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Review

# Nutritional role of the leucine metabolite β-hydroxy β-methylbutyrate (HMB)

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*This review develops the hypothesis that a metabolite of leucine termed β-hydroxy β-methylbutyrate (HMB) plays a key role in animal metabolism and that in certain circumstances insufficient amounts of HMB are either consumed in the diet or produced endogenously to supply tissue needs. The origin and metabolism of HMB is reviewed including the role of HMB in cholesterol biosynthesis. HMB feeding studies in animals are reviewed, which indicate that dietary supplementation of HMB can improve immune function and health and can increase the fat content of milk in lactating animals. Seven human studies are reviewed where HMB was fed. The results of both animal and human studies indicate that dietary supplementation of HMB is safe, as evidenced by lack of physical adverse effects and a lack of effect on blood hematology and chemistry. The only consistent change in blood chemistry was a decrease in LDL cholesterol, which changed 7% ($P < .01$). In humans undergoing resistance training, HMB supplementation increased lean mass gains from 50 to 200%, with similar percentage increases in strength when compared with unsupplemented subjects. The effects of HMB on muscle size and function seems to result from a diminution of exercise-related muscle damage and muscle protein breakdown. A general hypothesis is proposed that HMB is metabolized to HMG-CoA in tissues such as muscle, mammary tissue, and certain immune cells and is used for de novo cholesterol synthesis. In times of stimulated growth and/or differentiation, HMG-CoA may be rate-limiting for cholesterol synthesis, which could limit cell growth or function. It is proposed that feeding HMB can provide a saturating source of cytosolic HMG-CoA for cholesterol synthesis and in turn allow for maximal cell growth and function.* (J. Nutr. Biochem. 8:300–311, 1997)


**Keywords:** muscle; immune; cholesterol; health

## Introduction

The amino acid leucine has always held a classic position in amino acid biochemistry and metabolism in that it is the only purely lipogenic amino acid and it seems to have effects on metabolism beyond the need for protein synthesis. Although the regulatory and metabolic effects of leucine have been known for some 30 years,[1] as yet, there is no clear understanding of how leucine or its ketoacid, α-ketoisocaproate (KIC),[2] interacts with protein metabolism. This review develops the hypothesis that a metabolite of leucine and KIC termed β-hydroxy β-methylbutyrate (HMB) is responsible for the metabolic effects of leucine and KIC. Furthermore, it is proposed that HMB derived from leucine is converted to β-hydroxy-β-methylglutaryl CoA (HMG-CoA) in some tissues and serves as a key carbon source for cholesterol synthesis in these tissues which is necessary to maintain maximal cell function.

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## Leucine, KIC, and HMB metabolism

### *Leucine metabolism*

The tissue supply of the amino acid leucine is dependent on either exogenous (dietary) or endogenous (protein



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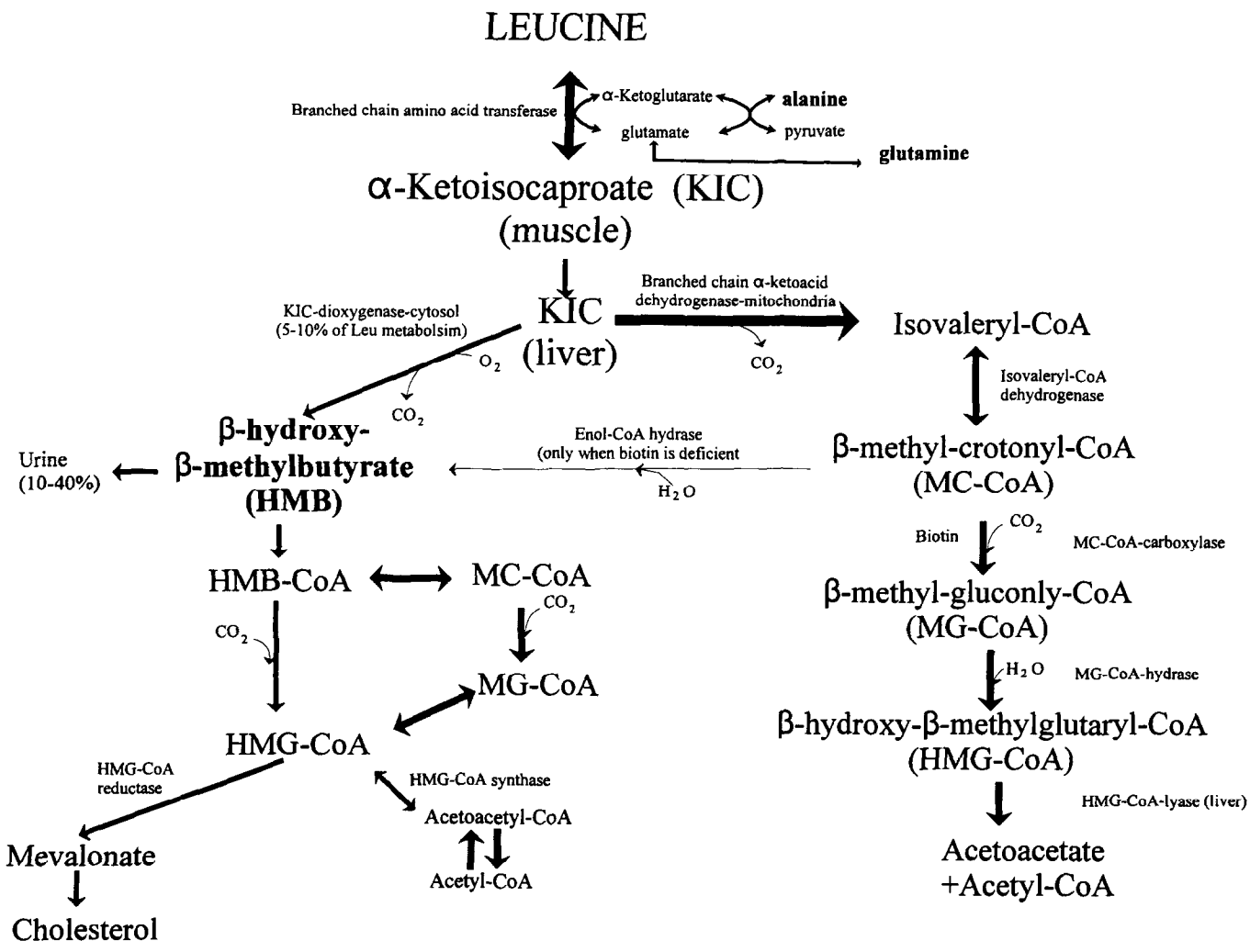


