-100mg. "Fat burner supplements" may contain 100-200mg caffeine per serve (Burke, *et. al.*, 2000), and 1-3 serves per day are recommended. The National Heart Foundation of New Zealand recommends that consumption of caffeinated beverages should not exceed the equivalent of five cups of coffee (Mattheson, 2002). There is increased risk of side effects at 9-13mg/kg body mass such as headaches, insomnia, and gastrointestinal problems (Hawley & Burke, 1998). Recent research indicates an influence of caffeine on plasma homocysteine concentration, and a possible relationship between caffeine and increased blood pressure in hypertensive individuals (Mattheson, 2002).

4.6.2 Smoking Habits

Four (12.9%) study participants were smokers. There was no significant difference in distribution of smokers between the treatment groups; in addition there were no correlations found between smoking and strength performance during this study. This differs from previous studies that have found detrimental effects of cigarette smoking on performance such as greater perceived exertion, symptoms of fatigue, exhaustion, shortness of breath, and leg pain with exercise compared to non-smokers (Huie, 1996). It was unlikely with the low number of smokers and low smoking frequency in this study that any significant relationship would be found.

4.6.3 Alcohol Consumption

Alcohol is considered to be one of the most commonly used drugs in New Zealand (Matheson, 2002). The Alcohol Advisory Council of New Zealand advises men to drink no more than 21 standard drinks per week, and no more than 6 standard drinks per drinking session. Binge drinking is defined as males consuming more than six standard drinks in a single drinking session (Matheson, 2002).

The percentage of study participants who consumed alcohol (78.1%) was actually less than expected, based on the percentage of New Zealand males who drink alcohol (88%) (Matheson, 2002). However the percentage of study participants who binge drank (25%), was greater than New Zealand population norms (17%). There are several reasons for these differences, as alcohol intake patterns tend to vary with age, gender, ethnicity (Matheson, 2002), current enrolment in tertiary education, and involvement in sport (Leichliter, *et. al.*, 1998; O'Brien & Lyons, 2000). In addition young males between the ages 18-24 years have an increased incidence of problem drinking (O'Brien & Lyons; 2000 Matheson, 2002).

The alcohol consumption of participants was not thought to adversely affect the results of the present study. There was no significant difference found in alcohol consumption between the treatment groups, no relationship between amount of alcohol usually consumed and strength or strength change during the study, and no reduction in dietary carbohydrate intake due to high alcohol consumption, a concern in some studies (Burke, 1996; Burke 2001). Bias may be introduced into these results in two ways; both serve to underestimate actual quantities of alcohol consumed. A standard drink contains 10g of alcohol, generally less than the usual serving per alcoholic beverage. In addition, self reported alcohol intakes are also thought to underestimate actual intakes (Matheson, 2002).

Alcohol is an ergolytic or performance impairing drug (O'Brien & Lyons, 2000). Alcohol consumption 24 hours prior to physical activity has been shown to have a significant negative effect on aerobic performance, dehydration, and decreased muscle glycogen levels

due to inhibition of gluconeogenesis by alcohol dehydrogenase (O'Brien & Lyons, 2000). Alcohol ingestion immediately prior to, and during exercise has been shown to decrease performance (O'Brien & Lyons, 2000) via impaired psychomotor skills (Ebert, 2000; Burke, 2001), vestibular (balance) system, temperature regulation (Burke, 1996), and possibly weakened heart pumping force (O'Brien & Lyons, 2000). Acute or chronic alcohol consumption decreases protein synthesis, especially protein synthesis of Type II muscle fibres (DiPasquale, 2000), repair of muscle damage and recovery (Ebert, 2000), decreases testosterone levels, and increases cortisol levels (DiPasquale, 2000). In addition, athletes who consume alcohol at least once a week have almost twice the injury rate of non-drinkers (O'Brien & Lyons, 2000).

However there were some concerns about the deleterious effects of alcohol on the long term health of study participants and their exercise performance. Two subjects went so far as to describe their drinking habits as "a blow-out of spirits, etc once a month", and "until I can't walk, and pass out". Long term health effects of excessive alcohol consumption include direct damage of organs such as brain, liver, intestines, and pancreas; increased risk of disease such as breast cancer, stroke, and cardiomyopathy; increased risk of traffic related morbidity and mortality; and increased incidence of some mental health disorders (Matheson, 2002).

4.7 Limitations of This Study

This study hoped to avoid some of the methodological problems of previous studies, in particular the limited supplementation duration of many previous studies. However due to time constraints, the supplementation period of this study was reduced to 9 weeks and not the 10-12 weeks originally planned. Another methodological problem of previous studies was low sample numbers, this study hoped to overcome this problem as well. Unfortunately, response to this study was not as high as hoped, and compliance was less than expected also. The problem with small sample numbers is the low statistical power of results.

As discussed before, a high proportion of study subjects were students. This study was advertised in numerous gyms throughout Auckland, including two on University campuses. However there was a disproportionately high response rate from students. Unfortunately this may bias the results as the dietary and exercise habits of students may not reflect that of the general population RTM.

The methods used to estimate body composition in this study also had limitations. The equation used to estimate percent body fat and fat free mass from skinfolds was the Durnin & Womersley four-site method (Hawes & Martin, 2001; Durnin & Womersley, 1974), developed on a Scottish population. However this study also included four Asian (12.5%) and two Indian subjects (6.3%), therefore reducing the validity of this equation in this instance. Body fat and fat free mass were also estimated using the bioelectrical impedance analyser, however reliability was reduced as the regression equation used by the bioelectrical impedance analyser software was unknown.

Lack of significant strength increases may have reflected low training intensity during the study. Training sessions were not supervised, and subject compliance was assessed via records kept by subjects in the training log books provided. Training loads may not have been intense enough to promote strength increase, and training logs not representative of actual training volume (Panton, *et. al.*, 2000). In addition, not all training records were returned at the end of the study, therefore training compliance reflected only those records that were returned. In addition strength testing was performed the same day as BIA testing, and after a four hour fast (required for BIA). Possible lowering or depletion of muscle glycogen stores with the four hour fast may have reduced strength performance. However the same effect would have been present in both treatment groups.

A further limitation of this study was that limb cross sectional area was not measured to estimate muscle size in response to resistance training with HMB supplementation.

Subject dietary intake was assessed using estimated food records. This is not an accurate measurement of food intake or dietary habits, merely an estimation of food intake over a defined period of time. Disadvantages of this method include; bias due to misreporting (there is thought to be a correlation between BMI increase and underreporting) (de Vries, *et. al.*, 1994); all subjects must be literate and motivated to keep records (Todd, *et. al.*, 1983); the validity of this method is unknown, and estimated energy intakes from this method is lower than doubly labelled water or supervised feeding methods (de Vries, *et. al.*, 1994); limited record periods don't include seasonal variations in food intake (Beaton, 1994); the act of recording food intake may alter subject's food intakes and underestimate usual intakes (Lee, & Nieman, 1996).

Use of food composition tables and databases introduce errors when foods chosen from the database differ in growing, storing, processing, and/or cooking methods; bioavailability of individual nutrients due to season or region; substitution of similar food for food item or recipe not contained in the database; and incorrect identification of food item in the database (Lee, & Nieman, 1996).

The FoodWorks database used to analyse and estimate the nutrient intake of participants has inherent errors associated with its use. Nutrient analysis via a food composition database gives estimations only, as the nutrients contained in food vary with agricultural practices, location, season, storing, and cooking processes. However these types of variation in nutrient content of food are random (Deakin, 2000). Foods contained in the database are limited; when information on a food or meal type was unavailable a substitute food or meal was chosen, decreasing the accuracy of the actual nutrient values (Deakin, 2000). Use of nutrition content from supplement labels was also inaccurate as labels did not represent the entire nutrient composition of the supplement (Deakin, 2000). Measurement of serving sizes and amounts actually consumed was difficult as this relied on subject estimation or measurement. Human error when entering food and measures into the database was also found to occur.

One food substitution common in this study was the use of canned tuna in brine, as there was no canned tuna in spring water in the database. This may result in an overestimation of the sodium content of the diets analysed when tuna was consumed. Another limitation found when using the FoodWorks nutrient database, were that leucine intakes could not be monitored. Leucine intake affects natural HMB production in the body. However it was unlikely that HMB production in the body would be high enough to affect the results of the study even with high leucine intakes.

Participants were asked to discontinue use of other sports supplements prior to beginning the study, and during the study period, including other HMB products, protein powders, protein shakes, protein bars, and products containing creatine to reduce the possibility of a synergistic effect on HMB from other sports supplements. However, during the study subjects were not monitored for compliance and a few subjects actually consumed protein bars and protein drinks and recorded these in their diet records. One subject had consumed a sports drink containing creatine and leucine, and his results were removed from the study. A final limitation was that the study did not measure plasma HMB concentrations for supplementation compliance, due to resource constraints. This study relied on subject recall of number of capsules remaining at the end of the 9 week supplementation period, a less accurate measure of supplementation compliance.

Chapter 5 Conclusions

Recreational resistance training is a sport redolent with misconceptions and inaccurate nutrition knowledge (Naylor & Garg, 1996), and therefore a good market for emotively advertised, yet un-trialled "sports supplements" (Philen, *et. al.*, 1992; Burke, *et. al.*, 2000). HMB may be one of these over-hyped and cleverly marketed products, as it seems that not all of the marketing claims made about HMB have been satisfactorily proven.

From the results of this study, the following conclusions have been reached:

- There was no beneficial effect of HMB supplementation on change in body composition as indicated by body weight, individual skinfolds, fat mass, fat free mass, or percent body fat. These results agree with the majority of previous studies, which also found no beneficial effect of HMB supplementation on body composition change in response to resistance exercise.

- There was a significant increase in strength with HMB supplementation in response to resistance exercise. Percent change in leg extension strength increased significantly greater (P=0.041) in the HMB group compared to the placebo group.

- There was strong resistance to change in eating habits in this group of RTM; none of the subjects significantly changed their dietary intakes during the study. This was despite individual dietary counselling and provision of a handout, on dietary recommendations appropriate for RTM, in an effort to standardise participant intakes.

- There were significant differences found in sugar, glucose, fat, saturated fat, monounsaturated fat, sodium, and calcium intakes between participants of European and non-European ethnicities. Participants of European ethnicity tended to consume greater amounts of the above nutrients.

- There were also significant differences found in consumption of percent energy from carbohydrates, percent energy from fats, intake of maltose and cholesterol between the HMB and placebo groups. The HMB group consumed significantly greater percent energy from carbohydrates, and maltose intakes.

- Most (96.2%) participants consumed greater protein than the Recommended Dietary Allowances (0.8g/kg/d), and more than half (60.7%) also consumed greater protein than study recommendations for RTM (1-1.4g/kg/d). Two participants consumed greater than four times the Recommended Dietary Intake (>2.8g/kg/d). Excessive protein intakes were not as common as predicted prior to this study.

- Most (71.4%) participants failed to consume within the Acceptable Macronutrient Distribution Ranges (45-65%) of total energy as carbohydrates. Most participants (85.7%) also failed to reach the study recommendations (5-7g/kg/d) for RTM.

- Eleven (39.3%) of the study participants failed to consume two thirds of the Adequate Intakes of fibre.

- Quarter (25.0%) of the study participants consumed less than the Recommended Dietary Allowances of Vitamin A, and two (7.1%) participants consumed less than the Lower Reference Nutrient Intake (300μg). This study indicates a greater incidence of inadequate vitamin A status than the 1997 National Nutrition Survey of the New Zealand population.

- Six (21.4%) of participants consumed below two thirds of the Recommended Dietary Allowances of Vitamin C.

- Eleven (39.3%) of participants consumed less than the Recommended Dietary Intake of vitamin E (10mg), and 17.9% failed to consume two thirds of the Recommended Dietary Intake.

- One (3.6%) participant consumed less than two thirds of the Recommended Dietary Allowances and less than the Lower Reference Nutrient Intake of vitamin B6 (1.0mg).

- Six (21.4%) of participants consumed below two thirds of the Recommended Dietary Allowances of folate.

- Most (82.1%) of participants exceeded the Tolerable Upper Intake Level of sodium (2300mg).

- Six (21.4%) subjects failed to consume two thirds of the Adequate Intakes, and one (3.6%) subject failed to consume the Lower Reference Nutrient Intake of potassium (2000mg), due to low total energy intake.

- Four (14.3%) subjects consumed less than two thirds of the Recommended Dietary Allowances of magnesium and one subject (3.6%) consumed less than the Lower Reference Nutrient Intake of magnesium (190mg).

- Five (17.9%) participants failed to consume two thirds of the Adequate Intakes of calcium and one subject (3.6%) failed to consume the Lower Reference Nutrient Intake of calcium (400mg).

- One (3.6%) subject failed to consume the minimum selenium intake recommended to New Zealanders (35μg) to reach two thirds of maximum plasma glutathione peroxidase activity. The same subject also consumed below two thirds of the Recommended Dietary Allowances.

- This group of RTM risk some potential health risks associated with their habitual alcohol intakes and binge drinking habits. The majority (78%) of participants regularly consume alcohol, and one quarter (25%) consume greater than six standard drinks per drinking session on average.

- This study also highlighted some potential health risks associated with habitual caffeine intakes. There was some risk of excessive caffeine consumption when "fat burner" supplements were combined with high caffeinated beverage consumption. Two (6.3%) participants were found to consume five or more cups of caffeinated beverages per day. The deleterious effects on long term health and resistance exercise performance may be prevented by increasing the awareness of this potential problem in RTM.

5.1 Study Recommendations

From the results of this study and previous studies in the literature review, several recommendations can be made to improve the nutritional status of RTM for both long term health and optimal resistance exercise performance.

- Results from this study suggest that supplementing with HMB in trained individuals may act to improve strength in response to resistance training.

- RTM need to be made more aware of the recommended protein intakes to promote muscle hypertrophy in response to resistance exercise, these intakes are easily achievable in a normal New Zealand diet, and protein intakes above these recommendations will not promote additional muscle hypertrophy. In addition some education on the dangers associated with excess protein intake is advisable.

- RTM need to be educated on the optimal carbohydrate intakes required to promote long term health and for fuelling and recovery following resistance exercise, and the dangers to health of not abiding by these.

- Data obtained during this study on the dietary intakes of RTM indicate, RTM should be encouraged to consume a diet providing adequate sources of vitamin A, vitamin C, vitamin E, vitamin B6, folate, potassium, magnesium, calcium, and selenium; the vitamins and minerals found to be lacking in the diets of some study participants. In addition RTM need to be made aware of the importance of adequate vitamin and mineral intakes for long term health, as well as exercise performance. RTM may need to be educated on the food sources of these nutrients and how to include them in a healthy and varied diet.

- RTM need to be made more aware of the risks of excessive cholesterol consumption, and the sources of cholesterol in their diets. The high cholesterol intake of most participants and excessively high intake of some participants may have an appreciable effect on their blood cholesterol (Mann, 2000). High cholesterol intake combined with high sodium, inadequate fibre and vitamin E intakes found in over half of study participants may lead to the development of atherosclerosis and heart disease or stroke over time (Mann, 2000).

- RTM need to be made more aware of the risks of excessive sodium consumption, and the sources of sodium in their diets. The high sodium intake of some participants puts them at risk of increased calcium urinary excretion (Matheson, 2002). High sodium intake combined with high protein and low calcium intakes of participants may produce negative calcium balance, and over time lead to decreased bone mineral density and osteoporosis. High sodium intake combined with intense resistance exercise also puts participants at increased risk of hypernatremia leading to dehydration, oedema, high blood pressure, and at worst, brain stem injury (Driskell, 2000).

- RTM need to be made more aware of the risks of excessive caffeine consumption, and the sources of caffeine in their diets.

- RTM need to be made aware of the adverse effects of alcohol consumption on general health and resistance exercise performance. Education of RTM on the adverse effects of alcohol on resistance training performance and retardation of muscle protein synthesis may help to improve their nutrition status and long term health. In addition education of university students on the dangers of binging on alcohol may improve their nutrition status and health.

5.2 Further Research

Further research is required to assess the diets of resistance trained individuals to confirm the potential deficiencies, excesses, and other nutritional problems associated with this group, found in this study. The inclusion of women would indicate any gender effects on the diets and exercise habits of resistance trained individuals. It is supposed that the motivation for performing resistance exercise may differ between the genders, and therefore this may affect the resistance training, other exercise, and eating habits of resistance trained individuals.

Further study of HMB supplementation in RTM should include some estimation or measure of muscle growth during the supplementation period, for instance measurement of limb cross sectional area, Dual X-Ray Absorptiometry (DEXA), Computed Tomography (CT), and Magnetic Resonance Imaging (MRI).

Further research is also required to elucidate any effect or relationship between HMB supplementation, endurance exercise, and immune function. A few studies have investigated the effects of HMB supplementation on markers of muscle damage in endurance exercise, and the effects of HMB supplementation have been investigated in animals in stressed conditions, however little has been done on the possible effects of HMB on immune system function in humans during stressed conditions. Therefore it may be useful to investigate the effects of a period of HMB supplementation on markers of immune system function and muscle damage, following a marathon or other endurance event.

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Appendix 1

6.1 Ethics Application

(Revised 3 July 2001) **AKL/PN/WGTN Protocol**

MASSEY UNIVERSITY HUMAN ETHICS COMMITTEE

To:		OR		OR	
	Secretary Human Ethics Committee Principal's Office Albany, Auckland		Secretary Human Ethics Committee Old Main Building Turitea, Palmerston North		Secretary Human Ethics Committee Block 5 Wellington

Please send/deliver this original (1) application plus eleven (11) copies

APPLICATION FOR APPROVAL OF PROPOSED TEACHING/RESEARCH PROCEDURES INVOLVING HUMAN SUBJECTS

APPLICANT(S):

Name: Patsy Watson for Jasmine Thomson

Department: Institute of Food Nutrition and Human Health, Albany Campus

Contact Email/Number:p.watson@massey.ac.nz

Status: Programme Leader in Human Nutrition
(e.g. lecturer, PhD/masterate student)

Name of Employer: Massey University

PROJECT:

Title: Pilot study of HMB supplementation of resistance trained men.

Status: Masterate
(e.g. staff research, doctorate/masterate)

Funding Source: Not applicable

Clinical Trial Status: yes ❑ no ❑***

ATTACHMENTS:
(e.g. Information Sheet(s), Consent Form(s), Questionnaire, etc)

Information sheets, Consent forms, Data collection sheets, Questionnaires, Feedback forms.

SUPERVISOR(S):

Name: Patsy Watson, Megan Merrilees (Assistant)

Department: Institute of Food Nutrition and Human Health, Albany Campus

SIGNATURE(S):

Applicant(s): .....

Supervisor(s): P E Watson .....

(required for all projects involving student research, implies satisfaction with application)

HUMAN ETHICS APPLICATION

1. DESCRIPTION

1.1. Justification

This study will compare a group of 25 body builders given the dietary supplement, HMB (beta-hydroxy beta-methyl butyrate), with a control group of 20 bodybuilders given a placebo, in a randomised fashion, over the 10-week study period. The supplementation group will receive 1g of HMB, 3 times per day in a capsule. The control group will receive a similar capsule 3 times per day containing a safe inert substance such as starch. The same weight-training programme will be performed by all participants at the same frequency and intensity, and dietary intake will be standardized as much as possible. Results for muscle strength, body composition, and body fat distribution will be compared in both groups.

HMB is produced naturally in the body during the metabolism of the amino acid leucine. Leucine occurs naturally in the diet in foods such as dairy products, meat, fish, eggs, cereals and fruits. Normal production of HMB in the body is thought to be around 0.2-0.4 grams per day, depending on the leucine content of the diet. HMB also occurs in small amounts in the diet, in such foods as catfish, and some citrus fruits.

Commercial advertising for the HMB dietary supplement has been aimed at strength athletes, with claims of; increased anabolic effects from exercise therefore increased gains in strength and muscle mass, increased fat oxidation by muscles causing increased body fat loss with exercise, and anti-catabolic activity and therefore inhibition of muscle tissue breakdown with intense exercise.

Many studies of HMB have been done prior to this study. Several studies of HMB supplementation in animals have shown increases in lean muscle mass, but HMB supplementation in humans has shown indeterminate evidence. There have been concerns regarding the methodology of previous human supplementation trials such as short study period, small study population numbers, lack of dietary control during the study, use of untrained subjects or unspecified length of previous training experience. The purpose of this study is to validate or dispute claims of increased strength and muscle mass gain, and body fat loss, with exercise, due to supplementation with HMB in resistance trained men.

Previous problems in study methodology hope to be avoided by increasing the study period, increasing the study population number, assessing subjects dietary intakes, using previously trained subjects (at least one year of weight training experience) to avoid any training effect, and randomly allocating participants to the supplementation or control group.

1.2. Objectives

The objectives of this study follow:

- To assess and compare muscular strength in the HMB and control groups before, during and at the end of a 10-week weight training programme.
- To assess and compare body composition in the HMB and control groups before, during and at the end of a 10-week weight training programme.
- To assess and compare body fat distribution in the HMB and control groups before, during and at the end of a 10-week weight training programme.
- To assess the effectiveness of the standardization of the dietary intake regime.
- To determine whether HMB does indeed increase muscle strength and mass in resistance trained men.

1.3. Procedures for Recruiting Participants and Obtaining Informed Consent

Jasmine will contact the New Zealand Federation of Body Builders' committee about advertising the study in their monthly Auckland newsletter. Flyers and brochures will be distributed throughout Auckland gyms and health centres (See pp.1-3). People who are interested in participating may contact the researcher by phone, or post forms included with the brochure, to request more information. The researcher will mail a detailed outline of the study to all those interested (See pp.4 - 8), along with an 'invitation to participate' letter (See p.9), 'consent' form (See pp.10 - 11), and stamped and self-addressed envelope. People wishing to participate may contact the researcher by phone or return the completed 'consent' forms. People who contact the researcher by phone wishing to participate, will have their signed consent forms collected by the researcher on her first visit. A non-random convenience sample of 45 men with previous weight training experience of greater than one year will be recruited.

1.4. Procedures in which Research Participants will be Involved

There will be four visits to each study subject. The procedures carried out during each visit are outlined below.

Week 0,Visit One (approximately one hour required)

- A 3-day dietary record sheet will be given out to each study participant, which will be collected in a week time, at the next visit. The researcher will explain what the dietary record sheet involves, and how to fill them out to each participant (See pp.12 - 14).
- Training log books will be made up for each participant for weeks one to five, and handed out during visit one. The weight-training programme has been designed by a qualified Personal Trainer (Network Fitness New Zealand NZQA accredited). The programme will be explained individually, including instruction on how to perform each exercise. This will enable all subjects to familiarise themselves with the exercises, and weight loads required, before commencing the programme (See pp. 15- 43).
- Muscular strength will be measured during a series of trials assessing grip strength, back strength, and leg strength. These trials will be done in a safe environment and under supervision, using hand and back/leg dynamometers, which are safe and easy to use (See pp.44 - 45).

Week 1,Visit Two (approximately one hour required)

- A questionnaire will be administered to assess demographic details, eating and exercise habits, and previous supplement usage (See pp.46 -62).
- The 3-day dietary records will be assessed and dietary aims for the study duration will be discussed individually (See pp. 12-14).
- The following anthropometric measurements will be taken; eight skinfold measurements will be made using skinfold calipers (this causes a slight pinching of the skin, but no pain), girth (circumference) measurements, bone breadths, height, and body weight will also be measured (See pp. 63 - 64).
- Bioelectrical Impedance (BIA), used to assess body composition, will be measured. This device uses electrodes placed on the hands and feet, to transmit a minute current throughout the body. The measurement takes only a few minutes, and is completely safe and painless (See p.65).
- Each subject will be given a 10 week supply of either the placebo capsule or the HMB capsule depending on whether they are in the control or intervention group.

Week 5,Visit Three (approximately one hour required)

- Assessment of muscular strength (as in visit 1) (See pp.66 - 67).
- Training log books will be made up for each participant for weeks six to ten, and distributed during this visit. The weight-training programme will again be explained individually, including instruction on how to perform each exercise, if required (See pp.15 - 43).

Week 11,Visit Four (approximately one hour required)

- 3-day dietary records (as in visit 1) (See pp.12 - 14).
- Anthropometric measurements (as in visit 2) (See pp.68 - 69).
- Bioelectrical Impedance Assessment (as in visit 2) (See p.70).
- Assessment of muscular strength (as in visit 1) (See pp.71 - 72).

1.5. Procedures for Handling Information and Material Produced in the Course of Research, Including Raw Data and Final Research Reports

All information will be collected as hard copy, no audio or video records will be used.

All participants will be given a code number. A separate master file will be kept, linking subject name and address to code numbers. This master file will be kept under lock and key and stored in a separate location to other study data. Only the supervisor and researcher will have access to this list.

Data collection forms will be identified by code number only. When organising interviews, the researcher will place removable name and address labels on each subject's data collection forms. These labels will be removed and destroyed once the interview in complete and data collected. All data will be stored on the researcher's hard drive or personal H: drive on the network and will be accessible by password only, by the researcher or her supervisor. The password will be changed regularly to maintain security. All completed data collection forms will be stored in locked filing cabinets in the nutrition research rooms, which is locked and alarmed when no one is present

No subject will be identified either by name or code number in the final research report, or in any conference presentations or scientific papers that may result from this work.

Each subject will receive a brief summary of the findings of the study, and individual fitness assessments, when complete (See pp.73 - 80).

1.6. Procedures for Sharing Information with Research Participants

At the completion of the study, each subject will receive a summary of their personal results, dietary assessments, body measurements, and strength measurements (See pp.73 - 80).

1.7. Arrangements for Storage and Security, Return, Disposal or Destruction of Data

The hard copy of all data will be kept in a locked data storage room, in a building fitted with a burglar alarm. Only authorised personnel have access to this room. The file connecting the subject's names, addresses, and phone numbers, will be kept in a separate file in a separate room, under lock and key by Patsy Watson, Programme Leader in Human Nutrition. The subject's identity will not be revealed in any results, thesis, or research papers resulting from this work. Original data will be destroyed after 10 years.

2. ETHICAL CONCERNS

2.1. Access to Participants

People who are interested in participating may contact the researcher by phone, or post forms included with the brochure, to request more information. The researcher will mail a detailed outline of the study to all those interested (See pp.4 - 8), along with an 'invitation to participate' letter (See p.9), 'consent' form (See pp.10 - 11), and stamped and self-addressed envelope. People wishing to participate may contact the researcher by phone or return the completed 'consent' forms. People who contact the researcher by phone wishing to participate, will have their signed consent forms collected by the researcher on her first visit. The information sheet (See pp.4 - 8) will explain the rights of all participants, including the right to decline to take part in any part of the study, at any time. Assurance of confidentiality is clearly stated. Participants will have the opportunity to ask questions of the researcher and/or supervisor at any time, before signing the consent form.

The consent form will be signed by the participant in the presence of a witness (other than the researcher), and by the above witness. The consent form includes the name of the researcher and her supervisor (See pp.10 - 11).

