

## EFFECTS OF BETA-HYDROXY- BETA-METHYL BUTIRATE (HMB) SUPPLEMENTATION ON THE BODY-COMPOSITION AND MUSCLE POWER OUTPUT OF NON- COMPETITIVE SPORTING MALES

M. Muller, P.J. du Toit and P.E. Krüger

### ABSTRACT

This study was directed at establishing the effect of beta-hydroxy-beta-methylbuturate(HMB) supplementation on the body-composition and muscle-power output of non- competitive sporting males between 19 and 24 years, who performed resistance weight training three times a week for 8 weeks. Physically active men and women are less likely to become overweight than their inactive peers. Exercise has a favourable effect on body fat distribution, with a reduction in waist-to-hip ratio with increased exercise. Physical activity can directly affect both total energy intake and total energy expenditure. Physical activity can also affect fat balance, and it is increasingly clear that imbalances in total energy are largely owing to imbalances in fat. Therefore, exercise testing provides a basis for the design of training programmes and allows for monitoring progress throughout the training programme. Used properly, testing and monitoring is useful to both trainers and athletes. Exercise in conjunction with an appropriate diet is of benefit to overweight persons, and provided that feasible methods and motivation are available, exercise is recommended as an important part of a weight-control programme. The effects of  $\beta$ -Hydroxy- $\beta$ -methylbuturate were tested in a clinical trial. Two homogenous groups of 20 males were evaluated for initial strength capabilities and body composition. For 8 weeks the subjects lifted weights three times a week and followed a balanced diet. Creatine-kinase activity decreased with HMB supplementation. Gains in muscle power output were greater in the experimental group. Fat percentage decreases were recorded with HMB supplementation. By evaluating the creatine-kinase values and the weightlifting values of the two different groups, it can be concluded that HMB supplementation does lower protein breakdown and the fat percentage in subjects, in conjunction with resistance training.

**Key words:** Exercise, overweight, diet, HMB supplementation.

### INTRODUCTION

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

Nutrient balance exists for both protein and carbohydrates. Excess is oxidised and not converted to fat, whereas excess fat is stored in adipose tissue. Weight control is achieved by maintaining the fat balance. Diets with a high fat-to-carbohydrate ratio are linked to obesity. A reduction in caloric intake by dieting or fasting can decrease the BMR, while physical activity is important in maintaining it (Mole, 1990).

The thermic effect of food represents a small part of total energy expenditure and is not predictive of obesity.

Moderate exercise promotes the expenditure of large amounts of fat and calories, achieving weight loss. Other advantages of exercise include increased cardiorespiratory fitness, HDL cholesterol, etc. (Hirsch, 1995).

A subject's response to endurance exercise is determined by the resultant response of several interrelated control systems. This response (performance) is finally determined by a tightly attached partnership (ratio) between oxygen transfer to and carbon dioxide removal from the exercising cells, and secondly (not less importantly), the capacity of the cells to produce ATP aerobically (Weber, 1984; Brown, Wiener & Brown, 1985). The accurate assessment of body composition in particular fat mass is of considerable importance to practitioners of sports medicine. Physical activity is an important component of life, contributing to quality and function, and reflects a multi-factorial, closely regulated integration of the functions of several organ systems (Van de Walle, Peres & Monod, 1987; Sue, 1994). The body is put under stress during resistance exercise (gym), because various forces (like lifting

a weight against gravity) are applied to the different muscles and joints (Tiidus & Ianuzzo, 1983). Intense exercise (weightlifting) can cause disruption of and/or degradation of structural proteins in the muscle fibres as well as in the connective tissue (Tiidus & Ianuzzo, 1983). More intense training has an even greater effect on the body. Excessive exercise, or an unaccustomed exercise, is the most common cause for delayed onset muscle soreness (DOMS) (Knitter, Panton, Rathmacher, Petersen & Sharp, 2000). Downhill running and resistance training (weight training) are forms of exercise that involve numerous eccentric contractions (contractions of the muscle while being stretched) that have a severe effect on the muscle fibres, because eccentric contractions cause micro tears in muscle fibres (Knitter *et al.*, 2000). Trying to lessen the amount of protein breakdown during high-intensity exercise could be beneficial to athletes in training (Armstrong, 1986; Hough, 1992; Evans & Cannon, 1992). The body can physiologically adapt to exercise up to a point, but needs some assistance in diminishing the amount of protein breakdown taking place (Knitter *et al.*,

2000). It seems as if additional nutrients may be needed to aid muscle and strength gains, and enhance recovery from resistance exercise which disrupts muscles (Florini, 1985). Both professional and amateur sport competitors do make use of extra nutrients to aid in either maintaining or gaining strength and/or stamina, depending upon the requirements of the particular sport (Steven, Nissen & Sharp, 2003).