**Figure 1** Overview of leucine, α-ketoisocaproate (KIC), and β-hydroxy-β-methylbutyrate (HMB) metabolism in mammals. The enzymes and major co-factors are listed with each reaction. The metabolism of HMB is based upon isotopic data which suggested that HMB is converted to β-hydroxy β-methylglutaryl-CoA (HMG-CoA) in the cytosol and ultimately to cholesterol.

breakdown) sources. Leucine is then transaminated to KIC, both in the cytosol and in the mitochondria of muscle[3] (See *Figure 1* for leucine metabolic pathways). The majority of KIC oxidation, however, occurs in the liver.[3] In the liver mitochondria, KIC is irreversibly oxidized to isovaleryl CoA via the enzyme branched-chain keto acid dehydrogenase (BCKAD). Further catabolism occurs within the mitochondria to yield other metabolites, leading to the formation of HMG-CoA ultimately yielding acetoacetate and acetyl-CoA, again inside the mitochondria.

An alternate cytosolic pathway of leucine/KIC metabolism was suggested as early as 1981 by Sanbourn.[4] In this alternate metabolic scheme, KIC is oxidized to HMB in the cytosol of at least the liver, and possibly other tissues, by the enzyme KIC dioxygenase.[5–8] This enzyme requires molecular $O_2$ and is distinct from other enzymes in the leucine catabolic pathway. The $K_m$ of this enzyme is in the 120 μM range, whereas the $K_m$ of BCKAD is in the 10 to 40 μM range. Recent data suggest that the dioxygenase is similar, if not identical, to *p*-phenylpyruvate dioxygenase, an enzyme in tyrosine catabolism.*

Further in vivo studies showed that, at least in the pig, HMB is derived exclusively from leucine.[9] Additionally, it has been shown that feeding leucine and KIC to pigs** caused plasma HMB to increase from basal concentrations of 2 to 4 μM to 15 to 30 μM (*Figure 2A*). In this same study, 20 gm of isovaleric acid (IVA) was also fed to these pigs, which also significantly increased HMB concentration to more than 15 μM (*Figure 2A*). Production of HMB from IVA is consistent with studies showing that HMB can be made from methylcrotenoic acid (MCA) when concentrations of MCA are elevated, such as in genetic inborn errors or when biotin is deficient.[10,11] In these instances, MCA is thought to be hydrated by the enzyme, enol-CoA hydrase, of the isoleucine catabolic pathway.[10] Thus, feeding IVA to

*Abstract: *FASEB J.* 9:A1318, 1995.
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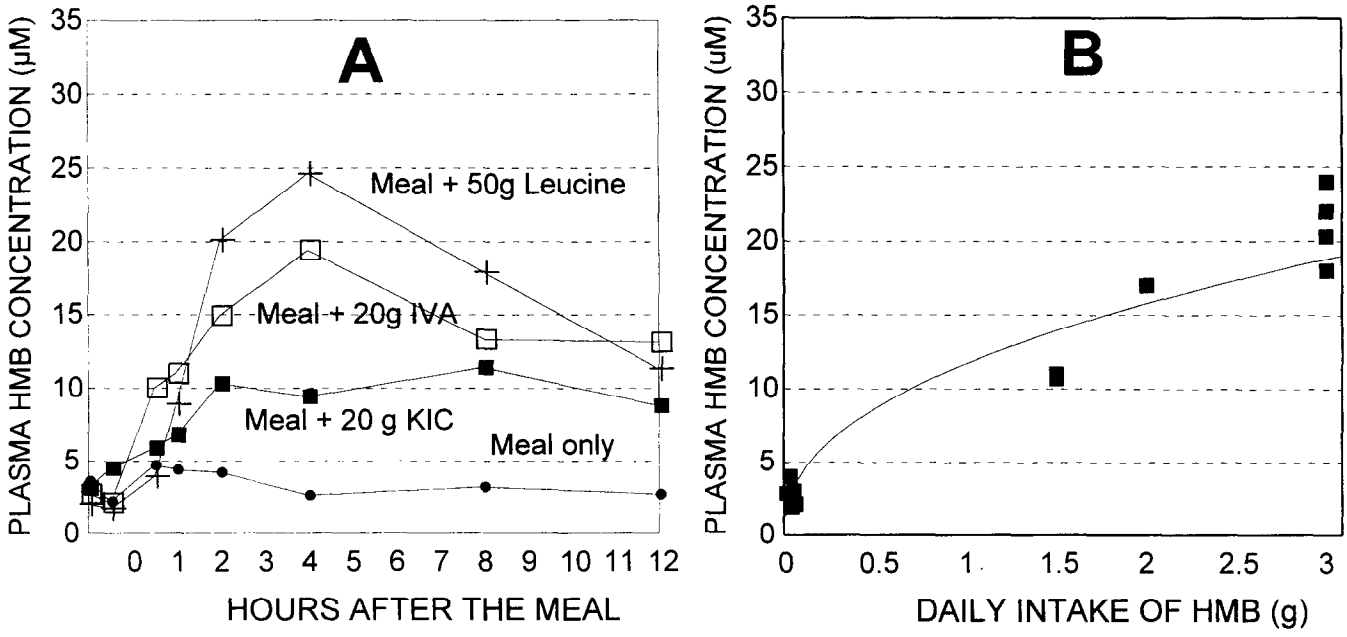


**Figure 2** *Panel A* depicts the plasma HMB concentrations in pigs before and after feeding a mixed meal (●) or a meal containing either 50 gm of leucine (+), 20 gm of isovaleric acid (□), or 20 gm of α-ketoisocaproate (KIC) (■). *Panel B* depicts the experimental averages of plasma HMB concentrations after feeding varying levels of HMB to humans.

pigs likely resulted in increased concentrations of MCA and subsequent conversion to HMB. Feeding 2 gm of HMB to these same pigs (2 gm/23 kg) increased plasma HMB to a peak of 240 μM 1 hr after feeding, which then decreased rapidly to less than 50 μM after 8 hr. This suggests that the half-life of HMB is about 2 hr in pigs.

Presented in *panel B* for *Figure 2* are the blood levels of HMB after chronic ingestion of HMB. As can be seen the basal HMB levels are similar between pigs and humans (2 to 5 μM) and that blood levels of HMB after feeding HMB are similar to the levels seen after ingesting large amounts of leucine.