2.3. Anonymity and Confidentiality

The measures taken in 1.5 and 1.7 will be used to ensure the anonymity and confidentiality of all participants.

2.4. Potential Harm to Participants

No adverse effects have been found in previous studies of HMB supplementation in humans, using either acute or chronic supplementation patterns. HMB is a legal supplement for use in sports, accepted by the International Olympic Committee (IOC). The training log book, given out to all participants, includes a section on each page for recording any adverse effects experienced by participant during any stage of the study. Participants are advised to immediately contact the researchers if any adverse side-effects are experienced, so that the cause may be determined. If side-effects continue, participants will be advised to withdraw from the study.

There is no risk of harm to any of the participants involved in this study, if all exercises are conducted in a safe environment and according to instructions given. The weight-training programme has been designed by a qualified Personal Trainer (Network Fitness New Zealand NZQA accredited), who has also checked all exercise descriptions to ensure the instructions are clear, and the exercises are safe to perform. The researcher herself, has already followed the weight training programmes that will be used in the study for about six weeks without harm or any side effects. If any participant is injured during the course of this study, they will be covered by ACC.

All strength tests will be supervised, and done in a controlled manner. When the skinfold measurements are taken, only a slight pressure is felt by subjects. The Bioelectrical Impedance Assessment transmits a minute current throughout the body, only taking a few minutes, and is completely safe and painless.

All participants have the right to decline to take part in any part of the study if they feel uneasy.

2.5. Potential Harm to Researcher(s)

The study methodology involves no harm to the researcher. All mail will be forwarded through an address at the university, and the researcher's home address will not be made known to participants. In case of emergency, the researcher will carry a mobile phone when she visits subjects in the study. If she feels threatened, she will leave the premises immediately.

2.6. Potential Harm to the University

This study can bring no potential harm to the university. The strict anonymity, confidentiality and professional attitude during collection and handling of data should avoid any potential embarrassment to the University.

The dietary supplement HMB will be provided in powder form by Musashi, including certificates of analysis of purity, at reduced price to the University for the purposes of this study.

2.7. Participant's Right to Decline to Take Part

The information sheet and consent form, clearly state that the participant can decline to take part in the study, can decline to take part in any section of the study, can decline to answer any question, or can withdraw from the study, at any time. This message will be repeated verbally during phone calls and

visits during the study period. Experience has shown us that if a subject decides to withdraw from a study after it has started, and this is declined, they are no longer cooperative and their results are affected and cannot be used. Hence we prefer to let them leave at any time, although this is inconvenient to the researcher.

2.8. Uses of the Information

The information obtained from the study will be analysed and written up as a research report (thesis), presented as a conference paper, and if suitable written up as a paper for publication in a scientific journal. Each participant will receive a brief summary of his individual results, and study results as a whole.

2.9. Conflict of Interest/Conflict of Roles

There is no conflict of interest for either the researcher or the supervisor involved in this study.

2.10. Other Ethical Concerns

If the researcher finds any participant with any serious social or family problems, she will listen attentively, and then refer the participant involved to the appropriate support organisations. If the researcher finds any participant with serious financial problems, she will refer the participant involved to Work and Income Support Services. If the researcher finds any participant with any health problems, she will suggest the participant involved see their General Practitioner.

3. LEGAL CONCERNS

3.1. Legislation

3.1.1. Intellectual Property Legislation e.g. Copyright Act 1994

Any scientific material will be appropriately referenced. The data collected will belong to Massey University.

3.1.2. Human Rights Act 1993

Any questions and procedures have been carefully designed to contain no verbal or physical abuse, and contain no insulting or derogatory remarks directed at any section of the community.

3.1.3. Privacy Act 1993

The information required will be collected directly from the participant, and recorded as hard copy. No video or audio records will be used. Measures to ensure confidentiality for participants are detailed in sections 1.5 and 1.7. These confidentiality measures will also cover those who choose to withdraw from the study at any stage.

The information collected will only be used for the purposes outlined in the information sheet. Publications will contain none of the participant's names or any information that may identify then. Massey University is clearly identified as the body responsible for this study.

3.1.4. Health and Safety in Employment Act 1992

No potential health hazards are foreseen.

3.1.5. Accident Rehabilitation Compensation Insurance Act 1992

The researcher will be covered by ACC.

3.1.6. Employment Contracts Act 1991
Not applicable.

3.2. Other Legal Issues

Not applicable.

4. CULTURAL CONCERNS

We don't see any cultural concerns or conflicts being raised with this research.

5. OTHER ETHICAL BODIES RELEVANT TO THIS RESEARCH

5.1. Ethics Committees

None.

5.2. Professional Codes

Not applicable.

6. OTHER RELEVANT ISSUES

None that we perceive at this time.

Watson, Patsy

From: Turner, Merle
Sent: Friday, 26 July 2002 13:44
To: Watson, Patsy
Cc: Chamberlain, Kerry
Subject: HUMAN ETHICS APPROVAL APPLICATION: MUAHEC 02/063

"Pilot Study of HMB Supplementation of Resistance Trained Men"
Jasmine Thomson
Department: Institute of Food Nutrition and Human Health
Supervisors: Patsy Watson, Megan Merilees

Thank you for the above protocol which was received and considered by the Massey University, Albany Campus, Human Ethics Committee at its meeting held on 25 July 2002.

The Committee would like to commend you on your full and detailed application.

Your protocol is provisionally approved, subject to you confirming formally

1. That there is no conflict of interest between the researcher and Musashi as suppliers of HMB.

2. That this is clearly not a clinical trial, and participants are covered by ACC.

Please note that from 2002, the Committee is requiring that the following statement be included at the bottom of Information Sheets:
"This project has been reviewed and approved by the Massey University Regional Human Ethics Committee, Albany Campus, Protocol MUAHEC 02/063. If you have any concerns about the conduct of this research, please contact Associate-Professor Kerry Chamberlain, Chair, Massey University Regional Human Ethics Committee, Albany, telephone 09 443 9799, email K.Chamberlain@massey.ac.nz."

Yours sincerely

Associate-Professor Kerry Chamberlain, Chair

Massey University Regional Human Ethics Committee: Albany

Merle Turner
Secretary
Massey University Human Ethics Committee, Albany
Study Centre, Massey University at Albany
ph (09) 443 9799, extn 9539
Fax (09) 414 0814, internal 9414
email M.L.Turner@massey.ac.nz
www.massey.ac.nz/~muhec

2 August, 2002

Ms Merle Turner,
Secretary
Massey University Human Ethics Committee,
Albany Campus

Dear Merle,

Re: MUAHEC 02/063 'Pilot study of HMB Supplementation of Resistance trained men.' – Jasmine Thomson

Thanks you very much for your email of 26/7/02. We appreciate the comments of the ethics committee, and answer them as follows.

1. I confirm that there is no conflict of interest between the researcher and Musashi as suppliers of HMB. Musashi has agreed to supply the HMB at cost price, and have asked for nothing in return as they feel the advantages of their product are well documented. However we are in the process of developing a memorandum of understanding with Musashi via the research office to protect the intellectual property of the researcher and the University, in case Musashi change their mind and ask to use the results.
2. This project involves a nutritional intervention, and is not a clinical trial. All subjects will be covered by ACC.
3. The statement that the ethics committee has reviewed the project has been included at the bottom of the information sheets. (See attached pp. 1,3,8)

Thanks you for your consideration. We look forward to receiving your approval for our project.

Yours sincerely,

Patsy Watson
Programme Leader in Human Nutrition.

Massey University

Office of the Principal
Massey University
Albany Campus
Private Bag 102 904,
North Shore MSC,
Auckland, New Zealand
Principal: 64 9 443 9700 ext
Regional Registrar: 64 9 443
ext 9516
Facsimile: 64 9 414 0814

6 August 2002

Jasmine Thomson
C/o Patsy Watson
Institute of Food Nutrition and Human Health
Massey University
Albany

Dear Jasmine

HUMAN ETHICS APPROVAL APPLICATION – MUAHEC 01/063
"Pilot Study of HMB Supplementation of Resistance Trained Men"

Thank you for your application. It has been fully considered, and approved by the Massey University, Albany Campus, Human Ethics Committee.

If you make any significant departure from the Application as approved then you should return this project to the Human Ethics Committee, Albany Campus, for further consideration and approval.

Yours sincerely

Associate-Professor Kerry Chamberlain
Chairperson,
Human Ethics Committee
Albany Campus

cc Patsy Watson
Institute of Food Nutrition and Human Health,
Massey University, Albany

Appendix 2

6.2 Study Flyer and Brochure

WANTED

MEN AGED 20-30 YEARS
WHO WANT TO GAIN LEAN MUSCLE!!

Have you ever wondered if all those expensive 'body-building' supplements actually work? Can you trust supplement companies using persuasive advertising gimmicks such as 'before and after' photos rather than real scientific evidence?

FREE 10-WEEK WEIGHT TRAINING PROGRAMME

Designed by a qualified personal trainer from Les Mills

FREE STRENGTH TESTING

FREE DIETARY ASSESSMENT

FREE BODY FAT AND SKINFOLD MEASUREMENTS

Using BIA Instrumentation and ISAK approved skinfold callipers

If you are male, aged between 20-30 years, and have been training with weights for at least 1 year, I need you. My name is Jasmine Thomson, and as part of my Master in Science degree in Human Nutrition, I will be researching **HMB** supplementation of resistance trained men. In addition to all of the above, you may also receive **free HMB** supplementation for the duration of this study. See the **free** brochure for more details, or

Contact Details

Name _____

Address _____

_____

_____

Phone (home) _____

Phone (work) _____

For more information, please send this completed form to;

Jasmine Thomson
C/O Patsy Watson
Programme Leader in Human Nutrition
Institute for Food, Nutrition, & Human Health
Massey University
Albany Campus
Private Bag 102 904
North Shore Mail Centre
AUCKLAND

What will I have to do during the study?

More good news!

To study the effects of HMB supplementation, we are asking for 45 volunteers to participate in a 10-12 week study. Each participant should be male, aged from 20-30 years, with at least 1 year weight training experience.

Each of you will receive our 10-week weight training programme, designed by a qualified personal trainer, with the aim of increasing muscle mass and strength. Also, at certain stages throughout the study period, we will do free strength testing using dynamometers, free body fat and skinfold measurements using BIA instrumentation and ISAK approved skinfold callipers, and free dietary assessment and advice.

But wait! There's more! 25 volunteers will get free HMB supplementation (20 will receive a placebo).

MASSEY UNIVERSITY

INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH

Pilot Study of HMB Supplementation of Resistance Trained Men

Wanted
Men Aged 20-30 Years Who Want to Gain Lean Muscle!

What is HMB?

The full name of this product is beta-hydroxy beta-methylbutyrate. HMB is produced naturally in the body when the amino acid leucine is metabolised. The body only produces about 0.2 to 0.4 grams of HMB per day, and this depends on how much leucine you eat. HMB is also found in low levels in some of the foods you eat, such as catfish (I don't know anybody who eats this!), and some citrus fruits.

HMB is currently being sold as a 'body-building' supplement in health and nutrition stores throughout New Zealand. In fact you have probably already heard of it.

Why Study HMB?

The advertising for HMB supplements is aimed at body builders and weight lifters. HMB supplementation is claimed to;

Increase the anabolic effects of exercise, therefore increase strength and muscle gains.

Increase fat oxidation by the muscles, therefore increase body fat loss.

Have anti-catabolic activity, therefore decrease muscle tissue breakdown during intense exercise.

There is some evidence for these claims in animal studies. But human studies of HMB supplementation done so far do not conclusively support these claims.

The good news is, there is no evidence of adverse effects associated with consumption of HMB supplements in humans. And, in this study we will be using the recommended dosage of 3g/day, to ensure the safety of all involved.

Who is Doing This Research?

My name is Jasmine Thomson. As part of my Master of Science degree in Nutritional Science at Massey University, I am doing research on HMB supplementation of resistance trained men.

Patsy Watson, the Programme Leader in Human Nutrition at Massey University is also involved in all aspects of this research.

If you want to participate in this exciting study, or for more information, please contact Jasmine on;

Ph. 413 8977 Ph. 021 210 6269

Email; thomson_jasmine@xtra.co.nz

This project has been reviewed and approved by the Massey University Regional Human Ethics Committee, Albany Campus, Protocol MUAHEC 02/063. If you have any concerns about the conduct of this research, please contact Associate-Professor Kerry Chanberlain, Chair, Massey University Regional Human Ethics Committee, Albany, Telephone 09 443 9799, email K.Chamberlain@massey.ac.nz.

Appendix 3

6.3 Invitation to Participate and Study Information Sheet

Pilot Study of HMB Supplementation of Resistance Trained Men

Invitation To Participate

To _____,

As a requirement of my Master of Science Degree in Nutrition, at Massey University, Albany Campus, I am conducting a 'Pilot Study of HMB Supplementation of Resistance Trained Men'. You will have read a little about this study in the articles, notices, and brochures advertising for men who have trained with weights to participate.

Thankyou for your reply to be included as a participant in this study. This letter is to let you know that you fulfil the criteria we are looking for, and should you accept this invitation you will be randomly selected for either the HMB or placebo supplementation groups. This letter is an invitation, and as an invitation, you have a choice of accepting or declining to take part.

Attached is an information sheet for participants, that outlines what is required of you during the 10-week study period. Please read through this thoroughly before making your decision.

I will contact you over the next week to confirm your decision. If you have any further questions, please feel free to contact me on [redacted]

Thankyou for your time.

Yours Sincerely

Jasmine Thomson

MASSEY
UNIVERSITY

INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH

Pilot Study of HMB Supplementation of Resistance Trained Men

STUDY OUTLINE FOR PARTICIPANTS

This Pilot Study is being conducted by Jasmine Thomson, as part of her Master of Science degree in Nutritional Science, at Massey University, Albany campus. Patsy Watson, Programme Leader in Human Nutrition at Massey University, will be involved in the supervision of all aspects of this study.

HMB (BETA-HYDROXY BETA-METHYLBUTYRATE) DIETARY SUPPLEMENT

HMB (beta-hydroxy beta-methylbutyrate) is produced naturally in the body during metabolism of the amino acid leucine. Leucine occurs naturally in the diet in foods such as dairy products, meat, fish, eggs, nuts, chocolate, cereals, vegetables, and fruits. Normal production of HMB by the body is thought to be from 0.2 to 0.4 grams per day, depending on the amounts of leucine in the diet. HMB may also occur naturally in the diet in small amounts, in foods such as catfish, and some citrus fruits.

The dietary supplement HMB, has recently become available over the last few years in health and nutrition stores throughout New Zealand. HMB advertising has been aimed at weight lifters and body builders, with claims of:

- Increased anabolic effects of exercise, therefore increased gains in strength and muscle mass.
- Increased fat oxidation by the muscles causing increased body fat loss with exercise.
- Anti-catabolic activity, therefore inhibition of muscle tissue breakdown during intense exercise.
- A possible role in the immune system enhancing muscle recovery from exercise.

Several animal studies have shown HMB to increase lean muscle mass. But, overseas studies of HMB supplementation in humans has been equivocal, some support the above claims, others do not. Concern has been raised about the methodology of some of these studies.

No adverse effects of HMB supplementation have been reported, either using acute (large amounts over a short time) or chronic (smaller amounts over long periods) supplementation patterns. HMB is a legal supplement for sports use. It is not banned by the International Olympic Committee (IOC).

STUDY OUTLINE

The aim of this study is to find the effect of HMB supplementation, if any, on gain of fat free mass, compared to a placebo. We are using resistance trained men rather than sedentary individuals (starting a resistance training programme), as we feel that the HMB supplement is marketed to this group.

All participants of the study will be given the same training programme. Regular strength and body composition measurements will be taken during the study period, as outlined below.

Participants will be randomly divided into two groups (HMB supplementation or placebo).

REQUIREMENTS OF PARTICIPANTION

The following gives an outline of the requirements of the study, and what will be involved for those of you that agree to participate. The researcher will explain the requirements of the study individually, and answer any questions you may have at each stage. Participation in this study is voluntary, and it is entirely up to you to decide if you want to be included.

The study will involve four visits by the researcher. There is an initial consultation and education visit, and three additional visits to obtain measurements and data. The study duration will be 10-12 weeks in total, 10 weeks of this involves the weight training programme.

(1). VISIT 1:

Initial Consultation and Education

The initial consultation will occur about 1 week before you begin the weight training programme.

(a). Three Day Dietary Record

A 3-day dietary record sheet will be given out to each study participant to be collected in a week time, for initial dietary assessment. The researcher will explain this to you, instructions also are included on the record sheets themselves.

(b). Weight Training Instruction

Training log books will be made up for everyone participating in the study. These will be handed out to you at the initial education session. The weight training programme will be explained individually, and instruction on how to perform each exercise will be given. This will enable you to familiarise yourself with the exercises, and experiment with weight loads to find your individual levels, before commencing the programme.

(c). Assessment of Muscular Strength

A series of trials will be performed to assess strength.

Grip Strength - This is tested using a hand dynamometer. It is a measure of static hand grip strength. The dynamometer is clenched with as much effort as possible for 2-3 seconds. Both right and left hand grips will be tested over 2-4 trials each.

Back Strength - This test is done with the back/leg dynamometer. It will be done over 2 trials, with a 1 minute rest period between each trial. You will be asked to stand on a platform, hold the dynamometer handbar, and use your back to make a lift.
Leg Strength - This test is also done with the back/leg dynamometer. It will be done over 2-3 trials, with a 1 minute rest period between each trial. You will be asked to stand on a platform, hold the dynamometer handbar, and use your legs to make a lift, similar to a squat exercise.
1 Repetition Maximum Bench Press - The one repetition maximum (1RM) is the heaviest weight you can lift for one attempt. We will select a starting weight that is close to your perceived 1RM. For each attempt where you are able to perform more than one lift, the weight is increased in 5, 2, or 1kg increments, after short rest periods. The first 1RM test will be carried out on the bench press.
1 Repetition Maximum Leg Extension - This test is also done using the 1RM principles discussed above. This second 1RM test will be carried out on the leg extension machine.

(2). VISIT 2:
Beginning of Weight Training Programme and Supplementation
The second visit will involve the following;

(a). Questionnaire
A questionnaire will be administered to each subject participating in the study.

(b). Dietary Advice Session
After the 3 day dietary records have been collected, the researcher will discuss with you, your individual dietary aims for the study duration. You will be asked not to consume any other dietary supplements over the period of the study. This will especially include other HMB products, protein powders, protein shakes, protein/sports bars, and products containing creatine.

(c). Anthropometric Measurements
Anthropometric profiles involve three parts, skinfolds, girths, and breadths/lengths.
Skinfold Measurements -
- The anatomical landmarks required for the skinfolds will be marked on your body with a felt tip pen.
- 8 skinfold measurements will be taken along the right hand side of your body including; triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh, and medial calf.
- You will feel a slight pinch of the skin fold callipers, for 2-3 seconds.
- Each skinfold measurement may need to be taken 3 or 4 times to ensure accuracy.

This entire process will take about half an hour.
Girths - The girths that will be taken are as follows:
- The arm-relaxed girth is a measure of the circumference of the arm while the arm is relaxed at the side of your body.
- The arm flexed and tensed girth is a measure of the maximal girth of the arm, while the biceps muscle is fully contracted.
- The waist girth, is a measure of the narrowest circumference of the waist. This measurement is taken with normal expiration.

- The gluteal girth is a measure of the largest protruding area of the buttocks, while you stand with feet together, gluteal muscles relaxed.
- The final girth measurement is the maximal calf circumference.

Breadths/Lengths -

- The width of the humerus, and femur will be measured using sliding callipers.
- Height will be measured while you stand without shoes, heels together, back straight, buttocks, shoulders, and head against the wall.
- Weight will be measured on a scale, while you stand straight, looking ahead, feet together.

(d). Bioelectrical Impedance Monitor (BIA)

The BIA gives a measurement of body fat. You will be asked to lie stationary, while electrodes are attached to your right hand and left foot. The measurement will only take a few minutes, and is completely painless.

(3). VISIT 3:

Mid-Point of Study Duration

Visit 3 will involve the following;

(a). Weight Training Instruction

The weight training programme for weeks 6 through 10, will be explained to each subject, with instruction on how to perform each new exercise. The weight training programme has been split up with a different set of exercises for weeks 1 through 5, and weeks 6 through 10, to keep the programme interesting for you, and prevent your muscle gains reaching a plateau due to lack of stimulation.

(b). Assessment of Muscular Strength

As in visit 1.

(4). VISIT 4:

Final Visit

This is the final visit by the researchers for data collection purposes.

(a). Three Day Dietary Record

As in visit 1.

(b). Anthropometric Measurements

As in visit 2.

(c). Bioelectrical Impedance Monitor (BIA)

As in visit 2.

(d). Assessment of Muscular Strength

As in visit 1.

SUDY CONFIDENTIALITY

All information provided, and measurements taken are for the purpose of this thesis. Your identity will be kept confidential, and will not be published in any results, theses, research papers, or other publications. Your identity will only be recorded or published as a code number, which only the researcher can identify. All of your

personal details will be kept separate from other information in the study in a locked filing system, by Patsy Watson, Programme Leader in Human Nutrition and supervisor of this study.

YOUR RIGHTS

Participation in this study is voluntary, you are under no obligation to participate. If you decide to take part in this study you have the right to the following conditions;

- You may refuse to answer any question(s).
- You may choose not to have any particular measurement(s) taken.
- You may cease to participate in this study at any time.
- You may ask any questions you have, at any time during the study.
- You may have a family member, or friend present for moral support during any of the visits by the researcher.

SUMMARY OF YOUR MEASUREMENTS AND RESULTS

Once the study has been completed, the results of your measurements and dietary assessments will be made available to you, along with an explanation of all such results.

CONSENT

Before the study commences, you will be asked to sign the attached consent form stating that you are aware of all requirements of the study, your rights as a participant, and have accepted the invitation to participate.

CONFIRMATION OF STUDY PARTICIPATION

Please take the time to read through and understand all of the information presented here on what will be involved should you choose to participate in this study. Once you have made your decision, please contact the researcher, Jasmine Thomson on 413 8977, or 021 210 6269, or simply mail the completed consent form to the address below.

RESEARCHER CONTACT DETAILS

Jasmine Thomson
Address: Jasmine Thomson
C/O Patsy Watson
Programme Leader in Human Nutrition
Institute for Food, Nutrition, & Human Health
Massey University
Albany Campus
Private Bag 102 904
North Shore Mail Centre
AUCKLAND
Phone: [redacted]
Email: [redacted]

This project has been reviewed and approved by the Massey University Regional Human Ethics Committee, Albany Campus, Protocol MUAHEC 02/063. If you have any concerns about the conduct of this research, please contact Associate-Professor Kerry Chamberlain, Chair, Massey University Regional Human Ethics Committee, Albany, Telephone 09 443 9799, email K.Chamberlain@massey.ac.nz.

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INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH

Pilot Study of HMB Supplementation of Resistance Trained Men

Consent Form

I _____, assert that:

I have read through and understand all information given to me regarding my participation in this study.

I have had the opportunity to ask questions, and any questions I have at this time have been answered adequately. I understand that I may ask additional questions throughout the study should any arise.

I am aware that participation in this study is confidential and no information that may identify me will be published or released to persons outside of this study.

I understand that participation in this study is voluntary, and I may decline to answer any question, or cease participation in this study at any time.

If I should suffer any side effects while participating in this study I know who I should contact.

I have no known medical condition/s, that may be aggravated by taking part in weight training exercise. Therefore, I release those conducting this research of any liability for direct or indirect injury or loss, sustained by me or any parties associated with me, resulting from my participation in this aspect of the study.

I hereby freely give my consent to participate in the Pilot Study of HMB Supplementation of Resistance Trained Men, that will be conducted with the approval of the Massey University Human Ethics Committee, Albany Campus, Auckland.

Participant Signature _____ Date _____

Witness Signature _____ Date _____

Please fill out your contact details, over the page.

Contact Details

Name _____

Address _____

_____

_____

_____

Phone _____(home)

Phone _____(work)

Mobile (optional) _____

Email (optional) _____

Please send this form to;
Jasmine Thomson
C/O Patsy Watson
Programme Leader in Human Nutrition
Institute for Food, Nutrition, & Human Health
Massey University
Albany Campus
Private Bag 102 904
North Shore Mail Centre
AUCKLAND

Appendix 4

6.4 β-hydroxy-β-methylbutyrate Certificate of Analysis and Gelatin Capsule Receipts

Certificate of Analysis

DATE: DEC.14,2001
INVOICE NO.NTNS01376

PRODUCT : HYDROXY METHYL BUTYRATE (CA SALT)
BATCH NO. 20011118
QUANTITY: 300KGS (12DRUMS)
P.O.No.: T5681

Results:

Appearance	:	white crystalline powder
Ca content	:	13.70%
HMB content	:	84.69%
Loss on Drying	:	1.52%
Pb	:	<10ppm max.
As	:	<2ppm max

Newsmart(Shanghai) International Trading Company
上海新亦美特国际贸易有限公司
NEWSMART (SHANGHAI)
INTERNATIONAL TRADING COMPANY

TRANS CHEM PTY LTD
ABN 96 064 545 473
Unit 8, 112-118 Talavera Rd
North Ryde NSW 2113
PO Box 1864
Macquarie Centre NSW 2113
Australia
Ph: 02 9887 1688 Fax: 02 9887 1117

Massey University

MASSEY UNIVERSITY
Institute of Food, Nutrition & Human Health
PRIVATE BAG 102 904
North Shore Mail Centre
AUCKLAND

SUPPLIER: Pharmaceutical Compounding Birkenhead	
Customer No:	

Order No: 1120 3646	**Date:** 26 November 2002
Ship To: IFNHH, Building 22 Albany Campus Eastborne Rd, off Old Albany Highway **ALBANY**	
ATTN: Kay Rowbottom	

Supply To: Kay Rowbottom	Authorised by Patsy Watson				
Account	Item Code	Coding	Description	QTY	Estimated $ Value
1120	1222	NUT	Double O gelatin capsules For: Jasmine Thomson Postgrad Student	1,000	~~$200.00~~ 36$

Christina or Colin

Tax Invoice / Statement

Invoice Date	27/11/2002
Tax Invoice	24182
Reference	
Page	1

PHARMACEUTICAL COMPOUNDING

35L Enterprise Street, PO Box 348
Birkenhead, Auckland, New Zeala
Ph: (09) 480 2660 Fax: (09) 480 26
www.pharmaceutical.co.nz

[redacted]

Invoice To:	Deliver To:
Cash Sale	Cash Sale DIMITRI - MASSEY UNIVERSITY

Prescribing Doctor: Dispensing Pharmacist: Denis Kay

Description	Quantity	Unit Price (ex GST)	Disc %	Am
Empty Gelatin Caps No.00-1000	1.0	32.000 EA		3

Payment Terms: Please pay on receipt of goods.

