EFFICACY OF BETA-HYDROXY-BETA-METHYLBUTYRATE (HMB) SUPPLEMENTATION IN HEMODIALYSIS PATIENTS

BY

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DISSERTATION

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ABSTRACT

Patients with renal failure undergoing maintenance hemodialysis (MHD) therapy suffer from a number of co-morbidities including skeletal muscle loss, reduced physical function, a significantly increased fall risk, and reduced quality of life (QOL). Therefore, interventions to combat these co-morbidities are needed. Beta-hydroxy-beta-methylbutyrate (HMB) is a metabolite of the amino acid leucine that has been shown to improve lean mass and physical function in the elderly and clinical populations, but had not previously been studied in MHD patients. Approximately 25 percent of supplemental HMB is cleared by the kidney; therefore, we first performed an acute study to determine the clearance of supplemental HMB in hemodialysis patients. MHD patients (n=8) consumed 3g HMB prior to a standard hemodialysis session. Following supplementation with HMB, a majority of supplemental HMB was cleared within 48hrs and plasma HMB levels returned to baseline within 7 days in all participants. These results suggest that supplemental HMB is cleared in patients with impaired renal function.

Based upon these results, we performed a double blind, placebo controlled, randomized trial to assess the effects of daily HMB supplementation on co-morbidities in MHD patients. MHD patients were recruited and assigned to either daily supplementation with HMB (n=16) or placebo (n=17) for 6 months. No significant effects of HMB on lean mass, strength, physical function, fall risk, or quality of life were found using an intent-to-treat analysis. However, upon analysis of plasma HMB concentrations, 5 of 16 patients (31%) who completed the study in the HMB group were found to be non-compliant at 3 or 6 months. Therefore, we performed a per-protocol analysis with compliant participants only. Although this analysis was underpowered, we observed a trend for improvements in chair stand and timed up-and-go tests with HMB

supplementation. However, no effects of HMB were observed for lean mass, strength, fall risk, or quality of life. As a whole, these results do not support the efficacy of HMB to attenuate muscle loss and declines in physical function in MHD patients. However, the observed low-compliance with study pills may have affected results. Moreover, it highlights the need for future interventions targeted at reducing pill burden and improving pill compliance in this population.

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CHAPTER 1

INTRODUCTION

The number of patients with renal failure requiring maintenance hemodialysis (MHD) treatment is expected to double within the next 30 years, primarily due to the increasing rates of hypertension and diabetes [1]. Once on hemodialysis, patients experience a number of a co-morbidities including skeletal muscle wasting, which has been shown to be a strong predictor of mortality in this population [2-4]. In addition, this decline in skeletal muscle mass contributes to reductions in physical function [5], a significantly increased fall risk [6], and reduced quality of life (QOL) [7]. Pharmacological agents have been investigated to treat muscle loss in dialysis patients; however, many of these treatments are expensive and have undesirable side effects [8, 9]. As a result, low-cost interventions designed to attenuate declines in muscle mass are needed in this critically ill patient population.

Beta-hydroxy beta-methylbutyrate (HMB) represents a potential low-cost nutritional intervention to attenuate muscle loss in hemodialysis patients. HMB is a metabolite of the amino acid leucine that has been shown to safely attenuate muscle mass loss in other clinical populations with accelerated muscle loss, such as the elderly [10], cancer [11], and AIDS patients [12], primarily through reductions in skeletal muscle protein catabolism (as reviewed by [13]). A recent study examining the effects of 4 weeks of HMB supplementation in diabetic hemodialysis patients observed beneficial effects on wound healing [14]; however, lean mass, strength, physical function, and quality of life were not measured. Therefore, the primary purpose of this trial is to determine if oral supplementation with HMB attenuates muscle loss and declines in muscle strength and physical function in hemodialysis patients.

CHAPTER 2

LITERATURE REVIEW: MUSCLE LOSS IN HEMODIALYSIS PATIENTS

Chronic kidney disease

Chronic kidney disease (CKD) affects 13 percent of adults in the United States and the prevalence is increasing rapidly [15]. The severity of CKD is determined by glomerular filtration rate (GFR), a measure of creatinine clearance. A GFR of less than 15 mg/min/1.73m represents end stage renal disease (ESRD) at which point a patient must receive dialysis for the remainder of their life, unless a kidney transplant is received. However, the number of patients in need of a kidney transplant far exceeds the number of kidney donors, resulting in more than 350,000 patients currently on hemodialysis in the United States [16]. Moreover, the number of hemodialysis patients is increasing and expected to double within the next 30 years, primarily due to the increasing rates of hypertension and diabetes, the leading causes of CKD in the United States [1].

Once on hemodialysis, patients experience a number of a co-morbidities including skeletal muscle wasting [2] which results in reduced physical function [5] and an increased fall risk [6]. Due to these co-morbities, hemodialysis patients are typically less active than sedentary healthy adults [17] and have a reduced quality of life [7]. Moreover, approximately 2/3 of hemodialysis patients will die within 5 years of initiation of hemodialysis [18] and this extremely high mortality rate has not improved in the last two decades [19]. This suggests that alternative therapies to attenuate declines in skeletal muscle mass, physical function, and quality of life in hemodialysis patients are needed.

Skeletal muscle loss: prevalence and association with mortality

Protein Energy Wasting (PEW), defined as "a state of decreased body stores of protein and energy fuels that is often associated with diminished functional capacity related to metabolic stress," is a common co-morbidity in hemodialysis patients [20]. Due to the high prevalence of PEW, incident MHD patients lose approximately 1-3 kg lean mass annually resulting in a low skeletal muscle mass in many hemodialysis patients [2]. Indeed, Carrero et al. [21] reported at least some degree of atrophy in 39% of a cohort of hemodialysis patients and a skeletal muscle mass of less than 90% of predicted was observed in 62% of dialysis patients in a French cohort [22]. Likewise, MacDonald et al. [23] observed a reduced appendicular lean mass in a cohort of hemodialysis patients, as compared with age-matched healthy controls.