In the same pig study, the uptake and output of HMB was measured across the hind limb of the pig before and during the feeding of a meal or a meal plus either leucine, KIC, or HMB. Across a wide array of HMB concentrations, it seems that muscle does not take up or release HMB in significant quantities* (there is no significant artery-vein difference, although there seems to be some uptake at low concentrations). Therefore, it seems the liver is the most likely site for HMB production, which is consistent with the enzyme data indicating that the liver is the major organ in the body with oxygenase activity.[7] Other studies with muscle homogenates and perfusates[12,13] also indicate that muscle and other tissues produce HMB at low rates if at all. Production of HMB seems to increase in diabetes[14] although it is not known if this is from an increase in dioxygenase activity or through hydration of MCA.

*Abstract: *FASEB J.* 7:A71, 1993.

### *Metabolic fate of HMB*

The major metabolic pathway of HMB metabolism seems to be conversion to HMG-CoA (see *Figure 1*). The experimental basis for this conversion was described extensively between 1949 and 1955. At this time, the precursor for cholesterol synthesis was not known and HMB was considered a likely candidate for the precursor. The most likely pathway of HMB metabolism is for direct conversion of HMB to HMG-CoA by either dehydration to MCA-CoA or direct carboxylation to HMG-CoA (*Figure 1*). The cytosolic HMG-CoA produced from HMB should provide a convenient substrate for HMG-CoA reductase, which is the committed step in cholesterol synthesis. In fact, HMB metabolism seems to be the only source of HMG-CoA other than condensation of Acetyl-CoA and Acetoacetyl-CoA. Indeed, there is an extensive body of literature that shows HMB carbon is readily incorporated into cholesterol.[15–19]

Although it is clear that HMB can be converted to cholesterol, it is unclear what percentage of cholesterol carbon is derived from HMB. In vivo studies with leucine indicate that indeed leucine carbon is converted to cholesterol.[20,21] Additionally, these studies also indicate that leucine may be an important source for cholesterol synthesis in muscle and possibly for export from muscle to other tissues. Again these studies did not measure the quantitative contribution of HMB to cholesterol synthesis.

An alternative fate of HMB is that it is excreted in urine. In pigs and sheep it has been shown that one-third of the HMB is lost this way.[9] In humans fed HMB, up to one half of the dosage fed was excreted in urine.

Additionally, HMB seems to be produced by the kidney.[22] Kidney excretion may partly account for the relatively short half-life of HMB, which seems to vary by species. The half-life of HMB was found to be about 1 hr in rats (unpublished observations), 2 hr in pigs (see graph in *Figure 2A*), and 3 hr in sheep (unpublished observations with deuterated HMB).

## Role of leucine in regulating protein metabolism

### *Role of leucine itself*

Several in vitro studies have shown that certain select amino acids, and particularly the branched-chain amino acids (BCAA), leucine, isoleucine, and valine, together exert anabolic effects similar to those of insulin. Chua et al. (1979)[23] proposed that leucine is a regulator of protein metabolism. These investigators demonstrated that the synthesis of heart protein and in particular myosin was increased by approximately 40% when either all of the amino acids or leucine alone were increased from 1 to 5 times the normal plasma concentrations in the culture medium. Similar findings were later observed in liver slices and in skeletal muscle preparations.[24,25] The first proposed clinical use of leucine was in the treatment of chronic liver disease. The early reports showed that the infusion of high doses of the BCAA improved recovery from liver failure[26–28] and hepatic encephalopathy.[29,30] Later reports, however, including a randomized multicenter European trial, failed to show any beneficial effects of either leucine or any combination of the three branched-chain amino acids in alleviating the symptomatology associated with liver failure.[26,27] Other studies examining the use of leucine (in combination with the other two BCAA, isoleucine and valine) during other stressful situations, such as infections[31,32] and trauma,[31,33,34] have in general showed benefits of supplementation; however, because of the differing levels of stress and the lack of proper controls the results have been variable over the studies.

The importance of BCAA in regulating protein metabolism *in vivo* has also been inferred from changes in their plasma concentrations.[35,36] Classically, it has been shown that insulin-deprived diabetics have markedly elevated plasma BCAA. Similarly, early starvation (up to 7 days) in humans was also found to be associated with marked elevations in BCAA.[37,38] These observations have led several investigators to postulate a role for plasma amino acids, and in particular BCAA in regulating the rates of whole-body and regional protein turnover. Recent studies showed that the availability of amino acids in the presence of elevated levels of plasma insulin resulted in a more pronounced suppression of whole-body proteolysis and in restoration of protein synthetic rates to near normal.[35] Additional studies by Frexes et al. attributed all the beneficial effects of amino acids to excessive consumption of leucine.[39] These findings are consistent with other studies in the rat that showed that the availability of amino acids in the diet, particularly the BCAA, were necessary to decrease myofibrillar and muscle proteolysis and to enhance protein synthesis.[40,41]

In pigs,[42,43] fish,[44] and lambs,[45] dietary leucine supplementation failed to show an effect of leucine to increase growth of muscle. Experiments with leucine supplementation in normal humans are largely related to studies with leucine infusion or feeding in stressful conditions such as trauma,[31,33,34] burns,[31] and uremia.[46] Limited studies with feeding leucine to normal exercising humans indicate little or no effect of leucine on muscle mass or strength.[47] Therefore, these studies do not support an anabolic role of leucine in normal physiology.

### *Role of "down-stream" leucine metabolites*

The variable effects of leucine on protein metabolism have led to speculation that leucine itself is not responsible for the observed metabolic effects attributed to BCAA in the tissues. Two lines of investigation were used to address the relative importance of leucine and BCAA in regulating protein turnover. The first examined the role of leucine-nitrogen in protein metabolism and the second examined the influence of leucine carbon(s) to these pathways. The first step in leucine metabolism involves the transfer of the amino group ($NH_2$) from leucine onto α-ketoglutarate (αKG) yielding glutamate which then is in most instances metabolized to glutamine (Gln) and KIC. The majority of this conversion occurs in skeletal muscle. It is through this latter pathway that the metabolism of leucine in skeletal muscle was linked closely to the glutamine cycle. Studies by Aoki et al.[48] and Abumrad et al.[49] showed that either ingestion or intravenous administration of high doses of leucine resulted in excessive release of Gln from skeletal muscle, which suggested a detrimental effect of a high load of leucine on muscle metabolism in humans. These findings in humans are consistent with the in vitro findings that the activity of the enzyme responsible for the transfer of the amino group, the branched chain amino acid transferase (BCAAT), is markedly enhanced with either a high-protein diet, high availability of leucine or KIC, or in the presence of elevated levels of glucocorticoids.[50]

Because the first byproduct of leucine metabolism is KIC, this led several investigators to postulate that KIC was the active controller of the action of leucine on protein metabolism. Chua et al.[23] noted that in rat heart muscle KIC decreased proteolysis and increased protein synthesis without increasing intracellular concentrations of leucine. In perfused rat livers, Mortimore et al.[51] found that both leucine and KIC decreased proteolysis by 63%. Tischler et al.[52] noted that inhibition of leucine transamination in rat diaphragms also resulted in the inhibition of muscle proteolysis.