Sub Total		3
GST		
Invoice Total	$	3
Paid		
Balance Due	$	3

Please return this portion with payment to:

PHARMACEUTICAL COMPOUNDING NZ LTD
PO Box 34897
Birkenhead
Auckland New Zealand

Customer:	CASHS
Invoice Number:	24182
Date:	27/11/2002
Amount:	$ 36.00

Please indicate if receipt required: ☐

Appendix 5

6.5 Resistance Training Log Books One and Two

Official Weight Training Log Book 1

For the Pilot Study of HMB Supplementation of Resistance Trained Men

Code Number _____

If this Training Log Book has been lost or misplaced ....
If found, please contact Jasmine Thomson [redacted]

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Weight Training Programme Instructions

Weight Training Programme

This programme has been designed by Mark Woodgate, a qualified Personal Trainer for Les Mills, Hamilton. The aim of this programme is to maximise muscle growth and strength.

Weight training is required to be performed 3 times per week. The weight training programme has been designed to take no longer than 40-50 minutes on each of these days. Each major body part is trained once a week, except for abdominals which are trained on 2 days, workouts 1 and 3.

Warm-Up

A warm-up of 3-5 minutes should be done prior to any weight training session. This can be done on the 'cardio' equipment at the gym, jogging, or other warm-up exercise. Also prior to performing the first set of exercise for each major body part , a warm-up set of the first exercise may be done at 50% of the normal starting weight for 15-20 repititions.

Weight Training Structure

Each set of weight training exercise should be done to muscular failure, i.e. you are not able to do any more repetitions with good form at that weight. If you are able to do more than the required repetitions, do so, but put the weight up for next time!

The programme is set out in a pyramid design, which means that with every subsequent set, the weight will be raised, and repetitions decreased.

Rest Periods

The rest times between sets are given in the training log sheets, later in the book. Rest times are generally 90 seconds between sets except for abdominals and calves, which only require 30 seconds.

The other exception is, prior to the final 2 sets of each body part, which are performed as a dropset, there is a rest period of only 30 seconds. While performing the dropset, there is no rest period between these final 2 sets. As soon as you have completed the second to last set, immediately decrease the weight by about 10%, and start the final

exercise set. In some cases the dropset is done on the same exercise for both sets, in other cases the dropset may use 2 different exercises for the same body part.

The rest time between body parts is always 120 seconds.

Repetitions Range

The number of required repetitions for each exercise will be given in the training log sheets, later in the book. The repetition range is generally 8-12 repetitions, except abdominals and calves, which require 15 repetitions. The other exception is while performing the dropsets, the first set of the dropset, is for 10 repetitions, the final set immediately following is for 5 repetitions.

Again, we are aiming for muscular failure! So if you are able to do more repetitions with good form, you should do so, and the next week put the weight up!

Repetition Tempo

During each repetition concentrate on counting 1 second for the lifting phase, pause and contract the muscle for 1 second, then 2-3 seconds for the lowering phase. When paused at the mid-point of the movement, make sure you give an extra squeeze of the muscle at this point, and concentrate on the particular muscle you are working.

Remember, the emphasis of this weight training programme is on the intensity not the duration!

On the following pages are some instructions and diagrams on how to perform each exercise used in this programme.

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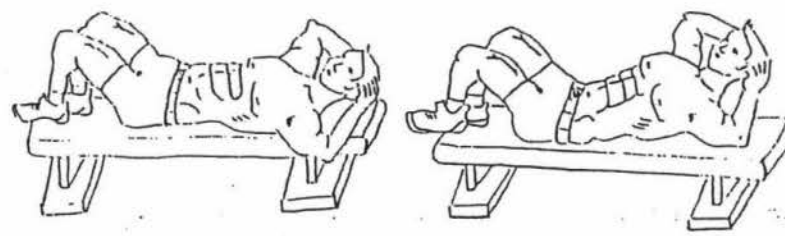
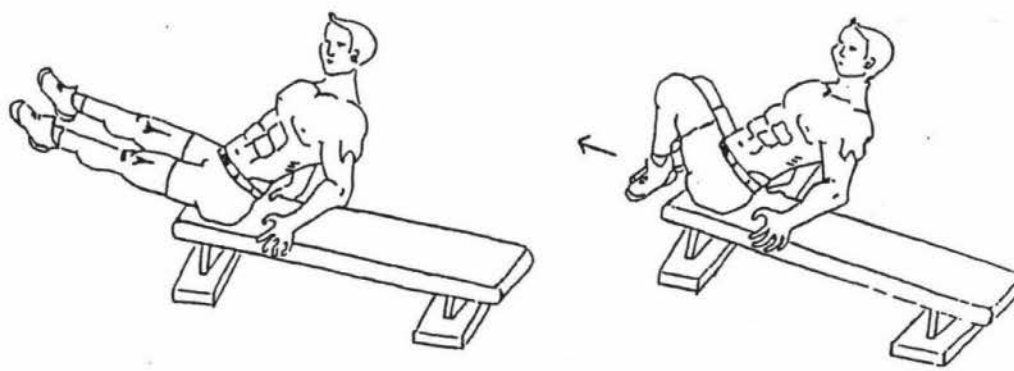
Exercise Instruction

Abdominals

Bench Crunch (Floor Crunch) - On a bench, or mat, position your knees together, feet flat about 30cm from your bottom, place your hands lightly behind your head. Push your lower back into the bench/mat, and raise your shoulders in a rolling motion. Pause and contract your abdominal muscles, then slowly lower your shoulders back to the start position. Your abdominal muscles should be tensed for the entire movement.

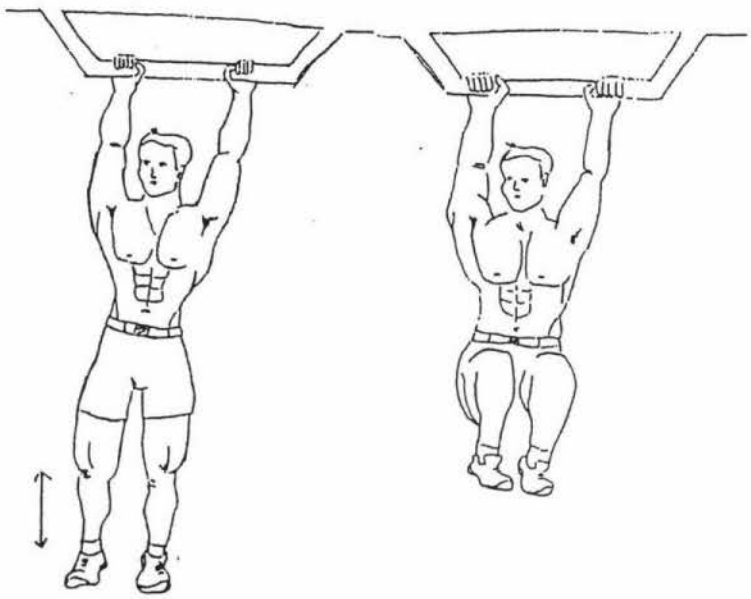


Bench Tuck (Seated knee-up) - Sit at the end of a bench, hands holding the bench directly behind your bottom. Stretch your legs out straight in front of you off the edge of the bench. Contract your abdominals and pull your knees in towards your chest, pause, then slowly extend your legs back to the start position again.

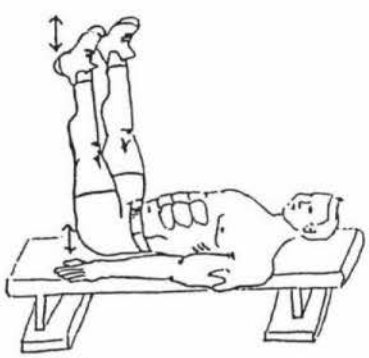


Hanging Leg Lift - Grip a chin-up bar with hands slightly wider than shoulder width apart. Use your lower abdominals to raise your knees as high as possible, pause, then

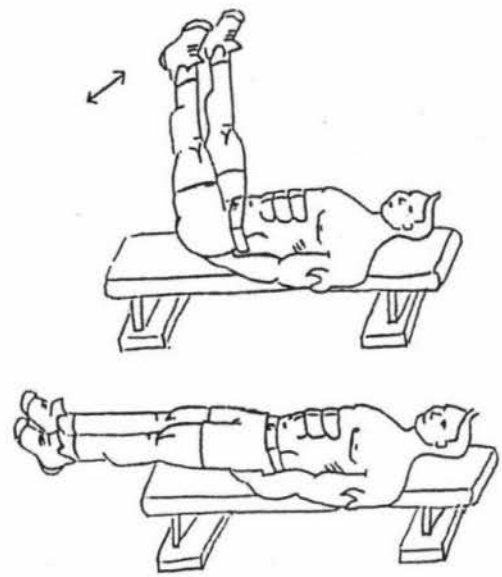
slowly return to the start position. Do this movement slowly, and don't use momentum to swing yourself.



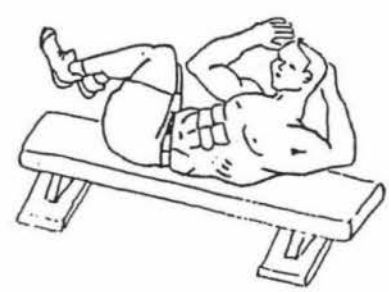
Leg Lift (Incline Leg Lift) - Lie with your arms at your sides, legs straight up in the air, perpendicular to the floor, and feet together, heels pointing up. Keep your shoulders firmly on the bench/mat, and slowly lift your hips to about 10-15cm off the bench/mat, pause, then lower back to the start position. You may increase the resistance by performing this movement on a incline bench.



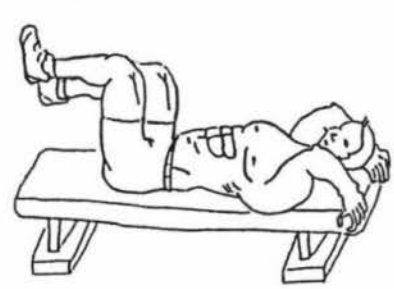
Leg Raise - Lie on a bench, hold the bench above your head, or position your hands under your hips. At the start position, your legs are straight up in the air, perpendicular to the floor, and feet together. Keep your legs straight, as you slowly lower you legs as far as you can without letting your lower back lift off the bench. Pause, then slowly raise your legs back to the start position. Throughout this movement use your abdominals to press your lower back firmly into the bench.

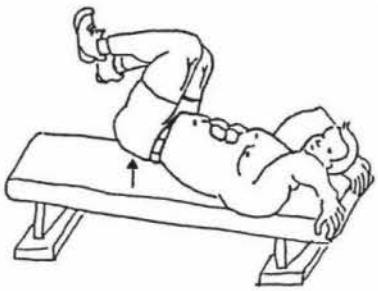


Oblique Bench Crunch - Position yourself the same as bench crunch above, or you may place your feet in the air with your knees bent at a 90^0 angle. Curl your shoulders up, use your oblique muscles twist your shoulders so that your left shoulder aims for your right knee. Pause, then slowly lower your shoulders to the start position. Repeat this for your right shoulder and left knee, this counts as one repetition.

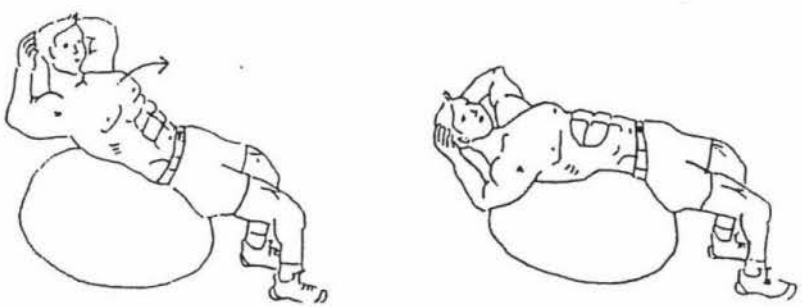


Reverse Crunch (Incline Reverse Crunch) - Place your arms above your head, and flatten your back against the bench/mat. Roll your pelvis and knees towards your chest. Exhale while contracting your abdominals. Slowly lower your pelvis back to the start position, maintaining tension on your abdominals the entire movement. To increase resistance change the start/finish position, so that your legs are extended and parallel to the bench/floor. Another way to increase resistance is to perform this movement on a incline bench.





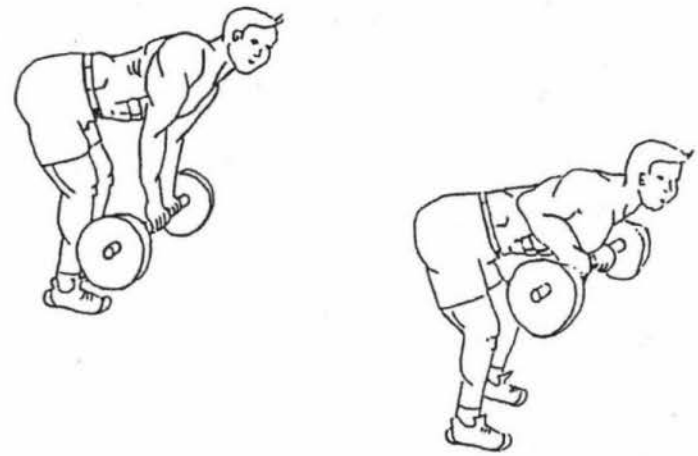
Swiss Ball Crunch - Lie on your back on Swiss ball, with the ball positioned in the small of your back. Hold your hands lightly at your head, elbows out. Slowly curl your body up into a crunch, pause, then slowly lower yourself until you are fully stretched over the ball. Concentrate on tensing your abdominal's for the entire movement for this exercise to be effective.



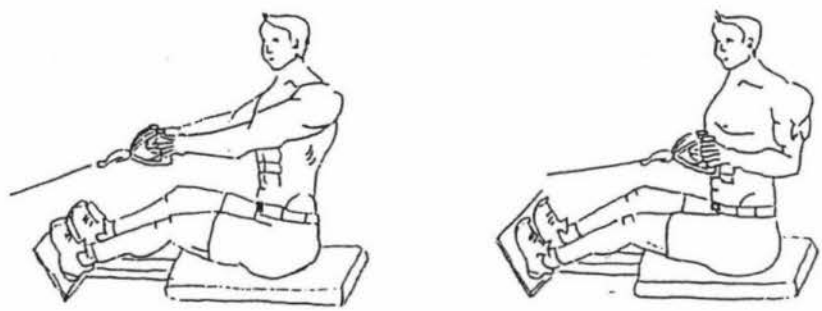
Swiss Ball Obliques - To work your obliques, position yourself as above on a Swiss ball. Slowly curl your body up into a crunch position, then twist to one side and reach towards your knee, pause and contract your obliques, then slowly lower yourself until you are fully stretched over the bail. Repeat for the other side. This counts as one repetition.

Back

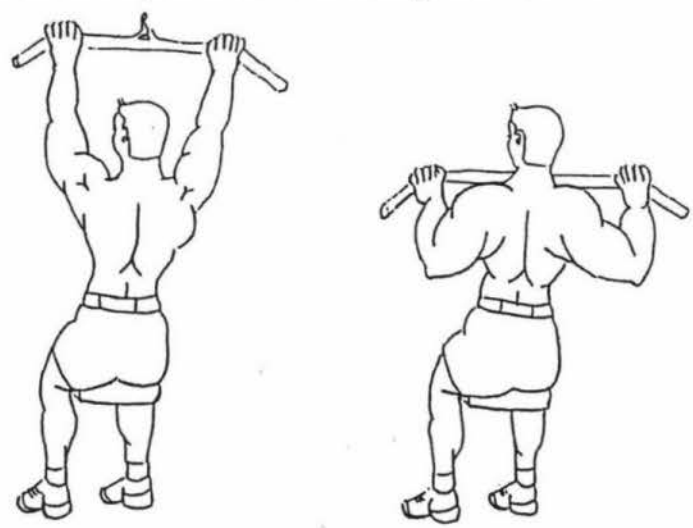
Barbell Bent-Over Row - Stand with your feet shoulder width apart, knees bent, back flat and parallel to the floor, and bottom out. At the start position, grip the barbell with hands shoulder width apart, arms extended to half-way between your knees and ankles. Raise the bar straight up, until level with the bottom of your ribcage, pause, then return to the start position.



Cable Row - Sit at a cable row machine, feet on the metal footrest, and knees slightly bent. Grip the handle of the pulley with palms facing inwards, and arms fully extended. Keep your back stationary, while you draw your arms towards you until the handle reaches your stomach, pause, then extend your arms slowly back to the start position.



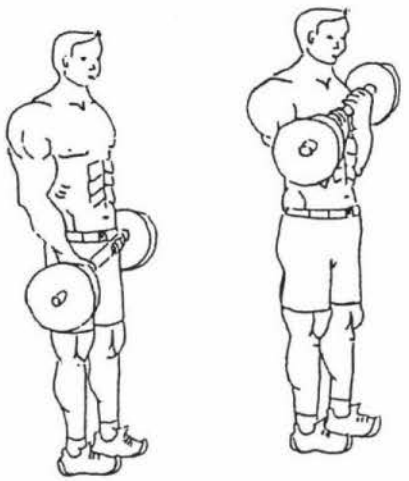
Lat Pull Down - Sit at a lat pull-down machine. Hold the pulldown bar with an overhand grip, about twice shoulder width distance apart. Pull down the bar until it just touches the top of your chest, you may arch your back slightly, but don't lean back. Pause, then slowly let the bar raise to the start position. Make sure you keep your elbows directly under the bar during this movement.



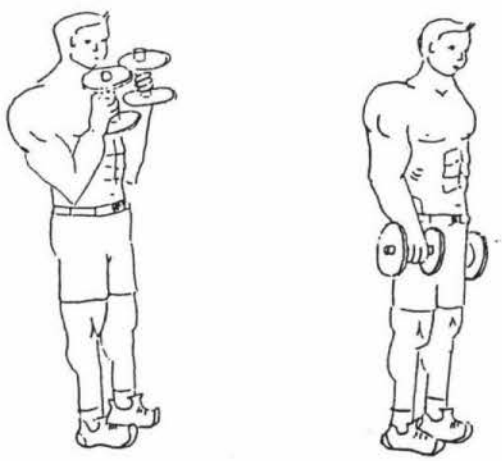
Biceps

Barbell Curl - Stand straight, with your feet shoulder width apart, chest out, and shoulders squared. Hold the bar with a palms up grip, arms extended downwards, the bar resting on your thighs. Use a curling motion to lift the bar towards your chest,

pause, then slowly lower the weight until your arms are almost straight, and your biceps still tensed. During this movement, keep your elbows stationary, and don't lean backwards or use momentum to swing the barbell.

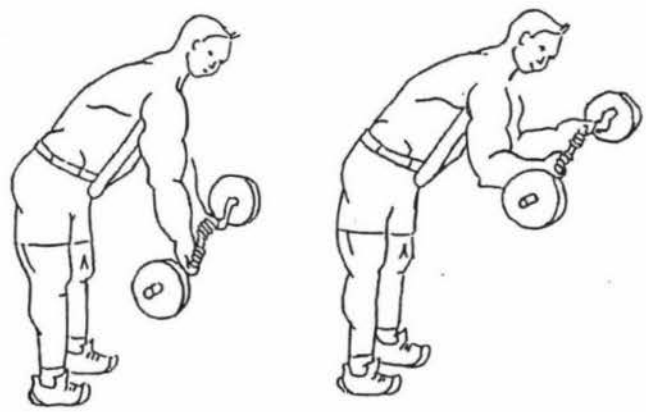


Hammer Curl - Grip dumbbells with palms facing in towards each other, arms fully extended down each side of your body. Curl both dumbbells upward in an arc, pause, then slowly lower the weights back to the start the start position. Your palms should remain facing each other throughout the movement. Don't lean forward or backward, or use momentum to swing the weights.



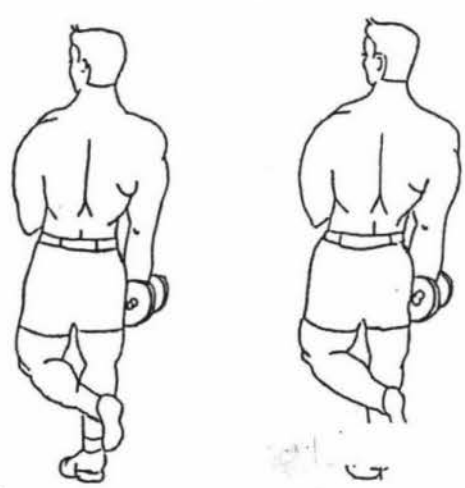
Preacher Curl - Use a preacher curl bench and machine, or a preacher curl bench and ezy bar to perform this exercise. If using an ezy bar, grip with your hands shoulder width apart, and palms up. Curl the bar upwards towards your chin, just to the point where the resistance starts to let up, pause, then slowly lower the bar until your arms are fully extended. Take care not to lean your shoulders forward.

Spider Curl (Reverse Preacher Ezy Bar Curl) - Use the opposite side of the preacher curl bench, in a standing position. Curl the bar upwards. Your body and shoulders should remain stationary. Then slowly lower the bar until your arms are fully extended.

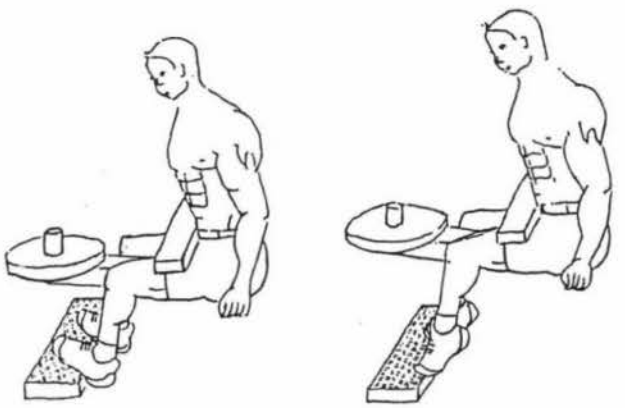


Calves

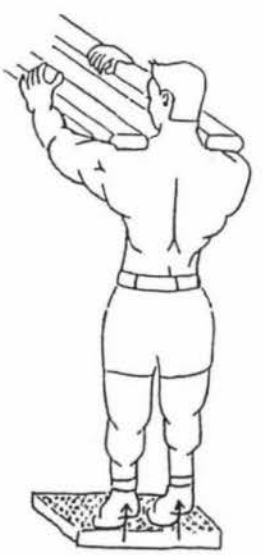
One Leg Calf Raise - Stand on a block, or on the floor. Stand on the ball of your right foot, with a dumbbell in your right hand, palm facing your body, with your left foot off the floor. You may hold onto a wall or sturdy piece of equipment for balance if needed. (If you are standing on a block, lower your right heel as far as possible) raise up on your toes as far as possible, pause and contract your calf, then lower to the start position. Finish all repetitions on your right leg, then repeat with your left leg.



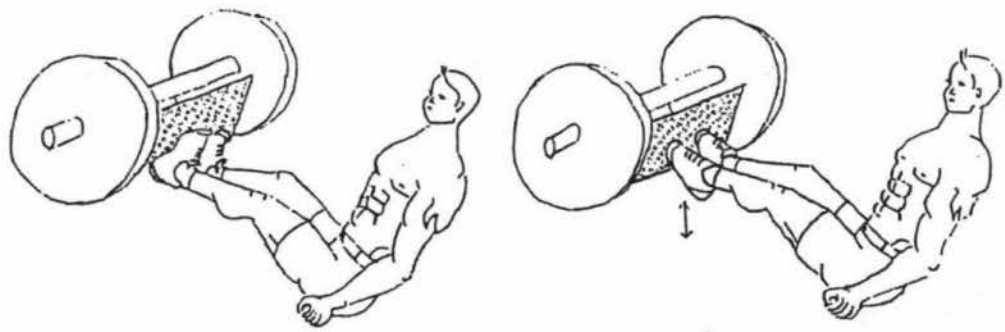
Seated Calf Raise - Sit on a seated calf raise machine. Slowly lower your heels until your calf muscles are stretched, pause, raise your heels pressing with the balls of your feet as high as possible, pause, slowly lower back to the start position.



Standing Calf Raise - Stand straight, knees slightly bent, your weight resting on the balls of your feet, on the platform of a standing calf raise machine. Slowly lower your heels until your calf muscles are stretched, pause, raise your heels pressing with the balls of your feet as high as possible, pause, and slowly lower back to the start position.

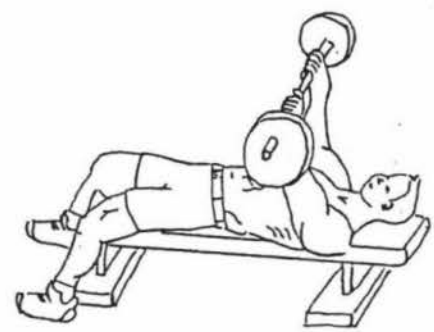


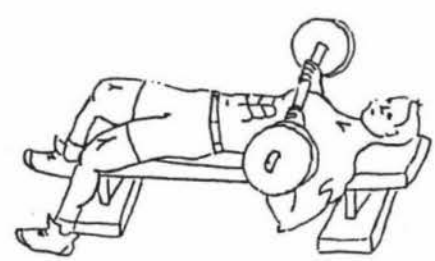
Toe Raise on Leg Press Machine - Sit at a leg press machine, position the balls of your feet on the foot plate, your heels extended over the bottom edge of the foot plate. Lift the weight until your legs are straight, but your knees not locked out, this is the start position. Release the safety stops. Press your feet up onto your toes raising the footplate, pause, then slowly lower back to the start position. If you point your feet straight you will work both heads of the gastronemius muscle, feet inwards you work the outer head, and feet outwards you work the inner head.



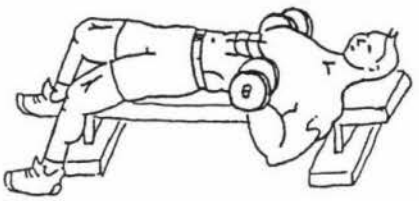
Chest

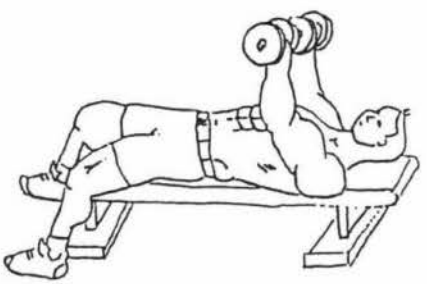
Barbell Bench Press - Lie on a bench, with your feet resting flat on the floor, a little wider than shoulder width apart. Your hips must remain flat on the bench, but your back may arch slightly. Hold the barbell with a grip slightly wider than shoulder width apart. At the start position, your arms should be fully extended, the barbell held above the middle of your chest, elbows locked. Lower the barbell to the middle of your chest, pause, then force the barbell back to the start position. Inhale as you lower the barbell, and exhale as you raise it.