Skeletal muscle mass also has been correlated with mortality in this population. Several studies have shown a correlation between mortality and a low skeletal muscle mass, measured by creatinine levels [3, 4, 24, 25], mid-arm circumference [26, 27], or DXA [28]. Moreover, skeletal muscle loss, measured by reductions in blood creatinine levels, has been shown to be a strong predictor of mortality in this population [3, 4]. Although skeletal muscle loss is not the primary cause of death in this population, the relationship between skeletal muscle loss and mortality is hypothesized to exist because skeletal muscle loss may increase risk of death from cardiovascular disease and infection, the leading causes of death in this population [29]. Taken together, these data suggest that skeletal muscle loss is a significant problem in hemodialysis patients and interventions are needed to combat this prevalent co-morbidity.

Causes of skeletal muscle loss in hemodialysis patients

Skeletal muscle loss is a common comorbidity in hemodialysis patients. Rates of muscle loss as high as 1-3 kg annually have been observed in incident MHD patients [2]. Dialysis patients experience skeletal muscle loss for several reasons including: metabolic acidosis, inflammation, insulin resistance, decreased nutrient intake, hormonal abnormalities, an elevated metabolic rate, and loss of amino acids into the dialysate (as reviewed by [30, 31]). Moreover, the dialysis process has been shown to up-regulate expression of genes involved in skeletal muscle protein degradation and result in a breakdown of skeletal muscle proteins [32]. The following section will review the causes of skeletal muscle loss in hemodialysis patients.

In end-stage renal disease patients, metabolic acidosis is caused by reduced bicarbonate production in the kidney, resulting in an inability to neutralize blood pH. Acidosis in hemodialysis patients has been shown to stimulate skeletal muscle protein degradation and result in a negative nitrogen balance [33, 34]. The mechanism by which metabolic acidosis stimulates protein degradation is not completely understood, but may involve impairment of insulin signaling [35], reduction in sodium coupled neutral amino acid transporter 2 (SNAT2) activity resulting in decreased amino acid transport into skeletal muscle cells [36], and/or up-regulation of the ubiquitin-proteasome pathway in skeletal muscle [37]. Correction of metabolic acidosis with bicarbonate has been shown to decrease protein degradation and amino acid oxidation [33, 34], increase intramuscular concentration of branched chain amino acids [38], and increase bodyweight and mid-arm muscle circumference in hemodialysis patients [39]. Thus, metabolic acidosis represents one of many causes of muscle loss in hemodialysis patients.

Elevated inflammation is common in dialysis patients and contributes to skeletal muscle loss. Several studies have shown that dialysis patients have increased levels of interleukin-6 (IL-

6) [40-44], tumor necrosis factor alpha (TNF-α) [41, 42] and C-reactive protein (CRP) [41, 44] compared with healthy controls. Inflammation in dialysis patients is caused by many factors including: increased oxidative stress, decreased clearance of pro-inflammatory cytokines, bio-incompatibility of dialysis membranes, impurities in dialysis water and dialysate, and back-filtration of contaminants during dialysis (as reviewed by [45]). In addition, the hemodialysis process has been shown to increase levels of plasma IL-6 [40-42, 46] which is believed to originate from either peripheral blood mononuclear cells [47] and/or skeletal muscle [44]. Moreover, the increase in IL-6 during a hemodialysis session has been correlated with an up-regulation of genes associated with skeletal muscle protein catabolism [42] and an increased rate of skeletal muscle protein degradation [46, 48]. The amino acids released from skeletal muscle during the hemodialysis process are believed to participate in acute-phase protein synthesis in the liver, which is elevated during hemodialysis [41]. In addition to correlations with protein catabolism and acute-phase protein synthesis during hemodialysis, inflammatory cytokine concentration has also been correlated with skeletal muscle mass in this population. Indeed, Kaizu et al. [43] showed that levels of IL-6 and CRP were inversely correlated with thigh muscle area, as measured by computer topography (CT), in hemodialysis patients. Taken together, these findings suggest that elevated inflammation in hemodialysis patients is, at least in part, responsible for the increase in skeletal muscle loss in this population.

Insulin resistance in hemodialysis patients occurs for several reasons including: metabolic acidosis, elevated inflammation, increased oxidative stress, accumulation of uremic toxins, excessive body fat, vitamin D deficiency and reduced physical activity (as reviewed by [49]). Insulin prevents skeletal muscle loss primarily through inhibition of catabolic pathways in skeletal muscle [50]. Indeed, Pupim et al. [51] observed an increased rate of skeletal muscle

protein degradation in diabetic hemodialysis patients compared with non-diabetic patients. This increase in protein degradation resulted in a significantly greater loss of skeletal muscle mass in diabetic hemodialysis patients. In a 1 year observational study of incident MHD patients, non-diabetic hemodialysis patients lost 1.1 kg lean mass while diabetic hemodialysis patients lost 3.4 kg lean mass [2]. Moreover, in non-diabetic non-obese hemodialysis patients, insulin resistance is evident and inversely correlated with net balance of skeletal muscle protein turnover [52]. Therefore, insulin resistance may be contributing to skeletal muscle loss in both diabetic and non-diabetic hemodialysis patients.

Anorexia is present in 35-50 percent of hemodialysis patients and results in a reduced nutrient intake [53]. In a cohort of 1,901 adults receiving hemodialysis in the United States, average caloric and protein intakes of 23.2 kcal/kg bodyweight/day and 0.96 g protein/kg bodyweight/day, respectively, have been reported [54]. This level of calorie and protein intake is well below the Kidney Disease Outcomes Quality Initiative (KDOQI) recommendation of 35 kcal/kg bodyweight/day and 1.2 g protein/kg bodyweight/day for hemodialysis patients [55]. Moreover, calorie and protein intakes are significantly lower on days in which patients undergo hemodialysis treatment [54] and are reduced the longer a patient is on hemodialysis [56]. Reduced caloric intake in hemodialysis patients occurs for several reasons including: dry mouth [57], changes in taste and smell [58], dental problems [59], and altered GI function [60]. Additionally, elevated inflammation has been shown to decrease appetite. Significant negative correlations between appetite and inflammatory markers, IL-6 and CRP, have been observed in hemodialysis patients [61, 62]. Moreover, abnormalities in appetite regulating hormones are also common. Elevated levels of deacyl-ghrelin [63] and leptin [64] are present in dialysis patients and have been correlated with a reduced skeletal muscle mass in this population [65, 66].