Walser and colleagues were the first to examine in vivo the effects of KIC on nitrogen metabolism. The early studies explored the use of KIC to moderate the nitrogen loss with obesity,[2,53] kidney failure,[54,55] and liver failure.[56] Cersosimo et al. compared the nitrogen sparing effects of leucine and KIC in normal volunteers subjected to 4 days of caloric deprivation. Daily intravenous administration of leucine (nearly 5.0 gm per hour) to normal volunteers did not alter the rate of whole-body protein breakdown, nor did it alter the extent of muscle wasting.[57] On the other hand, infusion of an equivalent dose of KIC resulted in moderate improvement in nitrogen loss and, by inference, a decrease in

muscle wasting. In addition to its effects on protein metabolism, KIC also seems to exert other metabolic effects. Intravenous infusion of a similar dose of KIC has been shown to spare glucose utilization and decrease glucose oxidation by skeletal muscle of normal, healthy volunteers.[58] In both studies, however, the doses of KIC used wee large, exceeding 60 gm per day, and the effects achieved on protein sparing and glucose oxidation by skeletal muscle were minor. These and other observations led several investigators to examine whether or not the beneficial effects of leucine and KIC noted in the in vitro studies could be related to metabolites of leucine downstream from KIC.

### *Role of "down-stream" KIC metabolites*

There are at least five metabolites of leucine/KIC that could be implicated in regulating protein metabolism in vivo. As shown in *Figure 1*, acetoacetate is the primary metabolite produced from leucine. β-Hydroxy butyrate (BHB) is also produced, only at much lower rates than acetoacetate. The BHB ketone body has been the subject of several investigations examining its nitrogen-sparing effects in humans. In these studies, volunteers were given nearly 100 gm of BHB per day.[59] Equivocal results were observed with some showing improved nitrogen retention[60–63] and others showing no effect.[64] The discrepancy in the data could be related to several confounding factors. One such factor relates to the use of a mixture of D and L isomers of BHB in many of these studies. Additional factors relate to the significant alterations in acid-base homeostasis associated with the use of BHB, and their independent influence in modulating protein metabolism in vivo.

Isovaleryl-CoA (IVA-CoA) is the major product of KIC oxidation in cells. IVA has been shown to inhibit protein breakdown in isolated muscle preparations.[65] Animal studies investigating the effects of IVA have been limited to sheep[45] and lactating cattle[66] and showed no effects on growth or body composition, but there was evidently an increase in total milk production with IVA treatment. The administration of IVA to humans has not been reported.

HMG, another metabolite of leucine, has not been studied extensively in animals relative to growth. Most of the reported studies in animals and humans showed that feeding HMG was associated with significant decreases in cholesterol levels.[67–70] There are no reported studies addressing the nitrogen-sparing effects of HMG in either humans or animals. It is predicted that such effects are unlikely to occur. It is also well established that the flux rates of HMG and BHB are quite large. Feeding of either HMG or BHB would not be expected to impact such flux rates, thus minimizing the effect of any direct or indirect contribution of either BHB or HMG on protein metabolism.

## Metabolic effects of HMB

### *In vitro studies with HMB*

The direct effects of HMB on muscle metabolism have recently been examined.* In this study, isolated muscle strips from rats and chicks were exposed to various concentrations of HMB (up to 1,000 μM) and the rates of proteolysis and protein synthesis were measured. HMB inhibited proteolysis by an average of 80% in both rat and chick muscle. Concurrently, protein synthesis was also increased about 20% in the muscle strips.

**Table 1** Effect of added leucine and leucine metabolites on ovine lymphocyte blastogenesis

| Metabolite | Percent change ± SEM | *P*< | Replicates |
|---|---|---|---|
| Leucine | −12 ± 6 | 0.10 | 8 |
| α-ketoisocaproate | −3 ± 7 | 0.60 | 8 |
| Isovalerate | −1 ± 7 | 0.99 | 6 |
| HMB | +78 ± 13 | 0.01 | 13 |
| Hydroxy methyl glutarate (HMG) | +28 ± 9 | 0.20 | 6 |
| β-hydroxy butyrate (BHB) | +25 ± 6 | 0.01 | 15 |

All metabolites were added to the cultures at a concentration of 1000 μM. The percentage change for each metabolite is expressed as the change compared with no metabolite addition. The number of replicate experiments indicates the number of individual sheep-lymphocyte isolates used for the particular metabolite. Other metabolites (and hydroxy acids) tested that did not alter blastogenesis were butyrate, acetate, acetone, acetoacetic acid, α-hydroxy-butyrate, γ-hydroxy-butyrate, citramalic acid and α-hydroxy-isovalerate (see text).

In addition to its effects on protein metabolism, our studies have established a major role for HMB in modulating immune responses, especially during periods of stress. The data in *Table 1* are the results of an in vitro experiment designed to look at the effect of HMB and other leucine metabolites on blastogenesis of isolated sheep lymphocytes.[71] Freshly isolated lymphocytes from sheep were cultured with phytohemagglutinin-P (PHA-P) in combination with and without the specified metabolite at a concentration of 1,000 μM. Radioactive thymidine uptake was used to measure blastogenesis. After 48 hr, the cells were washed and counted for incorporated radioactive thymidine.

The results (*Table 1*) showed that the only direct leucine metabolite to affect lymphocyte blastogenesis was HMB. BHB significantly increased blastogenesis, but to a lesser degree than HMB. Although HMG treatment affected blastogenesis to essentially the same extent as BHB, it was not significant probably because of the lower number of replications that contained HMG. Addition of either leucine or KIC resulted either in no change or in decreased blastogenesis. These data strongly suggest that, at least relative to immune function, HMB, and to a lesser extent HMG and BHB, were the only metabolites of leucine and KIC to be involved directly with increased immune cell function of sheep lymphocytes. Additional dose-response studies with sheep lymphocytes indicated that HMB also increased blastogenesis significantly at concentrations from 0.1 μM to 100 μM. Additionally, HMB concentrations up to 10,000 μM showed no inhibition of blastogenesis.[72] Further studies with cattle lymphocytes showed that HMB also increased lymphocyte blastogenesis in vitro.[73] Additional studies were performed using chicken macrophages.* In these studies, HMB was added to chicken macrophage cultures

*Abstract: *J. Anim. Sci.* **74(Suppl. 1)**, 138, 1996.

