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Paul Raymond Vulcan
Iowa State University

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Role of β -hydroxy- β -methylbutyrate (HMB) on inflammation after eccentric exercise

by

Paul Raymond Vulcan

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Kinesiology

Program of Study Committee:

Rick L. Sharp, Major Professor

Marion Kohut

Lance Baumgard

Iowa State University

Ames, Iowa

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Abstract

When exposed to resistance exercise, untrained muscle can develop micro-tears and disruptions in the sarcomeres. This is followed by swelling, pain and decreased muscle performance, which can be attenuated by taking β -hydroxy- β -methylbutyrate (HMB). The acute phase response is the inflammatory change that occurs to repair damaged tissue, and is mediated by cytokines. It is the purpose of this study to determine if HMB can influence the acute phase response. 32 untrained subjects (16 men, 16 women) completed three sets of 50 eccentric leg extensions on each leg. The subjects were group matched and randomly assigned to one of three treatment groups: placebo (CON), HMB pre exercise (PRE) or HMB pre-exercise and for 4 days following the exercise protocol (PRE-POST). We observed a drop in serum concentration of IL-1ra and TNF- α for the CON, that was attenuated by HMB at 48h and 72h post exercise ($p < .05$). The observed reduction in acute phase response suggests that HMB may moderate inflammatory response to exercise induced muscle damage.

Manuscript

Introduction

When untrained muscle is exposed to intense exercise, there is the potential to cause physical damage to the myofibers as demonstrated by sarcomere disruption (18) and myosin contractile protein leakage (61). The amount of damage is larger following eccentric contractions than concentric contractions and is associated with decreased range of motion (ROM) and peak force(19). Delayed onset muscle soreness (DOMS) occurs 24-72 h after the exercise and correlates with increases in plasma creatine phosphokinase (CK), lactate dehydrogenase (LDH) and 3-methyl histidine (3-MH) (41).

Following a damaging bout of exercise, inflammation sets in to repair damaged tissue. Inflammation is mediated chemically by several cytokines. Interleukin (IL) 1 beta (IL-1 β) and tumor necrosis factor alpha (TNF- α) have been linked to increased proteolysis and may modulate protein turnover (11). IL-1 β , TNF- α , and IL-6 are commonly regarded as pro-inflammatory cytokines because they initiate and amplify immune cell function. Anti-inflammatory cytokines (IL-10 and IL-1 receptor antagonist (IL-1ra)) work to regulate and resolve the inflammatory response.

The inflammatory response to strenuous endurance exercise has been examined previously, but the response to eccentric induced damage has returned mixed results. One study used elbow flexors and found increases in serum increases in IL-6 and IL-1ra (50), but another failed to find any rise with a similar protocol (22). It is possible that using the elbow flexors does not induce enough damage to increase serum levels to detectable differences. It is also possible that tissue accumulation of the cytokines occurs to limit serum increases (6, 13). Buford et al. (4) was unable to detect serum levels of TNF- α , IL-1 β , or IL-6 following leg resistance training but did find increases in the mRNA for those cytokines, signifying an up-regulation that was not detected in the serum.

The amount of inflammation is dependent upon the intensity of the bout, and training status. Trained subjects have higher resting cytokine levels, but do not have the up-regulation following a single bout experienced by untrained subjects (12). Repeated bouts also results in less muscle

damage as found by serum CK(9). In addition to training, β -hydroxy- β -methylbutyrate (HMB) may decrease the amount of muscle damage caused by exercise induced muscle damage (EIMD)(9). HMB is a leucine metabolite taken as a supplement and is associated with less proteolysis (58), reduced 3-MH, CK and LDH (38) following a bout of resistance training in untrained subjects. It is believed that increased availability of HMB, a de novo cholesterol synthesis precursor, increases the membrane integrity of skeletal muscle (57).

Increased muscle integrity and decreased muscle breakdown allows the muscle to experience less EIMD and recover faster. HMB has the potential to reduce the amount of inflammation that occurs. It is the goal of this study to examine the inflammatory response to EIMD by using eccentric leg extension and to determine if HMB is able to reduce the amount of inflammation that accrues.

Methods

Subjects. Sixteen females and 16 males volunteered to participate in this study. The mean \pm SE of the age, body mass and height were as follows: 23 ± 0.30 y, 67.5 ± 0.9 kg, and 172.2 ± 0.7 cm (table 1.). All subjects were free from injury and illness as determined by a medical questionnaire. None of the subjects had engaged in resistance training in the 3 months prior and were not taking any dietary supplements. They were advised to refrain from exercising outside of the protocol and to not consume anti-inflammatory medication (NSAID, etc.). They were also provided a list of foods high in 3-MH, that they were advised not to consume. Written informed consent was given to subjects prior to their involvement. This study was approved by the Iowa State University IRB.

Table 1. Age, Height and Mass. Mean (SEM).

	N	AGE (y)	Ht. (cm)	Mass (Kg)
CON	6	24 (2)	172 (3)	66.1 (4.9)
PRE	13	22 (1)	171 (4)	66.8 (3.4)
PRE/POST	13	24 (1)	173 (3)	68.8 (3.4)

Experimental protocol. Prior to the start, subjects came to the lab for a familiarization trial. They were measured for maximal isometric force on both legs. Maximal isometric leg extension was performed for 3 repetitions for 5 sec. each with 5 sec. rest between repetitions at an angle of 110°. Subjects were randomly assigned to 5 experimental groups and experimental groups were matched based on dominant leg average peak force so that the average between groups was the same.

Muscle damage and inflammatory response was determined with the following indicators: maximal isometric force, muscle soreness, mid-thigh leg circumference, urinary 3-MH, serum CK, LDH, IL-1ra, IL-1 β , C-reactive proteins (CRP), IL-6, and TNF- α . All measures were taken in the morning (5am-9am) at the same time day for each subject. Subjects were fasted (12 h) but allowed water. Maximal isometric force was taken 30 min after the morning supplement. The remaining measures were collected prior to the supplement. Measures were performed before the exercise protocol and for the 4 days following the exercise protocol. A chart outlining the procedure is displayed in Table 3.

Supplementation/Experimental groups. Supplementation was given 3 times throughout the day. The first occurred early morning in the lab under supervision and the remaining two occurred at mid day and evening time points. Supplements came in two forms, a capsule of HMB calcium salt, and a 3ml gel syringe of HMB free acid, both taken orally. Subjects were advised to consume the capsule, then the gel, and then 500ml of water. Empty syringes were collected to ensure compliance.

Experimental groups received supplement in one of the following manners: placebo pre- & post-exercise (CON), HMB pre-exercise (PRE) in either a calcium salt form or a free acid gel, HMB pre- & post-exercise (PRE/POST) in either a calcium salt form or a free acid gel. Supplements were given in a double-blind protocol so that investigators were also blind to the contents of the supplements throughout the study. Originally, the study called for 5 treatment groups with a separation of calcium salt groups from the free acid gel groups. Due to sample sizes, groups receiving the same quantity of HMB were consolidated for statistical reasons (see Table2.)

Table 2. Consolidation of groups. Treatment group name and type of HMB supplement.