The anticatabolic effects of the branched chain amino acid, leucine, and certain of its metabolites, e.g.  $\alpha$ -ketoisocaproate (KIC), have been known for over 30 years (Nissen, Sharp, Ray, Ratmacher, Rice, Fuller, Connelly & Abumrad, 1996). Animal studies showed that leucine and KIC were nitrogen sparing (van Koevering & Nissen, 1992). HMB is a metabolite of the branched chain amino acid leucine and is produced from KIC (Nair, Schwartz & Wells, 1992; Krieder, Ferreira, Greenwood & Wilson, 2000). Both leucine and KIC are proposed to decrease nitrogen and protein loss by inhibiting protein breakdown (Van Koevering & Nissen, 1992; Nair,

Schwartz & Wells, 1992; Nissen *et al.*, 1996; Steven *et al.*, 2003). Studies showed an increase in muscle cell and immune cell production, but the effect was only noticed in animals and was caused by severe stress or trauma (starvation) on the muscle tissue, during which proteolysis was greatly elevated (Ferreira, 1960; Greenwood, 1983; Wilson, 1989; Krieder, 1991). Plasma concentrations of HMB range between 1 to 4 $\mu$ M but can increase up to 10-fold after leucine has been added to a diet (Wilson, 1989). Data suggests that a 60-gram dose of leucine or 20-gram dose of KIC produces these anabolic effects (Nissen, Van Koevering & Webb, 1990; Zhang, Talleyrand & Ratmacher, 1993).

HMB, a normal product of human metabolism, is naturally present in mother's milk and is found (in small quantities) in the foods we eat, e.g. grapefruit and catfish (Steven *et al.* 2003). HMB is a water-soluble compound and is excreted in the urine in proportion to dietary intake (Zhang *et al.*, 1993). Therefore, based on the chemistry of HMB, it may be predicted that HMB is a safe compound. It is classified as a

dietary supplement (Nissen *et al.*, 1996). HMB is derived from KIC when KIC is oxidized in the liver by the enzyme KIC-dioxygenase (Van Koevering & Nissen, 1992).

Owing to concerns the public may have about the unhealthy manner in which young adults try to attain healthy looking bodies, and the unhealthy products they use to achieve this aim (Nissen, Sharp, Panton & Vukovich, 2000), researchers have studied HMB to determine its safety. Every human study included an extensive safety profile that screened products for adverse reactions and detrimental organ function (Nissen *et al.*, 1996). Psychological profiles and physical examinations were performed in each study. There were no indications in any of the tests that HMB presented any danger to the subjects (Greenwood, 1983; Baxter, Carlos, Thurmond, Rehani, Bultman & Frost, 2005). The only change in blood chemistry was related to a decrease in the total and low-density-lipoprotein (LDL) cholesterol levels.

The mechanism of HMB is not exactly known, but the latest research indicates

that HMB might supply a precursor to muscle and to the immune system that supports maximal cellular repair (Steven *et al.*, 2003). This hypothesis reasons that the muscle membrane is more rapidly repaired after exercise-induced muscle damage. Muscle growth is supported by having an adequate supply of this substrate in the muscle for membrane expansion. Another hypothesis is that HMB supports a decrease in protein turnover or muscle damage (Van Koevering & Nissen, 1992). This decrease in muscle breakdown could in turn result in more rapid recruitment of neural motor units by muscle fibres and may act as a catalyst to faster strength increases. HMB works in the laboratory (Nissen *et al.*, 1996), but does it work in the real world? It seems that the role of HMB is to shift the balance of protein metabolism in favour of new muscle growth; it also appears to minimize the breakdown of muscle tissue (Steven *et al.*, 2003). HMB has been shown to reduce the effects of DOMS 24 hours after exercise and reduce the signs and symptoms of exercise-induced muscle damage in non-resistance trained males, following a single bout of eccentrically-

biased resistance training (Sabourin & Bieber, 1983). This decrease in muscle breakdown could result in more rapid neural recruitment by the muscle fibres and may act as a catalyst to faster strength increases (Nissen et al., 1996). In doing so, HMB can help support a consistent increase in muscle-tissue growth and it appears that HMB supplementation may enhance the muscle-building and fat-burning effects of exercise (Nissen *et al.*, 1996; Gallagher, Carrithers, Godard, Schulze & Trappe, 2000; Steven *et al.*, 2003).