*Abstract: *Poult Sci* **75 (Suppl. 1)**, 7, 1996.

**Table 2** Summary of animal studies with HMB supplementation

| Species | # Exps/ # HMB-fed animals | Dose HMB mg/kg/d | Weeks fed | Blood chemistry/ Hematology | Mortality | Morbidity | Ref |
|---|---|---|---|---|---|---|---|
| Hamster | 3/80 | 20–200 | 4 | Lower cholesterol | 1 exp decrease | none[1] | LDL cholesterol lowered 20% Nissen et al. (1994) U.S. Patent 5,348,979 |
| Pig (weanling) | 1/20 | 60–100 | 6 | nm[2] | none | none | Immune function tended to increase with HMB Gatnau et al. (1995) *J. Anim. Sci.* **73,** 159–165 |
| Pig (lactating) | 6/100 | 10–50 | 3–4 | nm | ~10% decrease baby pig mortality | none | Increased milk fat production 10%- baby pigs larger Nissen et al. (1994). *J. Anim. Sci.* **72,** 2332–2337 |
| Pig (20 kg)[3] | 1/5 | 5,000 | 1 | nc[4] | none | none | No gross or histological lesions |
| Cattle (400 kg) | 2/300 | 8 | 16 | nc | none | none | No effect on growth, less subcutaneous fat Van Koevering et al. (1994). *J. Anim. Sci.* **72,** 1927–1935 |
| Cattle (200 kg-stress) | 4/200 | 20 | 4 | nm | 50% less | up to 40% less | Less respiratory disease Van Koevering et al. (1993). *Oklahoma State Univ. Res. Rep.* 312–316 |
| Sheep[5] | 4/150 | 10–40 | 16 | nc in any enzymes- lower cholesterol | none | 50% less in 1 exp | Increased lean growth, less fat, increased immune function |
| Goats[6] | 1/6 | 50–100 | 2 | nm | none | none | Increased milk fat Moschini, M. (1993). M.S. thesis, Iowa State University |
| Chicks | 8/7,800 | 10-growth 60-immune | 4–9 | nm | 32% lower overall & 09% lower the 1st 10d | | Increase in growth, increased muscle and increased immune function Nissen et al. (1994). *Poultry Sci.* **73,** 137–155 |

[1]None indicates that no mortality or morbidity occurred during the study.
[2]nm indicates the parameter was not measured.
[3]Unpublished observations. See text for description of materials and methods.
[4]nc indicates no change was measured with HMB supplementation.
[5]Abstract: *J. Anim. Sci.* 77, 243, 1994.
[6]Abstract: *FASEB J.* 7, A70, 1993.

(cell line MQ-NCSU) at concentrations from ~100 to 1,000 μM. Number of macrophages was increased by 20% ($P < 0.003$) and nitrite production was increased by 29% ($P < 0.06$) when HMB was added to the cultures. Additionally, the number of Sephadex-elicited macrophages from chick peritoneal fluid increased 2 to 3 fold in chicks fed HMB ($P < 0.05$).

Studies with isolated human lymphocytes seemed to show a biphasic response to HMB addition: At low concentrations, HMB addition increased blastogenesis, whereas at high concentrations blastogenesis was decreased by HMB. At 50, 100, and 500 μM, HMB altered blastogenesis by 43%, 5%, and −11%, respectively ($P < 0.03$ linear) (Unpublished observations by Dr. James Roth, Iowa State University).

Together, these in vitro data are consistent with the theory that HMB is responsible for the non-protein synthetic functions of leucine and KIC in vivo and that HMB is needed in very small amounts for maximal immune cell function in vitro.

### *Feeding studies with HMB in animals*

A variety of animal experiments have been conducted with HMB and are summarized in *Table 2*. Where possible in the animal studies, safety and toxicity of HMB were evaluated. In terminal animal studies, each animal was examined, dissected, and organ weights were recorded. Mortality, morbidity, and clinical signs of disease were observed and quantified when possible.

*Table 2* also contains the results of an unpublished toxicity study conducted in young pigs. In this study, three pigs weighing about 20 kg were fed a diet supplemented with 100 gm of Ca-HMB/d for a period of 4 days, and two control pigs were fed an unsupplemented diet. At the end of the period, the pigs were killed and blood and tissues were collected. Blood chemistry and hematology measurements were made on each pig, as well as gross organ pathology and histology. This dosage of HMB is approximately 100 times higher than is typically fed to humans. None of the pigs exhibited untoward signs related to HMB consumption. There were no changes in blood cell numbers, percentages, or organ weights, nor were there any histological lesions present in either control or HMB-fed pigs. Thus, it seems that even at very high intakes there are no adverse effects of HMB consumption, at least over a short period of time.