Dietary restrictions may also be, in part, responsible for the reduced caloric intake. In the United States, dialysis patients are placed on a restrictive diet which may leave patients feeling limited on food selection [67]. Furthermore, patients are not allowed to eat during hemodialysis, a period in which skeletal muscle protein catabolism peaks [68]. Finally, dietary counseling to lower protein intake during earlier stages of CKD may contribute to an insufficient protein intake after dialysis initiation [69]. For all of the aforementioned reasons, a reduced nutrient intake is common in hemodialysis patients and contributes to skeletal muscle loss in this population.

In addition to abnormalities in appetite regulating hormones, several other hormone systems are dysregulated in hemodialysis patients and may contribute to skeletal muscle loss. Cortisol increases during hemodialysis [70] and is correlated with proteolysis [71]. Cortisol may contribute to skeletal muscle loss, in part, through inhibition of insulin signaling [72]. In addition, adrenalectomy studies in rodent models of CKD have shown that glucocorticoids are necessary for metabolic acidosis induced skeletal muscle degradation [73]. Furthermore, in humans, hypercortisolemia has been shown to blunt the anabolic response to amino acids [74]. Taken together, these results indicate that cortisol may increase skeletal muscle loss through a variety of mechanisms. Testosterone production and metabolism are also dysregulated in male dialysis patients and correlated with a reduced muscle mass in this population [75, 76]. Moreover, Carrero et al. [76] observed an increased risk of all-cause mortality in male hemodialysis patients with low serum testosterone levels even after adjustment for age, sex hormone binding globulin, cardiovascular disease, diabetes and inflammation. However, this relationship was lost after adjustment for muscle mass, as measured by serum creatinine, suggesting that testosterone levels are related to muscle wasting and subsequent mortality risk in this population. In addition, vitamin D is reduced in hemodialysis due to a decreased production

of 1, 25-dihydroxyvitamin D3, the active form of vitamin D, in the kidney. Vitamin D may play a role in insulin sensitivity in hemodialysis patients. Indeed, Mak et al. [77] supplemented hemodialysis patients with intravenous 1,25-dihydroxyvitamin D3 for 4 weeks and observed an increase in insulin sensitivity, as measured by euglycemic clamp technique. Taken together, these studies suggest that endocrine abnormalities may contribute to skeletal muscle loss in hemodialysis patients.

Hemodialysis patients have an increased resting energy expenditure (REE) which may contribute to a negative energy balance and skeletal muscle loss. Indeed, an increased REE has been shown in dialysis [78] and pre-dialysis [79] patients compared to age and BMI matched healthy controls. Moreover, REE increases during a hemodialysis session [78]. The mechanism for the elevated REE in dialysis patients is not completely understood; however, inflammation has been shown to be positively correlated with REE in both dialysis [80] and pre-dialysis patients [81]. Therefore, elevated inflammation in dialysis patients may lead to increases in REE which may further contribute to a negative energy balance and decrease skeletal muscle mass in this population.

Plasma amino acid loss during the hemodialysis process contributes to skeletal muscle loss in this population. Ikizler et at. [82] observed a 6-8g loss in amino acids through the dialysis membrane during a hemodialysis session. This loss is equivalent to an approximately 2 kg loss annually in patients undergoing thrice weekly hemodialysis [32] and is similar to annual changes in lean mass that have been observed in this population [2]. Thus, intradialytic amino acid loss represents yet another mechanism by which skeletal muscle is lost in hemodialysis patients.

Molecular mechanisms of proteolysis in skeletal muscle of hemodialysis patients

Skeletal muscle protein turnover is the product of skeletal muscle protein synthesis and skeletal muscle protein degradation. When synthesis exceeds degradation, there is a net synthesis of skeletal muscle protein. However, when degradation exceeds synthesis, there is a net breakdown of skeletal muscle protein. The hemodialysis process has been shown to increase skeletal muscle protein degradation, without an adequate increase in protein synthesis, resulting in a negative net balance of skeletal muscle protein turnover [70]. Moreover, several signaling pathways involved in skeletal muscle protein turnover are differentially expressed in hemodialysis patients compared with healthy adults (Figure 1). This section will review abnormalities in skeletal muscle signaling pathways involved in protein synthesis and degradation in hemodialysis patients.