HMB is a natural by-product of the body. HMB is a metabolite of the amino acid leucine and is produced from KIC. If there is damage to the muscle, it can be reasoned that muscle damage could lead to a decrease in physical performance. When combined with exercise, HMB might be able to up-regulate the body's endurance (tempo of exercise) and accelerate the rate of muscle gain and fat burning.

The aim of this study was to determine whether HMB supplementation would increase the Lean Body Mass (LBM) and

muscle power output (measured as the load a subject can bench press) of males who gym for recreational purposes, after a combination of resistance weight training, eating a balanced set diet and supplementation with HMB for 8 weeks.

## MATERIALS AND METHODS

### Research design

The University's Research and Ethics Committee approved the study. The study was a double-blind clinical trial. A total number of 40 subjects (male, ages 19 – 24) participated. Each participant had to complete a series of pre- and post-tests which consisted of the following:

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

Before each evaluation, the subjects did not eat for 4 hours, exercise for 12 hours, or drink alcohol for 24 hours. The subjects participated in a supervised exercise programme for 1 hour, three times a week.

This programme was followed for 8 weeks. In addition, each subject received a dosage of 3g of HMB each day for 6 days of each week. During the 8 weeks they also followed a set diet (Protein/Carbohydrate/Fat) to lessen the chance of any external factors that could influence the study. After 8 weeks, the subjects were re-evaluated. Results were compared between pre- and post-test values.

### Materials

HMB – supplied by EAS, a company that distributes nutritional sport supplements. EAS was formerly known as NSA (Natural Supplement Association), but the name has now been changed to EAS.

Placebo – supplied by EAS. The active ingredients are listed in milligrams for each capsule:

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

HMB capsules – supplied by EAS. The HMB capsules all contain the same active ingredients as the placebo. There

is 250mg of HMB in each capsule.

Resistance weight-training programme (8 weeks) that was specifically developed for the study.

Isokinetic testing apparatus (Cybex Machine) supplied by Institute of Sports Research (ISR) at University of Pretoria (UP)

Gym equipment supplied by the ISR at UP

### Sample

Male students who live in residences at the University of Pretoria were the focus for volunteers to determine a study population. This was done to control external factors that could affect the study population. To control any extraneous factors from having any effect on the result of the study, all volunteers were screened before they could participate. The process involved completing a questionnaire to establish physical background information and a physical evaluation. The evaluation consisted of body-composition measurements, initial evaluation of strength (weightlifting test) and blood creatine kinase quantification. Potential subjects were excluded if they showed any evidence or history of diabetes mellitus, cardiac, renal, liver or pulmonary disease.



They were also excluded if they had sustained any joint, muscle or bone injury within the last three months prior to the study. No subjects were accepted if they used another anabolic product or had participated in any other training programmes within the three months prior to the trial.

### ADMINISTRATION OF TESTS

1. After completing the pre-tests, the 40 subjects were divided into two groups in such a manner that the two groups were as homogenous as possible. Stratification rules were applied on body composition and initial strength capability to insure that the two groups were homogenous. The experimental and control groups were selected randomly.
2. Each participant underwent a series of pre-tests to evaluate muscle-power output and total body composition:

Isokinetic (Cybex) test to evaluate quadriceps and hamstring strength, 1 repetition maximum test on all the major muscle groups (Latissimus dorsi, Quadriceps, Hamstrings/Biceps femoris, Trapezius, Pectoralis

major)

Body composition measurements (anthropometric measurements including height, weight of participant, and skinfolds of Triceps, Biceps, Subscapula, Iliac crest, Front thigh and Abdomen, using the Heath-Carter method)

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

3. Blood sample collections were taken only after the Cybex test, before and after the 8 weeks of the trial, to evaluate the creatine-kinase activity in the body. Only 1ml of anti-cubital blood from each subject was required for testing. The sample was cooled off to between 4-8 degrees Celsius. It was then centrifuged and 200µl of serum was then used to determine the creatine-kinase value. Creatine-kinase is an indicator of metabolism. It is the muscle enzyme that catalyzes the reaction during which creatine-phosphate is hydrolysed to donate a single phosphate from ADP to ATP. This reaction takes place during the contraction of skeletal muscles.

