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# Dietary toxicity of calcium β-hydroxy-β-methyl butyrate (CaHMB)

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**Abstract**

HMB, 3-hydroxy-3-methyl butyrate, is of interest as a dietary supplement and a possible component of functional and medical foods. The purpose of this study was to evaluate the toxicity of the calcium salt of HMB, calcium 3-hydroxy-3-methyl butyrate (CaHMB, monohydrate, food grade), when administered daily in the diet of rats for at least 90 days.

Male and female Crl:CD® (SD)IGS BR animals were assigned to four groups. Each group received diets containing the carrier or 1%, 2%, or 5% of CaHMB mixed with diet. Assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, and clinical and anatomic pathology evaluations.

Administration of CaHMB in basal diet for 91 days was tolerated well. There were no unscheduled sacrifices or deaths. There were no CaHMB-related adverse effects on clinical observations, body weights, food consumption, clinical chemistry, hematology, absolute or relative organ weights, or macroscopic or microscopic observations. A statistically significant increase in inorganic phosphorous was observed in male animals in the 5% feeding group; however, this effect was not considered adverse. Based on the results of this study, the no-observed-adverse-effect level (NOAEL) was considered to be 5% of CaHMB mixed with diet (3.49 g/kg BW for males and 4.16 g/kg BW for females).


*Keywords:* Calcium β-hydroxy-β-methyl butyrate; CaHMB; Rat; Toxicity; Oral

## 1. Introduction

β-Hydroxy-β-methyl butyrate (HMB) is a normal metabolite of the amino acid leucine and is produced endogenously in both animals and humans. Leucine is an important amino acid in muscle metabolism, serving in a regulatory role and as a precursor for protein synthesis and energy metabolism. The first step in the metabolism of leucine is transamination to α-ketoisocaproate (KIC). HMB can then be produced from KIC by the action of KIC-dioxygenase. This is a cytosolic enzyme and under normal conditions approximately 5% of leucine oxidation proceeds via HMB. The bulk of KIC is metabolized by KIC-dehydrogenase, which is exclusively found in mitochondria. A review with potential nutritional implications has been published (Nissen and Abumrad, 1997).

Studies have shown that supplemental HMB can magnify exercise-related improvements in performance, increase muscle mass and reduce body fat relative to

*Abbreviations*: ANOVA, analysis of variance; BW, body weight; CaHMB, calcium β-hydroxy-β-methyl butyrate; CFR, Code of Federal Regulations; CK, creatine phosphokinase; EPA, eicosapentaenoic acid; FDA, Food and Drug Administration; GLP, good laboratory practices; HMB, β-hydroxy-β-methyl butyrate; HMG-CoA, β-hydroxy-β-methylglutaryl coenzyme A; HPLC, high performance liquid chromatography; KIC, α-ketoisocaproate; NFκ B, nuclear factor κ B; NOAEL, no observed adverse effect level; RBC, erythrocyte cell count; SD, standard deviation.

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exercise alone (Nissen and Sharp, 2003). In a summary of nine clinical studies in humans, HMB had no adverse effects when supplemented at 3 g per day in men, women, young, and elderly subjects (Nissen et al., 2000). In all nine studies, lean mass increases were observed over baseline when supplemental HMB was provided. The mechanism of this effect is thought to involve decreases in muscle protein breakdown.

Cholesterol plays a crucial role in membrane structure, enhancing membrane fluidity and thus reducing lability to stretch rupture. Muscle cannot supply its cholesterol needs via absorption from the circulation, and must produce its own, usually via β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) from fatty acid β-oxidation and/or glycolysis. Biochemical studies have shown that HMB is a precursor of cholesterol (Bloch, 1944; Rabinowitz, 1955; Rudney, 1957; Zabin and Bloch, 1951). HMB in the cytosol of liver and muscle is first converted to cytosolic HMG-CoA, which can then be used for cholesterol synthesis. In situations where the normal routes of synthesis are inadequate, supplemental HMB can also serve as a precursor for cellular cholesterol in tissues such as muscle that rely solely on de novo synthesis. Because supplemental HMB does not increase circulating cholesterol (Nissen et al., 2000), de novo synthesis in this case seems to be restricted to local use.