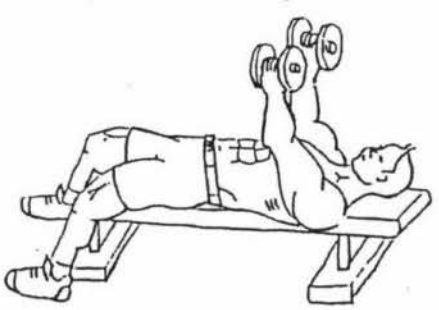


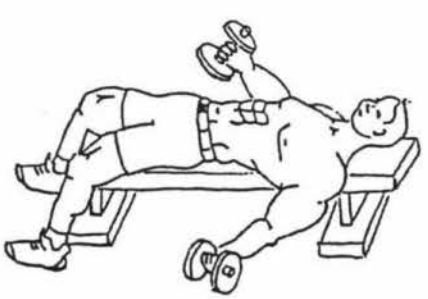
Dumbbell Bench Press - Lie on a bench, with your feet resting flat on the floor, a little wider than shoulder width apart. At the start position, your arms should be bent, elbows out, holding a dumbbell in each hand, palms facing your feet. Press the dumbbells straight up, pause, then slowly lower to the start position. Inhale as you lower the dumbbells, and exhale as you raise them.





Dumbbell Flys - Lie on a bench, with your feet resting on the floor, a little wider than shoulder width apart. At the start position, your arms should be extended, holding a dumbbell in each hand, palms facing each other, elbows slightly bent. Slowly lower the dumbbells to each side, keeping your elbows only slightly bent, until your arms are horizontal, no lower than level with the bench. Pause, then lift the dumbbells back up to the start position. The dumbbells should follow an arc-like motion. Inhale as you lower the dumbbells, and exhale as you raise them.

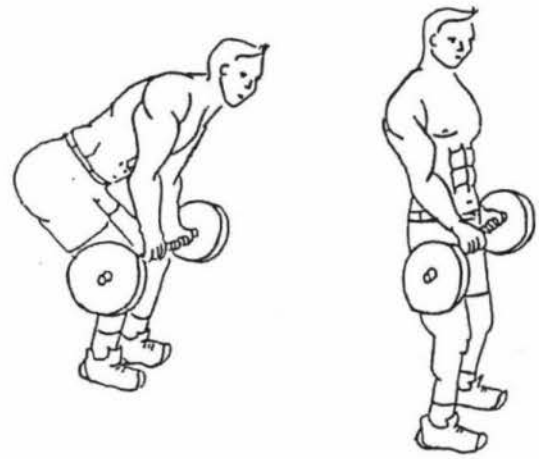




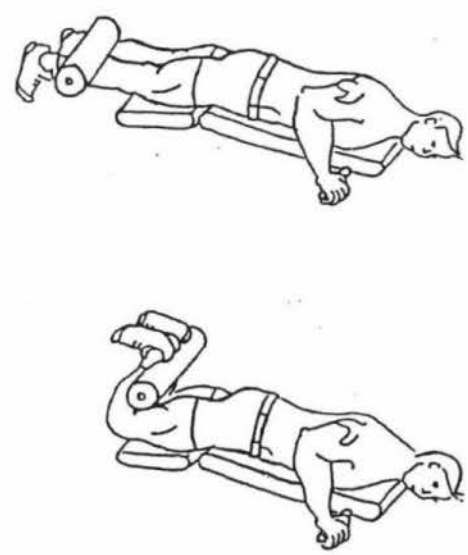
Hamstrings

Bent Knee Deadlifts - Hold a barbell with hands shoulder width apart (one hand palm down, the other hand palm up), arms extended, barbell resting across your thighs. Position your feet about shoulder width apart. Bend your knees and sink down to a half squat position, while keeping your torso upright, and lower the bar to a point half

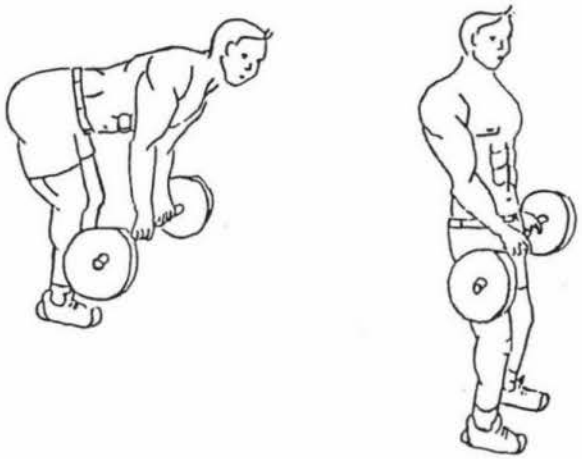
way between your knees and ankles. Keeping your back flat, and head up. Pause, then return to the starting position focussing on squeezing your hamstrings and glutes'.



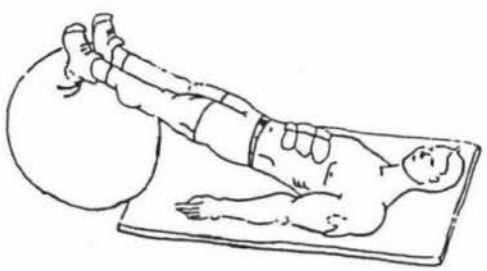
Lying Leg Curls - Lie on a lying leg curl machine, the machine pad on the back of your ankles. Curl your legs up in an arc, until the machine pad reaches the top of your hamstrings. Pause and contract your hamstring muscles, then lower the weight slowly back to the start position. Keep your hips down throughout the movement.



Stiff Legged Deadlifts - Hold a barbell using a pronated grip, arms fully extended, barbell resting across your thighs. Your legs should be very slightly bent, your body straight, shoulders back, and head up looking forward. Bend forward at the hips, back straight, push your bottom out and lower the bar until you feel a good stretch in your hamstrings. Pause, then return to the starting position focussing on squeezing your hamstrings and glutes'.



Swiss Ball Hamstring Curls - Lie on a mat on the floor, with your heels on the middle of a swiss ball. Raise your hips off the floor, so you are resting on your shoulders. Bend your knees, and use your hamstrings and gluteal muscles to roll the ball towards you until it reaches your bottom, and your feet are flat on the ball. Pause and contract your hamstrings, then straighten your legs back to the start position. You must ensure that your hips are raised off the floor for the entire movement. You should also concentrate on contracting your hamstrings as hard as you can for this exercise to be effective.



Quadriceps

Barbell Lunges (Front Lunges) - Position a weighted barbell across the back of your shoulders, and stand with your feet approximately 20cm apart, pointed straight ahead. Keep your back straight, shoulders square, and your head up. Step your right leg forward until your knee is at a 90^0 angle, your thigh parallel with the floor, and shin perpendicular with the floor. Using your quadriceps and hamstrings, lift your right leg back to the start position. Repeat this procedure with your left leg. This is counted as one repetition. Inhale as you lower yourself, and exhale as you raise yourself back to the start position.

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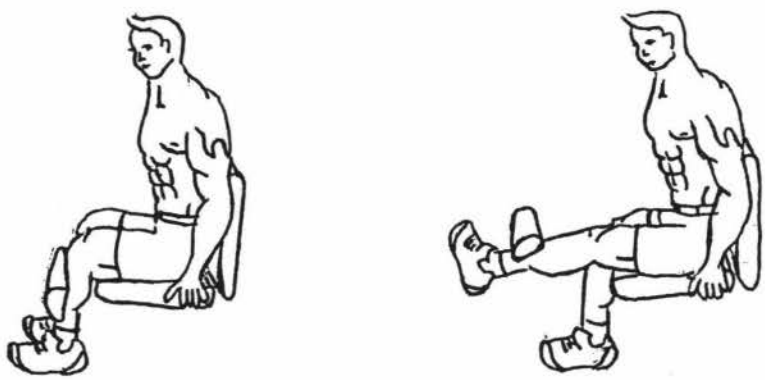
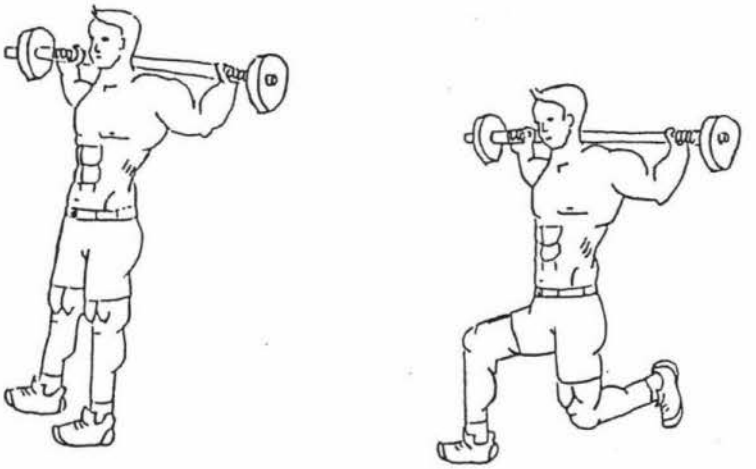
INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH

Pilot Study of HMB Supplementation of Resistance Trained Men

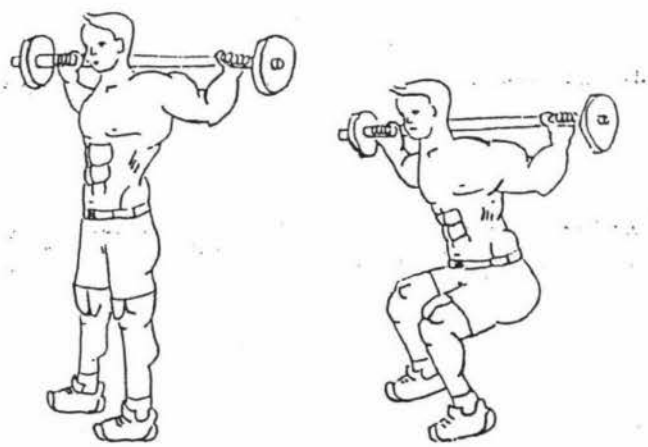
Exercise Instruction

Leg Extension - For this exercise you will work one leg at a time. Position the back of your knees at the edge of the padded seat, and position the leg you are working, under the extension pad, with the pad resting on the lower part of your shin. Hold the seat or handles of the machine to stabilise your body, and make sure your lower back is pressed into the padding of the seat behind you. Slowly raise the weight for a count of one, until your knee and hip is parallel to the floor, then lower the weight for a count of one, until your knee is bent at 90^0. Inhale on the upward motion and exhale on the downward motion. Finish all repetitions on one leg, then do the same number of repetitions for the other leg.

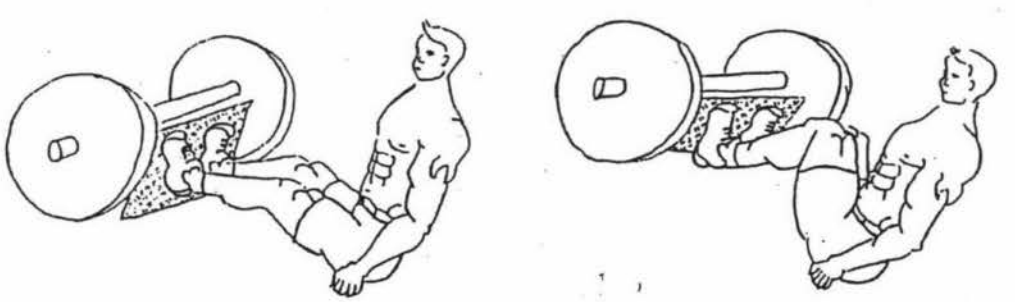




Barbell Squat - For safety it is recommended that a squat rack is used. Position the barbell across the back of your shoulders. Hold the bar with your hands spaced about double shoulder width apart. Stand straight, look straight ahead, space your feet about shoulder width apart, toes facing slightly outward, your weight resting on the balls of your feet. Slowly lower your hips until your thighs are parallel with the floor. Immediately press the weight up again, from the heels of your feet, to the start position. Inhale as you squat down, and exhale as you rise.



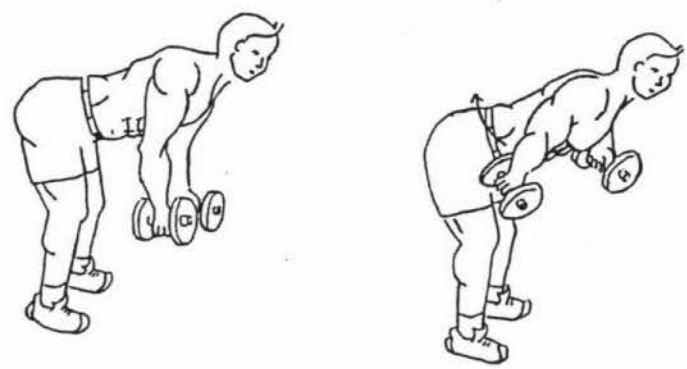
Leg Press - Sit at a leg press machine, with your feet on the foot plate at shoulder width apart, and pointed slightly outward. Bend your knees and lower the weight as far as possible, but don't let your hips rise off the seat. Pause, then slowly raise the weight to the start position pushing through your heels until just before your knees are locked out.



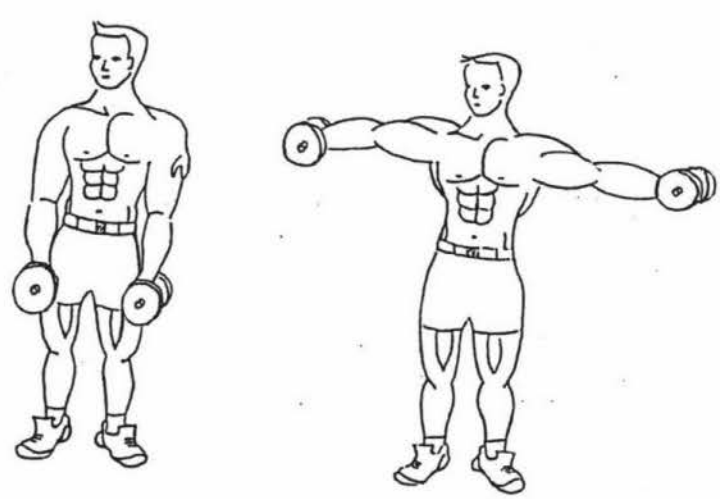
Walking Lunge - Stand straight, with your feet approximately 20cm apart, pointed straight ahead. Hold a dumbbell in each hand, palms facing your body. Stepping with alternate legs, lunge across the floor with each repetition. Remember, when you lunge, your knee should be at a 90^0 angle, your thigh parallel with the floor, and shin perpendicular with the floor.

Shoulders

Bent Over Dumbbell Raise (Bent Over Deltoid Rise) - Stand with your feet about 30cm apart, and knees slightly bent. Bend at your waist until your back is parallel to the floor, holding a pair of dumbbells in each hand, arms extended to the floor. This is the start position. Bend your elbows and raise the dumbbells until your upper arms are in line with your back, pause, then slowly lower the weights to the start position. Inhale as you raise the weights, exhale as you lower them. You may also do this exercise with straight arms, and a slightly lighter weight.

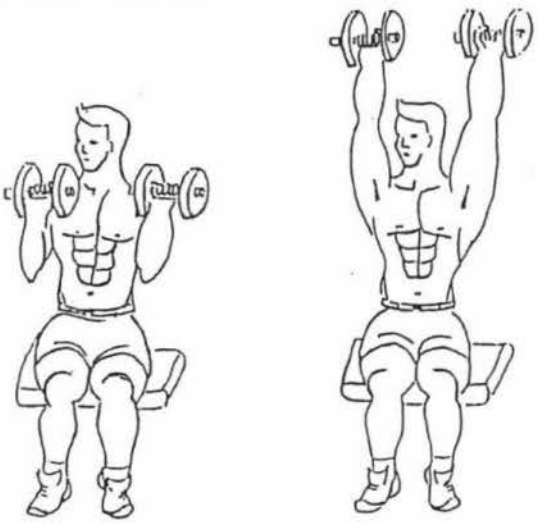


Dumbbell Side Raise (Lateral Raise) - Stand straight, with your feet shoulder width apart, arms at your sides, holding a dumbbell in each hand, with palms facing your body. Lift the weights up and out to the side, until parallel with the floor, level with your shoulders. Pause, then slowly lower to the start position. Keep your arms straight and your palms down throughout the movement.

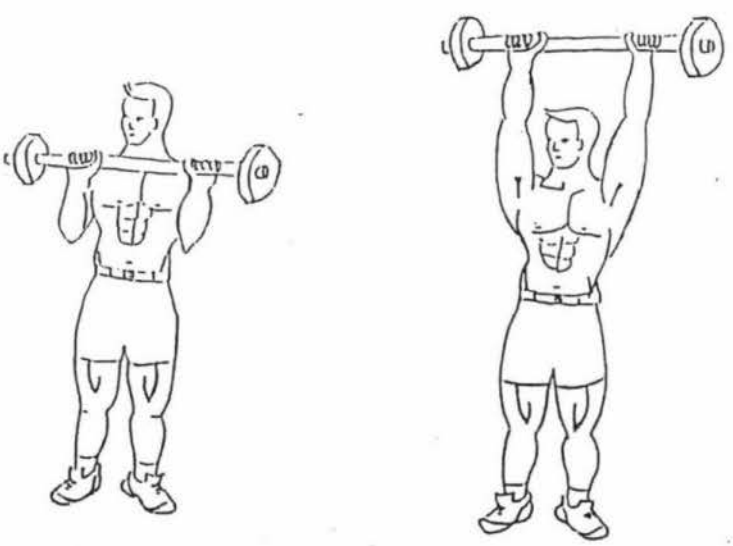


Seated Dumbbell Shoulder Press - Sit on a bench that has a short straight back. Grasp a dumbbell in each hand, at shoulder level, palms facing forward, elbows out. Press the dumbbells up and inwards in an arc until they almost touch, elbows just short

of lockout, pause, then slowly lower the dumbbells back to the start position. Keep your back pressed into the back of the bench.

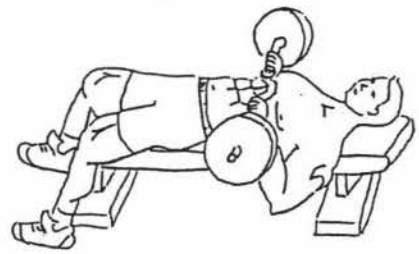


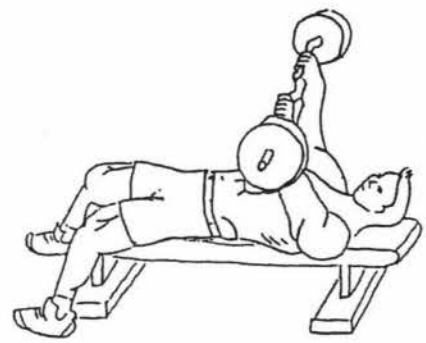
Standing Barbell Press - Stand straight, feet shoulder width apart, knees slightly bent. Hold a barbell with a palm up grip, just wider than shoulder width apart, positioned in line with your collarbone. Press the weight up, until your arms are fully extended, pause, then slowly lower to the start position again. Be careful not to arch your back as you do this movement, and keep your elbows in.



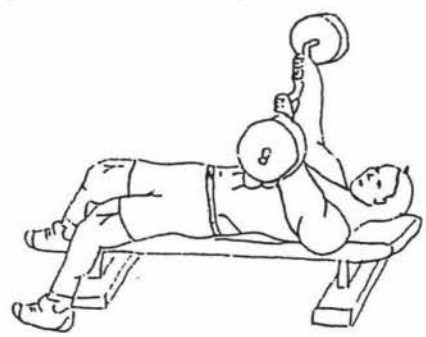
Triceps

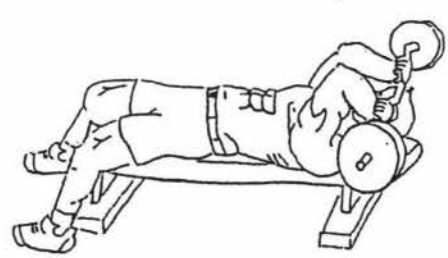
Close Grip Bench Press - Lie on a bench, feet resting flat on the floor, a little wider than shoulder width apart. Use an ezy bar or hold a straight bar with a grip about 20-30 cm apart. Start with arms fully extended straight upholdong the bar level with your chest, lower the bar slowly to your upper chest, pause, then press the bar back up to the start position. Take care not to flare your elbows out during this movement, keep them straight. Inhale as you lower the bar, exhale as you raise the bar.



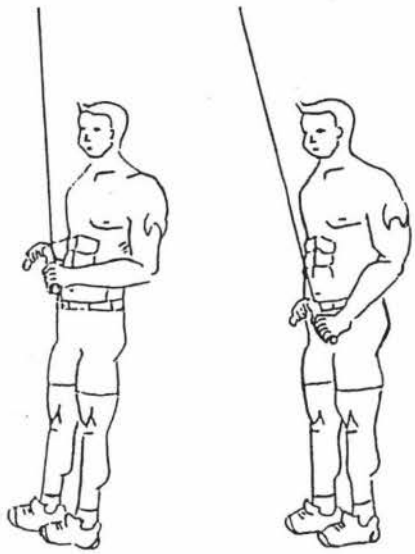


French Press - You may wish to use a spotter for this exercise. Lie on a bench, holding an ezy bar using an overhand grip. Your arms extended straight up, the bar in line with your eyes. Slowly lower the bar until it is almost touching your forehead, then raise the bar back to the start position in an arc-like motion, and lock elbows out at the top. Take care not to flare your elbows out during this movement, keep them straight and your upper arms stationary.

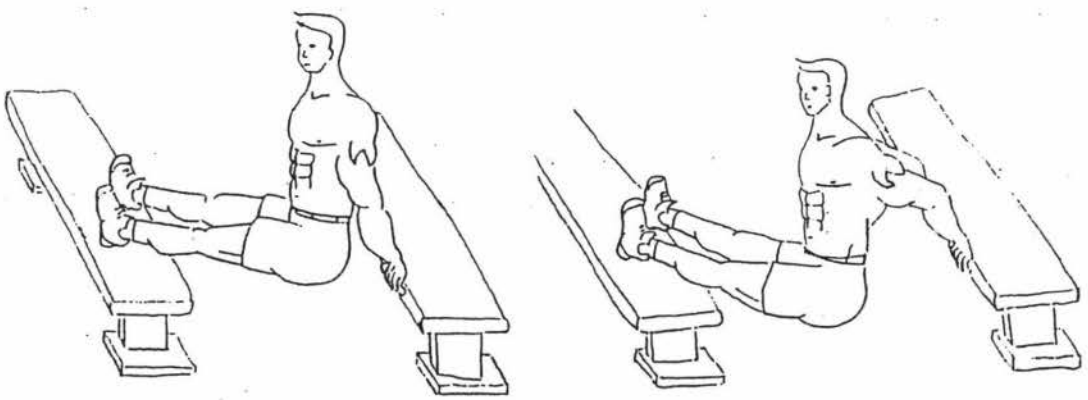




Triceps Pushdown - Grip the bar with an overhand grip, your feet should be shoulder-width apart, and knees slightly bent. At start position your forearms should be parallel with the floor, keep your upper arms steady, and elbows close to your body. Push the bar down as far as possible, pause and contract your triceps muscles, then slowly raise the bar to the start position.



Weighted Bench Dips - Position two benches parallel to each other. Balance between the benches in a sitting position, supporting your body weight with your hands on one bench on either side of your body, and feet on the other bench. Keep your body close to the bench where you have placed your hands. Slowly lower your body, bending your elbows to a 90^0 angle, pause, then raise yourself to the start position. To increase the resistance, place a weight on your lap as you do this exercise, or raise your feet by placing them on a Swiss ball instead of the bench.



Weight Training Workout 1
Weeks 1 through 5

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Abdominals	Bench Crunches	1	15			30
|	|	2	15			30
|	Swiss Ball Oblique Crunches	1	15			30
|	|	2	15			30
|	Reverse Crunches/Bench Leg Raises	DS	15/10			0/120
Chest	Bar Bell Bench Press	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Dumb Bell Flys	1	10			90
|	|	2	8			30
|	Dumb Bell Bench Press/Dumb Bell Bench Press	DS	10/5			0/120
Triceps	Close Grip Bench Press	1	10			90
|	|	2	8			90
|	French Press	1	10			90
|	|	2	8			30
|	Tricep Pushdowns/Tricep Pushdowns	DS	10/5			0/120
Biceps	Bar Bell Curls	1	10			90
|	|	2	8			90
|	Preacher Curls	1	10			90
|	|	2	8			30
|	Hammer Curls/Hammer Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Weeks 1 through 5

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Calves	Standing Calf Raise	1	15			30
"	"	2	15			30
"	Seated Calf Raise	1	15			30
"	"	2	15			30
"	Toe Press/Toe Press	DS	15/10			0/120
Quadraceps	Leg Extension	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Bar Bell Squat	1	10			90
"	"	2	8			30
"	Leg Press/Leg Press	DS	10/5			0/120
Hamstrings	Lying Leg Curls	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Stiff Legged Deadlift	1	10			90
"	"	2	8			30
"	Lying Leg Curl/Lying Leg Curl	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Weight Training Workout 3
Weeks 1 through 5

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Abdominals	Incline Leg Lift	1	15			30
|	|	2	15			30
|	Bench Crunch	1	15			30
|	|	2	15			30
|	Hanging Leg Raises/Bench Tuck	DS	15/10			0/120
Back	Cable Row	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Lat Pull down	1	10			90
|	|	2	8			30
|	Bar Bell Bent Over Row/Bar Bell Bent Over Row	DS	10/5			0/120
Shoulders	Seated Dumb Bell Shoulder Press	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Bent Over Dumb Bell Raise	1	10			90
|	|	2	8			30
|	Dumb Bell Side Raise/Dumb Bell Side Raise	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Physical Activity Record Sheet

Monday	Tuesday	Wednesday	Thursday
Friday	**Saturday**	**Sunday**	***Notes***

An example on how to fill this Physical Activity record, appears at the front of the Training Log Book

INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH

Official Weight Training Log Book 2

For the Pilot Study of HMB Supplementation of Resistance Trained Men

Code Number _____

If this Training Log Book has been lost or misplaced ....
If found, please contact Jasmine Thomson [redacted]

Weight Training Workout 1
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Abdominals	Bench Oblique Crunches	1	15			30
"	"	2	15			30
"	Reverse Crunches	1	15			30
"	"	2	15			30
"	Incline Leg Lift/Incline Leg Raise	DS	15/10			0/120
Chest	Bar Bell Bench Press	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Dumb Bell Bench Press	1	10			90
"	"	2	8			30
"	Dumb Bell Flys/Dumb Bell Flys	DS	10/5			0/120
Triceps	Tricep Pulldowns	1	10			90
"	"	2	8			90
"	Weighted Bench Dips	1	10			90
"	"	2	8			30
"	French Press/French Press	DS	10/5			0/12(
Biceps	Preacher Curls	1	10			90
"	"	2	8			90
"	Spider Curls	1	10			90
"	"	2	8			30
"	Bar Bell Curls/Bar Bell Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note side effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc.