4. Three capsules (HMB/Placebo) were taken with 200ml of water, three times a day (during main meals), with no less than 4 hours in-between following dosages. The prescribed dosages added up to 3g of HMB/day. This protocol was followed for six days in a week and then suspended on the seventh day. The dosage prescription has been studied and proven (Nissen et al., 1990; Nissen *et al.*, 1996, Nissen *et al.*, 2000).
5. Both groups followed a prescribed weight-training programme for 8 weeks. As the weeks passed, each participant had to increase the resistance accordingly to ensure that the level of exertion was maintained. A 2-by-2 rule was applied to determine the increase in load. If the subject could do 2 more reps than recommended in two consecutive sessions, the weight was increased. Each exercise and the load of all the subjects were recorded.
6. For the duration of the experiment (8 weeks), the subjects followed a prescribed diet that was provided by the food services of the University of Pretoria's Residential Housing. Three meals were taken each day. The distribution of the meals was recorded by a staff member from Residential Housing of the University of Pretoria. The subjects also recorded any between-meal snacking. This allowed the monitoring of external factors during training sessions, and provided evidence for exclusion of further participation, if necessary. After 8 weeks, subjects were re-evaluated to compare the results with the pre-test values.
7. Three 1-hour training sessions were scheduled for each week. Each session concentrated on a different part of the body to insure no overtraining or straining of a muscle group. Session 1 focused on pectorals (chest), triceps and calve muscles. Session 2 focused on the muscles of the upper legs (quadriceps, hamstrings) and shoulders (deltoids). Session 3 focused on the back (trapeziums and trapezoids), biceps and calves.



### Data analysis

Descriptive statistics included mean, standard deviation, and range. The primary analysis addressing the outcome variables of interest employed an analysis of covariance comparing groups with respect to change from baseline and using the baseline as a covariate. Within the groups, change from baseline was tested by using the student's paired t-test. Testing was done at the 0.05 level of significance and should data not confirm to normality, data analysis resorted to non-parametric procedures.

The data were captured on Microsoft excel and converted to SPSS for analysis. The data analysis had the following aims:

To determine whether significant differences existed between the two groups on all variables measured.

To determine whether significant differences existed between the pre- and post-intervention measurements within the same group.

### RESULTS

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

outcomes: maximal weight lift (bench press 1 repetition maximum), anthropometric measurements, and blood creatine-kinase activity.

The sample size calculations were based on maximal weight-lifting capability (bench press) which was of primary interest in this study. Three major muscle groups are involved and it is reasonable to assume that the effect on these muscle groups would follow similar trends, hence bench press was considered. Of interest is to compare groups with respect to improvement from baseline and the outcome used will be weight as a ratio of body mass.