The working hypothesis for HMB action presumes that stressed or damaged muscle cells may not be able to make sufficient HMG-CoA to support adequate cholesterol synthesis. Supplemental HMB could be a convenient source of HMG-CoA production in such muscle cells to maintain adequate cholesterol synthesis and support membrane function and structural integrity. This hypothesis is supported by the observation that supplemental HMB can markedly decrease muscle damage as evidenced by reduced leaking of creatine phosphokinase (CK) out of muscle cells, for example, after strenuous exercise (Nissen et al., 1996).

Other mechanisms of action of HMB in reducing muscle loss have been the subject of recent studies in a tumor-bearing mouse model. In this highly cachectic animal-tumor model, Tisdale and coworkers demonstrated that oral supplementation of HMB can slow or partially prevent muscle wasting (Smith et al., 2005) by suppressing upregulation of the ubiquitin–proteosome system and by enhancing protein synthesis rates (Smith et al., 2004). This effect is distinct from that reported for eicosapentaenoic acid (EPA) (Whitehouse and Tisdale, 2003): while oral EPA supplements prevent activation of phospholipase $A_2$ and subsequent arachadonic acid release, HMB exerts its effect significantly downstream from this at protein kinase C. With the involvement of NFκ B (Nuclear Factor κ B) in these pathways, numerous questions obviously arise as to possible impacts on the inflammatory response are obvious.

Immune function may be weakened during times of stress. Studies in animal models suggest that HMB can nutritionally support both innate and specific immune function, as indicated, for example, by enhanced macrophage function (Ostaszewski, 1998; Peterson, 1996) and increased production of antibody to specific vaccine (Peterson et al., 1999; Siwicki, 1998), respectively. The relevance of these findings may be demonstrated by the decreased incidence and severity of morbid conditions caused by infectious agents in animals fed HMB (see for example, Nissen et al., 1994). This immune-response enhancement has not yet been demonstrated in humans.

Thus, for several reasons, HMB is of interest for possible addition to a variety of functional foods, medical foods, and dietary supplements. It is therefore advisable to look carefully at the safety of introducing this compound more widely into the food supply. The purpose of the study reported here was to assess certain aspects of the safety profile of this compound that had not yet been adequately studied. We report here the results of experiments designed to evaluate the general toxicity of HMB. This study was conducted in compliance with the Food and Drug Administration (FDA), Good Laboratory Practice (GLP) Regulations, 21 CFR 58.

## 2. Materials and methods

### 2.1. Animals and feed

Male and female Crl:CD® (SD)IGS BR rats were obtained from Charles River Laboratories, Portage, MI. One hundred and sixty animals were randomly assigned to study groups as noted in Table 1. At initiation of treatment, the animals were 48–54 days old, and their body weights ranged from 237 to 303 g for the males and 159 to 221 g for the females.

The carrier, basal diet, was Certified Rodent Diet #8728CM, Harlan, Teklad. CaHMB was obtained from Technical Sourcing International, Inc., Missoula, MT.

Rodent diet was prepared in-house (Covance) and tested for HMB content by reverse-phase HPLC, essentially as described by Snowden et al. (2002). For Group 1, the specified amount of basal diet was dispensed into labeled storage containers. For Groups 2–4, the appro-

Table 1
Description of diet preparation

| Group | Dose level (%) | CaHMB weight (g) | Basal diet weight (kg) |
|---|---|---|---|
| 1 (control) | 0 | 0 | 169.0 |
| 2 (low) | 1 | 1719.2 | 167.3 |
| 3 (mid) | 2 | 3438.5 | 165.6 |
| 4 (high) | 5 | 8596.1 | 160.4 |