Weight Training Workout 2
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Calves	Toe Press	1	15			30
"	"	2	15			30
"	One Legged Calf Raises	1	15			30
"	"	2	15			30
"	Standing Calf Raises/Standing Calf Raises	DS	15/10			0/120
Quadraceps	Leg Extension	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Leg Press	1	10			90
"	"	2	8			30
"	Walking Lunge/Walking Lunge	DS	10/5			0/120
Hamstrings	Bent Knee Deadlift	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Swiss Ball Leg Curls	1	10			90
"	"	2	8			30
"	Lying Leg Curls/Lying Leg Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Note *Swiss Ball Leg Curls should be done to musculat failure, you may be able to complete more than 10 reps, the average is usually 15 reps.*

Weight Training Workout 3
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (
Abdominals	Reverse Crunch	1	15			30
|	|	2	15			30
|	Swiss Ball Crunch	1	15			30
|	|	2	15			30
|	Bench Crunch/Oblique Bench Crunch	DS	15/10			0/12
Back	Bent Over Bar Bell Rows	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Cable Row	1	10			90
|	|	2	8			30
|	Lat Pull Down/Lat Pull Down	DS	10/5			0/1
Shoulders	Standing Bar Bell Shoulder Press	1	12			9(
|	|	2	10			9
|	|	3	8			9
|	Dumb Bell Side Raise	1	10			9
|	|	2	8			3
|	Bent Over Dumb Bell Raise/Bent Over Dumb Bell Raise	DS	10/5			0

Note any effects on muscle soreness, strength, and/or recovery time

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Monday	Tuesday	Wednesday	Thursday
Friday	**Saturday**	**Sunday**	***Notes***

An example on how to fill this Physical Activity record, appears at the front of the Training Log Book

Weight Training Workout 1
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s
Abdominals	Bench Oblique Crunches	1	15			30
"	"	2	15			30
"	Reverse Crunches	1	15			30
"	"	2	15			30
"	Incline Leg Lift/Incline Leg Raise	DS	15/10			0/120
Chest	Bar Bell Bench Press	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Dumb Bell Bench Press	1	10			90
"	" 328	2	8			30
"	Dumb Bell Flys/Dumb Bell Flys	DS	10/5			0/12
Triceps	Tricep Pulldowns	1	10			90
"	"	2	8			90
"	Weighted Bench Dips	1	10			90
"	"	2	8			30
"	French Press/French Press	DS	10/5			0/12
Biceps	Preacher Curls	1	10			90
"	"	2	8			90
"	Spider Curls	1	10			90
"	"	2	8			30
"	Bar Bell Curls/Bar Bell Curls	DS	10/5			0/

Note any effects on muscle soreness, strength, and/or recovery time

Note side effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc.

Weight Training Workout 2
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Calves	Toe Press	1	15			30
|	|	2	15			30
|	One Legged Calf Raises	1	15			30
|	|	2	15			30
|	Standing Calf Raises/Standing Calf Raises	DS	15/10			0/120
Quadraceps	Leg Extension	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Leg Press	1	10			90
|	|	2	8			30
|	Walking Lunge/Walking Lunge	DS	10/5			0/120
Hamstrings	Bent Knee Deadlift	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Swiss Ball Leg Curls	1	10			90
|	|	2	8			30
|	Lying Leg Curls/Lying Leg Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Note *Swiss Ball Leg Curls should be done to musculat failure, you may be able to complete more than 10 reps, the average is usually 15 reps.*

Weight Training Workout 3
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Abdominals	Reverse Crunch	1	15			30
|	|	2	15			30
|	Swiss Ball Crunch	1	15			30
|	|	2	15			30
|	Bench Crunch/Oblique Bench Crunch	DS	15/10			0/120
Back	Bent Over Bar Bell Rows	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Cable Row	1	10			90
|	|	2	8			30
|	Lat Pull Down/Lat Pull Down	DS	10/5			0/120
Shoulders	Standing Bar Bell Shoulder Press	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Dumb Bell Side Raise	1	10			90
|	|	2	8			30
|	Bent Over Dumb Bell Raise/Bent Over Dumb Bell Raise	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Monday	Tuesday	Wednesday	Thursday
Friday	**Saturday**	**Sunday**	***Notes***

An example on how to fill this Physical Activity record, appears at the front of the Training Log Book

Weight Training Workout 1
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Abdominals	Bench Oblique Crunches	1	15			30
|	|	2	15			30
|	Reverse Crunches	1	15			30
|	|	2	15			30
|	Incline Leg Lift/Incline Leg Raise	DS	15/10			0/120
Chest	Bar Bell Bench Press	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Dumb Bell Bench Press	1	10			90
|	|	2	8			30
|	Dumb Bell Flys/Dumb Bell Flys	DS	10/5			0/120
Triceps	Tricep Pulldowns	1	10			90
|	|	2	8			90
|	Weighted Bench Dips	1	10			90
|	|	2	8			30
|	French Press/French Press	DS	10/5			0/12(
Biceps	Preacher Curls	1	10			90
|	|	2	8			90
|	Spider Curls	1	10			90
|	|	2	8			30
|	Bar Bell Curls/Bar Bell Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note side effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc.

Weight Training Workout 2
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Calves	Toe Press	1	15			30
"	"	2	15			30
"	One Legged Calf Raises	1	15			30
"	"	2	15			30
"	Standing Calf Raises/Standing Calf Raises	DS	15/10			0/120
Quadraceps	Leg Extension	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Leg Press	1	10			90
"	"	2	8			30
"	Walking Lunge/Walking Lunge	DS	10/5			0/120
Hamstrings	Bent Knee Deadlift	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Swiss Ball Leg Curls	1	10			90
"	"	2	8			30
"	Lying Leg Curls/Lying Leg Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Note *Swiss Ball Leg Curls should be done to musculat failure, you may be able to complete more than 10 reps, the average is usually 15 reps.*

Weight Training Workout 3
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (
Abdominals	Reverse Crunch	1	15			30
"	"	2	15			30
"	Swiss Ball Crunch	1	15			30
"	"	2	15			30
"	Bench Crunch/Oblique Bench Crunch	DS	15/10			0/12
Back	Bent Over Bar Bell Rows	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Cable Row	1	10			90
"	"	2	8			30
"	Lat Pull Down/Lat Pull Down	DS	10/5			0/1
Shoulders	Standing Bar Bell Shoulder Press	1	12			9
"	"	2	10			9
"	"	3	8			9
"	Dumb Bell Side Raise	1	10			9
"	"	2	8			3
"	Bent Over Dumb Bell Raise/Bent Over Dumb Bell Raise	DS	10/5			0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Physical Activity Record Sheet

Monday	Tuesday	Wednesday	Thursday
Friday	**Saturday**	**Sunday**	***Notes***

An example on how to fill this Physical Activity record, appears at the front of the Training Log Book

Weight Training Workout 1
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Abdominals	Bench Oblique Crunches	1	15			30
|	|	2	15			30
|	Reverse Crunches	1	15			30
|	|	2	15			30
|	Incline Leg Lift/Incline Leg Raise	DS	15/10			0/120
Chest	Bar Bell Bench Press	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Dumb Bell Bench Press	1	10			90
|	|	2	8			30
|	Dumb Bell Flys/Dumb Bell Flys	DS	10/5			0/120
Triceps	Tricep Pulldowns	1	10			90
|	|	2	8			90
|	Weighted Bench Dips	1	10			90
|	|	2	8			30
|	French Press/French Press	DS	10/5			0/12(
Biceps	Preacher Curls	1	10			90
|	|	2	8			90
|	Spider Curls	1	10			90
|	|	2	8			30
|	Bar Bell Curls/Bar Bell Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note side effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc.

Weight Training Workout 2
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Calves	Toe Press	1	15			30
|	|	2	15			30
|	One Legged Calf Raises	1	15			30
|	|	2	15			30
|	Standing Calf Raises/Standing Calf Raises	DS	15/10			0/120
Quadraceps	Leg Extension	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Leg Press	1	10			90
|	|	2	8			30
|	Walking Lunge/Walking Lunge	DS	10/5			0/120
Hamstrings	Bent Knee Deadlift	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Swiss Ball Leg Curls	1	10			90
|	|	2	8			30
|	Lying Leg Curls/Lying Leg Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Note *Swiss Ball Leg Curls should be done to musculat failure, you may be able to complete more than 10 reps, the average is usually 15 reps.*

Weight Training Workout 3
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Abdominals	Reverse Crunch	1	15			30
"	"	2	15			30
"	Swiss Ball Crunch	1	15			30
"	"	2	15			30
"	Bench Crunch/Oblique Bench Crunch	DS	15/10			0/120
Back	Bent Over Bar Bell Rows	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Cable Row	1	10			90
"	"	2	8			30
"	Lat Pull Down/Lat Pull Down	DS	10/5			0/12(
Shoulders	Standing Bar Bell Shoulder Press	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Dumb Bell Side Raise	1	10			90
"	"	2	8			30
"	Bent Over Dumb Bell Raise/Bent Over Dumb Bell Raise	DS	10/5			0/(

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Physical Activity Record Sheet

Monday	Tuesday	Wednesday	Thursday
Friday	**Saturday**	**Sunday**	***Notes***

An example on how to fill this Physical Activity record, appears at the front of the Training Log Book

Weight Training Workout 1
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Abdominals	Bench Oblique Crunches	1	15			30
"	"	2	15			30
"	Reverse Crunches	1	15			30
"	"	2	15			30
"	Incline Leg Lift/Incline Leg Raise	DS	15/10			0/120
Chest	Bar Bell Bench Press	1	12			90
"	"	2	10			90
"	"	3	8			90
"	Dumb Bell Bench Press	1	10			90
"	"	2	8			30
"	Dumb Bell Flys/Dumb Bell Flys	DS	10/5			0/120
Triceps	Tricep Pulldowns	1	10			90
"	"	2	8			90
"	Weighted Bench Dips	1	10			90
"	"	2	8			30
"	French Press/French Press	DS	10/5			0/12(
Biceps	Preacher Curls	1	10			90
"	"	2	8			90
"	Spider Curls	1	10			90
"	"	2	8			30
"	Bar Bell Curls/Bar Bell Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note side effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc.

Weight Training Workout 2
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Calves	Toe Press	1	15			30
|	|	2	15			30
|	One Legged Calf Raises	1	15			30
|	|	2	15			30
|	Standing Calf Raises/Standing Calf Raises	DS	15/10			0/120
Quadraceps	Leg Extension	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Leg Press	1	10			90
|	|	2	8			30
|	Walking Lunge/Walking Lunge	DS	10/5			0/120
Hamstrings	Bent Knee Deadlift	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Swiss Ball Leg Curls	1	10			90
|	|	2	8			30
|	Lying Leg Curls/Lying Leg Curls	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Note *Swiss Ball Leg Curls should be done to musculat failure, you may be able to complete more than 10 reps, the average is usually 15 reps.*

Weight Training Workout 3
Weeks 6 through 10

Code Number	
Date	

Muscle Group	Exercise	Set No.	Rqd. Reps.	Wt.	Reps.	Rest (s)
Abdominals	Reverse Crunch	1	15			30
|	|	2	15			30
|	Swiss Ball Crunch	1	15			30
|	|	2	15			30
|	Bench Crunch/Oblique Bench Crunch	DS	15/10			0/120
Back	Bent Over Bar Bell Rows	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Cable Row	1	10			90
|	|	2	8			30
|	Lat Pull Down/Lat Pull Down	DS	10/5			0/12(
Shoulders	Standing Bar Bell Shoulder Press	1	12			90
|	|	2	10			90
|	|	3	8			90
|	Dumb Bell Side Raise	1	10			90
|	|	2	8			30
|	Bent Over Dumb Bell Raise/Bent Over Dumb Bell Raise	DS	10/5			0/0

Note any effects on muscle soreness, strength, and/or recovery time

Note any side-effects such as nausea, dizzyness, stomach cramps, headaches, increased thirst, etc

Monday	Tuesday	Wednesday	Thursday
Friday	**Saturday**	**Sunday**	***Notes***

An example on how to fill this Physical Activity record, appears at the front of the Training Log Book

Appendix 6

6.6 Study Questionnaire

Pilot Study of HMB Supplementation of Resistance Trained Men

Study Questionnaire

Code Number	
Date	
Time of Day	

Questionnaire Part 1. Demographic Questionnaire

Please answer each question carefully.
Tick the boxes following all answers that apply, unless instructed otherwise.
Answer the questions on the left side of the page, the right side will be used by the researcher.

Fill in your answers below; **1.** Please state your date of birth _____	*Researcher Use Only* **1. Age** _____
2. (a) How would you describe your ethnic group? **i.** European ☐ *Go to question 3* **ii.** Maori ☐ *Go to question 3* **iii.** Pacific Islander ☐ *Go to question 3* **iv.** Asian ☐ *Go to question 3* **v.** Indian ☐ *Go to question 3* **vi.** Other ☐	**2(a). Ethnicity** i. = 1 ii. = 2 iii. = 3 iv. = 4 v. = 5 vi. = 6
2. (b) If you answered other, please use your own words to describe your ethnic group _____	**2(b). Ethnicity** _____
3. What is the highest level of education you have completed? _____ _____	**3. Level of Education** **Secondary** 4th From = 1 5th Form = 2 6th Form = 3 7th From = 4 **Work Training** = 5 **Tertiary** = 6 Certificate = 7 Diploma = 8 Degree = 9

4. If you are currently employed full-time, how would you describe your occupation? _____ _____ *i e.g. Legislators, Senior Business Administrators Special Organisation Administrators, Corporate Managers, General Managers, etc* *ii e.g. Physical, Mathematical, Engineering, & Science Professionals, Life Science & Health Professionals, Teaching Professionals, and Other Professionals* *iii e.g. Physical Science & Engineering Associate Professionals, Life Science & Health Associate Professionals, Other Associate Professionals* *iv e.g. Office clerks, Customer Service Clerks, etc* *v e.g. Personal & Protective Services Workers, Salespersons, demonstrators, & models, etc* *vi e.g. Market Orientated Agricultural, Fishery Worker, i.e. Farmer, Crop Owner, Forestry Worker, Fishery Worker, Hunter/Trapper, etc* *vii e.g. Building Trade Worker, Metal/Machinery Trade Worker, Precision Trade Worker, and Other Craft Related Workers* *viii e.g. Industrial Plant Operators, Machine Operator/Assembler, Driver & Mobile Machinery Operator.* *ix e.g. Labourer & Related Elementary Service Worker, i.e. Caretaker, Cleaner, Messenger, Doorkeeper, Refuse Collector, Packer/Freight Handler, Other Labourer.*	**4. Occupation ISCO Coding 1999** i Legislators, Administrators, and Managers = 1 ii Professionals = 2 iii Associate Professionals & Technicians = 3 iv Clerks = 4 v Service & Sales Workers = 5 vi Agriculture & Fishery Workers = 6 vii Trades Workers = 7 viii Plant/Machine Operators/ Assembler = 8 ix Elementary Occupations = 9 x Armed Forces = 10
5. (a) Do you have a history of any long term medical condition? Yes ☐ No ☐ *Go to question 6* Don't Know ☐ *Go to question 6*	**5(a). Long Term Medical Conditions** Yes = 1 No = 0 DK = 8

5. (b) If you answered yes above, please specify _____ _____ _____ _____	**5(b). Medical Condition** _____
6. (a) Over the last three months have you taken any medications? Yes ☐ No ☐ *Go to question 7* Don't Know ☐ *Go to question 7*	**6(a). Medication Use** Yes = 1 No = 0 DK = 8
6. (b) If you answered yes above, please specify _____ _____ _____ _____	**6(b). Medication Use** _____
6. (c) If you answered yes to question 6(a), were the medications Prescribed by your doctor? ☐ Prescribed by your chemist? ☐ Self prescribed ☐ Other ☐	**6(c). Prescription** Doctor = 1 Chemist = 2 Self = 3 Other = 9
7. (a) Over the last three months have you taken any herbal preparations, dietary supplements, or sports supplements? Yes ☐ No ☐ *Go to question 8* Don't Know ☐ *Go to question 8*	**7(a). Supplement Use** Yes = 1 No = 0 DK = 8

7. (b) Please record below, all the information you can regarding supplements you are currently taking.

7(b). Supplements

Name	Brand	Dose *(amount taken each time, e.g. number of tablets, weights, volumes)*	Freq. *(how often the supplements are taken per day)*

7. (c) *Feel free to supply interviewer with an empty bottle or container, or photocopy of the supplement label.*

8.	Are you currently, or have you ever used steroids? Yes ☐ No ☐ Don't Know ☐	**8. Steroid Use** Yes = 1 No = 0 DK = 8

Questionnaire Part 2. General Dietary Questionnaire

9. (a) How would you describe your diet?

- **i.** Eat a variety of all foods, including animal products ☐
- **ii.** Eat eggs, dairy products, fish and chicken, but avoid other meat ☐
- **iii.** Eat eggs, dairy products, fish, but avoid chicken and other meat ☐
- **iv.** Eat eggs and dairy products, but avoid all meats and fish ☐
- **v.** Eat eggs, but avoid dairy products, meats, fish ☐
- **vi.** Eat dairy products, but avoid eggs, meats, fish ☐
- **vii.** Eat no meat, fish, milk, or eggs ☐
- **viii.** Other ☐

9(a). Eating Habits

i.	= 1
ii.	= 2
iii.	= 3
iv.	= 4
v.	= 5
vi.	= 6
vii.	= 7
viii.	= 8

9. (b) If you answered other above, please specify

_____

_____

9(b). Eating Habits

_____

10. (a) Are there any foods or drinks you don't consume due to allergies or beliefs?

Yes	☐	
No	☐	*Go to question 11*
Don't Know	☐	*Go to question 11*

10(a). Food Aversions

Yes	= 1
No	= 0
DK	= 8

10. (b) If you answered yes above, please specify

_____

_____

_____

_____

10(b). Food Aversions

_____

11. Do you think that a diet consisting only of whole foods (no vitamin, mineral, or other dietary supplements) adequately provides your body with all the nutrients it requires? Yes ☐ No ☐ Don't Know ☐	**11. Food Beliefs** Yes = 1 No = 0 DK = 8
12. On average, how many servings of fruit do you eat per day? *Includes fresh, frozen, canned preserved, or stewed.* *1 serving equals 1 medium piece of fruit, 2 small pieces of fruit, or 1/2 cup stewed fruit.* **i.** Don't eat fruit ☐ **ii.** Less than 1 serving a day ☐ **iii.** 1 serving a day ☐ **iv.** 2 servings a day ☐ **v.** 3 or more servings a day ☐	**12. Fruit Consumption** i. = 5 ii. = 4 iii. = 3 iv. = 2 v. = 1 DK = 8
13. On average, how many servings of vegetables do you eat per day? *Include fresh, frozen, and canned.* *1 serving equals 1 medium potato or kumara, 1/2 cup cooked vegetables, 1 cup salad vegetables* **i.** Don't eat vegetables ☐ **ii.** Less than 1 serving a day ☐ **iii.** 1 serving a day ☐ **iv.** 2 servings a day ☐ **v.** 3 servings a day ☐ **vi.** 4 or more servings a day ☐	**13. Vegetable Consumption** i. = 6 ii. = 5 iii. = 4 iv. = 3 v. = 2 vi. = 1 DK = 8
14. On average, how many servings of cereals such as noodles, pasta, or rice do you eat per day? *1 serving equals 1/2 cup of cooked noodles, pasta, or rice.* **i.** Don't eat cereals ☐ **ii.** Less then 1 serving a day ☐ **iii.** 1-2 servings a day ☐ **iv.** 3-4 servings a day ☐ **v.** 5-6 or more servings a day ☐ **vi.** 7 or more servings a day ☐	**14. Cereal Consumption** i. = 6 ii. = 5 iii. = 4 iv. = 3 v. = 2 vi. = 1 DK = 8

15. On average, how many servings of breakfast cereals do you eat each day? *1 serving equals 2 weetbix, 1/2 cup museli, 1 cup cooked porridge, or 1 cup other breakfast cereal* **i.** Don't eat breakfast cereals ☐ **ii.** Less then 1 serving a day ☐ **iii.** 1-2 servings a day ☐ **iv.** 3-4 servings a day ☐ **v.** 5-6 or more servings a day ☐ **vi.** 7 or more servings a day ☐	**15. Breakfast Cereal Consumption** i. = 6 ii. = 5 iii. = 4 iv. = 3 v. = 2 vi. = 1 DK = 8
16. On average, how many servings of breads and bakery products do you eat per day? *1 serving includes 1 slice, bun, roll, or pita of bread or toast, 1 medium muffin, or pastry.* **i.** Don't eat bread or bakery products ☐ **ii.** Less then 1 serving a day ☐ **iii.** 1-2 servings a day ☐ **iv.** 3-4 servings a day ☐ **v.** 5-6 servings a day ☐ **vi.** 7 or more servings a day ☐	**16. Bread and Bakery Product Consumption** i. = 6 ii. = 5 iii. = 4 iv. = 3 v. = 2 vi. = 1 DK = 8
17. On average, how many servings of dairy products do you eat per day? *Include milk, cream, yoghurt, cottage cheese, cheese, etc.* *1 serving equals 1 cup milk, cottage cheese, 1 tub of yoghurt (~150g), 2 slices of cheese.* **i.** Don't eat dairy products ☐ **ii.** 1 serving a day ☐ **iii.** 2 servings a day ☐ **iv.** 3 servings a day ☐ **v.** 4 or more servings a day ☐	**17. Dairy Product Consumption** i. = 5 ii. = 4 iii. = 3 iv. = 2 v. = 1 DK = 8

18. On average, how many servings of meat do you eat per day? *Include redmeat, poultry, fish, and seafood. 1 serving equals 2 slices of cooked meat (~100g), 3/4 cup mince or casserole, (~195g), 1 medium fish fillet (~100g), 1 medium steak (~120g), 2 chicken drumbsticks or 1 chicken leg (~110g), 1 medium paua (120g), 3 medium muscles (30g), 1 kina (100g)* **i.** Don't eat meat or meat products ☐ **ii.** Less than 1 serving a day ☐ **iii.** 1-2 servings a day ☐ **iv.** 3-4 servings a day ☐ **v.** 5 or more servings a day ☐	**18. Meat Consumption** i. = 6 ii. = 5 iii. = 4 iv. = 3 v. = 2 DK = 8
19. Is salt normally added during the preparation or serving of your food? Yes ☐ No ☐ Don't Know ☐	**19. Salt Consumption** Yes = 1 No = 0 DK = 8
20. (a) Do you drink caffeinated drinks? *Include coffee, tea, cola, and energy drinks* Yes ☐ No ☐ *Go to question 21* Don't Know ☐ *Go to question 21*	**20(a). Caffeinated Drinks** Yes = 1 No = 0 DK = 8
20. (b) If you answered yes above, how many caffeinated drinks would you consume per day? **i.** <1 caffeinated drink ☐ **ii.** 1 caffeinated drink ☐ **iii.** 2-4 caffeinated drinks ☐ **iv.** 5 or more caffeinated drinks/day ☐	**20(b). Caffeinated Drink Consumption** i. = 1 ii. = 2 iii. = 3 iv. = 4
21. (a) Do you usually drink alcoholic drinks? Yes ☐ No ☐ *Go to question 22* Don't Know ☐ *Go to question 22*	**21(a). Alcoholic Drinks** Yes = 1 No = 0 DK = 8

21. (b) If you answered yes above, how often do you consume alcoholic drinks? **i.** < 1 time/month ☐ **ii.** 1-2 times/month ☐ **iii.** 2-3 times/week ☐ **iv.** 1 time/week ☐ **v.** daily ☐	**21(b). Alcoholic Drink Consumption** i. = 1 ii. = 2 iii. = 3 iv. = 4 v. = 5
21. (c) When you drink alcohol, what type of alcoholic drink do you usually consume? *Such as beer, wine, spirits, etc.* _____ _____ _____	**21(c). Type of Alcohol Usually Consumed** Beer = 1 Wine = 2 Spirits/Liquers = 3 Other = 4
21. (d) When you drink alcohol, how much do you usually drink? *One standard drink equals 300-350mL of beer, 1 glass of wine (100mL), a measure of spirits (30mL)* Number of standard drinks _____	**21(d). Amount of Alcohol Usually Consumed** Number of standard drinks _____
22. (a) Have you ever smoked? Yes ☐ No ☐ Don't Know ☐	**22(a). Past Smoking Habits** Yes = 1 No = 0 DK = 8
22. (b) Do you currently smoke? Yes ☐ No ☐ *Go to question 23* Don't Know ☐ *Go to question 23*	**22(b). Present Smoking Habits** Yes = 1 No = 0 DK = 8

22. (c) How many cigarettes would you usually smoke per day?		22(c). Smoking Quantity
i. Less than 5		i. = 1
ii. 5-9		ii. = 2
iii. 10-14		iii. = 3
iv. 15-20		iv. = 4
v. 20 or more		v. = 5

Questionnaire Part 3. Physical Activity Questionnaire

23. How long have you been training with weights? _____	**23. Resistance Training Experience** Years = _____
24. How long have you been following the same weight training programme? _____	**24. Pattern of Resistance Training** \< 4 weeks = 1 \< 8 weeks = 2 \< 12 weeks = 3 > 12weeks = 4 > 6 months = 5
25. (a) Do you regularly perform any other exercises or sports other than weight training? Yes ☐ No ☐ *Go to question 26* Don't Know ☐ *Go to question 26*	**25(a). Physical Activity Habits in Addition to Resistance Training** Yes = 1 No = 0 DK = 8
25. (b) If you answered yes above, please describe this physical activity in your own words _____ _____ _____ _____	**25(b). Physical Activity Habits in Addition to Resistance Training** _____
26. (a) What is the average frequency of weight training you usually perform each week? _____	**26(a). Frequency of Resistance Training** Frequency _____

26. (b) What is the average frequency of other physical activity you usually perform each week? _____	**26(b). Frequency of Other Physical Activity** Frequency _____
27. (a) What is the average duration of each weight training session? _____	**27(a). Average Duration of Resistance Training** Minutes _____
27. (b) What is the average duration of other physical activity you usually perform? _____	**27(b). Average Duration of Other Physical Activity** Minutes _____
28. (a) Who designed your previous weight training programme? **i.** Sports Coach ☐ **ii.** Personal Trainer/Gym Staff ☐ **iii.** You designed it yourself ☐ **iv.** Training Partner/Friend ☐ **v.** Other ☐	**28(a). Source of Resistance Training Programme** i. = 1 ii. = 2 iii. = 3 iv. = 4 v. = 5
28. (b) If you answered other, please explain _____ _____ _____	**28(b). Source of Resistance Training Programme Continued** _____
28. (c) Please describe, in your own words, your current weight training programme. *Include exercises, reps, and sets where you can.* _____ _____ _____ _____ *Continued over the page*	**28(b). Resistance Training Programme**

28. (d) *Feel free to attach a photocopy of your training programme to this questionnaire*

29. Do you plan to compete in a bodybuilding competition in the next 6 months?

Yes ☐
No ☐
Don't Know ☐

29. Competitive Bodybuilder

Yes = 1
No = 0
DK = 8

30. (a) Is your bodyweight relatively stable? Yes ☐ No ☐ Don't Know ☐	**30(a). Stable Bodyweight** Yes = 1 No = 0 DK = 8
30. (b) How long have you been at your current body weight? **i.** Less than 6 months ☐ **ii.** Greater than 6 months ☐ **iii.** Greater thn one year ☐ **iv.** Greater than two years ☐	**30(b). Current Body weight** i. = 4 ii. = 3 iii. = 2 iv. = 1
31. How long have you been following your current eating habits? **i.** Less than 6 months ☐ **ii.** Greater than 6 months ☐ **iii.** Greater thn one year ☐ **iv.** Greater than two years ☐	**31. Current Eating Habits** i. = 4 ii. = 3 iii. = 2 iv. = 1

32. (a) Since beginning weight training, have you ever undergone restrictive dieting practices, for example dieting for competitive bodybuilding or to make a body weight category for sport? Yes ☐ No ☐ *Go to question 33* Don't Know ☐ *Go to question 33*	**32(a). Restrictive Dieting Practices** Yes = 1 No = 0 DK = 8
32. (b) If you answered yes above, please explain the restrictive dieting practices you have followed _____ _____ _____ _____ _____ _____	**32(b). Restrictive Dieting Practices Continued** _____

33. (a) Have you recieved any dietary advice in the past?