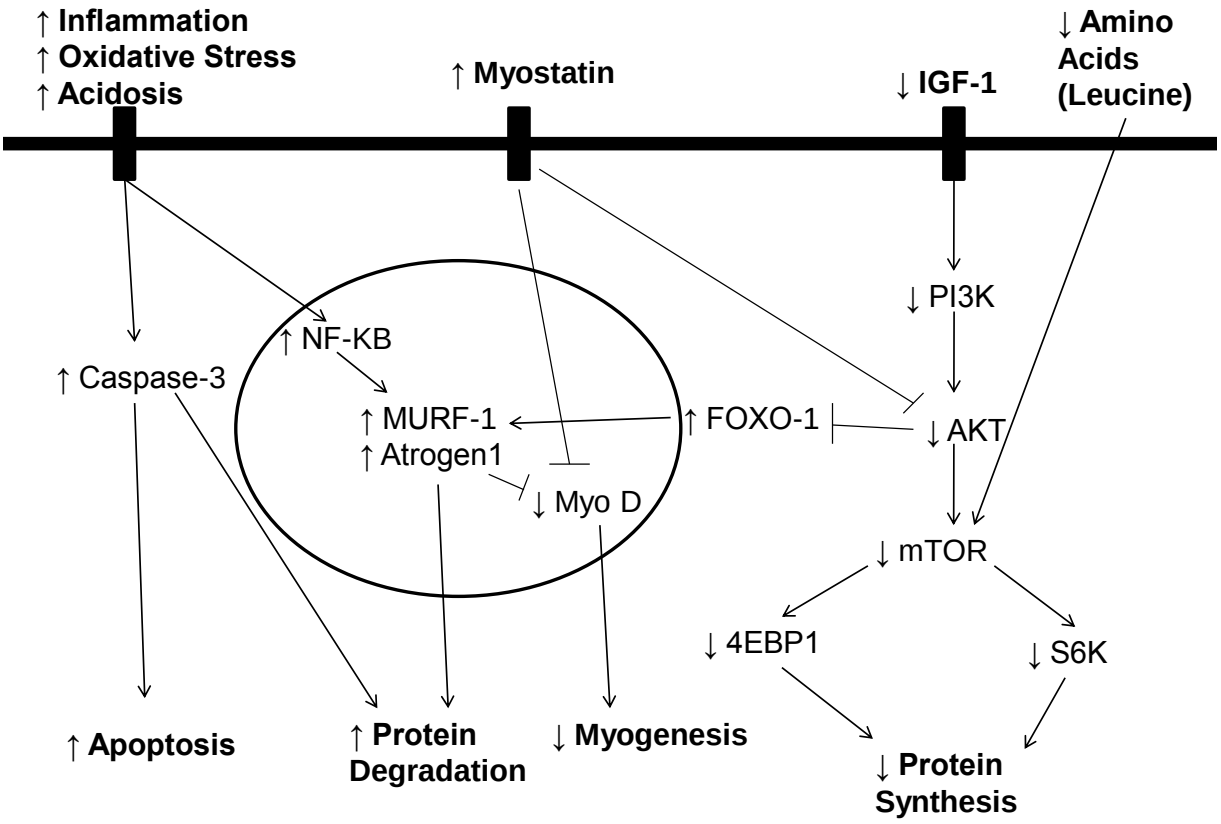

Figure 1. Anabolic and catabolic pathways in skeletal muscle of patients with chronic kidney disease. Arrows represent an increase or decrease in a protein or pathway compared with healthy adults. Increased inflammation, oxidative stress, and metabolic acidosis stimulate protein degradation and apoptosis through activation of caspase and ubiquitin-proteasome pathways. Elevated myostatin inhibits myogenesis and protein synthesis. Reduced IGF-1 lowers protein synthesis and myogenesis and increases protein degradation. Reduced amino acid intake lowers protein synthesis. Abbreviations: NF-KB – nuclear factor –KB; MURF-1 – muscle ring finger-1; IGF-1 – insulin like growth factor-1; IRS-1 – insulin receptor substrate-1; mTOR – mammalian target of rapamycin; 4EBP1 – eukaryotic initiation factor 4E binding protein-1; S6K – ribosomal S6 kinase; MyoD – myoblast determination protein 1; FOXO-1 – forkhead box O1.

The insulin-like growth factor-1 (IGF-1) pathway is the primary anabolic pathway in skeletal muscle tissue. Binding of IGF-1 to the IGF-1 receptor activates in a signaling cascade that results in the phosphorylation of Akt and the mammalian target of rapamycin (mTOR), ultimately, stimulating protein synthesis (as reviewed by [83]). However, in the skeletal muscle of hemodialysis patients, IGF-1 mRNA [84-86] and protein [23, 86] are reduced. Likewise, in animal models of CKD reduced intramuscular IGF-1 has also been observed [87-89]. In addition to a reduced quantity of intramuscular IGF-1, IGF-1 resistance may be present in CKD. In an animal model, Zhang et al. [90] injected IGF-1 into the skeletal muscle of CKD and wild-type rats. Reduced Akt phosphorylation was observed in the skeletal muscle of CKD rats after IGF-1 injection, suggesting possible IGF-1 resistance. Taken together, these results suggest that there is decreased expression and sensitivity to IGF-1 in skeletal muscle of CKD patients.

In addition to simulating protein synthesis, IGF-1 also inhibits skeletal muscle protein degradation. As mentioned previously, activation of the IGF-1 pathway increases phosphorylation of Akt and leads to stimulation of protein synthesis. However, phosphorylated Akt also phosphorylates forkhead box O1 (FOXO-1), preventing translocation to the nucleus. When IGF-1 signaling is reduced (e.g. in hemodialysis patients) FOXO-1 is primarily dephosphorylated and can translocate to the nucleus, increasing expression of E3 ligases, muscle like ring finger-1 (MURF-1) and atrogin-1. This signaling cascade ultimately results in skeletal muscle protein degradation through the ubiquitin-proteasome pathway (as reviewed by [91]). Therefore, it is not surprising that skeletal muscle ubiquitin, MURF-1 and atrogin-1 mRNA are increased in animal models of CKD [87, 92]. Likewise, in human CKD patients, increases in inflammation [42] and metabolic acidosis [37] have been shown to up-regulate components of the ubiquitin-proteasome pathway and increase skeletal muscle protein degradation.

Caspases induce skeletal muscle proteolysis through apoptosis of myonuclei and are involved in the initial cleavage of actomyosin, resulting in a 14 KD actin fragment [93]. Similar to the ubiquitin-proteasome pathway, IGF-1 signaling can inhibit caspase activation. However, CKD patients have reduced IGF-1 signaling and increased caspase-3 activity compared with healthy controls [48]. Moreover, skeletal muscle caspase-3 expression and activity increase over the course of a hemodialysis session increasing skeletal muscle protein degradation [42, 48] and this increase in caspase-3 has been correlated with the rise in inflammation that occurs during hemodialysis [42]. Furthermore, increased 14KD actin fragment has been detected in both human [48] and animal [87, 92, 93] models of CKD, indicating increased actomyosin cleavage by caspase 3.