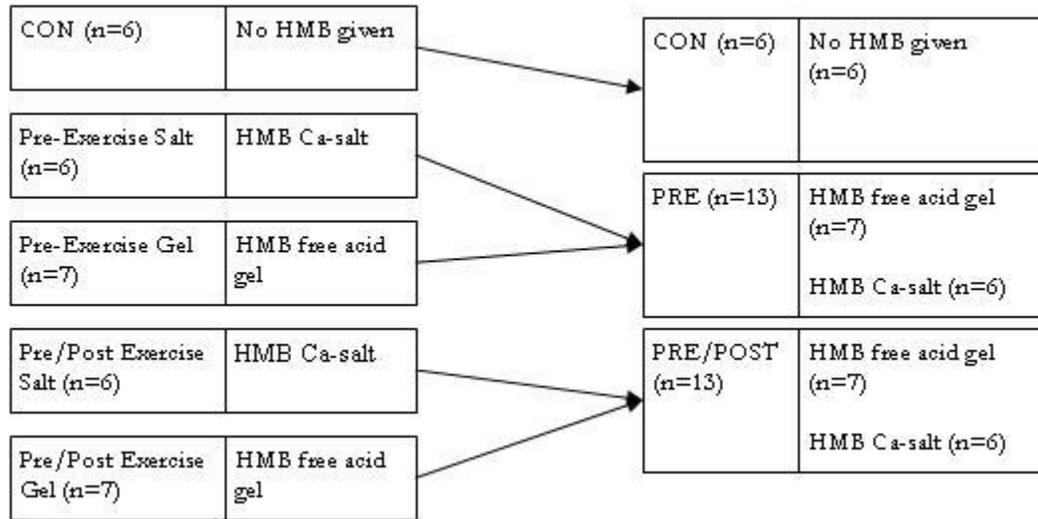


Table 3. Experimental Protocol.

~1 wk Prior	Pre-Exercise	24 h Post	48 h Post	72 h Post	96 h Post
1. Informed Consent	1. Pre-Test				
2. Pre-Test	2. Supplement				
3. Strength Test	3. 30 min rest				
	4. Strength Test				
	5. Exercise Protocol				
	Lunch - supplement Dinner - supplement				
Pre-Test		Supplement		Strength Test	
○ Urine collection		1. Capsule		○ 3 maximal contractions for 5 sec.	
○ Muscle Soreness		2. 3 ml gel syringe		○ 5 sec. rest between contractions	
○ Leg circumference		3. 500 ml water		○ R. & L. leg	
○ Ht. and Wt.				○ Isometric leg extension at 110°	
○ Blood collection (not during screening)					

Muscle Soreness/Leg circumference. Muscle soreness was determined by a visual analog scale (43). A 10 cm horizontal line was presented with “no pain” at one end and “extreme pain” on the other. Subjects would draw a vertical line along the horizontal to establish the degree of muscle soreness. Mid-thigh leg circumference was measured by tape measure half-way between the inguinal crest and top of the patella while subjects were seated and relaxed with knees flexed at

90°. Measurement sites were labeled with semi-permanent marker, and all measurements were taken by the same investigator.

Exercise protocol. Subjects were seated and performed 3 sets of 50 eccentric leg extensions on both legs. The leg extensions went from 0° to 90° at a rate of 60°/sec. Each repetition took approximately 3 sec. to complete and contraction occurred on the downward swing only. Subjects were given 2 min rests between sets.

Cytokines. Whole blood was collected into two 10 ml glass tubes and allowed to coagulate for at least 30 min at RT before being centrifuged at 2,800 rpm for 10 min. Serum was manually extracted by pipette and separated into 600 µl aliquots. Samples were stored at -80°C until analysis and all measures were taken to limit freeze-thaw cycles. Cytokines measured were IL-6, IL-1β, IL-1ra, TNF-α, and CRP. All samples were measured in duplicates and all time points for a given subject were analyzed on the same plate. Serum cytokines were measured using commercially available enzyme linked immunosorbent assays (ELISA; R&D Systems, R&D Systems, Minneapolis, MN, USA). High sensitivity kits were used for TNF-α, IL-6 and IL-1β.

Statistical Analysis. All measured samples were tested using a repeated measures ANOVA. The p-value for significance was set at 0.05. Analysis of the deltas from baseline were analyzed for IL-6, IL-1ra, TNF-α and CRP.

Results

Muscle soreness, leg circumference, peak leg force. Peak muscle soreness for all groups was reached 48h after the exercise bout with a 1200% increase in perceived muscle soreness. There was no difference in muscle soreness found between treatment groups. Leg circumference was unchanged over time and there was no difference between treatment groups. The decrease in peak leg force of the right leg peaked at 72h post-exercise and decreased by 10.5% from pre-exercise to 72h post-exercise (p-value = 0.008). The decrease in peak leg force of the left leg peaked at 48h post-exercise and decreased by 10.5% (p-value = 0.023). No difference in the loss of peak force was found between the treatment groups. Results are displayed in table 4, and figures 4 & 5.

CK, 3-MH, LDH. Serum CK concentration peaked at 96h post exercise ($p= 0.037$) for all three treatment groups (CON = 3487.5 U/ml, PRE = 4067.6 U/ml, PRE/POST = 1592.6 U/ml). There was no difference in peak CK between treatment groups. Peak serum LDH was reached at 72h post-exercise ($p= 0.022$) but no difference was found between groups (CON = 269.8 U/ml, PRE = 212.7 U/ml, PRE/POST = 180.9 U/ml). 3-MH was not significantly different between groups and failed to show a time effect. Results are displayed in table 4.

IL-1 β and IL-6. The concentration of IL-1 β , for most subjects, was below detection minimums. Therefore we were unable to obtain reliable data regarding this cytokines. IL-6 showed no change over time and there was not an observed difference between groups (table 4).

TNF- α , CRP, IL-1ra. The change in serum concentration of IL-1ra, as shown in figure 1, was significantly different from the control group at 48h (PRE $p= 0.007$, PRE/POST $p= 0.038$) and 72h (PRE $p= 0.005$, PRE/POST $p= 0.040$) for both treatment groups, with PRE/POST remaining elevated at 96h as well ($p= 0.018$). Serum concentration of CRP was elevated from baseline with PRE group at 48h ($p= 0.002$), 72h ($p= 0.019$), and 96h ($p= 0.048$), but failed to show any significant difference between the other treatment groups. A graph of the change in CRP is displayed in figure 2. A decrease in serum TNF- α within the control group is shown in figure 3. The treatment attenuated the depression and was significantly different from CON at 48h (PRE/POST $p= 0.047$) and 72h (PRE $p= 0.010$, PRE/POST $p= 0.008$).