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

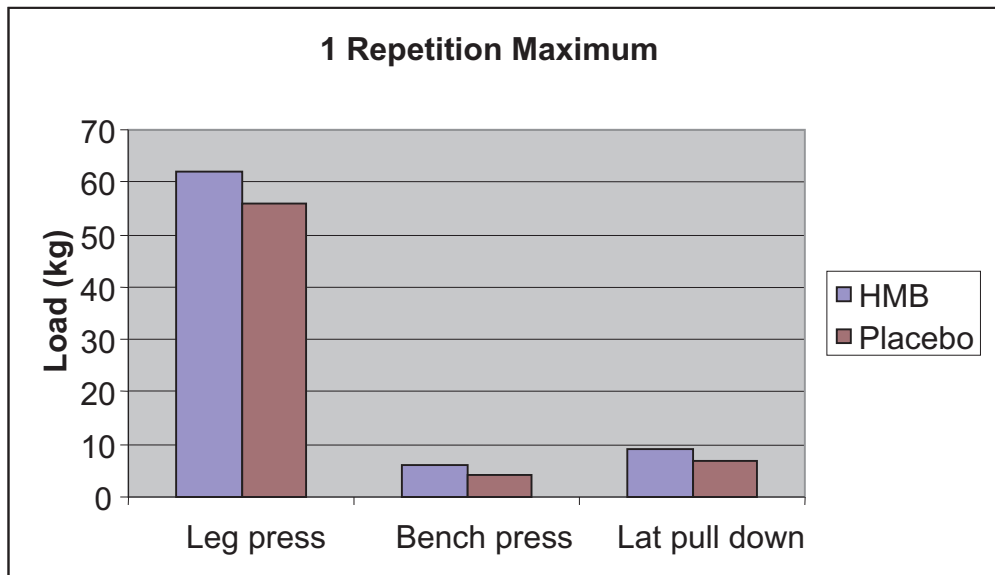


Figure 1: Increase of weightlifting with and without HMB supplementation.

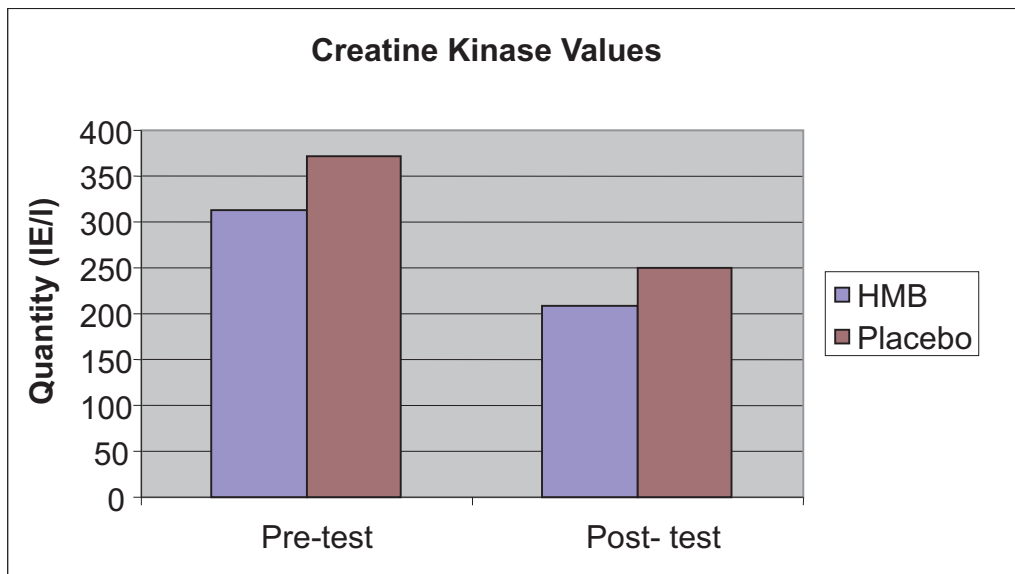


Figure 2: Activity of creatine-kinase with and without HMB supplementation .