Muscle regenerative capacity is impaired in hemodialysis patients. In animal models of CKD, a reduced number of key proteins in skeletal muscle regeneration (myoblast determination protein 1 (MyoD) and myogenein) have been observed [87, 90]. This correlates with a decrease in embryonic myosin heavy chain in skeletal muscle of CKD rodents compared with wild-type controls [87, 90]. Moreover, skeletal muscle regeneration is blunted in response to injury [90] and treadmill running [87] in animal models of CKD. Although muscle regenerative capacity has been shown to be reduced in animal models of CKD, to date, the effects of CKD and/or hemodialysis on skeletal muscle regenerative capacity in humans have not been investigated.

Myostatin is a negative regulator of skeletal muscle growth and proliferation. Myostatin knockout animals and humans exhibit an extreme amount of muscle mass [94, 95]. However, increased myostatin expression has been observed in dialysis patients [86] and animal models of CKD [85, 88, 96], although not all studies have observed an increased myostatin expression [84].

Future studies are needed to determine to what extent myostatin affects skeletal muscle loss in hemodialysis patients.

In summary, CKD has been shown to result in a net negative balance of skeletal muscle protein turnover though reduced protein synthesis and increased of protein degradation. CKD patients have a reduced expression and sensitivity to IGF-1. In addition, inflammation and acidosis up-regulate caspase and the ubiquitin-proteasome pathways to increase skeletal muscle protein degradation. Moreover, skeletal muscle from animal models of CKD has an impaired regenerative capacity. Together, these dysregulations result in skeletal muscle loss in hemodialysis patients.

Physical function, fall risk and quality of life in hemodialysis patients

Loss of muscle mass is common in hemodialysis patients and results in reduced strength, physical function, and quality of life [5, 23, 97, 98]. Indeed, reduced strength [5, 23, 99], slower gait speed [99-101] and poorer performance on a sit-to-stand test [23, 99, 101], have been observed in HD patients compared with age-matched healthy controls, even in high functioning dialysis patients [99]. Moreover, dialysis patients have increased muscle fatigue [102], poorer balance [99], and an increased dual-task cost [100] compared with healthy age-matched controls, resulting in an increased fall risk. Indeed, 1.18 falls/patient-years were reported in a cohort of over 300 hemodialysis patients (median age 70.9 yrs, approximately 5 percent nursing home residents), a rate similar to elderly nursing home patients (average age approximately 80yrs) [6]. Moreover, hemodialysis patients are less active than age-matched healthy sedentary controls [17], particularly on dialysis days [103], which may further decrease physical function and increase disability. As a result, approximately 20 percent of advanced kidney diseases patients

are classified as frail [104] and many hemodialysis patients experience a significantly reduced quality of life [105].

Effects of nutritional interventions on muscle loss, physical function, and quality of life in hemodialysis patients

Several nutritional interventions have been investigated to prevent declines in muscle mass, physical function and quality of life in hemodialysis patients. Most of these interventions have included a protein supplement from a high-quality protein source and many provided supplementation during the hemodialysis session (as reviewed by [106]). Acutely, intradialytic oral protein supplementation stimulates protein synthesis and results in a net positive balance in skeletal muscle and whole body protein turnover [107, 108]. Chronically, many studies have found increases in serum albumin, a marker of nutritional status, after oral protein [109-112] or essential amino acid [113, 114] supplementation in malnourished hemodialysis patients. Moreover, improvements in physical function [113, 114] and quality of life [110, 112, 115] have also been observed after oral supplementation in malnourished patients. However, the effects of oral protein supplementation on patients who do not meet the criteria for malnutrition is less studied. Our lab recently performed a 6 month intervention in which non-malnourished hemodialysis patients were supplemented with 27g whey or soy protein for 6 months. We found that intradialyitc protein supplementation improved physical function and quality of life in patients who do not meet the traditional criteria for malnutrition [116].

Although oral protein supplementation has been shown to increase markers of nutritional status and improve physical function and quality of life in hemodialysis patients, to date, nutritional supplementation alone has predominately had little effect on skeletal muscle mass.

Many intervention studies have not measured skeletal muscle mass and those that did have not observed significant increases in lean mass, as measured by mid-arm circumference [113], serum creatinine concentration [112] or DXA [116]. To date, an improvement in lean mass has only been observed in one nutritional supplementation study. Hiroshige et al. [117] supplemented elderly malnourished hemodialysis patients with either 12 g branched chain amino acids (BCAA) or a placebo daily for 6 months in crossover design and observed an increase in lean mass, as measured by bioelectrical impedance analysis. However, these results have not been replicated with more precise measurements of body composition and no additional studies supplementing BCAA, or their metabolites, have been performed in hemodialysis patients.

Conclusion

Skeletal muscle loss is a common co-morbidity in end stage renal disease patients that is likely caused by a number factors including: acidosis, inflammation, insulin resistance, anorexia, hormone dysregulation, changes in energy expenditure, and changes in skeletal muscle molecular signaling. Declines in skeletal muscle mass have been shown to result in reductions in strength, physical function, an increased fall risk, reduced quality of life, and increased mortality. Nutritional interventions have successfully improved nutritional status, physical function, and quality of life in this population. However, to date, nutritional interventions to target muscle loss in this population have not been successful. Therefore, future studies are needed to determine successful interventions to attenuate skeletal muscle loss in this critically ill patient population.