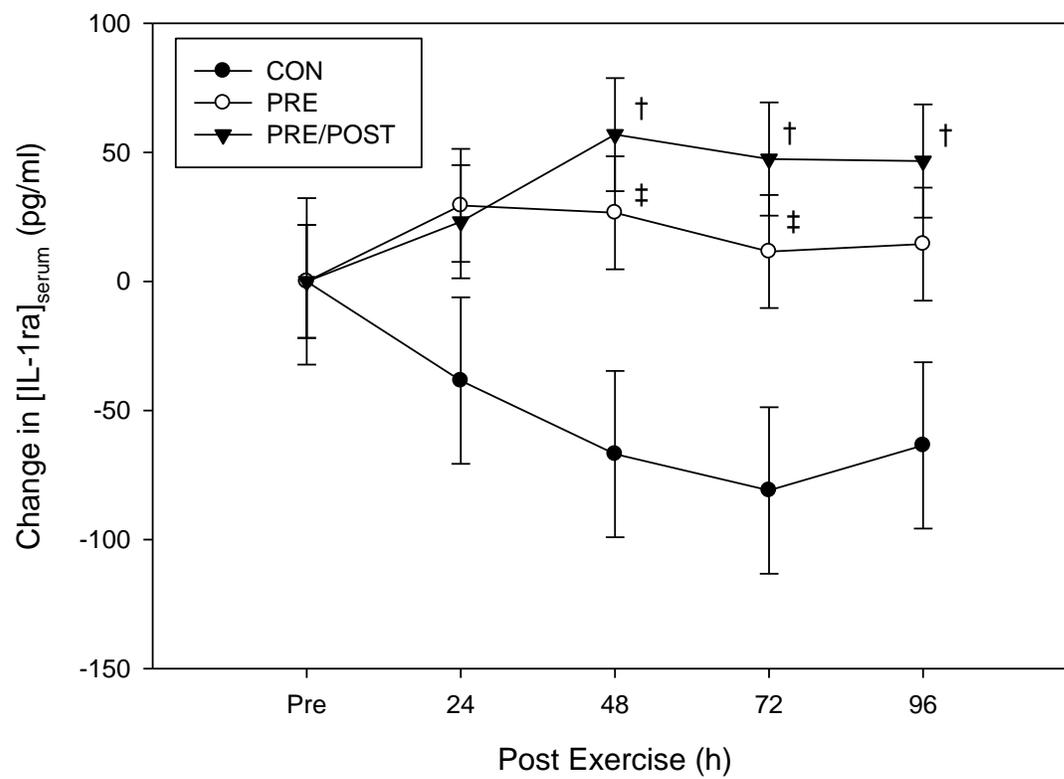


Figure 1. Change in IL-1ra. CON vs. PRE/POST (†, $p \leq 0.05$), CON vs. PRE (‡, $p \leq 0.05$).

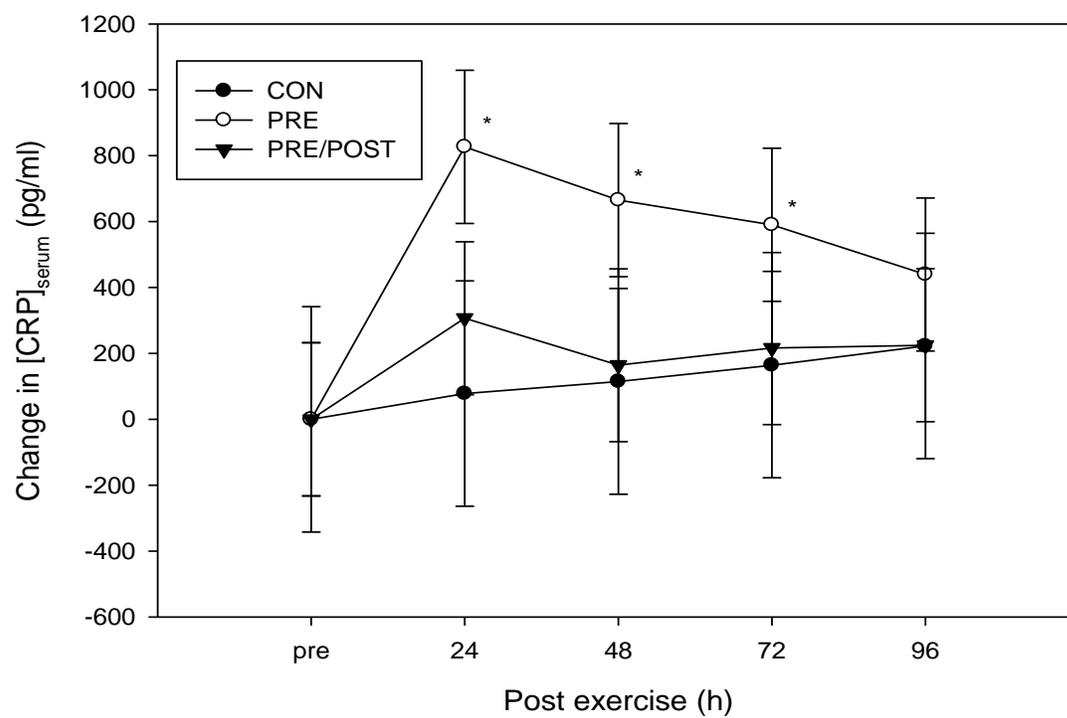


Figure 2. Change in CRP. Different from baseline (*, $p \leq 0.05$).

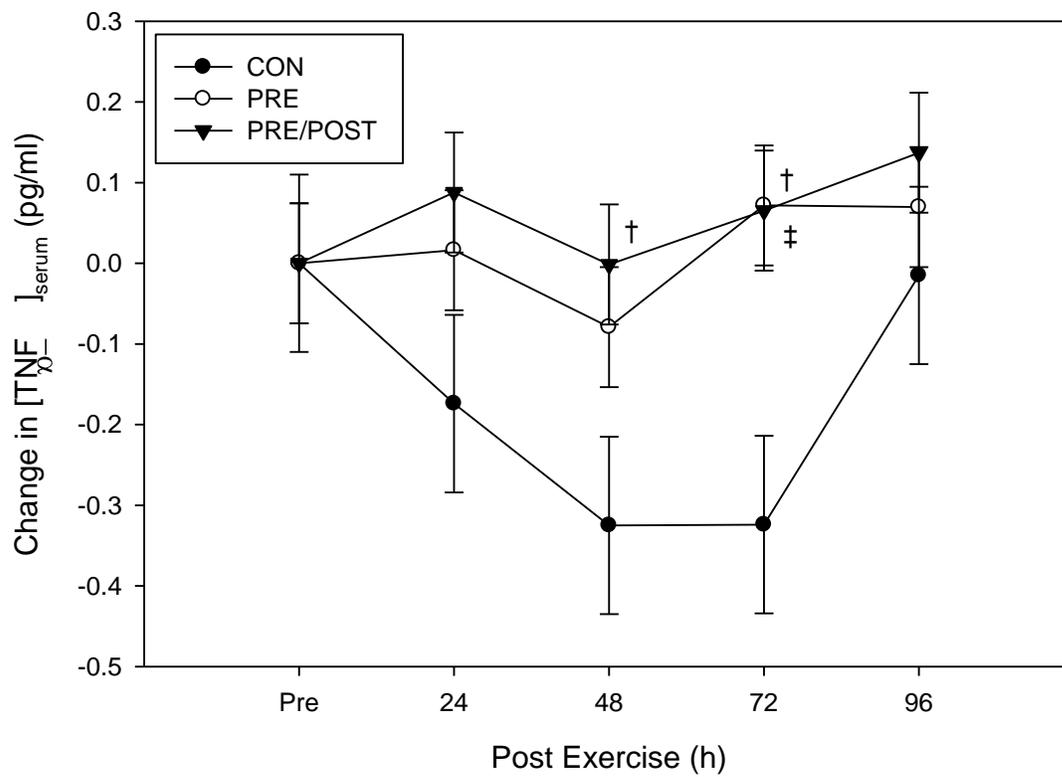


Figure 3. Change in TNF- α . CON vs. PRE/POST (†, $p \leq 0.05$), CON vs. PRE (‡, $p \leq 0.05$).