Across all animal studies (*Table 2*), no adverse effects of

**Table 3** Summary of changes in blood chemistry and hematology values due to HMB supplementation

| | Study no. | | | | | | Average | |
|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | Unweighted | Significance |
| *n* placebo/HMB | 5/5 | 12/12 | 15/15 | 18/19 | 19/20 | 18/19 | | |
| HMB dose (g/d) | 2 | 1.5 & 3 | 3 | 3 | 3 | 3 | | |
| Gender | Male | Male | Male | Male | Female | Female | | |
| Blood chemistry | | | | | | | | |
| CPK | nm | −2% | 26% | −16% | 28% | −70% | −7% | 0.68 |
| Glucose | 5% | −2% | −5% | −1% | 3% | 4% | 1% | 0.75 |
| Uric acid | nm | 9% | −11% | 1% | 6% | 7% | 2% | 0.47 |
| Urea | nm | −6% | – | 9% | −2% | −11% | −2% | 0.50 |
| Creatinine | nm | 0% | −5% | 0% | 7% | 4% | 1% | 0.55 |
| Sodium | nm | 0% | −1% | −1% | 1% | −1% | 0% | 0.17 |
| Potassium | nm | 0% | 1% | −2% | −2% | −2% | −1% | 0.20 |
| Chloride | nm | 2% | −1% | −1% | 1% | −1% | 0% | 0.79 |
| Calcium | nm | 0% | 0% | −1% | −1% | 1% | 0% | 0.75 |
| Phosphorus | nm | −3% | −12% | −2% | 5% | −9% | −4% | 0.15 |
| Protein | −0.7% | 1.5% | 3.0% | 1.9% | 0.9% | −1.2% | 1% | 0.20 |
| Albumin | −0.7% | 0.0% | 2.8% | 1.8% | 0.4% | 0.4% | 1% | 0.15 |
| Globulin | nm | 3.7% | 3.6% | 1.6% | 1.6% | −3.8% | 1% | 0.30 |
| Liver function tests | | | | | | | | |
| Bilirubin | 3% | −5% | 9% | −4% | 9% | 16% | 5% | 0.18 |
| Alkaline phosphatase | −4% | 3% | 4% | 2% | −2% | −4% | 0% | 0.98 |
| Lactate dehydrogenase | 6% | −4% | 7% | 1% | 6% | −10% | 1% | 0.76 |
| SGOT | 15% | −1% | 15% | −4% | 8% | −3% | 5% | 0.19 |
| SGPT | 19% | 17% | −25% | −6% | −3% | −9% | −1% | 0.87 |
| GGT | nm | 10% | −5% | −18% | 5% | −5% | −2% | 0.61 |
| Iron | nm | 0% | 24% | −2% | −3% | 6% | 5% | 0.30 |
| Blood lipids | | | | | | | | |
| Total cholesterol | −6% | −5% | −4% | −1% | −2% | −2% | −3% | 0.002 |
| Triglycerides | 10% | −1% | −17% | 17% | −2% | −5% | 0% | 0.94 |
| HDL cholesterol | 11% | −14% | −1% | 0% | −1% | 1% | −1% | 0.84 |
| VLDL cholesterol | nm | 7% | −17% | 17% | −2% | −5% | 0% | 0.98 |
| LDL cholesterol | −14% | −10% | −6% | −5% | −3% | −3% | −7% | 0.003 |
| Chol./HDL chol. | | 11% | −5% | 0% | −2% | −4% | 0% | 0.98 |
| Hematology | | | | | | | | |
| Total WBC | −5% | −4% | −13% | 0% | −3% | −12% | −6% | 0.02 |
| Total RBC | nm | −2% | 2% | −2% | −1% | −2% | −1% | 0.26 |
| Hemoglobin | nm | −4% | 2% | −1% | 0% | −1% | −1% | 0.29 |
| Hematocrit | nm | −1% | 1% | −1% | −1% | −1% | −1% | 0.28 |
| MCV | nm | 0.0% | −0.5% | 0.0% | −0.7% | 0.9% | 0% | 0.90 |
| MCH | nm | −1% | 0% | −5% | 4% | 1% | 0% | 0.89 |
| MCHC | nm | 0% | 1% | −5% | 1% | 0% | −1% | 0.47 |
| Platelets | nm | 5% | −4% | −7% | −5% | 4% | −1% | 0.56 |
| Neutrophils | −3% | −1% | −10% | −3% | −2% | −22% | −7% | 0.06 |
| Lymphocytes | 1% | −10% | −17% | 7% | 0% | −6% | −4% | 0.24 |
| Monocytes | −20% | 156% | −22% | −6% | 2% | 15% | 21% | 0.47 |
| Eosinophils | −48% | −12% | −4% | 9% | −41% | −8% | −18% | 0.08 |
| Basophils | −5% | −6% | 27% | −19% | 0% | 36% | 6% | 0.53 |

The unweighted average and overall significance of the HMB effect are presented.

HMB were noted in any organs or tissues or in behavior or growth. The most notable effects in animals seem to be an increase in milk fat production by the mammary glands of sows (10%) and goats and improved immune function in stressful situations, which results in significantly less morbidity and mortality in mammalian and avian species.

### *Human studies with HMB*

The following is a summary of human studies conducted with HMB supplementation. This overview is not intended to summarize all the aspects of the studies, but instead highlight the major effects of HMB related to safety in humans (*Table 4*) and changes in muscle metabolism with exercise. In human studies 3 through 7, extensive blood work, adverse effect questionnaires and psychological tests[74] were given every week.

**Human study 1.** Normal human subjects were allowed food ad libitum and were given various dosages (0.5, 1.0, 2.0, or 4.0 gm) of HMB (250 mg capsules divided in equal daily doses). Each subject was examined before and immediately after 8 days of HMB intake at the designated dosage. Blood tests were obtained before the test and every other day of the treatment to determine biochemical and hematological parameters and to assess hepatic and kidney functions.

**Table 4** Summary of human studies conducted in which HMB was supplemented

| | Study no. | | | | | | | Overall mean |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| *n* placebo/HMB | | 5/5 | 12/12 | 15/15 | 18/19 | 19/20 | 18/19 | |
| HMB dose (gm/d) | .5–4 | 2 | 1.5 & 3 | 3 | 3 | 3 | 3 | |
| Gender | Male | Male | Male | Male | Male | Fem | Fem | |
| Exercise protocol | No | No | Yes | Yes | Yes | No | Yes | |
| Body wt change (gm/wk) | nm | nm | 244 | 231 | 84 | 5 | 22 | 117* |
| Body fat loss (gm/wk) (%/week) | nm | less[1] | −57 (−.27) | −151 (−1.07) | −79 (−1.00) | −5 (−.08) | −37 (−.70) | −66* |
| FFM gain (gm/wk) (%/week) | nm | more[2] | 185 (.19) | 365 (.39) | 164 (.48) | 9 (.02) | 68 (.13) | 158* |
| Gain in strength (% change) | nm | nm | 300% | 250% | 50% | nm | 54% | 164* |
| Adverse effects[3] | nc | nc | nc | nc | nc | nc | nc | |
| Psychological[4] | nm | nm | nc | nc | nc | nc | nc | |

The mean values represent the net change over the period divided by the weeks of study compared with the control group. No significant change relative to controls is noted by nc. A notation of nm indicates when a parameter was not measured.
*Indicates a significant effect of HMB on a given parameter ($P < 0.05$).
[1]Body fat was measured in only half of the subjects. In these subjects there was a numerical increase in body fat relative to controls.
[2]Urinary nitrogen was measured in this study and HMB decreased urinary nitrogen by 18%, which suggests lean tissue was increased.
[3]Weekly questionnaires were given to each subject related to major organ function and symptoms. No significant effects of HMB were noted in any study or across all studies.
[4]A psychological profile of mood was given to each subject each week (see text for details). No significant effects of HMB were noted.