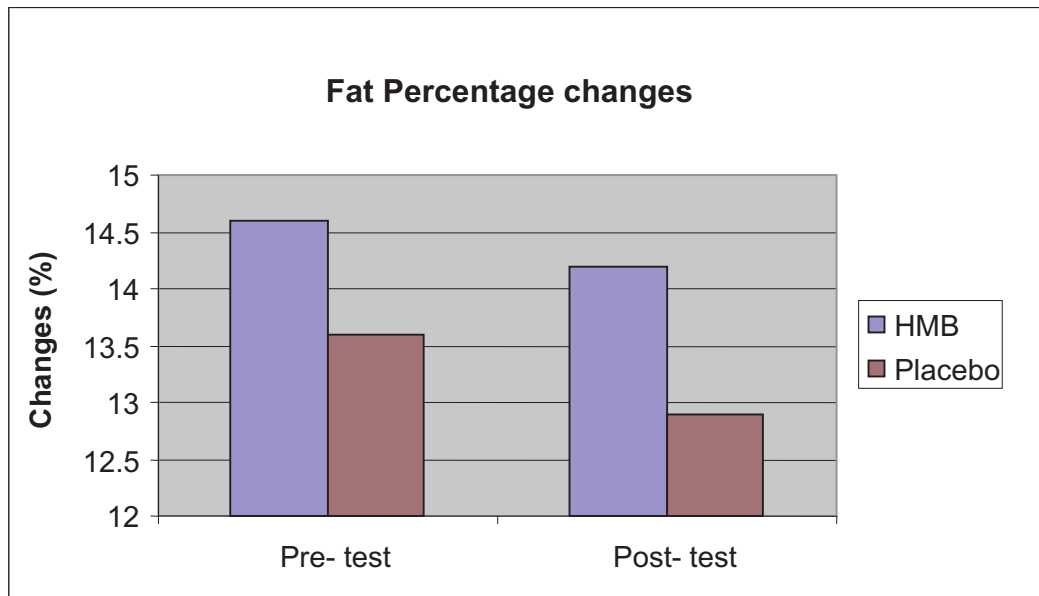


Figure 3: Fat percentage decrease with and without HMB supplementation.

HMB supplementation resulted in a higher increase of weightlifting capabilities from a baseline value than in the control group.

With HMB supplementation the activity of creatine-kinase was lowered by a greater margin than without the supplementation. This indicates that less protein breakdown occurs with HMB supplementation.

When protein breakdown decreases, the amount of fat in the body is also lowered and the fat percentage decrease is higher in conjunction with HMB supplementation.

## DISCUSSION

Exercise is generally considered to be

good for health and its effects on body composition have been widely studied (Aulin, 1995; Fielding, 1995; Lukaski, Bolonchuk, Siders & Milne, 1996; Terbizan & Seljevold, 1996; Wallberg-Henriksson, Rincon & Zierath, 1998). Regular, moderate exercise should be considered as a viable means of treating depression, anxiety and improving mental well-being, physical self-perception, and in some cases, the global self-esteem of the general public (Fox, 1999). Total energy expenditure varies as a result of differences in duration, frequency and intensity of physical activities (van Baak, 1999).

During moderate (< lactate threshold) exercise, and also with rapidly incremental work, pulmonary and muscle oxygen uptake ( $\text{VO}_2$ ) increases as a linear function of work rate (Poole & Richardson, 1997). In heavy muscular work the muscles have to be supplied with up to 500 times more oxygen ( $\text{O}_2$ ) than when the body is in a resting state, and at the same time, larger quantities of carbon dioxide ( $\text{CO}_2$ ) and lactate have to be removed (Despopoulos & Sibernagl, 1991).

Exercise as part of a weight-loss programme has been shown to help preserve, or even increase, fat-free mass (Saris, 1995; Kraemer, Volek, Clark, Gordon, Incledon & Puhl, 1997; Pérusse, Collier, Gagnon, Leon, Rao & Skinner, 1997), of which muscle is an important component. This increased ratio of fat-free mass to fat mass has important health consequences. Muscle is metabolically active and an increase in muscle mass results in an increase in the resting metabolic rate (Saris, 1995) and the  $\text{VO}_2$  max (Proctor & Joyner, 1997). Diseases known to be associated with obesity are decreased in subjects with less fat and an

increased fat-free mass, making fat loss more important than weight loss (Bryner, Toffle, Ullrich & Yeater, 1997).

An exercise programme can, therefore, contribute to good health by reducing the risk of some chronic diseases, for instance, coronary heart disease, non-insulin dependent diabetes and hypertension (Bjorntorp, 1995). It can also help keep older people active (Evans, 1995) and independent for a longer time.

These beneficial results are not only a result of a decrease in body fat, but also an increase in the muscle component of the body. Analyses showed that a person's level of physical activity can have an impact on body composition and that specific aerobic exercise, in conjunction with a restricted diet, resulted in significant differences in body composition (decrease in body fat). The associated increase in lean body mass and the muscle component affords added health benefits.

## **CONCLUSION**

By evaluating the creatine-kinase values and the weightlifting values of the two

different groups, it can be concluded that HMB supplementation does lower protein breakdown and the fat percentage in subjects, in conjunction with resistance training. There are greater increases in muscle power output with HMB supplementation.