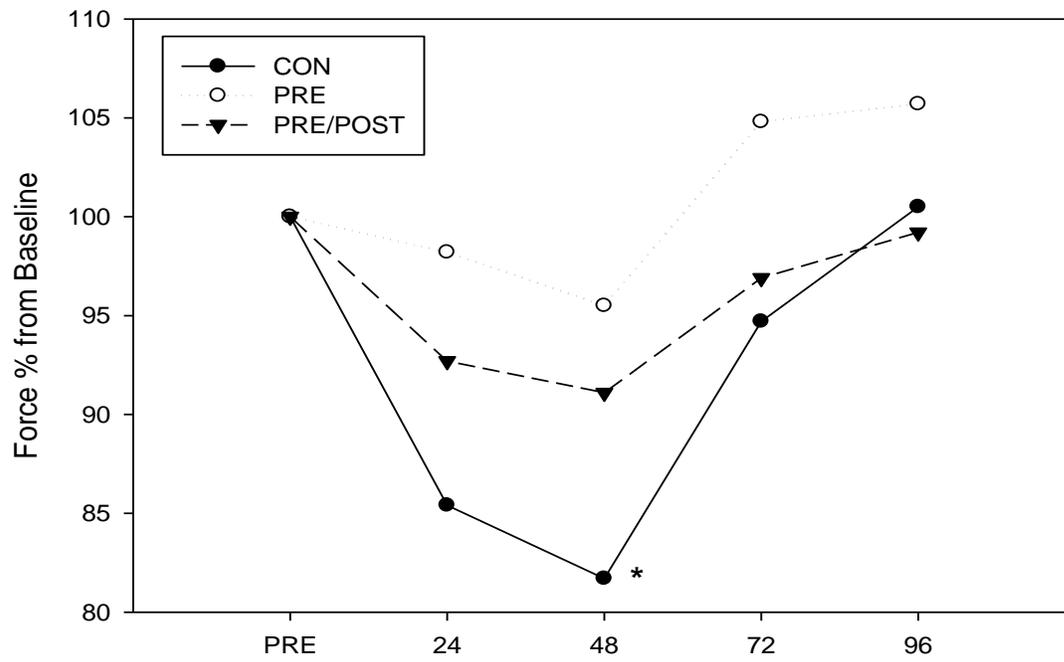


Figure 4. Left leg average peak force. Different from baseline (*, $p \leq 0.05$).

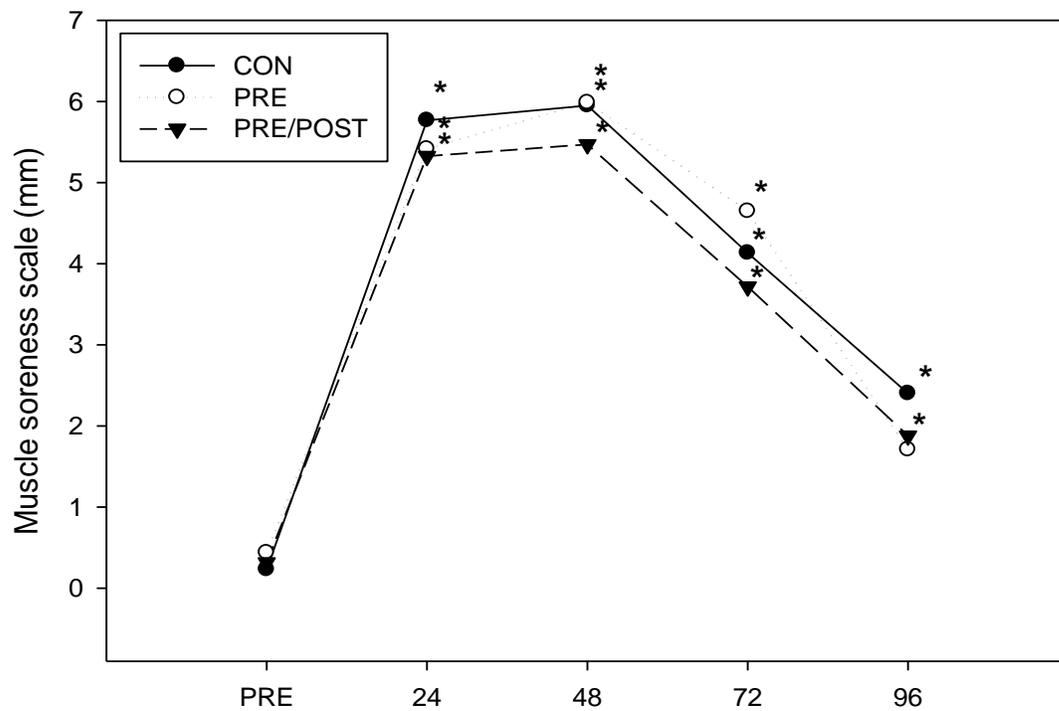


Figure 5. Muscle soreness scale. Different from baseline (*, $p \leq 0.05$).

Table 4. Serum concentrations and markers of muscle damage. CON vs. PRE/POST (†, $p \leq 0.05$); CON vs. PRE (‡, $p \leq 0.05$); Different from baseline (1, $p \leq 0.05$); different from 96h (2, $p \leq 0.05$); Different from 72h (3, $p \leq 0.05$).