CHAPTER 3

EFFICACY OF BETA-HYDROXY-BETA-METHYLBUTYRATE (HMB) SUPPLEMENTATION IN ELDERLY AND CLINICAL POPULATIONS

Introduction

Muscle loss is common throughout the ageing process and may begin as early as age 30 [118]. Approximately 30 percent of muscle mass is lost between the 5^{th} and 8^{th} decade of life and rates of muscle loss can reach up to 15 percent per decade by age 70 [119, 120]. Moreover, low-levels of muscle mass in the elderly have been correlated with reduced physical function [121], decreased quality of life [122] and increased mortality [123]. A similar relationship exists in many clinical populations such as cancer or Acquired Immune Deficiency Syndrome (AIDS) patients where muscle wasting is common. Indeed, low levels of lean mass in clinical populations have been correlated with reduced physical function, decreased quality of life, poorer response to treatment and increased mortality [124-127]. Therefore, interventions to maintain or potentially increase lean mass in elderly and clinical populations are needed. Recently, beta-hydroxy-beta-methylbutyrate (HMB) has been researched for its muscle sparing properties in these populations. The following review summarizes the evidence for use of HMB in human elderly and clinical populations.

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Methods

Searches were performed using the Pubmed database using terms such as "HMB," "beta-hydroxy-beta-methylbuytrate," "HMB muscle," "HMB supplementation," and "HMB exercise." Results were thoroughly reviewed for primary research studies on HMB supplementation in clinical and elderly populations, HMB supplementation in animal models of disease, HMB safety and dosage studies, and studies on the potential mechanism of action of HMB. In addition, review articles on HMB supplementation were obtained and the reference sections of all papers were thoroughly examined for appropriate papers. All papers meeting the inclusion criteria are discussed below.

Metabolism and dosage

HMB is a metabolite of the ketogenic amino acid, leucine. A small amount (~0.3-0.4 g/day) of HMB is produced endogenously through leucine metabolism. The first step in leucine oxidation is transamination to ketoisocaproate (KIC). The majority (approximately 95%) of KIC is metabolized to isovaleryl CoA by the mitochondrial enzyme, branched-chain α-keto-acid dehydrogenase (BCKDH), and ultimately enters the citric acid cycle. However, a small amount of KIC (approximately 5%) is converted to HMB by α-ketoisocaproate dioxygenase in the cytoplasm and ultimately metabolized into cholesterol [128].

Although HMB can be synthesized endogenously from leucine, approximately 60g of leucine would need to be consumed daily to reach the 3g of HMB per day dosage that has been used in most previous studies [129]. High leucine protein sources, such as dairy, eggs and meats contain roughly 7-10 percent leucine [130]. Therefore, in order to obtain 60g of leucine from the diet, one would have to consume at least 600g of protein from a high leucine protein source

daily. Clearly, this level of consumption is not practical; therefore, in order to obtain 3g HMB/day, supplementation of HMB is necessary.

When supplemental HMB is consumed, HMB peaks in circulation at 1-2 hours and reaches baseline levels by 9 hrs post-consumption, suggesting that consumption of HMB in multiple dosages throughout the day may be optimal [131]. Additionally, when 1-6 g HMB is supplemented, approximately 14-29 percent is excreted in the urine [131, 132]. Therefore, it appears that a majority of supplemental HMB remains in the body.

Most researchers seem to agree that the optimal dosage of HMB is 3g/day. This recommendation comes from previous research by Nissen et al. [133] which showed that HMB supplementation in strength training healthy subjects increased strength in a dose dependent manner in, up to 3g HMB daily. Furthermore, Gallagher et al. [132] showed that 3g HMB daily significantly increased total body strength in healthy strength training subjects compared to a control group that strength trained, but did not receive HMB; while, consumption of 6g HMB/day did not result in any additional increases in total body strength. As a result of these studies, the dosage of 3g HMB/day is the commonly agreed upon daily dosage of supplemented HMB. Unfortunately, no dose-response studies have been conducted in clinical or aging populations and it is not clear if 3 grams is the ideal under these conditions or if more may be required to optimize HMB's effects.

Safety of HMB

With consumption of any dietary supplement, safety is a concern. Accordingly, the safety of HMB supplementation has been widely studied [134-137]. Early studies in animals found that consumption of HMB in dosages as high as 100 g/day in pigs weighing 20 kg

(approximately 100 times the g HMB/kg bodyweight dose used in most human studies) for 4 days had no effect on changes in blood cell numbers, organ weights, or histological lesions [134]. Likewise in humans, consumption of dosages as high as 6 g HMB/day for 1 month had no effect on liver enzymes, kidney function, cholesterol, white blood cells, hemoglobin, or blood glucose [135]. Furthermore, two meta-analyses on HMB supplementation have concluded that HMB is safe and does not result in any major side effects [136, 137]. In fact, HMB may actually decrease blood pressures, total and LDL- cholesterol, especially in hypercholesterolemic individuals [136]. Moreover, 2 relatively short term studies in clinical populations did not have any major negative side effects [11, 12]. However, there is less research on the long-term effects of HMB supplementation in elderly and clinical populations. Recently, Baier et al. [10] examined the effects of 2-3g of HMB daily for one year in the elderly and found that HMB consumption did not result in any changes in blood or urine markers of hepatic or renal function or blood lipids. Therefore, it appears that up to one year of HMB supplementation is safe; however, future studies should investigate the long-term safety of HMB supplementation, especially in clinical populations.

Efficacy of HMB in healthy populations and athletes

Previous studies investigating HMB supplementation in athletes and healthy populations have shown mixed results. A meta-analysis of 9 studies found that HMB resulted in significant gains in muscle size and strength [138]. However, a more recent meta-analysis of 11 studies by Rowlands et al. [139] concluded that 3-9 weeks of HMB supplementation at a dosage of around 3g/day resulted in only small to trivial increases in muscle strength and only trivial increases in muscle size, regardless of training experience. As pointed out by Wilson et al. [129], the

discrepancies in the results of HMB supplementation studies in healthy populations may be due to many factors including clustering of data in these meta-analysis to include many studies from similar groups, small sample sizes, poorly designed, non-periodized training protocols, and lack of specificity between training and testing conditions. Moreover, thus far, direct measures of changes in muscle size have been poor and generally indirect, which makes it difficult to truly quantify HMB's effects in healthy populations. Thus, while the effects of HMB supplementation in athletes and healthy populations can be debated, it is beyond the scope of this review to do so. Interested readers are encouraged to read Wilson et al. [129].