Yes	☐	
No	☐	*Go to end*
Don't Know	☐	*Go to end*

33(a). Received Dietary Advice

Yes = 1
No = 0
DK = 8

33. (b) If so, please indicate the sources of the dietary advice you have received in the past?

Tick yes or no to each option below

	Yes	No
Family/Friends	☐	☐
Trainer/Gym Staff	☐	☐
Nutritionist/Dietitian	☐	☐
Doctor	☐	☐
Other Gym Users	☐	☐
Media/Websites	☐	☐
Other	☐	☐

33(b). Sources of Dietary Advice

Yes = 1
No = 0
DK = 8
Total Score _____

33. (c) If you answered other above, please explain

_____

_____

_____

_____

33(c). Sources of Dietary Advice Continued

_____

Your contribution to this study is greatly appreciated. Thankyou.

Appendix 7

6.7 Data Collection Form

	Visit 1	Visit 2	Visit 3	Visit 4
Code Number				
Date				
Time				

Anthropometric Restricted Profile

Skinfolds (mm)

	Visit 2				Visit 4			
Site	Trial 1	Trial 2	Trial 3	Mean	Trial 1	Trial 2	Trail 3	Mean
Triceps								
Subscapular								
Biceps								
Iliac Crest								
Supraspinale								
Abdominal								
Front Thigh								
Medial Calf								
Height (cm)								
Weight (kg)								

Bioelectrical Impedance Analysis

	BIA Visit 2	BIA Visit 4
Fat Mass		
%Fat Mass		
FFM (Fat Free Mass)		
%FFM (% Fat Free Mass)		

Assessment of Muscular Strength

Quadriceps

1RM Leg Extension	Right Leg Maximum	Left Leg Maximum
Visit 1		
Visit 2		
Visit 3		
Visit 4		

Chest

1RM Bench Press	Maximum Bench Press	
Visit 1		
Visit 2		
Visit 3		
Visit 4		

Biceps

1RM Single Arm Bicep Curl (Preacher Bench)	Right Arm Maximum	Left Arm Maximum
Visit 1		
Visit 2		
Visit 3		
Visit 4		

Appendix 8

6.8 Procedures for Testing Isotonic (Dynamic) Strength on the Leg Extension, Bench Press, and Preacher Curl Apparatus

Strength Testing Using the One Repetition Maximum Method

The 1RM method was chosen to test strength because it was useful in predicting performance in other dynamic activities (Wilson, 1994); the protocol has good reliability (Wilson, 1994); and used resistance exercise machines which reduced risk of accident.

Prior to testing strength, each subject completed a five minute warm up on their preferred aerobic exercise apparatus (Dowson, 1999). The strength testing apparatus was adjusted for each individual, and seat and bench positions noted for later retesting. In addition a warm up was done on each strength test exercise using a load estimated to be 40-60% of the individual's perceived 1RM, for 5-10 repetitions. Between each set subjects were given a one minute rest period, and allowed to stretch if required. Next, a weight close to 60-80% of the subject's perceived 1RM was chosen for 3-5 repetitions. Load increments became smaller (1-5 kg increments) as the weight came closer to the perceived 1RM of the subject. Each attempt where the individual was able to perform more than one lift, the weight was increased, until failure to complete full range of motion during a lift (Kraemer & Fry, 1995). The researcher observed subject technique to ensure full range of movement was completed for each exercise attempt, as well as offering encouragement to each subject.

Leg Extension Apparatus

A single leg extension exercise was used to assess strength of each leg during this exercise. The subject was positioned with the back of their knees on the edge of the padded seat, with the tested leg under the extension pad, the pad resting low on the shin of the leg. The subject was asked to hold the handles of the machine and was belted into the machine to stabilise the body. From a starting position where the knee was bent at a 90° angle, the participant was asked to raise the weight until knee and hip were parallel to the floor. This was counted as one repetition.

Bench Press Apparatus

The bench press barbell was previously marked at two points 40cm from the centre of the bar, so that hand positions of all participants would be tested in the same position each attempt. Each participant was asked to lie on the bench under the Smith Press machine and position themselves so the centre of their pectoral muscles was in line with the barbell. From chest position, the bar was raised until arms fully extended, for one repetition. With the aid of the spotter the barbell was returned to the rack.

Bicep Preacher Curl Apparatus

Each subject was positioned on the Preacher Curl bench, with triceps pressed firmly against the bench padding. Subjects were advised that elbows should not move to either side or come off the bench for the lift to be counted. The subject was asked to fully extend the arm to be tested, and the chosen dumbbell placed in position by a spotter. From the start position, the subject was asked to raise the weight as far as possible and pause at the top of the movement. This was counted as one repetition.

Appendix 9

6.9 Three Day Dietary Record Sheets

3-Day Diet Record

Instructions

On the following pages, spaces have been provided to record everything you eat and drink, over three days. These three days should include two weekdays, and one weekend day, but it is not necessary to be 3 days in a row.

(1). The record for each day may start at any time during the day, but must continue for 24 hours.

(2). Record as accurately as possible, all food and drink consumed on the diet record sheets provided.

(3). Record the volume of each food using weights or volumes that occur on the packaging of the food, or measure food with measuring cups, tablespoons, or teaspoons, weigh the food on kitchen scales, or record number of units eaten e.g. slices of bread, number of apples, etc.

(4). Describe the cooking method used.

(5). Record recipes of home prepared dishes where possible, and the proportion of the meal you consumed.

(6). Record the cuts, and type of meat, type of milk, bread, brand names etc.

(7). After any meal if there is edible food left over, the amount must be estimated and subtracted from the amount originally recorded. It is not necessary to include bones, fruit peel, cores, or stones, as these are already accounted for.

An example of a diet record is given over the page;

Code Number	XX
Date	10-07-02
Page Number	1

Time	Quantity	Food Type and Brand
7:30am	1	220g can of Watties Baked Beans
	2	Slices of Vogel's Mixed Grain Toast
	1 tsp	Butter
	1 pinch	Salt
	2	Whole eggs poached in water
	1 tsp	Instant Coffee
	1 tsp	White sugar
	1/4 cup	Trim milk, Anchor

Code Number	
Date	
Page Number	

Time	Quantity	Food Type and Brand

Appendix 10

6.10 Dietary Recommendations

Dietary Recommendations For the Duration of This Study
You are asked not to consume any other dietary supplements for four weeks prior to beginning the study, and for the duration of the study period. This especially includes other HMB products, protein powders, protein shakes, protein/sports bars, and products containing creatine. Dietary supplements such as creatine, have been shown in studies to influence the effect of HMB on the body, therefore continued use of additional dietary supplements, may invalidate the results of this study. By following the diet examples given over the following few pages, you will easily be able to achieve your protein, vitamin and mineral requirements by eating whole foods.

Dietary energy, protein, and carbohydrate recommendations for strength training athletes, such as yourselves, are given below.

Energy
There are several methods used to predict the energy requirements of individuals, but for the purposes of this study we will be using the recommendations from the American Dietetic Association for strength trained athletes and body builders. The energy intake recommended for male bodybuilders wanting to maintain current body mass is 44 kcal/kg/day; to increase body weight but minimise fat gain is 52-60 kcal/kg/day; to maintain muscle, increase muscle definition but lose body fat is 30 kcal/kg/day.

These are suggested values only, due to the large number of factors that influence the energy requirements of an individual. The basal energy requirement is the energy required to run body systems and is influenced by age, sex, body size (lean mass and fat mass), and some genetic influence. The thermic effect of food is the energy required to digest, absorb, transport, metabolise, and store the nutrients provided from food, and is influenced by energy provided during a meal and throughout the day, the percentage of protein, carbohydrate, and fat in a meal and throughout the day, and level of obesity. Energy required for physical activity depends on daily activities and exercise duration and intensity, and involuntary muscle contractions such as shivering and fidgeting. Other influences on energy expenditure include temperature, stress, and medications/drugs.

Protein

Protein requirements of strength athletes who have been training for greater than one year, are in the range 1-1.4 g/kg/day. Intakes of dietary protein above these requirements, provide no added benefit to muscle gain. There is not thought to be any adverse health effects from consuming a diet of less than 2g/kg/day of protein. But there is concern that excess intakes of protein over long periods increase calcium urinary excretion, and aggravate any existing renal disease.

Table 1; Examples of Food Portions Containing 15g of Protein

Red Meat		
50g lean cooked meat	75g lean cooked mince	1 hamburger patty
2/3 cup meat casserole/stew	1 large/2 small sausages	2 slices luncheon
2 slices bacon	1 thick slice of ham	
Poultry and Eggs		
60g cooked, skinned chicken/duck/turkey		2 large eggs
Fish		
1 small, baked fillet of fish	75g canned salmon/sardines	50g canned tuna
7 oysters	1/2 cup of mussels	
Milk and Dairy Products		
400mL milk	300mL yoghurt	60g hard cheese
100g cottage cheese	1 milkshake	
Soy Products/Beans/Lentils/Chickpeas		
450mL soy milk	3/4 cup tofu	1 cup soy beans
1 cup kidney/baked beans	1/2 cup humus	
1 1/2 cup lentils/haricot beans/chickpeas		
Nuts and Seeds		
1/2 cup almonds/cashew nuts	1 cup brazil nuts	1 1/2 cup walnuts/hazel nuts
3 Tblsp pumpkin seeds	8 Tblsp sunflower seeds	10 Tblsp sesame seeds
Breads and Cereals		
3 cups cooked rice/pasta	6 slices whole-grain bread	1 cup muesli (fruit and nut)

Carbohydrate

Carbohydrate requirements range from 5-7g/kg/day (50-55%) for strength-trained athletes to 12g/kg/day (60-70%) for endurance-trained athletes. The New Zealand Nutrition Taskforce recommends 6 or more servings of breads and cereals, 3 or more servings of vegetables, and at least 2 servings of fruits per day.

Table 1; Examples of Food Portions Containing 20g of Carbohydrate

Bread and Baked Products		
2 slices thin bread	1 slice thick bread	1 dinner roll
1/2 long roll	1/2-1 pita bread	1/2 bagel
1 crumpet/English muffin	1 scone/muffin	2 1/2 Gingernuts
5 Ryvita crackers/cream crackers	2 Salada crackers	1/2 fruit/iced bun
2 plain Digestives	2 thin pancakes	1 1/2 pikelets
1 Fruity-bix bar	1/2 Mother Earth/Uncle Toby's cereal bar	
Breakfast Cereals and Grains		
1 cup cooked porridge	2 Weet-bix	1/3-1/2 cup muesli
2 cups plain popcorn	1/2 cup cooked pasta/rice/noodles	
1 cup Cornflakes/Rice bubbles/Light 'n Tasty/Stamina, etc		
Vegetables		
1/2 cup corn/yams	1 cup beetroot/mixed veges/broad beans	
2 1/2 cup peas	1 medium potato/kumara/taro/parsnip	
1 cup pumpkin		
Fruit		
1 medium banana	1 large apple/pear/orange/peach	

2 large apricots/kiwifruit/grapefruit	20 cherries/grapes	12 strawberries
1 cup canned/stewed fruit	2 Tblsp raisin/sultanas	
Dairy Products		
400mL milk	300mL plain yoghurt	200mL fruit yoghurt
2 scoops ice-cream		
Confectionery and Drinks		
1 Tblsp jam/honey/sugar	300mL sports drink	

Meal Frequency

The timing of meals and snacks, especially protein intake is important for resistance trained athletes. To consume the amount of food required to fuel your training, eat frequently throughout the day, plan for 5-6 meals/snacks in a day. To maintain blood amino acid levels, try to include a serving of protein-rich foods in each of your meals and snacks throughout the day. Following training you should try to consume a snack or meal that provides high carbohydrate and moderate protein levels, as this will increase your levels of anabolic (protein synthesis) hormones, muscle protein synthesis, and decrease proteolysis (muscle protein breakdown).

Example 1

For a 70kg man, protein requirements are 70-98g per day, carbohydrates requirements are 350-490g per day, with the rest of the daily calories made up of fat. An example of a maintenance diet is included below.

Foods	**Amounts**	PRO(g)	FAT(g)	CARB(g)
Breakfast				
Cereal, mixed type	1cup	6	2	30
Trim milk	1/2 cup	6	1	7
Sliced Banana on cereal	1 banana	1	1	31
Multi-grain Bread, toasted	2 slices	4	0	21
Margarine spread on the toast	2 tsp.	0	7	0
Peanut butter spread on the toast	1 Tblsp	5	8	2
Berry Jam spread on the toast	1 Tblsp	0	0	14
Morning Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	4 crackers	3	5	24
Cottage Cheese, low fat	20g	4	0	0
Tomato, raw	1 tomato	1	0	3
Spread cottage cheese, and place tomato slices on the crackers, add pepper to taste.				
Lunch				
Multi-grain bread	4 slices	9	1	41
Tuna, canned in spring water	45g	11	1	0
Mayonnaise, reduced fat	1 Tblsp	0	6	7
Salad, no cheese or dressing	1/2-1/3 cups of salad	0	0	1
Olives, sliced	4 olives	0	1	0
Margarine, spread on the bread	4 tsp.	0	15	0
Make the above ingredients into sandwiches				
Potato, microwaved, no salt	1 medium potato	3	0	21
Chives, sprinkled on potato	1 tsp.	0	0	0
Mayonnaise, reduced fat	1 tsp.	0	1	1
Muesli bar, fruit flavour	2 bars	1	12	36
Afternoon Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	4 crackers	3	5	24
Smoked Salmon slices	1 slice	3	1	0
Tomato, raw	1 tomato	1	0	3
Cucumber slices	4 slices	0	0	1

Post-Workout Snack				
Muesli bar, fruit flavour	2 bars	1	12	36
Yoghurt, reduced fat, fruit flavour	1 small carton	7	3	22
Dinner				
Prime Beef Mince	45g	10	3	0
Drain the fat from the mince when cooking if possible.				
Olive Oil, used to cook the mince	1 Tblsp	0	14	0
Pasta sauce, tomato based	2 Tblsp	1	0	6
Add pasta sauce and additional spices to mince while cooking				
Fresh pasta, boiled	200g	14	1	48
Salad, no cheese	2-2 1/2-cups of salad	2	0	3
Italian style salad dressing, lite	1 serving	0	0	1
Soft Brown Bread Roll	1 roll	5	2	24
Margarine, spread on bread roll	1 tsp.	0	7	0

Example 2

For an 80kg man, protein requirements are 80-112g per day, carbohydrates requirements are 400-560g per day, with the rest of the daily calories made up of fat. An example of a maintenance diet is included below.

Foods	**Amounts**	**PRO(g)**	**FAT(g)**	**CARB(g)**
Breakfast				
Cereal, mixed type	1 1/2 cup	9	3	45
Trim milk	1/2 cup	6	1	7
Sliced Banana on cereal	1 banana	1	1	31
Multi-grain Bread, toasted	2 slices	4	0	21
Margarine spread on the toast	2 tsp.	0	7	0
Peanut butter spread on the toast	1 Tblsp	5	8	2
Berry Jam spread on the toast	1 Tblsp	0	0	14
Boiled Egg	1 size 7 egg	6	5	0
Morning Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	6 crackers	5	7	36
Cottage Cheese, low fat	20g	4	0	0
Tomato, raw	1 tomato	1	0	3
Spread cottage cheese, and place tomato slices on the crackers, add pepper to taste.				
Lunch				
Multi-grain bread	4 slices	9	1	41
Tuna, canned in spring water	45g	11	1	0
Mayonnaise, reduced fat	1 Tblsp	0	6	7
Salad, no cheese or dressing	1/2-1/3 cups of salad	0	0	1
Olives, sliced	4 olives	0	1	0
Margarine, spread on the bread	4 tsp.	0	15	0
Make the above ingredients into sandwiches				
Potato, microwaved, no salt	1 medium potato	3	0	21
Chives, sprinkled on potato	1 tsp.	0	0	0
Mayonnaise, reduced fat	1 tsp.	0	1	1
Muesli bar, fruit flavour	2 bars	1	12	36
Afternoon Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	6 crackers	5	7	36
Smoked Salmon slices	1 slice	3	1	0
Tomato, raw	1 tomato	1	0	3
Cucumber slices	6 slices	0	0	1
Post-Workout Snack				
Muesli bar, fruit flavour	2 bars	1	12	36
Yoghurt, reduced fat, fruit flavour	1 small carton	7	3	22
Creamed rice, canned, reduced fat	1 cup	9	6	33

Dinner				
Prime Beef Mince	45g	10	3	0
Drain the fat from the mince when cooking if possible.				
Olive Oil, used to cook the mince	1 Tblsp	0	14	0
Pasta sauce, tomato based	2 Tblsp	1	0	6
Add pasta sauce and additional spices to mince while cooking				
Fresh pasta, boiled	200g	14	1	48
Salad, no cheese	2-21/2-cups of salad	0	0	1
Italian style salad dressing, lite	1 serving	0	0	1
Soft Brown Bread Roll	1 roll	5	2	24
Margarine, spread on bread roll	1 tsp.	0	7	0

Example 3

For a 90kg man, protein requirements are 90-126g per day, carbohydrates requirements are 450-630g per day, with the rest of the daily calories made up of fat. An example of a maintenance diet is included below.

Foods	**Amounts**	**PRO(g)**	**FAT(g)**	**CARB(g)**
Breakfast				
Cereal, mixed type	11/2 cup	9	3	45
Trim milk	1/2 cup	6	1	7
Sliced Banana on cereal	1 banana	1	1	31
Multi-grain Bread, toasted	3 slices	6	1	31
Margarine spread on the toast	2 tsp.	0	7	0
Peanut butter spread on the toast	1 Tblsp	5	8	2
Berry Jam spread on the toast	1 Tblsp	0	0	14
Boiled Egg	1 size 7 egg	6	5	0
Morning Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	8 crackers	7	10	48
Cottage Cheese, low fat	20g	4	0	0
Tomato, raw	1 tomato			
Spread cottage cheese, and place tomato slices on the crackers, add pepper to taste.				
Lunch				
Multi-grain bread	4 slices	9	1	41
Tuna, canned in spring water	45g	11	1	0
Mayonnaise, reduced fat	1 Tblsp	0	6	7
Salad, no cheese or dressing	1/2-1/3 cups of salad	0	0	1
Olives, sliced	4 olives	0	1	0
Margarine, spread on the bread	4 tsp.	0	15	0
Make the above ingredients into sandwiches				
Potato, microwaved, no salt	1 medium potato	3	0	21
Chives, sprinkled on potato	1 tsp.	0	0	0
Mayonnaise, reduced fat	1 tsp.	0	1	1
Muesli bar, fruit flavour	2 bars	1	12	36
Afternoon Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	8 crackers	7	10	48
Smoked Salmon slices	1 slice	3	1	0
Tomato, raw	1 tomato	1	0	3
Cucumber slices	8 slices	0	0	1
Post-Workout Snack				
Muesli bar, fruit flavour	2 bars	1	12	36
Yoghurt, reduced fat, fruit flavour	1 small carton	7	3	22
Creamed rice, canned, reduced fat	11/2 cup	13	9	49
Dinner				
Prime Beef Mince	45g	10	3	0
Drain the fat from the mince when cooking				

Olive Oil, used to cook the mince	1 Tblsp	0	14	0
Pasta sauce, tomato based	2 Tblsp	1	0	6
Add pasta sauce and additional spices to mince while cooking				
Fresh pasta, boiled	200g	14	1	48
Salad, no cheese	2-2 1/2-cups of salad	2	0	3
Italian style salad dressing, lite	1 serving	0	0	1
Soft Brown Bread Roll	1 roll	5	2	24
Margarine, spread on bread roll	1 tsp.	0	7	0

Example 4

For a 100kg man, protein requirements are 100-140g per day, carbohydrates requirements are 500-700g per day, with the rest of the daily calories made up of fat. An example of a maintenance diet is included below.

Foods	**Amounts**	**PRO(g)**	**FAT(g)**	**CARB(g)**
Breakfast				
Cereal, mixed type	1 1/2 cup	9	3	45
Trim milk	1/2 cup	6	1	7
Sliced Banana on cereal	1 banana	1	1	31
Multi-grain Bread, toasted	3 slices	6	1	31
Margarine spread on the toast	2 tsp.	0	7	0
Peanut butter spread on the toast	1 Tblsp	5	8	2
Berry Jam spread on the toast	1 Tblsp	0	0	14
Boiled Egg	1 size 7 egg	6	5	0
Morning Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	10 crackers	9	12	60
Cottage Cheese, low fat	20g	4	0	0
Tomato, raw	1 1/2 tomato	2	0	5
Spread cottage cheese, and place tomato slices on the crackers, add pepper to taste.				
Lunch				
Multi-grain bread	4 slices	9	1	41
Tuna, canned in spring water	45g	11	1	0
Mayonnaise, reduced fat	1 Tblsp	0	6	7
Salad, no cheese or dressing	1/2-1/3 cups of salad	0	0	1
Olives, sliced	6 olives	0	2	0
Margarine, spread on the bread	4 tsp.	0	15	0
Make the above ingredients into sandwiches				
Potato, microwaved, no salt	1 1/2 medium potato	4	0	31
Chives, sprinkled on potato	1 tsp.	0	0	0
Margarine, spread onto hot potato	1 tsp.	0	4	0
Muesli bar, fruit flavour	2 bars	1	12	36
Afternoon Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	10 crackers	9	12	60
Smoked Salmon slices	1 slice	3	1	0
Tomato, raw	1 1/2 tomato	2	0	5
Cucumber slices	10 slices	0	0	1
Post-Workout Snack				
Muesli bar, fruit flavour	2 bars	1	12	36
Yoghurt, reduced fat, fruit flavour	1 small carton	7	3	22
Creamed rice, canned, reduced fat	2 cup	17	13	26
Dinner				
Prime Beef Mince	50g	11	3	0
Drain the fat from the mince when cooking if possible.				
Olive Oil, used to cook the mince	1 Tblsp	0	14	0
Pasta sauce, tomato based	2 Tblsp	1	0	6
Add pasta sauce and additional spices to mince while cooking				

Fresh pasta, boiled	200g	14	1	48
Salad, no cheese	2-2 1/2-cups of salad	2	0	3
Italian style salad dressing, lite	1 serving	0	0	1
Soft Brown Bread Roll	2 rolls	10	4	48
Margarine, spread on bread roll	2 tsp.	0	7	0

Example 5

For a 110kg man, protein requirements are 110-154g per day, carbohydrates requirements are 550-770g per day, with the rest of the daily calories made up of fat. An example of a maintenance diet is included below.