		Pre	24	48	72	96	SEM
IL-1ra (pg/ml)	CON	279.5	241.0	212.6	198.5	215.9	43.2
	PRE	161.9	191.3	188.5	173.5	176.4	29.3
	PRE/POST	189.4	212.5	246.2	236.7	236.0	29.3
d IL-1ra	CON	0.0	-38.4	-66.9	-81.0	-63.5	32.2
	PRE	0.0	29.4	26.6‡	11.6‡	14.4	21.9
	PRE/POST	0.0	23.1	56.9†	47.4†	46.6†	21.9
TNF-a (pg/ml)	CON	1.62	1.45	1.30	1.30	1.61	0.24
	PRE	1.49	1.50	1.41	1.56	1.56	0.17
	PRE/POST	1.03	1.12	1.03	1.10	1.17	0.17
d TNF-a	CON	0.00	-0.17	-0.33	-0.32	-0.02	0.11
	PRE	0.00	0.02	-0.08	0.07‡	0.07	0.07
	PRE/POST	0.00	0.09	0.00†	0.07†	0.14	0.07
IL-6 (pg/ml)	CON	0.99	0.92	1.13	1.13	1.38	0.28
	PRE	1.16	1.23	1.26	0.97	0.89	0.19
	PRE/POST	0.95	0.99	0.93	1.21	1.10	0.19
d IL-6	CON	0.00	-0.07	0.14	0.14	0.39	0.25
	PRE	0.00	0.07	0.10	-0.19	-0.27	0.17
	PRE/POST	0.00	0.04	-0.02	0.26	0.14	0.17
CRP (pg/ml)	CON	1088.5	1166.5	1203.3	1252.7	1311.2	576.5
	PRE	1219.8	2046.7	1885.1	1810.2	1659.3	391.7
	PRE/POST	1143.4	1450.1	1308.0	1360.0	1368.2	391.7
d CRP	CON	0.0	78.0	114.8	164.2	222.7	342.0
	PRE	0.0	826.9 ¹	665.3 ¹	590.4 ¹	439.5	232.3
	PRE/POST	0.0	306.7	164.6	216.5	224.8	232.3
CK (U/ml)	CON	170.8	395.3	1956.3	3513.8	3487.5	1503.3
	PRE	113.0	449.4	1121.5	2907.2	4067.6	1021.3
	PRE/POST	169.2	424.2	1025.7	1495.9	1592.6	984.1
LDH (U/ml)	CON	145.4	150.8	229.3	269.8	242.2	37.2
	PRE	145.9	153.7	167.8	212.7	222.9	25.3
	PRE/POST	138.3	146.7	163.8	180.9	177.6	24.3
3-MH (nmol/ml)	CON	169.7	219.5	176.7	126.0	163.5	32.0
	PRE	193.9	207.7	150.9	210.4	138.6	21.7
	PRE/POST	210.9	203.9	163.8	175.5	157.2	20.9
R-leg Peak Force (N)	CON	188.9	173.2	174.9	158.6	190.9	25.2
	PRE	216.6	194.3	196.5	197.6	203.1	17.1
	PRE/POST	210.5	197.9	196.8	195.0	209.2	17.1
L-leg Peak Force (N)	CON	178.5	159.7	153.1	178.1	183.1	24.3
	PRE	185.1	179.9	174.1	192.8	195.5	16.5
	PRE/POST	203.0	187.0	180.0	193.4	198.9	16.5
Muscle Soreness (mm)	CON	0.23	5.77 ^{1,2}	5.95 ^{1,2}	4.13 ¹	2.40 ¹	0.78
	PRE	0.44	5.42 ^{1,2}	5.99 ^{1,2}	4.65 ^{1,2}	1.71	0.53
	PRE/POST	0.32	5.32 ^{1,2,3}	5.47 ^{1,2,3}	3.72 ^{1,2}	1.88 ¹	0.53

Discussion

The objective of this study was to determine the effect of HMB on the inflammatory response to EIMD. HMB has been shown to improve recovery and decreases damage to muscle cells after intense exercise, especially in untrained subjects. It was predicted that HMB will reduce the magnitude of an inflammatory response to EIMD and the results provide evidence for a decrease in serum TNF- α and IL-1ra after exercise, which is not observed with HMB supplementation. These findings occurred despite no difference in CK and LDH, common indicators of muscle damage, between groups.

The largest limitation of this study was the low subject number, particularly with the CON group which had just half the number of subjects of each of the other two treatment groups. Time and economic constraints dictated our sample size. Also, due to the large variability of the markers used in this study, a large sample size is needed to establish statistical significance between the small variations observed. High sensitivity tests were used when available to ensure the best results.

There were several outcomes that were expected but not observed. We did not observe any difference between groups with regards to CK, LDH and 3-MH. Circulating concentrations of these muscle damage indicators have been shown to be reduced with HMB (38). In terms of CK, we did not detect nearly the same peak in the PRE/POST compared to PRE and CON, but it was not significant. PRE/POST peaked at 1593 IU compared to 3514 IU and 4068 IU in the CON and PRE groups respectively. The trend suggests less damage if HMB is given before and after the bout, and it is believed that with larger sample sizes that would have more evident. The decrease in right leg peak force was about 16% in the CON group compared to 9% and 7% in the PRE and PRE/POST groups, respectively. This difference was not significant but does suggest a trend towards a smaller loss of function. Muscle soreness was also not significantly different between groups, which has been supported by other studies (44), but both PRE and PRE/POST appear as if they may be closer to baseline at 96h than CON. In addition to sample size, the degree of muscle damage may have impacted our findings. A previous study (36) reported CK and LDH as high as 17,000 U/ml and 580 U/ml, respectively. Our results did not

reach those levels and it is possible that the amount of EIMD induced was insufficient to create a detectable change in the serum concentrations of CK and LDH.

Following a bout of resistance exercise, several studies have shown cytokines have a transient rise immediately after and return to pre-exercise levels by 24h (12, 24, 32, 50, 69), while others reported no change in serum cytokines detected (4, 22, 41). Only a few studies have shown elevated levels in the serum after +24h post-exercise (52, 60). During these first 24h following exercise, urine output of cytokines increases but is transient as well (62). Even without observed changes in serum concentrations, it is possible that cytokine production is enhanced as a previous study reported mRNA for TNF- α , IL-1 β and IL-6 to be up-regulated without the subsequent serum rise (4).

Our results showed a decrease in TNF- α and IL-1ra with the CON group. The depression in serum cytokines has been documented by other researchers as well. Hirose et al. (22) observed cytokines after eccentric resistance exercises of the elbow flexors. They reported plasma concentrations of TNF- α and IL-8 that were lower than pre-exercise levels for up to 4d. It is possible that the decreased serum cytokine concentration is a result of tissue accumulation. The accumulation of neutrophils, a source of cytokine production, and IL-1 β in exercise tissue has been reported (6, 13). The IL-1 β in the muscle tissue persisted for up to 5d and correlated to structural damage of the myofibers.

The results of TNF- α and IL-1ra support the theory that inflammation is affected by HMB. In both cases (fig. 1 & 2) the CON group experienced a decline in serum concentration and the dip was reduced or limited by supplementation of HMB. These results could be interpreted as an increase in the inflammatory response following HMB supplementation, and is further supported by the results of CRP at least in the PRE group. This suggests that HMB creates a greater inflammatory response which may improve recovery of damaged tissue. When exactly this occurs and how, whether direct or indirect, is not evident from this study. We already know that proteolysis is affected by HMB and it is also possible that inflammatory cytokines are mediating an optimal recovery.

For future investigations, it would be beneficial to measure early in the recovery to determine if HMB has an impact on the initial and transient rise of cytokines observed by others. Whether

or not IL-6 is affected would help suggest an inflammatory or metabolic response as HMB would not influence any metabolic response from a single bout. Without also identifying changes in mRNA or tissue accumulation, it is difficult to determine if HMB has a role in cytokine production or tissue accumulation. It is possible that HMB effects the production of both pro and anti-inflammatory cytokines to speed recovery and limit overstimulation of pro-inflammatory cytokines.

The goal of this study was to describe the role HMB had on the inflammatory response to a stressful bout of exercise. Our data showed that HMB does suppress the decrease of IL-1ra and TNF- α in the serum. This effect could be a result of cytokine production being influenced by HMB, or an indirect result of less damage occurring. Previous research suggests HMB is beneficial to recovery, at least in untrained individuals, and regulating the inflammatory response to facilitate optimal recovery could be the process influenced by HMB.