The results showed that at these dosages intake of HMB for one week was safe. No untoward effects were noted in any of the subjects. The liver enzymes, SGOT, LDL, alkaline phosphatase, and SGPT, did not change with any of the HMB dosages used. Moreover, there were no untoward effects of the use of HMB on kidney function. Blood cholesterol tended to decrease in a dose responsive manner, but the shortness of the study precluded definitive conclusive results. Plasma SGPT, alkaline phosphatase, SGOT, and LDH remained constant over the 8-day study in all groups.

**Human study 2.** The primary goal of this study was to determine, in a controlled double-blinded fashion, the effects of feeding HMB on loss of urinary nitrogen in normal humans. Blood was also obtained for estimating liver and kidney function as well as for estimates of tissue metabolism. Daily urinary nitrogen was measured at the beginning and at the end of the study to calculate the nitrogen balance. Five normal males, with an average age of 30, were studied twice in a random order using a switchback design to determine nitrogen balance both with and without HMB. All but one subject engaged in regular exercise, and all received their meals at a clinical research unit with the same meals being fed each day of the week. During one 2-week period, the subjects were given capsules containing HMB (250-mg capsules), and during the other 2-week period, they were given capsules containing 250 mg of calcium carbonate (placebo). The daily intake of HMB per subject was 2.0 gm administered in three divided doses.

During the control period when subjects consumed a calcium carbonate placebo, urinary nitrogen increased from a basal level of 14.5 to 16.1 g/d, an average increase of 1.6 ± 0.8 gm/day. During the HMB-supplemented period urinary nitrogen decreased from 16.7 to 15.4 g/d with an average decrease of −1.3 ± 0.9 gm/day ($P < 0.05$ vs control). Skin-fold estimates were conducted in three of the subjects. A slight change during the control period (12.4% fat to 12.0% fat) was observed, which was slightly accentuated during the HMB period (12.5% fat to 11.0% fat). Body weight did not change in controls but increased 1.4 kg in HMB supplemented subjects over the 2-week study (non-significant).

Again, this study indicated that HMB was safe as evidenced by no change in liver enzymes and no change in blood chemistry (*Table 3*). There was also no effect of HMB on CD3, CD4, and CD56 lymphocytes. Plasma levels of insulin, glucagon, and cortisol were measured but did not change with HMB treatment. None of the subjects reported any other untoward effects of HMB supplementation.

**Human study 3.** Based on previous work showing that leucine and KIC could decrease muscle protein breakdown[23,51] and preliminary in vitro studies showing HMB inhibited protein breakdown in isolated muscle strips, a study was conducted to test the hypothesis that HMB would decrease muscle proteolysis induced by resistance exercise. It was hypothesized that the decrease in muscle proteolysis should result in increased muscle mass and function.[75] Therefore this study examined the effects of dietary supplementation with HMB on muscle metabolism in normal male volunteers performing resistance training for 3 weeks. The study was composed of 41 male volunteers, divided into three groups, consuming HMB at either 0, 1.5, or 3.0 gm per day. Two levels of protein intake in the form of a protein-shake were also used.

The results of this study indicated that HMB supplementation caused a dose-responsive decrease in percentage muscle protein breakdown as (6.0, 5.5, and 4.5 in subjects consuming 0, 1.5, 3.0, gm/day, respectively) measured by urinary 3-methylhistidine. In addition, there were also decreased levels of the muscle enzymes creatine kinase

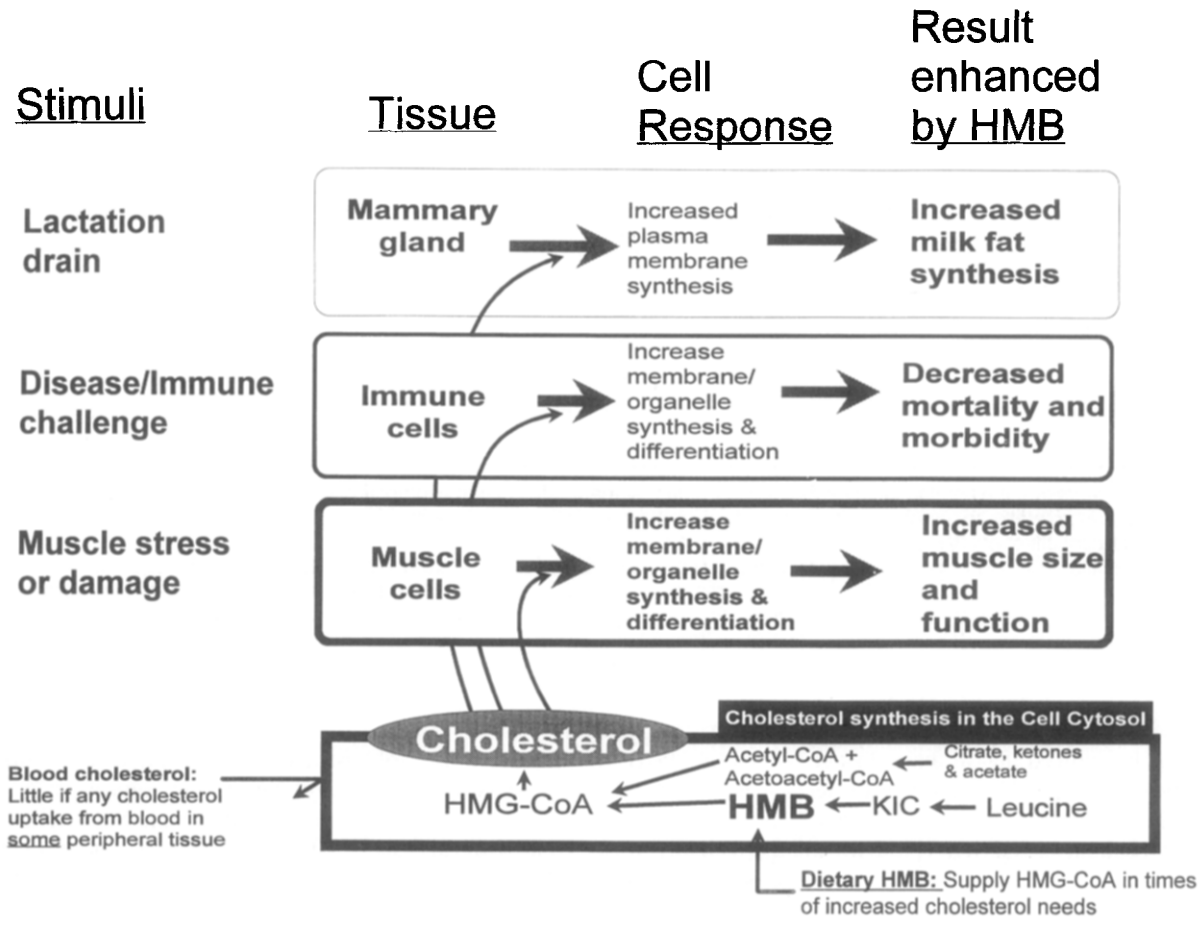


**Figure 3** Proposed model of HMB action on muscle, the immune system, and the mammary gland. This theoretical model depicts HMB as an important precursor of cholesterol metabolism in these tissues. In times of rapid growth, production, or repair, dietary supplementation of HMB could be used as a precursor source of HMG-CoA for cholesterol synthesis in these tissues.