## REFERENCES

- Armstrong, R.B. (1986). Muscle damage and endurance events. *Sports Medicine*, 3, 80-93.
- Aulin, K.P. (1995). Gender-specific issues. *Journal of Sports Sciences*, 13, 535-539.
- Baxter, J.H., Carlos, J.L., Thurmond, J., Rehani, R.N., Bultman, J. & Frost, D. (2005). Dietary toxicity of calcium beta-hydroxy-beta-methylbutyrate (CaHMB). *Journal of Applied Physiology*, 43(12), 1731-1741.
- Blair, S.N. (1993). Evidence for success of exercise in weight loss and control. *Annals of Internal Medicine*, 119, 702-706.
- Bryner, R.W., Toffle, R.C., Ullrich, I.H. & Yeater, R.A. (1997). The effects of exercise intensity on body composition, weight loss, and dietary composition in women. *Journal of the American College of Nutrition*, 16(1), 68-73.
- Bjorntorp, P.(1995). Evolution of the understanding of the role of exercise in obesity and its complications. *International Journal of Obesity and Related Metabolic Disorders*, 19, S1-4.
- Brown, S.E., Wiener, S. & Brown, R.A. (1985). Exercise performance following a carbohydrate load in chronic airflow obstruction. *Journal of Applied Physiology*, 58, 1340-1346.
- Calle, E.E., Thun, M.I., Petrelli, I.M., Rodriguez, C. & Heath, C.W. (1999). Body-mass index and mortality in a prospective cohort of U.S. adults. *New England Journal of Medicine*, 341, 1097-1105.
- Despopoulos, A. & Silbernagl, S. (1991). *Color Atlas of Physiology*. New York: Thieme Medical Publishers, pp. 46-50.
- Evans, W.J. (1995). Effects of exercise on body composition and functional capacity of the elderly. *Journals of Gerontology Series A*, 50A (Special Issue), 147-150.
- Evans, W.J. & Cannon, J.G. (1991). The metabolic effects of exercise-induced muscle damage. *Exercise and Sport Science Review*, 19, 99-125.
- Fielding, R.A. (1995). The role of progressive resistance training and nutrition in the preservation of lean body mass in the elderly. *Journal of the American College of Nutrition*, 14(6), 587-594.
- Florini, J.R. (1985). Hormonal control of muscle cell growth. *Journal of Animal Science*, 61, 21-37.
- Fox, K.R. (1999). The influence of physical activity on mental well-being. *Public Health Nutrition*, 2(3A), 411-418.
- Frexes-Steed, M., Lacy, D.B., Collins, J. & Abumrad, N. (1960). Role of leucine and other amino acids in regulating protein metabolism in vivo. *American Journal of Physiology*, 262(25), E925-E935.
- Garrow, J.S. & Summerbell, C.D. (1995). Meta-analysis: effects of exercise, with or without dieting, on the body composition of overweight subjects. *European Journal of Clinical Nutrition*, 49, 1-10.
- Gallagher, P.M., Carrithers, J.A., Godard, M.P., Schulze, K.E. & Trappe, S.W. (2000).  $\beta$ -hydroxy- $\beta$ -methylbutyrate ingestion. Part I: effects on strength and fat-free mass. *Medical Science of Sports Exercise*, 32, 2109-2115.
- Hill, J.O. & Commerford, R. (1996). Physical activity, fat balance and energy balance. *International Journal of Sports Nutrition*, 6, 80-92.