Review of Literature

Introduction

During exercise it is possible to cause damage to the cellular structure of muscle fibers. The extent of damage is dependent on the mode of contractions with eccentric causing more damage than concentric contractions (19). This exercise induced muscle damage (EIMD) triggers a training adaptation that leads to removal of cellular debris and repair of the disrupted myofibers. Trained muscle has adapted to be more resilient and has the ability to withstand exercise of higher intensity without subsequent damage. This process is mediated by the inflammatory system. Chemical regulators, known as cytokines, are generated locally and systemically to facilitate the process.

Training has been shown to decrease the muscle damage and inflammation that occurs during resistance exercise bouts (5). Muscle damage is also reduced by the supplementation of the leucine metabolite, β -hydroxy- β -methylbutyrate (HMB) (15, 38). The use of HMB following resistance exercise in which muscle damage occurs has been shown to reduce muscle soreness, loss of force production, and increase lean muscle mass. But the role of HMB on inflammation has not yet been investigated.

HMB, a precursor to de novo cholesterol synthesis, is believed to increase cholesterol availability during the rebuilding process of damaged muscle (57). HMB is also associated with decreased proteolysis following exercise (58). The decrease in proteolysis and increased membrane synthesis when HMB is supplemented, contributes to less damage and faster recovery.

HMB may also influence the inflammatory response to exercise bouts that cause damage. EIMD & other intense exercise bouts have been shown to release several inflammatory markers. Research on cytokine release following a resistance exercise bout with supplemented HMB is very limited. Due to HMB's effect on muscle damage, it is the goal of this study to provide evidence regarding the role of HMB on the inflammatory response. It is hypothesized that markers of inflammation will be reduced when HMB is taken following an eccentric exercise bout.

Muscle Damage and Proteolysis

Exercise-induced muscle damage

During contraction, muscle sarcomeres shorten. When the force is too great, the muscle fibers become stretched and damaged. This overload is the basis of most exercise training regimens. The damaged muscle cells contain micro-tears where the sarcolemma is disrupted. The ultra-structure of damaged muscle fibers show disrupted sarcomeres, widening and irregularity of cross striations, and Z-band streaming (14, 19, 28).

The EIMD that occurs is commonly associated with swelling of the tissue, pain, and a rise in muscle enzymes and is typically followed by an inflammatory response (1, 10). EIMD is also strongly associated with a decline in force production in the days following (31, 43). The loss of force is believed to be from disrupted excitation-contraction couplings associated with the physical separation of myofibrils. Range of motion (ROM) of the exercised limb has also been found to decrease after damaging bouts of exercise (43).

Delayed onset muscle soreness (DOMS) is commonly seen with EIMD and is identified as the pain present 24 to 72 hrs after the damaging exercise bout. DOMS is a subjective indicator of muscle damage and its correlation to EIMD is weak, but it is still commonly used to indicate some degree of muscle damage. The magnitude of DOMS and EIMD that is invoked is dependent on the load and the number of contractions. Eccentric contractions occur when contraction force is less than the resistance force and the muscle lengthens. They induce greater cell damage than concentric (muscle shortens) or isometric (muscle length stays the same) contractions (18, 19, 34). Many high load eccentric contractions cause the greatest muscle damage and subsequent DOMS compared to fewer eccentric contractions.

The physical separation of the sarcolemma causes gaps in the membrane (1). Common serum markers of muscle damage include creatine phosphokinase (CK), lactate dehydrogenase (LDH), and myoglobin (Mb) (9, 41). These enzymes are found in the muscle tissue, but when damage to the sarcolemma occurs they are able to leak out and enter the circulatory system. Mb is relatively small and can enter the blood system directly, so the increase is seen soon after the strenuous exercise bout. But Mb exists in the cytoplasm and indicates membrane leakage not structural

damage of contractile units. CK and LDH are larger and must enter the lymphatic system before entering the blood. This results in a delayed rise and may influence the high variability typically found with these markers (43). Additionally, Nosaka & Clarkson (42) did not find a correlation between CK release and the amount muscle mass that was used, which suggest CK may be a poor indicator of muscle damage.

To determine a more ideal marker of muscle damage, several proteins have been investigated. Myosin heavy chain (MHC) has been shown to increase with EIMD (61). MHC is a structurally bound contractile protein in muscle. The increase in MHC suggests both structural damage and membrane leakage, but the rise is delayed (~2 d) and not detectable early after the exercise. Additionally, MHC has several isoforms that make it difficult to determine if the damage is coming solely from skeletal muscle. Troponin I (TnI) is a contractile protein that is found only in muscle tissue. It has isoforms that exist in cardiac (cTnI) and skeletal (sTnI) muscle cells. Sorichter et al. (61) found rises in sTnI after 2-6 h from onset, and remains elevated for 1-2 d.

Recovery/training

Following muscle damage, the cells immediately begin repair. Muscle proteolysis begins to degrade damaged protein prior to adaptation. Three mechanisms of protein degradation occur: lysosomal, calpain activated and ubiquitin activated. Lysosomal and calpain activated protein degradation are broadly based with low specificity. The ubiquitin pathway is the intracellular mechanism for specific protein degradation. Ubiquitin pathway is stimulated following a strenuous exercise bout and is also common during increased muscle proteolysis such as during limb immobilization, starvation and de-nervation (54, 58, 59, 74).

Repeating the same strenuous exercise causes reduced damage with each subsequent bout (9, 10). Training decreases the activation of the ubiquitin pathway leading to less proteolysis following exercise (75). The diminished proteolysis is a training response in which the muscle cells become more resilient and require less tissue repair. Resistance training stimulates hypertrophy of muscle fibers, hyperplasia, and increased connective tissue surrounding the muscle fiber. The balance between degradation and synthesis is tightly regulated and research has suggested the mTOR receptors play a significant role in transcriptional regulation of protein

balance. The mTOR pathway is stimulated following an exercise bout to increase protein synthesis.

Inflammation

Cytokine action

Damage to skeletal muscle is followed by an inflammatory response that is geared towards removal of damaged tissue. Increased protein turnover is common in sepsis, trauma and EIMD. An acute phase response commonly expresses the following symptoms: heat production, protein degradation, fatigue, edema and higher urea excretion. The regulation of the acute phase is mediated by several inflammatory proteins. These circulating peptides (cytokines) were proposed by Clowes et al. (11) when he found a three-fold increase in normal protein turnover after plasma from septic patients was introduced to muscle tissue from non-septic subjects.

Cytokines are released by neutrophils, macrophages, fibroblasts, and damaged muscle cells (7). The acute phase response is identified as the systemic change in cytokines that follows a local inflammatory response. It initiates white blood cell (WBC) accumulation after EIMD occurs. The WBC accumulation mediates the repair process and is directed towards damaged tissue; non-damaged tissue has little to no increase in WBC concentration (31).

Two common WBC involved with the acute phase response are neutrophils and macrophages. Neutrophils enter damaged tissue and are involved with phagocytosis of necrotic myofibers and cellular debris (28). In rat muscle, the neutrophils enter the tissue within 2 h post-exercise and are present for up to four days. Humans have similar response times; neutrophils accumulate as quickly as 45 min (13) and persists for up to three days (21). Macrophages/monocytes enter human tissue within one day and are evident for several days after injury (21).