(666, 388, 304, μ/mL, respectively) and lactate dehydrogenase (187, 171, 169, μ/mL, respectively) released, indicating less muscle damage caused by the exercise protocol in subjects consuming 0, 1.5, or 3 gm HMB/day, respectively. Our results also showed no independent effect of protein intake on muscle metabolism or urinary 3-methyl histidine output. Similarly, protein intake had no additive effects to those of HMB on any of the parameters examined. These results underscored the independent beneficial effects of HMB on protein and muscle metabolism in exercising humans.

**Human study 4.** This study was carried out in healthy athletes who were subjected to stringent exercise schedules.[75] Briefly, 35 males between the ages of 20 and 28, weighing between 80 and 130 kg, were studied over a 7-week period. Subjects were supplemented with 3 gm of calcium HMB daily contained in the form of a protein-shake identical to that used in study 3. Control subjects received an isocaloric carbohydrate drink instead of the protein shake. Subjects did not know which drink contained the HMB supplement.

Body composition was determined at the onset of each experimental protocol, after an overnight fast, and weekly thereafter by TOBEC.[76] Strength was measured by one-repetition maximums of the bench press, the squat, and the hang-clean at the beginning and end of the 7-week study. Subjects exercised a total of 6 days a week with resistance exercise on 5 days each week. All subjects ate their normal meals at the athletic training center, and were allowed free access to another protein drink during the workouts.

The results of this study are depicted in *Table 4*. The HMB-supplemented group had a rapid and sustained increase in lean (fat-free) gain (365 gm/day) whereas the placebo group had virtually no lean gain over the entire 7-week study. In addition, HMB-supplementation resulted in a significant increase (250%) in muscle function. This is best exemplified by a significant 6.82 kg increase in the bench press of the HMB-supplemented group versus the 2.45 kg increase of the placebo group ($P < 0.01$).

**Human study 5.** In this study,* 17 trained and 24 untrained subjects were assigned to either placebo capsules or capsules containing 3 gm of HMB/day. Subjects underwent a strenuous resistance training regimen for 4 weeks. The trained subjects underwent regular resistance exercise at

*Abstract: *FASEB J.* 10:A287, 1996.

least 3 times per week, whereas the untrained subjects did not participate in any regular weightlifting for at least 4 months before the study.

The results of this study are summarized in *Table 4*. The results indicated that HMB supplementation significantly ($P < 0.05$) stimulated muscle accretion in both trained (those that participated previously in weight training) and untrained subjects (a combined net change of 164 gm/wk). Additionally, and more importantly, we found that there was no interaction of HMB with previous training in promoting muscle accretion.

**Human study 6.** This is an unpublished safety study using 40 women randomly assigned to either a control or HMB (3 gm per day mixed in orange juice) treatment. A complete physical examination was performed before and after the study. During the 4-week study, each subject underwent a weekly blood sampling, body composition measurement by TOBEC and filled out a questionnaire related to health and mood.

Similar to our previous studies utilizing males, HMB supplementation did not cause any significant changes in the measured parameters in the women (*Tables 3* and *4*). Because there was no exercise imposed in this study, no marked changes in body composition over the 4-week study occurred, but the changes were consistent with that found in other studies.

**Human study 7.** This study examined the effect of HMB supplementation on hematology and body composition of women undergoing resistance exercise. Blood was sampled and body composition measured (underwater weighing) at the beginning and end of 4 weeks of study.

The results of this study were similar to those in study number 5 with males. There were no changes in blood parameters, no adverse effects, and no change in psycholoical profiles (*Tables 3* and *4*). Body composition changes indicated increased lean and decreased fat content (68 and −37 gm/wk, respectively). Strength was also increased (54%, $P < 0.05$) with HMB supplementation.

## Summary of human studies with HMB

*Tables 3* and *4* summarize the effects of HMB in humans. The combination of HMB with resistance exercise resulted in enhanced muscle growth and improved strength. HMB supplementation was safe, did not result in any adverse physical or psychological effects, and did not alter any indicators of organ or tissue function.

The only other demonstrable effect of HMB beyond that of muscle metabolism, was a consistent lowering of LDL-cholesterol. This observation in humans is consistent with data collected in several animal studies suggesting an effect on cholesterol synthesis. If the theory that HMB supplies precursor carbon for cholesterol in peripheral tissues is valid it could be speculated some form of negative feedback on liver cholesterol synthesis is occurring resulting in a decrease in blood LDL cholesterol.[69,70]

## Conclusion

### *A nutritional role for HMB in cholesterol synthesis?*

Although HMB cannot fulfill the leucine requirement for protein synthesis, it seems that HMB is needed for maximal function of the immune system (in at least animals), for maximal milk-fat production in lactation and for maximal gains in muscle in response to resistance exercise. The biochemical mechanism tying all these effects together is the classic biochemical notion that leucine is obligatorily linked to fat metabolism. The current data support a working hypothesis that HMB is supplying a source of HMG-CoA for cholesterol synthesis in cells of the immune system, mammary gland, and muscle. In certain conditions these cells may require increased levels of cholesterol synthesis for either the synthesis of new cell membranes or to regenerate damaged membranes of existing cells. This may be particularly true in tissues such as muscle where de novo synthesis appears to be the major if not exclusive source of cholesterol (*Figure 3*). This could also explain why certain cholesterol synthesis inhibitors can cause severe muscle toxicity (myopathy).[77,78] Whatever the mechanism, it seems that supplementation of HMB can allow functionally-stimulated tissues such as muscle, mammary cells and immune cells to perform at maximal levels even under demanding situations.

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