Foods	**Amounts**	**PRO(g)**	**FAT(g)**	**CARB(g)**
Breakfast				
Cereal, mixed type	1 1/2 cup	9	3	45
Trim milk	1/2 cup	6	1	7
Sliced Banana on cereal	1 1/2 banana	2	1	46
Multi-grain Bread, toasted	4 slices	9	1	41
Margarine spread on the toast	2 tsp.	0	7	0
Peanut butter spread on the toast	2 Tblsp	10	17	3
Berry Jam spread on the toast	1 Tblsp	0	0	14
Boiled Egg	1 size 7 egg	6	5	0
Morning Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	10 crackers	9	12	60
Cottage Cheese, low fat	20g	4	0	0
Tomato, raw	1 1/2 tomato	2	0	5
Spread cottage cheese, and place tomato slices on the crackers, add pepper to taste.				
Lunch				
Multi-grain bread	4 slices	9	1	14
Tuna, canned in spring water	45g	11	1	0
Mayonnaise, reduced fat	1 1/4 Tblsp	0	8	9
Salad, no cheese or dressing	1/2-1/3 cups of salad	0	0	1
Olives, sliced	8 olives	0	2	0
Margarine, spread on the bread	4 tsp.	0	15	0
Make the above ingredients into sandwiches				
Potato, microwaved, no salt	2 medium potatoes	5	0	41
Chives, sprinkled on potato	1 tsp.	0	0	0
Margarine	1 tsp.	0	7	0
Muesli bar, fruit flavour	2 bars	1	12	36
Afternoon Snack				
Apple	1 apple	0	1	11
Cream Cracker, low-fat	10 crackers	9	12	60
Smoked Salmon slices	1 slice	3	1	0
Tomato, raw	1 1/2 tomato	2	0	5
Cucumber slices	10 slices	0	0	1
Post-Workout Snack				
Muesli bar, fruit flavour	2 bars	1	12	36
Yoghurt, reduced fat, fruit flavour	1 small carton	7	3	22
Creamed rice, canned, reduced fat	2 cup	17	13	66
Dinner				
Prime Beef Mince	50g	11	3	0
Drain the fat from the mince when cooking if possible.				
Olive Oil, used to cook the mince	1 Tblsp	0	14	0
Pasta sauce, tomato based	3 Tblsp	1	1	8
Add pasta sauce and additional spices to mince while cooking				
Fresh pasta, boiled	250g	17	1	61
Salad, no cheese	2-2 1/2-cups of salad	2	0	3
Italian style salad dressing, lite	1 serving	0	0	1

Soft Brown Bread Roll	2 roll		10	4	48
Margarine, spread on bread roll	2 tsp.		0	7	0

Appendix 11

6.11 Subject Compliance

Table 6.1 Subject Compliance

ID	CAPSULES COLLECTED DURING WEEK										CAPS REMAIN-ING	QUESTION-NAIRE	3DAY DIET 1	RED LOG BOOK	3DAY DIET 2	BLUE LOG BOOK	VISIT 1	VISIT 2	VISIT 3	VISIT 4
	1	2	3	4	5	6	7	8	9	10										
101	Y	Y	Y	Y	Y	Y	Y	Y	Y		0	Y	Y	Y	Y	Y	09/10	16/10	21/12	23/12
102	Y	Y	Y	Y	Y	Y	Y	Y	Y		0	Y	Y	Y			04/10	11/10	20/12	
103	Y	Y	Y	Y	Y	Y	Y	Y	Y		25	Y	Y	Y	Y	Y	03/10	10/10	21/12	23/12
105	Y	Y	Y	Y	Y	Y	Y	Y	Y		12	Y	Y	Y	Y	Y	07/10	15/10	17/12	20/12
106	Y	Y	Y	Y	Y	Y	Y	Y	Y		0	Y	Y	Y	Y	Y	08/10	15/10	18/12	23/12
107	Y	Y	Y	Y	Y	Y	Y	Y	Y		34	Y	Y	Y	Y	Y	08/10	15/10	17/12	20/12
108	Y	Y	Y	Y	Y	Y	Y	Y	Y		24	Y	Y	Y	Y	Y	04/10	15/10	17/12	21/12
109	Y	Y	Q								QUIT	Y					08/10	11/10		
111	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	32	Y	Y	Y		Y	08/10	15/10		23/12
112	Y	Y	Y	Y	Y	Y	Y	Y	Y		14	Y	Y	Y	Y		03/10	10/10	19/12	21/12
113	Y	Y	Y	Y	Y	Y	Y	Y	Y		18	Y		Y			03/10	10/10	16/12	20/12
114	Y	Y	Y	Y	Y	Q					QUIT	Y	Y				03/10	15/10		
115											QUIT						03/10			
116	Y	Y	Y	Y	Y	Y	Y	Y	Y		42	Y	Y				03/10	16/10	19/12	21/12
117	Y	Y	Y	Y	Y	Y	Y	Y	Y		16	Y	Y	Y	Y	Y	09/10	14/10	17/12	23/12
118	Y	Y	Y	Y	Y	Y	Y	Y	Y		0	Y	Y	Y	Y	Y	03/10	10/10	16/12	18/12
119	Y	Y	Y	Y	Y	Q					QUIT	Y					04/10	15/10		

ID	CAPSULES COLLECTED DURING WEEK										CAPS REMAI N-ING	QUEST ION- NAIRE	3DAY DIET 1	RED LOG BOOK	3DAY DIET 2	BLUE LOG BOOK[4]	VISIT 1	VISIT 2	VISIT 3	VISIT 4
	1	2	3	4	5	6	7	8	9	10										
120	Y	Y	Q								QUIT	Y	Y				04/10	11/10		
121	Y	Y	Y	Y	Y	Y	Y	Y	Y	Q	QUIT	Y	Y	Y			07/10	14/10		
122	Y	Y	Y	Y	Y	Y	Y	Y	Y		?	Y	Y	Y			07/10	14/10	16/12	18/12
123	Y	Y	Y	Y	Y	Y	Y	Y	Y		12	Y	Y	Y	Y	Y	07/10	15/10	16/12	18/12
124	Y	Y	Y	Y	Y	Y	Y	Y	Y		20	Y	Y	Y			07/10	16/10		19/12
125	Y	Y	Q								QUIT	Y					07/10	16/10		
126	Y	Y	Y	Y	Y	Y	Y	Y	Y		18	Y	Y				08/10	14/10	19/12	21/12
127	Y	Y	Y	Y	Y	Y	Y	Y	Y		4	Y	Y	Y	Y	Y	08/10	15/10	18/12	20/12
128	Y	Y	Y	Y	Y	Y	Y	Y	Y		24	Y	Y	Y	Y	Y	08/10	15/10	18/12	23/12
129	Y	Y	Y	Y	Y	Y	Y	Y	Y		0	Y	Y	Y	Y	Y	09/10	16/10	27/12	30/12
130	Y	Y	Y	Y	Y	Y	Y	Y	Y		7	Y	Y				09/10	14/10	19/12	30/12
131	Y	Y	Y	Y	Y	Y	Y	Y	Y		0	Y	Y	Y	Y		09/10	14/10	19/12	21/12
133	Y	Y	Y	Y	Y	Y	Y	Y	Y		21		Y	Y	Y	Y	10/10	16/10	19/12	21/12
134	Y	Q									QUIT	Y	Y				09/10	14/10		
135	Y	Y	Y	Y	Y	Y	Y	Y	Y		18	Y	Y	Y	Y	Y	09/10	16/10	19/12	23/12
137	Y	Y	Y	Y	Y	Y	Y	Y	Y	Q	QUIT	Y	Y	Y			16/10	17/10		
138	Y	Y	Y	Y	Y	Y	Y	Y	Y	Q	QUIT						16/10	17/10		

Note: Abbreviations used; Y = yes, N = no, and Q = quit.

Appendix 12

6.12 Individual Results Summary

Summary of Results for _____

Below are your results for all measurements taken during the duration of this study. Also included with these results, is an explanation of what the values for your results mean, and how they compare with population norms.

(1). Dietary Intake

The 3 day dietary records you completed as part of visits 1 and 4 have been analysed and summarised below. Included, is a comparison of the New Zealand Recommended Dietary Intakes (RDIs), and your personal daily nutrient intakes.

Table 1; Comparison of Personal Mean Daily Nutrient Intakes and the New Zealand Recommendations

Nutrient	Males 19-64 years	Average Intake/Day Visit 1	Average Intake/Day Visit 4
Energy (kJ)			
Energy (kcals)			
Protein (%)	11-15		
Carbohydrate (%)	50-55		
Fat (%)	30-33		
Saturated Fat (%)	\<12		
Monounsaturated Fat (%)	\<20		
Protein (g/kg/day)	1-1.4g/kg/day		

Nutrient	Males 19-64 years	Average Intake/Day Visit 1	Average Intake/Day Visit 4
Carbohydrate (g)	5-7g/kg/day		
Fibre (g)	25-30		
Total Sugars (g)	\<15%		
Alcohol (g)	\<60		
Water (g)	1500-2000mL		
Iron (mg)	7		
Calcium (mg)	800		
Sodium (mg)	920-2300		
Folate (mcg)	200		
Zinc (mg)	12		
Vitamin A (mcg)	750		
Vitamin C (mg)	40		
Vitamin B6 (mg)	1.3-1.9		
Vitamin B12 (mcg)	2.0		
Niacin (mg)	19		
Riboflavin (mg)	1.7		
Thiamin (mg)	1.1		
Vitamin E (mg)	10.0		
Magnesium (mg)	320		
Potassium (mg)	1950-5460		
**Selenium (mg)	85		
Phosphorus (mg)	1000		

* 1 standard drink equals 10g of alcohol.
** This is the New Zealand RDI only, not the Australian RDI which is 150 mcg/day.
*** This is the Australian RDI, New Zealand has not adopted these values. and currently has no selenium recommendations.

The New Zealand RDI uses values adopted from the Australian RDI, except where stated. These recommendations are mean daily nutrient requirements aimed at population groups, rather than the requirements of specific individuals. The RDI can be used for athletes due to the large safety margins built into the nutrient recommendations, with a few exceptions, such as percentages of energy from carbohydrate and protein, which will increase with physical activity. See below.

Summary of Good Food Sources of the Nutrients Discussed Above

Protein

Protein requirements of strength athletes who have been training for greater than one year, are in the range 1-1.4 g/kg/day. Intakes of dietary protein above these requirements, provide no added benefit to muscle gain. There is not thought to be any adverse health effects from consuming a diet of less than 2g/kg/day of protein. But there is concern that excess intakes of protein over long periods increase calcium urinary excretion, and aggravate existing renal disease.

Healthy food sources that are rich in protein include lean meats, chicken, fish, seafood, eggs, low-fat dairy products, dried peas, beans, and lentils.

Carbohydrate

Carbohydrate requirements range from 5-7g/kg/day (<55%) for strength-trained athletes to 12g/kg/day (60-70%) for endurance-trained athletes. The New Zealand Nutrition Taskforce recommends 6 or more servings of breads and cereals, 3 or more servings of vegetables, and at least 2 servings of fruits per day.

Good food sources of carbohydrate include fruits, vegetables, legumes, breads, and cereals (includes rice, pasta, breakfast cereals, etc).

Dietary Fat

Dietary fat is required for essential fatty acids omega-3 and omega-6, and fat soluble vitamins A, D, E, and K. Fats are required for an energy source, formation of membranes in the body, lubricants, and signalling molecules such as the sex hormones.

Saturated Fat - these fats occur in animal products such as dairy products, meats, as well as the vegetable oils palm oil, and coconut milk, be wary of the saturated fat content of processed foods. Saturated fats negatively effect total cholesterol and LDL cholesterol (bad cholesterol) levels.

Unsaturated Fats **Polyunsaturated Fatty Acids** - include omega-3, and omega-6 fatty acids. Good sources of omega-3 fatty acids include oily fish, soybean oil, mustard oil, and flax-seed oil. Healthful properties associated with omega-3 consumption include lowered blood pressure, and reduced risk of thrombosis (blood clots). Good sources of omega-6 fatty acids include sunflower seed oils, and corn oils. Healthful properties include lowering of total cholesterol, and LDL cholesterol.

Monounsaturated Fatty Acids - Good sources of monounsaturated fatty acids include olives, olive oil, and canola oils.

Iron

Haem iron, which is more easily absorbed by the body, is found in foods such as meats, chicken, fish, seafood. Non-haem iron is found in plant foods such as vegetables and some fruits, iron supplements, and iron fortified foods. Non-haem iron is not as well

absorbed by the body, with less than 5% being absorbed. Consumption of vitamin C increases iron absorption. Excessive intakes of some grains, soy products, legumes, nuts, and calcium may inhibit some non-haem iron absorption.

Calcium
Dairy products, green vegetables, whole grain cereals and breads, canned fish with bones, tofu, beans, nuts, and calcium fortified products are all a good source of calcium.

Sodium
High dietary intakes of sodium over long periods of time are associated with the development of hypertension (high blood pressure) which is a risk factor for heart disease, stroke, and renal failure. Also damage of the gastrointestinal tract, possible leading to gastric cancer. Be aware of high amounts of sodium (as salt) in processed foods.

Folate
Folate is commonly found in foods such as liver, egg yolk, nuts, green leafy vegetables, citrus fruits, peas, and fortified cereals.

Zinc
Rich sources of zinc include organ meats, other red meats, and shellfish. Zinc is also found in whole grain cereals, nuts, and legumes. Excessive intakes of some grains may reduce the absorption of zinc by the body.

Vitamin A
Animal fats and fish oils are good sources of vitamin A. Plant foods such as vegetables and fruits are good sources of carotenoids which may be converted to vitamin A in the body. Vitamin A is toxic in excessive amounts, via supplementation.

Vitamin C
Food sources of vitamin C include all fruits and vegetables, especially cantaloupes, kiwifruit, strawberries, lemons, oranges, broccoli, capsicums, cauliflower, spinach, tomatoes, brussels sprouts, and asparagus. Vitamin C enhances the absorption of iron in the gut.

Vitamin B6
Food sources include cereals, organ meats, other meats, chicken, fish, fruits.

Vitamin B12 (cobalamin)
Food sources include meat, dairy products, and seafood.

Niacin
Good food sources of niacin include meat, chicken, fish, legumes, peanuts, and some cereals.

Riboflavin
Some sources of riboflavin are milk, eggs, liver, other meats, green vegetables, and small amounts occur in cereals.

absorbed by the body, with less than 5% being absorbed. Consumption of vitamin C increases iron absorption. Excessive intakes of some grains, soy products, legumes, nuts, and calcium may inhibit some non-haem iron absorption.

Calcium
Dairy products, green vegetables, whole grain cereals and breads, canned fish with bones, tofu, beans, nuts, and calcium fortified products are all a good source of calcium.

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Vitamin B6
Food sources include cereals, organ meats, other meats, chicken, fish, fruits.

Vitamin B12 (cobalamin)
Food sources include meat, dairy products, and seafood.

Niacin
Good food sources of niacin include meat, chicken, fish, legumes, peanuts, and some cereals.

Riboflavin
Some sources of riboflavin are milk, eggs, liver, other meats, green vegetables, and small amounts occur in cereals.

Thiamin
Good sources include whole grain cereals, pork, dried peas and beans. Other sources include other meats, fish, vegetables, fruit, and dairy products. Cooking foods causes the loss of about 25% of thiamin form foods.

Vitamin E
Vitamin E is a fat soluble vitamin, therefore is absorbed with fats and oils such as vegetable oils, wheat germ, rice bran, and nuts and seeds.

Magnesium
Good sources come from green leafy vegetables, legumes, tofu, nuts, and whole grains.

Potassium
Sources include fruits such as avocado, banana, cantaloupe, orange juice, and watermelon. Vegetables such as lima beans, potatoes, tomatoes, spinach, and pumpkin. Potassium also occurs in fresh meats.

Selenium
Selenium in New Zealand soils is low, therefore selenium in plant and animal products is also low. Foods containing selenium include seafood, non-refined cereals, dried peas and beans, and chicken. Australian wheat products contain higher levels of selenium than New Zealand.

Phosphorus
Sources of phosphorus include meats, milk, eggs, and cereals.

(2). Assessment of Muscular Strength
The results of your strength assessments at visits 1 and 4, have been recorded below. This will allow you to see any strength gains you achieved during the course of this study.

Visit 1	**Visit 4**
Left Hand _____	Left Hand _____
Right Hand _____	Right Hand _____
Back _____	Back _____
Leg _____	Leg _____
Total Strength _____	Total Strength _____
Relative Strength _____	Relative Strength _____

Table 2; Table of Strength Test Norms

Male Strength Assessment	Left Hand (kg)	Right Hand (kg)	Back (kg)	Leg (kg)	Total Strength (kg)	Relative Strength
Excellent	>68	>70	>209	>241	>587	>7.50
Good	56-67	62-69	177-208	214-240	508-586	7.10-7.49
Average	43-55	48-61	126-176	160-213	375-507	5.21-7.09
Poor	39-42	41-47	91-125	137-159	307-374	4.81-5.20
Very Poor	<39	<41	<91	<137	<307	<4.81

Relative Strength is calculated from total strength (kg) divided by body weight (kg).

The results of your 1 Repetition Maximum for bench press and leg extension exercises during visits 1 and 4, have been recorded below.

Visit 1

Bench Press _____

Leg Extension _____

Visit 4

Bench Press _____

Leg Extension _____

(3). Anthropometric Measurements

Your anthropometric Measurements are as follows;

Visit 2

Skinfolds

*Triceps _____

*Subscapular _____

Biceps _____

Iliac Crest _____

*Supraspinale _____

*Abdominal _____

*Front Thigh _____

*Medial Calf _____

Sum of 6 Skinfolds _____

Visit 4

*Triceps _____

*Subscapular _____

Biceps _____

Iliac Crest _____

*Supraspinale _____

*Abdominal _____

*Front Thigh _____

*Medial Calf _____

Sum of 6 Skinfolds _____

*Indicates the skinfold measurements used to calculate the 'Sum of 6 Skinfolds'.

Girths

Arm (relaxed) _____

Arm (flexed/tensed) _____

Arm (relaxed) _____

Arm (flexed/tensed) _____

Table 2; Table of Strength Test Norms

Male Strength Assessment	Left Hand (kg)	Right Hand (kg)	Back (kg)	Leg (kg)	Total Strength (kg)	Relative Strength
Excellent	>68	>70	>209	>241	>587	>7.50
Good	56-67	62-69	177-208	214-240	508-586	7.10-7.49
Average	43-55	48-61	126-176	160-213	375-507	5.21-7.09
Poor	39-42	41-47	91-125	137-159	307-374	4.81-5.20
Very Poor	<39	<41	<91	<137	<307	<4.81

Relative Strength is calculated from total strength (kg) divided by body weight (kg).

The results of your 1 Repetition Maximum for bench press and leg extension exercises during visits 1 and 4, have been recorded below.

Visit 1 **Visit 4**

Bench Press _____ Bench Press _____

Leg Extension _____ Leg Extension _____

(3). Anthropometric Measurements

Your anthropometric Measurements are as follows;

Visit 2 **Visit 4**

Skinfolds

*Triceps _____ *Triceps _____

*Subscapular _____ *Subscapular _____

Biceps _____ Biceps _____

Iliac Crest _____ Iliac Crest _____

*Supraspinale _____ *Supraspinale _____

*Abdominal _____ *Abdominal _____

*Front Thigh _____ *Front Thigh _____

*Medial Calf _____ *Medial Calf _____

Sum of 6 Skinfolds _____ Sum of 6 Skinfolds _____

*Indicates the skinfold measurements used to calculate the 'Sum of 6 Skinfolds'.

Girths

Arm (relaxed) _____ Arm (relaxed) _____

Arm (flexed/tensed) _____ Arm (flexed/tensed) _____

Waist (minimum) _____	Waist (minimum) _____
Gluteal (maximum) _____	Gluteal (maximum) _____
Calf (maximum) _____	Calf (maximum) _____
Waist to Hip Ratio _____	Waist to Hip Ratio _____

Breadths/Lengths

Humerus _____	Humerus _____
Femur _____	Femur _____
Height (cm) _____	Height (cm) _____
Weight (kg) _____	Weight (kg) _____
Body Mass Index (BMI) _____	Body Mass Index (BMI) _____

Skinfolds - Individual skinfolds measurements give an indication of subcutaneous fat thickness (thickness of fat layer under the skin), and fat distribution on your body. The higher the skinfold measurement (mm), the more body fat is present. The sum of 6 skinfolds is also used as a measure of body fatness. There is increased risk of adverse health effects when the sum of 6 skinfolds values reach 106-130 or greater.

Girth measurements - give an indication of relative muscular development of certain body areas during the study period. These girth measurements will also give some indication of fat distribution on your body.

The waist to hip ratio is calculated using the circumference measurement of the waist, compared to that of the gluteal circumference. This provides a quick measure of body fat distribution, specifically central body fat, and the associated health risk. There is increased risk of adverse health effects when the waist to hip ratio exceeds 0.91-0.93. High measures of central body fat indicate an increased risk of morbidity and mortality from diseases such as heart disease, diabetes, hypertension, high blood cholesterol levels, and obesity.

Bone breadths - are used as a measure of body frame size. This is a more accurate measure than BMI (below), for individuals who have a high body weight due to large bone and muscle mass. Some norms for elbow breadths have been given below, but keep in mind that these values have been taken from American individuals, and may not apply to New Zealander's.

Table 3; Table of Humerus (elbow) breadth measurements and Associated Body Frame Size

	Male Body Frame Size		
Age	**Small**	**Medium**	**Large**
18-24	6.6 or less	> 6.6 to <7.7	7.7 or greater
25-34	6.7 or less	> 6.7 to <7.9	7.9 or greater
35-44	6.7 or less	> 6.7 to <8.0	8.0 or greater
45-54	6.7 or less	> 6.7 to <8.1	8.1 or greater
55-64	6.7 or less	> 6.7 to <8.1	8.1 or greater
65-74	6.7 or less	> 6.7 to <8.1	8.1 or greater

Body Mass Index (BMI) - is a ratio of body weight to height. This is used as an indication of obesity. In New Zealand, a BMI value of 25-29.9 is considered overweight, 30-40 obese, and >40 morbidly obese. This measure may not be useful for athletes with high muscle mass, such as body builders and other strength athletes, as for the sedentary population.

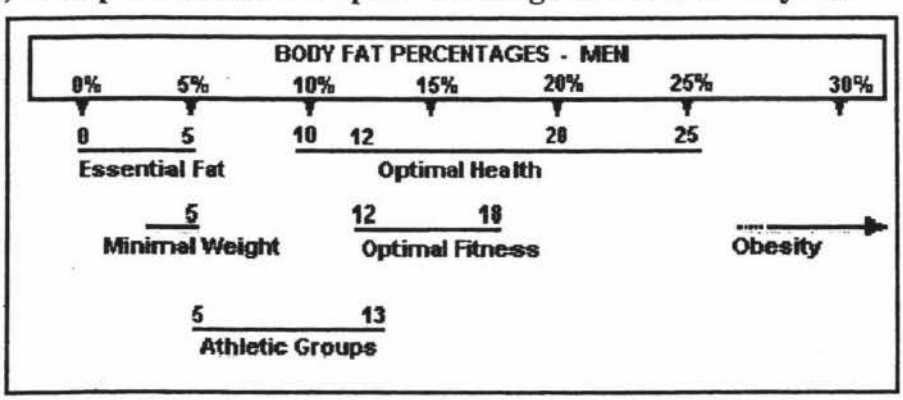
(4). Bioelectrical Impedance Analysis (BIA)

Your percent body fat as measured by the BIA Monitor is as follows;

Visit 2 **Visit 4**

% Body Fat _____ % Body Fat _____

Table 4; A Representation of Population Ranges of Percent Body Fat



Thankyou for your participation in this study. If you have any further questions on the meaning of your results, please contact, Jasmine Thomson on 413 8977 or 021 210 6269, or email thomson_jasmine@xtra.co.nz.

Appendix 13

6.13 Chemical Synthesis of HMB (β-hydroxy-β-methylbutyrate) From Diacetone Alcohol

Initially, HMB was chemically synthesised by Pressman and colleagues in 1940 to study the kinetics, mechanism, heat of reaction, reaction velocities, and influence of an acid or base catalyst on hydration, dehydration and decarboxylation reactions of β-hydroxy-isovaleric acid and β, β-dimethylacrylic acid. The study included a description of the chemical synthesis of β-hydroxy-isovaleric acid, from diacetone alcohol by the chloroform reaction. This process has been described in detail below.

A solution of 320g sodium hydroxide is made up to 1.5L with water. Once this solution has cooled, 230g chlorine, and 100g of diacetone alcohol is added, and mixed vigorously. The solution increases in temperature due to the exothermic nature of the reaction, and the chloroform is distilled out as it is formed. Excess chlorine is removed using sodium bisulfite (Pressman, *et. al.,* 1940).

A volume of 1250mL, of 6N Sulphuric acid is added to the solution. The solution is then extracted with 8 1L volumes of ether. The ether is then extracted using 20g of calcium hydroxide made up to 200mL with water, forming a thin paste. The extract is diluted to 400mL, then filtered and carbonated until neutral pH, tested by acid-base titration using phenolphthalein. The solution is filtered with 'hyflow' diatomaceous earth (Pressman, *et. al.,* 1940).

Calcium β-hydroxy-isovaleric acid crystals are separated and concentrated to 250mL. The crystals are then dried in a vacuum over phosphoric anhydride at a temperature of

70 °C. The crystals were found to be contaminated with chloride in this experiment. A solution of 34g silver nitrite made up to 500mL with water is brought up to boiling temperature, and then the calcium β-hydroxy-isovalerate salt is added. Silver β-hydroxy-isovalerate is precipitated immediately (Pressman, *et. al.*, 1940).

While still hot, this mixture is filtered with 3g 'hyflow'. As the mixture cools, crystals are separated. The mixture is extracted another two times. The crystals are dried at a temperature of 78 °C over phosphorus peroxide. This method produces 8.5g of Silver β-hydroxy-isovalerate ($C_5H_5O_3Ag$) (Pressman, *et. al.*, 1940).

Patents US5028440, US4992470, US5087472, US5348979, and US5360613

In the early '90's Nissen and colleagues began experimenting with HMB supplementation of animals. The process used to synthesise HMB for the purposes of these studies was similar to that described by Coffman and colleagues in 1958, above.

In a 5000mL round bottom flask, with a condenser attached, 3785mL of 5.25% sodium hypochlorite, 45g of sodium hydroxide powder, were added and mixed using a magnetic stirrer. Then 150mL of 1,4-Dioxane, and 93mL of 4-Hydroxy-4-Methyl-2-Pentanone were added. The mixture was refluxed for 40 minutes using the attached condenser (Nissen, 1990; Nissen, 1990; Nissen, 1991; Nissen, 1992; Nissen, 1993).

The solution was transferred from the flask to a waterbath or washtub under an extraction hood, and allowed to cool for 30 minutes. Using concentrated sulphuric acid, the acidity of the solution was adjusted to pH 5, to stabilise the HMB (Nissen, 1990; Nissen, 1990; Nissen, 1991; Nissen, 1992; Nissen, 1993).

Transfer the mixture to cooling pans, placed under an extraction hood, or use a steam table to increase evaporation. Sodium sulphate salts will precipitate out of the mixture overnight, to form slurry (Nissen, 1990; Nissen, 1990; Nissen, 1991; Nissen, 1992; Nissen, 1993).

Transfer the slurry from the cooling pans to a washtub and add concentrated sulphuric acid to adjust the acidity of the solution to pH 1. Depending on the volume of the mixture at this point, the salts and solution may be extracted separately. Transfer the solution to a 10L bottle, and wash with 2L ethyl acetate, about four times. Save the ethyl acetate layer, which contains the HMB, discard the final acid layer. The acetate layer is placed in a flask in a Roto Vap (Rotary Evaporator) which enables the rapid removal of solvents from solutions, at a temperature of 50°C. The salts may begin to precipitate out, and are solubilised in pure water. An equal volume of ethyl acetate is added to this solution and it is re-extracted. The ethyl acetate layer is saved, and again placed in the Roto Vap at 50°C. The temperature is increased to 70 °C (Nissen, 1990; Nissen, 1990; Nissen, 1991; Nissen, 1992; Nissen, 1993).

The remaining solution contains HMB. Calcium hydroxide powder is added to the HMB acid, while stirring until a neutral pH is reached. pH is measured using pH paper strips. At neutral pH the HMB solidifies, therefore is dissolved in hot 95% ethanol. Any substance that has not dissolved is removed via centrifugation or filtration. The solution is cooled at -20% freezing for 1-3 days, until the mixture crystallises. The HMB crystals are filtered under vacuum and excess liquid removed (Nissen, 1990; Nissen, 1990; Nissen, 1991; Nissen, 1992; Nissen, 1993).

The HMB crystals are redissolved in hot 95% ethanol, cooled and refiltered. This process generally takes 3 repetitions to purify the crystals and remove all yellow coloration. The HMB crystals are placed in a pan and freeze-dried to produce an anhydrous (no water present) calcium HMB powder (Nissen, 1990; Nissen, 1990; Nissen, 1991; Nissen, 1992; Nissen, 1993).

Commercial synthesis of HMB was thus far, uneconomic, used large volumes of solvents, produced large volumes of waste-products, had low yield recovery, and was an extremely slow process (Barac, 1998). Therefore in 1998 George Barac of Iowa State University began the development of a more efficient and environmentally friendly chemical process for the commercial manufacture of HMB.