Sepsis is the most extreme form of inflammation, usually caused by some form of infection. Cytokines that have been well documented as increasing during sepsis are interleukin (IL)-1 β , IL-10, IL-6, IL-1 receptor antagonist (IL-1ra), IL-8, and tumor necrosis factor- α (TNF- α) (67).

Cytokines are commonly referred as pro-inflammatory or anti-inflammatory. Pro-inflammatory cytokines cause neutrophil aggregation and swelling, and they amplify inflammatory responses.

Anti-inflammatory cytokines work against pro-inflammatory cytokines to control and regulate the acute phase response. Both types of cytokines are present in serum, but their responsiveness is very sensitive and small changes in plasma concentration can elicit large responses.

Common pro-inflammatory markers seen in the blood are TNF- α and IL-1 β . These pro-inflammatory cytokines are responsible for many of the acute metabolic responses that occur during sepsis and inflammation (11, 20). Protein turnover is mediated strongly by TNF- α as determined by larger 3-MH excretion (20), loss of muscle mass (30) and increased ubiquitin gene expression (17). Additionally, IL-1 β in the brain may be a significant contributor to fatigue, decreased performance and hyperalgesia following EIMD (8)

Anti-inflammatory makers typically include IL-4, IL-10 and IL-1 receptor antagonist (IL-1ra). IL-1ra specifically acts against the function of type 1 interleukins. When IL-1ra is given to animals, 3-MH excretion is reduced (78). IL-6 is a cytokine that is commonly referenced as anti-inflammatory because it triggers production of IL-10 and IL-1ra (3, 65) and may reduce IL-1 β and TNF- α release with exercise (33, 63). However, IL-6 has consistently been shown to increase due to low grade exercise where exercise induced damage is not believed to occur.

Cytokines and exercise

A rise in cytokine levels following marathons and prolonged strenuous exercise has been well-documented. This type of exercise has induced elevated plasma levels of IL-1 β (35, 47), IL-6 (35, 45, 64), TNF- α (35, 45, 64), IL-1ra (45, 47), IL-10 (47), IL-8 and MIP-1 β (46). But the physiological response to endurance type exercises and resistance type exercises are very different. Thus it is important to look at how cytokines respond specifically to resistance type exercises.

The most widely studied cytokine, IL-6, is associated with contracting muscle and increases following exercise (51). IL-6 increases even when muscle damage does not occur and circulating levels are increased based on exercise intensity, duration, muscle group, and glucose levels (51). The concentration of IL-6 in the plasma following heavy exercise can be attenuated by carbohydrate ingestion (37). The systemic increase in IL-6 occurs early following exercise with studies showing a significant rise immediately after but returning to normal by 24h (32, 50, 69).

Additionally, local production of IL-6 within muscle tissue has been shown to increase with active tissue and not inactive tissue (25, 66). It has been suggested that IL-6 is related to a metabolic response, because it occurs in a similar response with both eccentric and concentric exercise (25).

However, IL-6 has been found 2d post eccentric leg extensions (52). Any metabolic response would have been over by that point, so it is possible that under some situations IL-6 does act as an anti-inflammatory cytokine. IL-6 acts to increase IL-1ra, IL-10 and cortisol(65), all of which limit production and function of pro-inflammatory cytokines IL-1 and TNF- α (5).

The anti-inflammatory cytokine, IL-10 has been shown to be elevated (60) and absent (50) following strenuous exercise. A study by Izquierdo et al (24) that looked at the acute response and a training effect, found that IL-10 and IL-1ra had a more pronounced acute response after 7 weeks of resistance training. It is possible that untrained subjects do not see a rise in anti-inflammatory cytokines because the tissue has not adapted to repeated bouts and therefore less efficient at the repair process. Another resistance exercise study found increases in IL-1ra immediately after exercise but the levels returned to normal by 24h (69).

Following a bout of eccentric or resistance exercise in which EIMD occurs, there appears to be a transient rise in IL-1 β that returns to normal by 24h (12, 24). Following the transient rise in pro-inflammatory cytokines in the blood, serum concentrations of TNF- α and IL-1 β will show a decrease as early as 6h and may persist for up to 120h (22, 60). Not all studies show significant circulating changes in these cytokines (4, 60, 69). Corresponding with the transient systemic rise of the cytokines, is a rapid clearance in the kidneys (62). Clearance rates of IL-1 β and TNF- α return to normal by 24h along with systemic concentrations. Production of cytokines is enhanced despite seeing no elevation in the blood. Buford et al (4) found no increase in serum levels of TNF- α or IL-1 β but a significant increase in mRNA for those cytokines. Increased mRNA for TNF- α has been confirmed in rats after eccentric running as well (29). In the muscle tissue, cytokines can accumulate for up to 5d post exercise (6, 13).

It is worth noting that many of the studies that reported poor plasma cytokine response (22, 41, 50) used elbow flexors as the exercising muscle group. It is possible that the elbow flexors were

just too small of a muscle group to induce enough damage in which cytokines would raise to discernable levels.