Efficacy of HMB in the elderly

The effects of HMB supplementation in elderly populations have been examined in several studies (Table 1). Hsieh et al. [140] investigated the effects of HMB in elderly subjects receiving tube feeding. Subjects were assigned to either usual care (n=40) or two grams of HMB per day (n=39) for 28 days. All tube feeding protocols remained the same throughout the study. After 28 days, HMB supplementation increased weight, BMI, waist, hip, and calf circumference. Additionally, HMB supplementation resulted in a decrease in nitrogen excretion, suggesting that HMB either decreased protein breakdown and/or increased protein synthesis. The authors concluded that HMB was beneficial to malnourished elderly receiving tube feeding; however, this study did not measure body composition to determine if the increases in weight were from lean or fat mass.

The efficacy of HMB supplementation on lean mass and physical function in healthy elderly populations has also been investigated. Vukovich et al. [141] compared the effects of 8 weeks of HMB supplementation on body composition and strength in 70 yr old men and women.

Subjects were assigned to either 3g HMB per day (n=14) or a placebo containing 3g rice flour per day (n=17) and all subjects participated in 5 days of supervised exercise per week. Strength training was completed twice weekly and consisted of 2 sets of 10-15 repetitions on 8 exercises at 70 percent of 1 repetition maximum. On the other 3 days of exercise, subjects participated in 60 minutes of walking and stretching. At the end of 8 weeks, upper body strength increased by nearly 15 percent and lower body strength was increased approximately 20 percent in both groups; however, there was no difference in strength changes between groups. A near significant 0.8 kg increase in lean mass ($p=0.08$) was observed in the HMB group measured by skin fold calipers, while no change was observed in the placebo group. On a subset of subjects, lean body mass was also assessed via DXA; however, no difference was observed between groups. The HMB group also had an approximately 8 percent decrease in fat mass, measured via CT scan; however, no differences in lean mass change were observed between groups, as measured by CT. Overall, HMB decreased fat mass and may have increased lean mass over a relatively short term in exercising adults; however, this increase in lean mass did not appear to result in additional increases in muscle strength. Moreover, measurements of basic physical function and quality of life were not performed so it cannot be determined if the additional lean mass gained as a result of HMB supplementation resulted in significantly improvements clinically significant outcomes over strength training alone. Additionally, measurement of lean mass with skin fold calipers is not the gold standard method for measuring body composition and skin fold caliper measurements did not show the same results as the subset of subjects who received DXA scans. Although the results in this study provide mild support for the use of HMB in exercising elderly individuals, future studies investigating the effects HMB supplementation in strength training

elderly employing a longer duration intervention and using more precise measurements of body composition are needed to confirm these findings.

The effects of HMB supplementation in the elderly without an exercise intervention have also been examined. Flakoll et al. [142] investigated the effects of 12 weeks of HMB supplementation in subjects over 62 years living in nursing homes. Subjects were randomly assigned to either 2g HMB, 5g arginine, and 1.5g lysine daily (n=27) or a placebo (n=23). A near significant 0.7 kg increase in lean mass was observed (p=0.08), as measured by bioelectrical impedance and Bod Pod, while no changes in lean mass were observed in the placebo. Moreover, subjects in the HMB group significantly decreased "timed up and go" test time by 2.3 sec, increased leg extensor force by 3.0 kg, and increased handgrip strength compared to the placebo. These improvements in physical function suggest that HMB supplementation can improve clinically significant outcomes in the elderly. Moreover, all changes were observed without an exercise intervention suggesting that HMB alone may be able increase lean mass and improve physical function in the elderly.

To further investigate the ability of HMB to increase lean mass and physical function, independently of exercise, Baier et al. [10] investigated the effects of 1 year of HMB supplementation in elderly subjects age 65 or older. Subjects were given either 2-3g HMB/5-7.5g arginine/1.5-2.25g lysine (n=40) or an isonitrogenous control made up of non-essential amino acids (n=37), daily. One year of HMB supplementation increased lean mass by 0.88 kg as measured by BIA and 0.55 kg as measured by DXA while no significant changes in lean mass ever observed in the control group. However, HMB did not result in any differences in bone density, "timed up and go" test time, chair stand, handgrip strength, leg strength, or quality of life between groups. Overall, this study indicates that 1 year of HMB supplementation in

sedentary older adults could increase lean mass. Additionally, HMB supplementation did not improve physical function, which differed from the previous study by Flakoll et al. [142].

Interestingly, additional analysis of the Baier [10] study by Fuller et al. [143] found that vitamin D status affected strength gains. Subjects with adequate vitamin D status experienced a nearly 21 percent net gain in total body strength during the 1 year HMB intervention, while those who did not have adequate vitamin D status did not gain strength after HMB supplementation. This suggests that vitamin D deficiency may blunt the increase in strength gains observed with HMB supplementation.

A possible confounder in many previous HMB supplementation studies in elderly populations was that lysine and arginine were supplemented along with HMB so it is not possible to determine which of the supplements were effective or if there was a possible synergistic effect. To isolate HMB's effects from lysine or arginine our lab recently utilized a fisher 344 rat model (unpublished data) to investigate the effects of HMB supplementation from young to middle (44 to 60 wks) age and from old to very old age (86 to 102 wks) [144]. HMB was given as 1 percent of the diet. The supplementation period of 16 weeks represented approximately 16 % of the rats' lifespan. We found that body fat mass increased by nearly 50 percent from young to middle age, measured by DXA ($p<0.05$), but that HMB supplementation prevented this gain. This is important as research indicates that the onset of fat gain may increase whole body inflammation and initiate sarcopenia. We also found that supplementation with HMB throughout old age prevented any significant loss in muscle fiber dimensions and blunted the rise in the ubiquitin pathway typically seen with age in the rats' soleus muscles. Finally, HMB decreased fat mass and improved normalized limb strength by 23 percent as measured by the grip strength test [145] in the old group. Thus, it is possible that HMB alone

supplemented throughout old age can improve strength, maintain muscle size, and create an overall leaner phenotype.