- Hirsch, I. (1995). Role and benefits of carbohydrate in the diet: Key issues for future dietary guide-lines. *American Journal of Clinical Nutrition*, (Suppl.61), 996S-1000S.
- Hough, T. (1992). Ergographic studies in muscular soreness. *American Journal of Physiology*, 7, 76-92.
- Knitter, A.E., Panton, L., Rathmacher, J.A., Petersen, A. & Sharp, R. (2000). Effect of  $\beta$ -hydroxy- $\beta$ -methylbutyrate on muscle damage after a prolonged run. *Journal of Applied Physiology*, 89, 1340-1344.
- Kraemer, W.J., Volek, J.S., Clark, K.L., Gordon, S.E., Incledon, T. & Puhl, S.M. (1997). Physiological adaptations to a weight-loss dietary regimen and exercise programmes in women. *Journal of Applied Physiology*, 83(1), 270-279.
- Krieder, R.B., Ferreira, M., Greenwood, M. & Wilson, M. (2000). Effects of calcium  $\beta$ -HMB supplementation during training on markers of catabolism, body composition, strength and sprint performance. *Journal of Exercise Physiology*, 3(4), 48-57.
- Lukaski, H.C., Bolonchuk, W.W., Siders, W.A. & Milne, D.B. (1996). Chromium supplementation and resistance training: effects on body composition, strength, and trace element status of men. *Journal of Clinical Nutrition*, 63(6), 954-965.
- Mole, P. A. (1990). Impact of energy intake and exercise on resting metabolic rate. *Sports Medicine*, 10, 72-87.
- Nair, K.S., Schwartz, R.G. & Wells, S. (1992). Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am Journal of Physiology*, 263, 928-934.
- Nissen, S., Sharp, R., Ray, M., Rathmacher, J.A., Rice, D., Fuller, J.C., Connelly, A.S. & Abumrad, N. (1996). Effect of leucine metabolite  $\beta$ -hydroxy- $\beta$ -methylbutyrate on muscle metabolism during resistance-exercise training. *Journal of Applied Physiology*, 81(5), 2095-2104.
- Nissen, S., Van Koevinger, M. & Webb, D. (1990). Analysis of  $\beta$ -Hydroxy- $\beta$ -methyl Butyrate in plasma by gas chromatography and mass spectrometry. *Analytical Biochemistry*, 188, 17-19.
- Nissen, S., Sharp, R.L., Panton, L., Vukovich, M., Trappe, S. & Fuller, J.C. (2000). Beta-Hydroxy-beta-methylbutyrate (HMB) Supplementation in Humans Is Safe and May Decrease Cardiovascular Risk Factors. *Human Nutrition and Metabolism*, 130, 1937-1945.
- Perusse, L., Collier, G., Gagnon, J., Leon, A.S., Rao, D.C. & Skinner, J.S. (1997). Acute and chronic effects of exercise on leptin levels in humans. *Journal of Applied Physiology*, 83(1), 5-10.
- Poole, D.C. & Richardson, R.S. (1997). Determinants of oxygen uptake. Implications for exercise testing. *Sports Medicine*, 24(5), 308-320.
- Proctor, D.N. & Joyner, M.J. (1997). Skeletal muscle mass and the reduction of  $\text{VO}_2\text{max}$  in trained older subjects. *Journal of Applied Physiology*, 82(5), 1411-1415.
- Sabourin, P.J. & Bieber, L.L. (1983) Formation of  $\beta$ -hydroxyisovalerate by an  $\alpha$ -ketoisocaproate oxygenase in human liver. *Metabolism*, 32, 160-164.
- Saris, W.H.M. (1995). Exercise with or without dietary restriction and obesity treatment. *International Journal of Obesity*, 19(Supplement 4), S113-S116.
- Sorof, J. & Daniels, S. (2002). *Obesity hypertension in children: a problem of epidemic proportions. Hypertension*, 40(4), 441-447.
- Steven, L., Nissen, S. & Sharp, R. (2003). Effect of dietary supplements on lean mass and strength gains with resistance exercise: A meta-analysis. *Journal of Applied Physiology*, 94, 651-659.
- Sue, D.Y. (1994). Integrative cardiopulmonary exercise testing: basis and application. *Medicine, Exercise, Nutrition and Health*, 3, 32-55.
- Terbizan, D.J. & Seljevoold, P.J. (1996). Physiological profile of age-group wrestlers. *Journal of Sports Medicine and Physical Fitness*, 36(3), 178-185.

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

Van Baak, M.A. (1999). Physical activity and energy balance. *Public Health and Nutrition*, 12(3A), 335-9.

Van de Wolle, H., Peres, G. & Monod, H. (1987). Standard anaerobic exercise tests. *Sports Medicine*, 4, 268-289.

Van Koevering, M. & Nissen, S. (1992). Oxidation of leucine and  $\alpha$ -ketoisocaproate to  $\beta$ -hydroxy- $\beta$ -methylbutyrate in vivo. *American Journal of Physiology*, 262(25), E27-E31.

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

Weber K. (1984). Exercise testing in the evaluation of cardiopulmonary disease. *Clinics in Chest Medicine*, 5, 175.

Zhang, Z., Talleyrand, V., Ratmacher, J. & Nissen, S. (1993). Change in plasma beta-hydroxy-beta-methylbutyrate (HMB) by feeding leucine alpha-ketoisocaproate (KIC) and isovaleric acid (IVA) to pigs (abstract). *FASEB Journal*, 7, A392.