β -Hydroxy- β -Methylbutyrate

Leucine metabolite

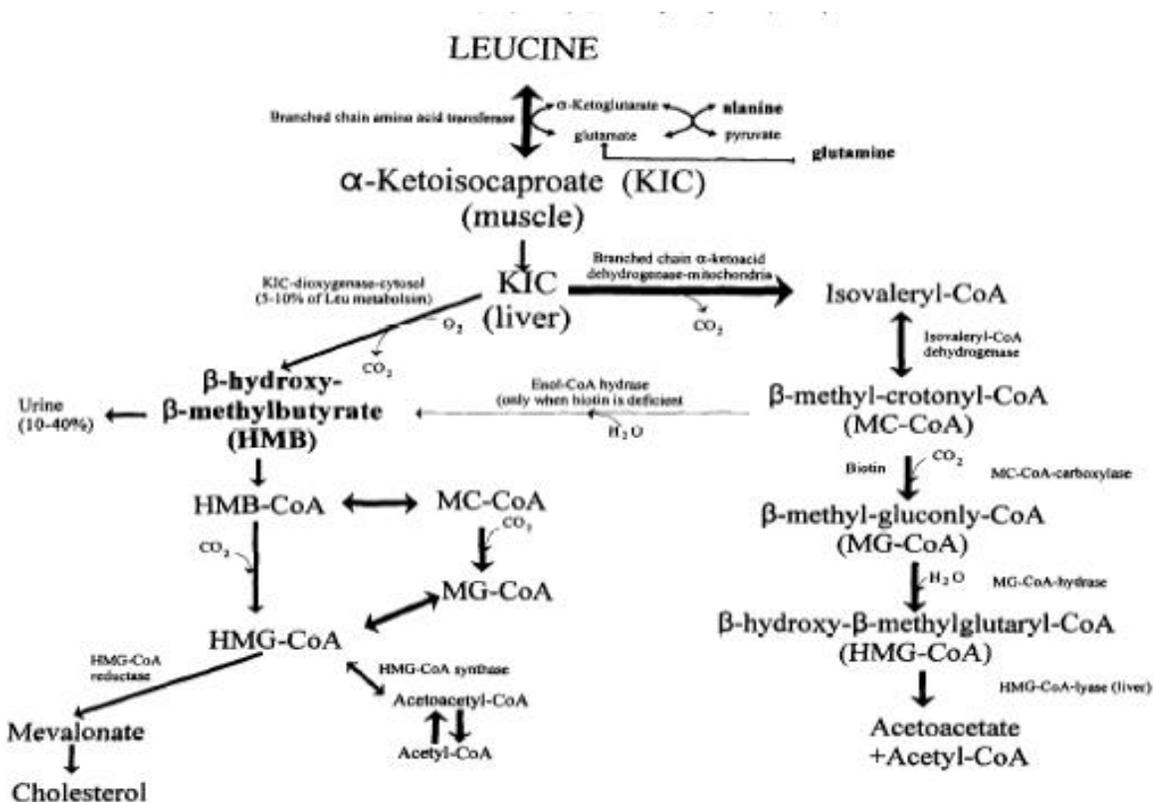


Figure 1. Overview of cholesterol synthesis from leucine. Taken from Nissen & Abumrad (40)

Leucine, an essential amino acid, has several fates. It can be incorporated into proteins, or it can be oxidized. Oxidation primarily leads to the formation of Acetyl-CoA where it can be used as an energy source or formed into free fatty acids. A secondary pathway is the formation of HMG-CoA, a necessary precursor to cholesterol.

The pathway to cholesterol synthesis begins with the oxidation of leucine into α -ketoisocaproate (KIC) primarily in the muscle. KIC, once transported to the liver, can be converted to acetyl-CoA via branched chain α -ketoacid dehydrogenase or it can form HMB through KIC-

dioxygenase. KIC to HMB accounts for approximately 5-10% of leucine metabolism. The K_m for branch chain α -ketoacid dehydrogenase is 10 to 40 μ M, which is considerably lower than the K_m for KIC-dioxygenase (120 μ M). This large difference in K_m is responsible for the low formation of HMB from leucine.

Evidence indicates that HMB is produced solely from leucine oxidation. In the liver, HMB is converted to β -hydroxy- β -methylglutaryl-CoA (HMG-CoA) where it is available for use by HMG-CoA reductase. Formation of mevalonate from HMG-CoA is the committed step in cholesterol synthesis and data demonstrate that the carbon in HMB is consistently found in de novo cholesterol. Marked leucine carbons have also been shown to appear in cholesterol synthesis in vivo (55), verifying that leucine oxidation leads to cholesterol synthesis.

Dose/Kinetics

Common dosage for HMB supplementation has been 3 g/day for humans (38). That dose causes a 10-fold increase in plasma HMB that peaks around 60-120 min (72). At the typical dose of 3 g/day, there has been no evidence of adverse effects (15, 16, 38, 73). Additionally, HMB does not impair renal function, hepatic enzyme function or immune system at doses up to 6 g/day. Doses beyond the typical 3 g/day have not shown any additional benefit in strength and lean body mass improvements (15).

The half-life of HMB seems to be relatively low, lasting only 2.5 hours in humans (72). Approximately 15-30% of supplemented HMB is lost through urine with the remaining being retained for further metabolism. Peak values following HMB administering are reached at 1-2 hr depending on the dose, and return to resting levels within 9 hrs. Plasma HMB reaches peak concentration at 60 min with 3 grams and at 120 min with 1 gram. Supplementation loading has been the norm (38-40, 71), with one study demonstrating a lack of significant effect without loading before damaging exercise (77). Markers of muscle damage are highly variable (source), and could have been the reason for the lack of significance.

Resistance Training

One of the most widely used reasons for taking supplemented HMB is its effect on muscle protein breakdown. It is commonly used by body builders and strength athletes (57).

Supplementation of HMB at varying dosages demonstrated that during a resistance training program, 3-methylhistidine is reduced in untrained individuals (38). Muscle function gains, measured as peak force, were also larger with HMB supplementation. Muscle damage prevention was greatest at the highest HMB dose and those subjects at the higher levels had the largest gains in strength. Studies have also found creatine kinase (CK) and lactate dehydrogenase (LDH) to be lower in the HMB supplemented groups (15, 26, 38, 49, 56, 73) when compared to controls.

Along with decreases in muscle damage, improvements in strength with untrained participants have been well documented (15, 26, 38, 49, 56, 73). These studies also reported increases in lean body mass. The increase in lean body mass is most likely attributed to decreased muscle proteolysis following EIMD. HMB suppresses DOMS after a single bout of eccentric muscle contractions (70).

Support for HMB improvements in strength with trained individuals is less than clear. Kreider et al. (27) did not find an improvement with 1 RM, an increase in fat free mass, or a reduction in training induced muscle damage when the subjects were experienced resistance trained athletes. However, this study did not monitor training routines and participants were advised to maintain their usual routine. Trained individuals require a larger stimulus than untrained due to their muscle structure. Failing to alter their training regimen may not have evoked enough of a stimulus for HMB's effects.

In addition to Kreider et al., studies have shown a lack of change in muscle damage markers (23, 44) suggesting that HMB does not have an effect. Evidence that DOMS is not reduced has also been documented (44, 48). Significance was not reached with these studies due to study size or training specificity, but most likely from the large variance seen with these measures.

Possible Mechanisms

One mechanism that is believed to occur is the cholesterol synthesis hypothesis. HMB is converted to HMG-CoA, a rate limiting cholesterol precursor. Because the muscle synthesizes its cholesterol *de novo*, the increased availability of HMG-CoA could provide the components for increased membrane integrity. With increased cholesterol synthesis the sarcolemma will be

stabilized and allow for more resilience during eccentric contractions and muscle damage. This theory is supported by the fact that muscle function is impaired with cholesterol synthesis inhibition (2). Cholesterol synthesis inhibition also results in increased muscle damage (53) and possible cell death (36).

It is also possible that HMB reduces the ubiquitin pathway for protein degradation. The ubiquitin pathway is responsible for specific degradation of proteins by proteasomes within the cell and is common during muscular proteolysis caused by exercise (68). HMB has been associated with decreased ubiquitin pathway activity in cachexic tumor bearing mice (58). Ubiquitin activity decreases with repeated bouts of the same exercise (75). This suppression of the ubiquitin pathway could account for the lack of evidence supporting HMB having a positive impact with trained subjects.

Conclusion

It is evident that HMB can reduce proteolysis and improve muscle function. Untrained subjects seem to benefit the most, but HMB can also benefit subjects who experience increased proteolysis. Decreased proteolysis and muscle mass retention is not clearly understood but believed to occur by increasing the integrity of muscle cells and decreasing the ubiquitin pathway protein degradation.

The role of HMB in inflammation resistance has not been identified. Muscle damage and breakdown is associated with an inflammatory response. It is believed that HMB can diminish inflammation by attenuating cytokine release, specifically the pro-inflammatory cytokines. The purpose of this study is to measure cytokine response to an acute resistance training session when HMB is given as a supplement to reduce exercise induced muscle damage.

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