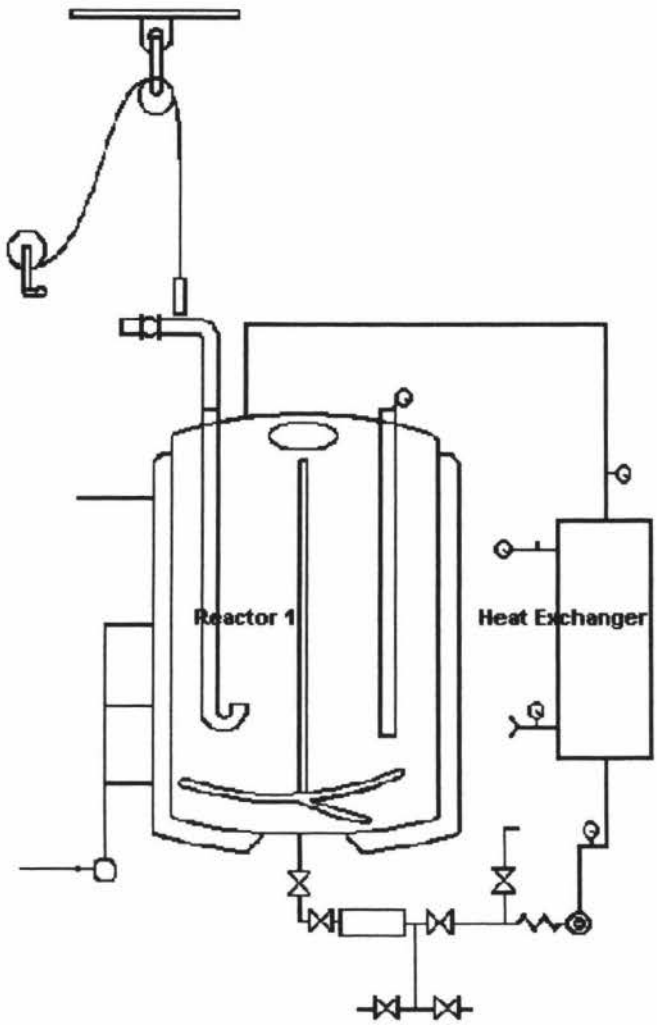
The HMB product yield was increased from 50% to 70% and operation costs substantially reduced, after changes were made in types of solvents used for separation of HMB, and reactor design and engineering. Several solvents were identified with superior partition coefficients and selectivity's for HMB, for example methyl-ethyl ketone, and hexanol which work as physical extractants, and methyl-ethyl ketone solutions of tertiary fatty amines and chloroform solutions of tri-octyl phosphine oxide which work as reactive extractants (Barac, 1998).

Patents US 6090978, US 6031000, US 6248922, and US 6392092

The first patent describing the commercial manufacture HMB was filed in 1996, it was titled, 'Process for manufacturing 3-hydroxy-3-methylbutanoic acid. This method is based largely on a change in reactor design to include an external heat exchanger, which regulates the temperature of the reaction mixture, and therefore ultimately increases the yield and decreases reaction time compared to previous processes (McCoy *et. al.,* 2000; McCoy *et. al.,* 2001; McCoy *et. al.,* 2002). This large scale manufacturing process is used in all four of the above patents, and HMB products displaying the patent number US5348979 on the label (www.hmb-mti.com, 2002). This process will be discussed in detail below.

Initially, reactor 1 is charged with an oxidant such as sodium hypochlorite, sodium hypobromite, sodium hypoiodite, calcium hypochlorite, and calcium hypobromite or calcium hypoiodite. In general, 12.5-15% sodium hypochlorite is used. The amount of oxidant used is greater than 757L, and most preferably greater than 4542L. The temperature of the oxidant is lowered to 0-5 °C using the cooling jacket and external heat exchanger on reactor 1. Once the temperature has been lowered, diacetone alcohol is added slowly over a 4-8 hour period. The amount of diacetone alcohol added, is in the ratio 1:12 pounds of oxidant, generally 900 or more pounds of diacetone alcohol is added. The addition of diacetone alcohol is slow, and the reaction mixture is put through the cooling loop to maintain a temperature below 10 °C, and preferably in the range 0-5 °C, as this is an exothermic reaction (McCoy *et. al.,* 2000; McCoy *et. al.,* 2001; McCoy *et. al.,* 2002).

Figure 1: Diagram Showing Reactor 1 (McCoy *et. al.*, 2000; McCoy *et. al.*, 2001; McCoy *et. al.*, 2002).



Once diacetone is added, the temperature of the reaction mixture is maintained at 3-10 °C. Carbon monoxide will begin to form, so it is vented off. Hydrochloric acid, preferably 1700-2120 pounds at 32% concentration, is added, and temperature maintained at 10-20 °C using the cooling loop and external heat exchanger. pH is maintained at 3.0-3.5, with stirring, using the agitator on reactor 1. Once the pH has reached this range, the mixture is allowed to settle, and two layers are formed. The bottom, haloform layer is drained off, then any remaining water is removed using vacuum which may be attached to reactor 1 (McCoy *et. al.*, 2000; McCoy *et. al.*, 2001; McCoy *et. al.*, 2002).

The reaction mixture is cooled to 25 °C, with stirring, to form thick, white slurry. Hydrochloric acid is added to adjust the pH to within the range 3.0-3.2, and the product is stirred for another 10-30 minutes using the agitator on reactor 1 (McCoy *et. al.,* 2000; McCoy *et. al.,* 2001; McCoy *et. al.,* 2002).

HMB is extracted from the slurry, using a polar organic solvent such as ethyl acetate. Once the mixture is allowed to settle into layers, the organic solvent is extracted via a decant tube. This procedure may be repeated 2-3 times. Each time the pH must be checked, as it tends to increase (McCoy *et. al.,* 2000; McCoy *et. al.,* 2001; McCoy *et. al.,* 2002).

The salt waste contained in reactor 1, is re-dissolved using water and heat under vacuum, then once it has cooled is pumped into the waste tank. The HMB-solvent solution contained in the extraction tank is placed under vacuum to recover the solvent by distillation and condensation, into the recovery tank (McCoy *et. al.,* 2000; McCoy *et. al.,* 2001; McCoy *et. al.,* 2002).

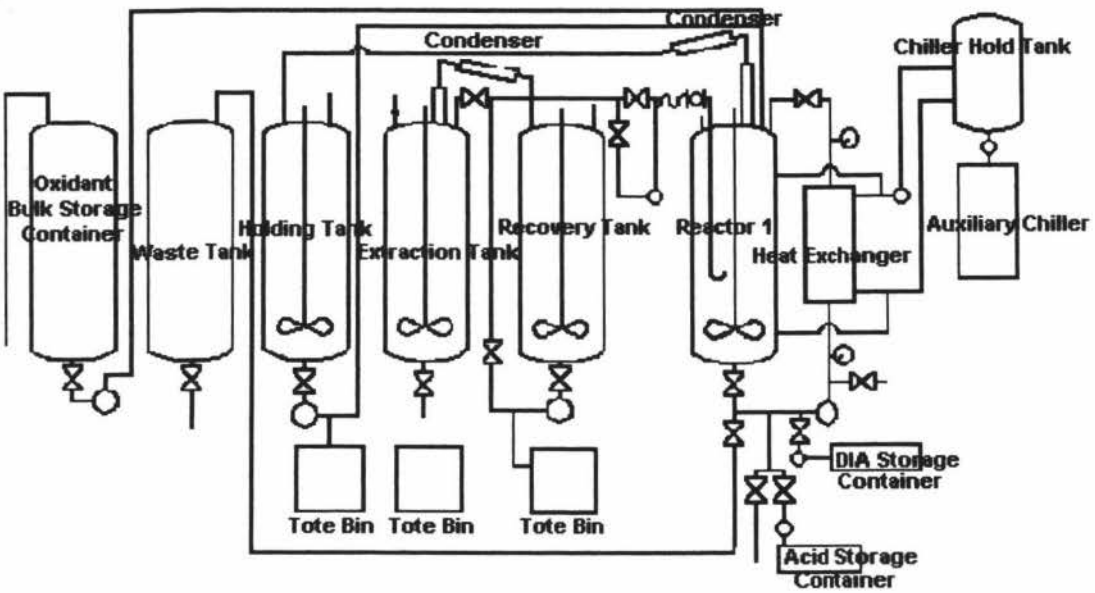
The HMB crude oil is transferred from the extraction tank to reactor 1 through a bag filter, where it is heated under vacuum to 60-70 °C to remove any remaining organic solvent via distillation. A sample of this mixture may be checked for remaining organic solvent, via high performance liquid chromatography analysis. HMB is removed from reactor 1, and stored in tote bags (McCoy *et. al.,* 2000; McCoy *et. al.,* 2001; McCoy *et. al.,* 2002).

Reactor 1 is charged with 5000-9000 pounds of ethanol or toluene, stirred by the agitator, and heated to 25 °C (McCoy *et. al.,* 2000; McCoy *et. al.,* 2001; McCoy *et. al.,* 2002).

The crude HMB is now transferred to the recovery tank, and heated to 30 °C. 500-800 pounds of calcium hydroxide, calcium oxide, calcium carbonate, or calcium acetate is added, slowly, with cooling to maintain the temperature at 30-45 °C. The pH of the mixture is adjusted to 6.5-7.0 using calcium hydroxide. Then the recovery tank is purged and the temperature maintained at 45 °C (McCoy *et. al.,* 2000; McCoy *et. al.,*

2001; McCoy *et. al.*, 2002).

Figure 2: Diagram Showing Relationship of Reactor 1 to the Recovery Tank, Extraction Tank, Holding Tank, and Waste Tank (McCoy *et. al.*, 2000; McCoy *et. al.*, 2001; McCoy *et. al.*, 2002).

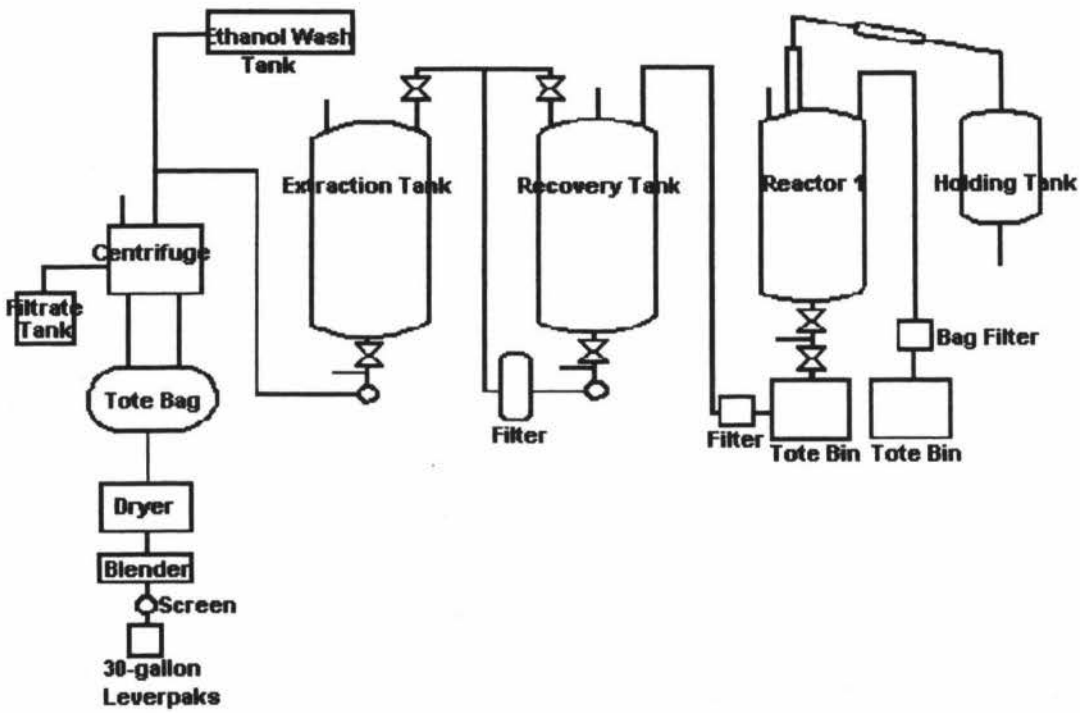


The extraction tank is purged with nitrogen. Celite, a diatomite or cellulose filter media, is added to the mixture in the recovery tank. The mixture is filtered using a 18-inch, 12-tray Hiagara filter, that has been preheated with atmospheric steam. Once the filtrate is clear, it is transferred to the extraction tank. The mixture is cooled to 30 °C, and then seeded with HMB. Seed crystals, in this case already formed HMB crystals, decrease the nuclei induction phase, therefore time taken to form crystals, also the crystals are often more uniform. The mixture is further cooled to 30 °C, over a period of 4 hours, and will begin forming milky, white slurry. The mixture cooled to 20-25 °C, over a 4 hour period, then cooled to 0-5 °C over a 2 hour period, and maintained at this temperature for an hour (McCoy *et. al.*, 2000; McCoy *et. al.*, 2001; McCoy *et. al.*, 2002).

Prior to use the centrifuge is purged with nitrogen. An ethanol wash is added to each centrifuge batch, and the filtrate waste product disposed of. After centrifugation at 600rpm, the entire batch is placed in a dryer at 45-50 °C for 24 hours. Once the first

sample shows a loss of 0.1% moisture, the batch is loaded into the blender and screener. The batch is put through a 600-micron screen to remove lumps, and then stored in drums as finished HMB product. This process has an average yield of 0.44 pounds of HMB for every 1 pound of diacetone alcohol (McCoy *et. al.,* 2000; McCoy *et. al.,* 2001; McCoy *et. al.,* 2002).

Figure 3: Diagram Showing Relationship of Reactor 1 to the Recovery Tank, Extraction Tank, Centrifuge, Dryer, Blender, and Screen (McCoy *et. al.,* 2000; McCoy *et. al.,* 2001; McCoy *et. al.,* 2002).



Microbial Synthesis of HMB

Galactomyces reessii has been found to produce HMB from β-methylbutyric acid (MBA). Up to 40g of HMB per litre has been produced experimentally when pH, dissolved oxygen, and substrate concentrations were controlled for (Lee, *et. al.,* 1997).

A two-step batch-fed fermentation process was used for this HMB production method. There were several reasons for this, initially, the Galactomyces reesii culture grows more efficiently in a slightly acidic media, but this was found to increase the

degradation of HMB. The inclusion of MBA in the culture medium was found to inhibit culture growth at concentrations of more than 5g/L. The addition of MBA in high concentrations was also found to inhibit HMB production (Lee, *et. al.,* 1997).

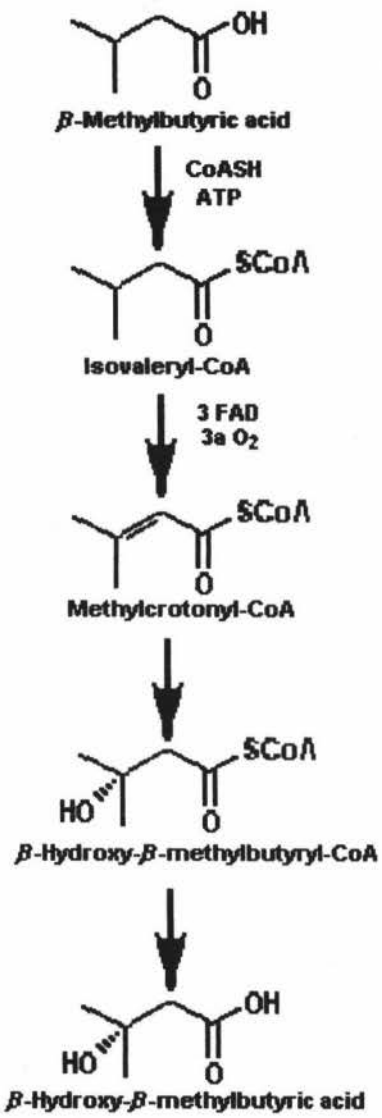
The culture media was a Luria broth, consisting of 10g yeast extract, 5g tryptone, 10g sodium chloride, and 10g of glucose per litre of broth. The Basel fermentation (GI) medium consisted of 40g glucose, 13g ammonium hydrogen phosphate, 7g potassium dihydrogen phosphate, 0.8g magnesium sulphate, 3g yeast extract, 1g sodium chloride, 0.1g iron(II) sulphate, 0.05g calcium chloride, and 10mL of trace element solution per litre of media (Lee & Rosazza, 1997; Lee, *et. al.,* 1997).

The Galactomyces reessii cells were cultivated at pH 6.5, with 28% ammonium hydroxide added to supply nitrogen, for 19 hours. The seed culture is harvested by centrifugation and inoculated into the fermentor medium. MBA is added, but kept to less than 20g/L. A glucose solution is continuously fed at constant flow into the fermentor to decrease the utilisation of HMB as an energy source and increase final yield of HMB. Dissolved oxygen (DO) is maintained at 20% air saturation, due to HMB sensitivity to DO (Lee, *et. al.,* 1997).

The maximum HMB yield produced experimentally was 38g/L after 136 hours, a conversion yield of greater than 50% (Lee, *et. al.,* 1997).

The conversion of beta-methylbutyric acid to beta-hydroxy-beta-methylbutyric acid is thought to be via *ß*-oxidation. *ß*-methylbutyric acid is transformed to isovaleryl-Coenzyme-A catalysed by acyl-Coenzyme-A synthetase. Isovaleryl-Coenzyme-A is converted to methylcrotonyl-Coenzyme-A catalysed by acyl-Coenzyme-A dehydrogenase. Methylcrotonyl-Coenzyme-A is hydrated to HMB-Coenzyme-A catalysed by enoyl-Coenzyme-A hydratase. The final step is the hydrolysis of *ß*-hydroxy- *ß*-methylbutyric-Coenzyme-A to *ß*-hydroxy- *ß*-methylbutyric (Lee & Rosazza, 1997; Lee, *et. al.,* 1997).

Figure 4: The Proposed Biosynthesis of HMB from MBA by the Micro-organism *Galactomyces reesii* (Lee & Rosazza, 1997).



For this method of HMB synthesis to become commercial, strains of Galactomyces reesii that utilise larger amounts of leucine, or alpha-ketoisocaproic acid as substrate, and decreased HMB degradation during this process, would be required (Lee & Rosazza, 1997).

Other Methods of HMB Synthesis

Throughout the years several other methods of HMB synthesis have been proposed to try to improve the economic processing on a large scale, increase the yield of HMB (Nissen, 2000), and decrease the production of large amounts of environmentally undesirable waste products (Lee, *et. al.,* 1997). However the most common synthetic process for the production of HMB for the use of animal or human supplementation appears to be the methods described above using diacetone alcohol (4-hydroxy-4-methyl-2-pentanone) as the substrate, and various catalysts and large scale reactor designs. Several patents describing alternative methods of HMB synthesis have been reviewed below.

Patent US2650936

In the year 1948, a patent was filed by Schmidt & Wuppertal-Vohwinkel in Germany, shortly afterwards in 1953, the patent was filed in the United States. The patent was titled 'Preparation of Hydroxy-Carboxylic Acids'. This patent describes the synthesis of polyhydroxymonocarboxylic acids via the oxidation of reducing sugars with oxygen and air, in an alkaline solution, to produce a polyhydroxymonocarboxylic acid with one less carbon atom than the substrate. Yield is increased with the use of catalysts nitrobenzene, *p*-nitrobenzoic acid, *m*-nitrobenzoic acid, *o*-nitrobenzoic acid, and *o*-nitrophenol. The process has been described in detail below.

Approximately 150g of saccharose sugar are dissolved in 1200mL water. Hydrochloric acid is added to invert the solution. With addition of the catalyst (see examples of catalysts used, above), the solution is poured into a 75mm vertical glass tube. Oxygen is introduced via a clay filter candle at the bottom of the glass tube. A funnel at the top of the glass tube is used to add 147.5g potassium hydroxide mixed with 950mL water, drop-wise for a 2 hour time period. Due to the exothermic nature of this reaction, the temperature of solution increases and is maintained at 40-42 °C. Oxygen is passed into the glass tube for a further 2 hours, then air for a period of 4 hours (Schmidt & Wuppertal-Vohwinkel, 1953).

Titration may be used to test how the oxidation has progressed. Once oxidation is complete, the solution has the pH neutralised with glacial acetic acid, and decreased in volume in a vacuum. Potassium arabonate is formed and crystallises out of solution. Further potassium arabonate may be crystallises out using a methanol wash (Schmidt & Wuppertal-Vohwinkel, 1953).

The final products obtained have melting points between 215-220 °C. Good yields are obtained with the conversion to calcium arabonate using calcium oxalate, thereby decreasing the production of by-products. Any residual catalyst remaining in the final product, not removed with the methanol wash, may be removed using animal charcoal (Schmidt & Wuppertal-Vohwinkel, 1953).

Patent GB647094

Meanwhile a patent was filed in England titled 'Improvements in and Relating to the Manufacture of Isovaleric Acid', by applicants Bing and colleagues, in 1950. This patent described improvements to be added to the method of synthesis of HMB to increase the yield, and decrease production of isomer by-products. This method of production of HMB (termed isovaleric acid in this patent), used the substrate methyl isobutyl ketone which has a high level of purity, and produces a high yield of isovaleric acid, and the useful by-products chloroform or bromoform. The theoretical yield of this synthesis method is 55-60% isovaleric acid with up to 60% chloroform or bromoform by-product.

Methyl isobutyl ketone oxidation is catalysed using a solution of alkali metal hypohalite such as alkali metal hypobromite, or more preferably, alkali metal hypochlorite. Or by treating a suspension of methyl isobutyl ketone, in aqueous alkali with bromine, or more preferably chlorine. External cooling is used to keep the temperature low during the addition of the alkali metal hypochlorite (Bing, *et. al.*, 1950).

The aqueous layer containing the isovalerate alkali salt is separated from solution. If an alkali metal hypochlorite is added to methyl isobutyl ketone, or chlorine is used to

treat a suspension of methyl isobutyl ketone and sodium hydroxide, the upper chloroform and unreacted-reacted ketone layer is separated by distillation at a boiling range of 110-120 °C. If bromine is used to treat a suspension of methyl isobutyl ketone and sodium hydroxide, the lower bromoform layer is drawn off (Bing, *et. al.,* 1950).

Isovaleric acid is further separated via concentration and acidification with dilute sulphuric acid, then distillation at boiling range 170-180 °C (Bing, *et. al.,* 1950).

Specific examples of catalysts and temperature ranges used have been given in this patent, but it is not necessary to cover this process in such detail for this thesis (Bing, *et. al.,* 1950).

Patent JP8198800

In the year 1996, a patent was published in Japan by Kunio and Takashi, titled 'Production of 3-substituted-3-methylbutanoic acid'. This patent outlined a process of synthesising HMB using an inexpensive substrate and catalyst, producing a high yield, for use as an intermediate in perfume, medicine, agro-chemical and other chemical production.

The substrate 3-hydroxy-3methylbutanol is oxidised with oxygen in the presence of water, and a platinum- or palladium-based catalyst to produce 3-hydroxy-3-methyl butanoic acid. The catalyst is prepared by supporting platinum or palladium and another metal, for example lead or tellurium on a carrier of active carbon or alumina. The reaction is carried out preferably at 70-90 °C and preferably in a 1-10kg/cm^2 oxygen gas atmosphere (Kunio & Takashi, 1996). This patent also discusses the preparation of 3-methyl-3-methoxybutanoic acid from 3-methyl-3-methoxybutanol using the same procedure, but this is not relevant to this thesis.

Patent JP9087270

A year later in 1997, Kunio and colleagues filed a second patent also titled 'Production of 3-substituted-3-methylbutanoic acid'. This patent outlined a different process to synthesise HMB, required as an intermediate in the manufacture of chrysanthemum-monocarboxylic acid ester for use as an insecticide.

The substrates used are 3-hydroxy-3-methylbutanal and/or 3-methoxy-3-methylbutanal, and are reacted with a hypochlorite such as sodium hypochlorite in the presence of an N-oxyl compound for example 4-benzoyloxy-2,2,6,6-tetramethylpiperidinyloxy at a temperature of 5-30 °C. The pH of the reaction mixture may need to be adjusted based on the substrate used, and is achieved by the addition of a salt such as potassium dihydrogen phosphate (Kunio, *et. al.,* 1997).

Appendix 14

6.14 Correspondence

thomson_jasmine

From: "Steven L Nissen" [redacted]
To: "thomson_jasmine"
Sent: Thursday, 23 May 2002 1:50
Attach: Meta-analysis manuscript AJCN.doc
Subject: HMB Research

Jasmine:

Thanks for your letter. Depending on the amount needed I can provide you with pure HMB either as a powder or in capsules. We also have placebo capsules. All I require is a completed protocol.

This product is of the highest grade and is what is used in t he commercial product. All the safety studies have been done with this product. As far as published safety data, the J of Nutrition article published about at year ago is the most up to date. A summary is on the HMB web site—HMB.org. I can send you a hard copy is you provide your address.

Lastly, are you sure you want to study HMB alone? There are 9 studies published on HMB in weight lifting already. The novelty of your study may be with trained subjects but that has also been done. I have attached a paper that is now being reviewed that summarizes all types of supplements in weight lifting that may give you a better picture.

I would look at adding something to HMB to examine the synergistic effects on muscle gains. We have some basic data (in vitro) to suggest that HMB and leucine may have some synergism. If this would be of interest again we could provide the treatments and placebo (may have to be a drink).

Let me know what you think.

Steve Nissen

thomson_jasmine

From: "Steven L Nissen" [redacted]
To: "thomson_jasmine"
Sent: Thursday, 30 May 2002 11:28
Subject:
Jasmine:

I am absolutely positive there is not am manufacturer of HMB in Australia. There are several companies that sell HMB but it is mostly imported from China and almost all of it is poor quality. In addition I am not sure you will get a good purity answer from an independent labs that are not familiar with the procedure for analysis. Our labs use a combination of HPLC, NMR, and GCMX as well as heavy metal analysis on all samples to assure purity. This is the only way to be sure. If you wish we can analyze the sample for you.

I assure you it will be no problem importing HMB from the US we have collaborated on many studied around the world will little difficulty.

Good Luck

Steve Nissen

thomson_jasmine

From: "Steven L Nissen" <[redacted]
To: "thomson_jasmine"
Sent: Thursday, 30 May 2002 11:28
Subject:
Jasmine:

To make thinks clear. Metabolic Technologies (MTI) has a licence from Iowa State University to market HMB world wide. Iowa State has patents in most parts of the world that protect the use of HMB by anyone other than MTI. Thus there is no legal source of HMB in the world other than through MTI. All other products are unlawful. Iowa State has taken legal action against several companies—some of which are in Australia to curtail unlawful use.

S to summarize, anything that is sold or manufactured outside of this patent license cannot be trusted. MTI is therefore the only company in the world that is licensed to manufacture and distribute HMB. The manufacturer of HMB varies but both the current manufactures are making HMB at the pharmaceutical level of purity.

I know you don't need to know all the details of the business structure on HMB but I hope this gives you an idea of how the process works and why I am concerned that you don't provide test subjects with something that will harm them. I hope this helps.

Steve Nissen.

YMCA North Shore

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E-mail: northshore@nzymca.com
Website: www.nzymca.com

2 October 2002

To whom it may concern:

Re: Jasmine Thompson

This letter is to confirm Jasmine Thompson and client will be using YMCA North Shore Fitness Centre free of charge during the following times:

Mom – Fri 12 noon – 4pm
Mon – Thur 7pm – 8.30pm

Jasmine is from Massey University and will be conducting specific exercises for study purposes only.

Yours sincerely

Shirley McKain
Fitness Centre Manager