Table 1. Summary of beta-hydroxy-beta-methybutyrate (HMB) supplementation studies in elderly humans.

Study	Dosage (Daily)	Length of Study	Exercise	Results and Comments
Vukovich 2001 [141]	3g HMB	8 weeks	2 days strength training and 3 days aerobic exercise	HMB (n=14) ↑ LBM[a] by 0.8 kg measured by calipers (p=0.08) No difference in LBM measured by DXA[b] or strength between HMB and placebo group (n=17)
Flakoll 2004 [142]	2g HMB 5g Arginine 1.5g Lysine	12 weeks	None	HMB (n=27) ↑ LBM by 0.7 kg measured by BIA[c] (p=0.08) HMB ↑ leg extensor strength by 3 kg, ↑ grip strength, and ↓ "timed up and go" test time by 2.3 sec No changes in LBM, leg strength, grip strength, or "timed up and go" test time in placebo (n=23) group
Baier 2009 [10]	2-3g HMB 5-7.5g Arginine 1.5-2.25g Lysine	1 year	None	HMB (n=40) ↑ LBM by 0.55kg measured by DXA No change in LBM in control group (n=37) No change in bone mineral density, strength, physical function, or quality of life in either group
Hsieh 2010 [140]	2g HMB	4 weeks	None	Subjects receiving tube feeding HMB (n=39) ↑ bodyweight, BMI[d], hip, and calf circumference HMB ↓ nitrogen excretion No changes in BMI, hip, or calf circumference in control group (n=40)
Fuller 2011[143]	2-3g HMB 5-7.5g Arginine 1.5-2.25g Lysine	1 year	None	Additional analysis of Baier [10] Vitamin D Status affected strength gains HMB + adequate vitamin D status ↑ total body strength by 21 percent No change in strength in HMB supplemented subjects with vitamin D deficiency or in placebo group

[a]LBM = lean body mass, [b]DXA = dual x-ray absorptiometry, [c]BIA = bioelectrical impedance, [d]BMI = body mass index

Efficacy of HMB in clinical populations

Patients with chronic disease such as cancer and AIDS experience significant muscle loss, which leads to decreased physical function, quality of life and survival [127]. Numerous nutritional interventions have been investigated in an attempt to counteract muscle wasting in these populations; however, many of these interventions have been unsuccessful in attenuating muscle loss (reviewed in Klein et al. [146]). Recently, HMB has been investigated for its anti-catabolic effects in clinical populations such as cancer, AIDS, and chronic obstructive pulmonary disease (COPD) (Table 2).

Several studies support the efficacy of HMB to attenuate muscle loss in cancer cachexia. Numerous animal models of cancer have shown benefits from HMB supplementation including attenuation of weight loss [147-149] and tumor growth [148], and prolonged survival time [150]. The reduction in tumor growth was thought to occur through a reduction in nuclear factor-κB p65 subunit expression (34). Likewise, the efficacy of HMB supplementation in human cancer patients has been previously demonstrated. For instance, May et al. [11] recruited cancer patients with solid tumors that had a documented weight loss of greater than 5% and a prognosis of greater than 3-month survival. Patients were assigned to either 3g of HMB, 14g arginine, and 14g glutamine (n=18) or an isonitrogenous mixture of nonessential amino acids (n=14) daily. HMB supplementation resulted in an approximately 1 kg increase of lean mass in 4 weeks, as determined by Bod Pod analysis. Despite these promising results, to our knowledge no additional human trials have been conducted in cancer patients. Future studies should investigate the long-term effects of HMB supplementation in cancer patients to determine if the gains in lean mass observed in the first 4 weeks are maintained long term and to determine if HMB can increase survival time in humans, as was shown in rats.

The efficacy of HMB supplementation to attenuate muscle wasting in AIDS has also been investigated. Clark et al. [12] recruited AIDS patients with a weight loss of greater than 5% over the previous 3 months. Subjects were assigned to either 3g of HMB, 14g arginine, and 14g glutamine (n=22) or an isocaloric maltodextrin control (n=21) daily. Similar to the results observed in cancer patients, 8 weeks of HMB supplementation in AIDS patients increased lean mass by 2.6 kg in 8 weeks, as determined by Bod Pod. Moreover, HMB supplementation increased CD3+, CD4+, and CD8+ cell numbers indicating that HMB may improve the immune system of immunocompromised AIDS patients. Despite these findings, no additional clinical trials have been performed in AIDS patients. Therefore, similar to HMB research in cancer patients, future studies should investigate the long-term effects of HMB supplementation in AIDS patients and also look into possible immune system benefits in AIDS patients as a result of HMB.

The effects of HMB supplementation in trauma patients have also been investigated. Kuhls et al. [151] recruited trauma patients that were candidates for enteral feeding. Subjects received a daily dose of either 3g of HMB, 14g arginine, and 14g glutamine per day (n=22), 3g HMB alone per day (n=28), or an isonitrogenous control (n=22). After 4 weeks, both groups receiving HMB supplementation reduced nitrogen excretion. The reduction in protein breakdown may have been observed as a result of a decrease in protein breakdown, increase in protein synthesis, or a combination of both events occurring simultaneously. However, no significant differences were observed in inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein (CRP), or pre-albumin, a marker of nutritional status. This indicates that HMB may reduce muscle protein breakdown in trauma patients; however, this does not appear to happen through a decrease in inflammation. Although these results support the use of HMB in

trauma patients, the efficacy of HMB supplementation to maintain lean mass has not been investigated in this population.

Although the previous study did not find a change in inflammation in trauma patients, there is some evidence that HMB may be anti-inflammatory in certain clinical populations. Hsieh et al. [152] assigned COPD patients to either 3g HMB (n=18) or a placebo (n=16) daily for 7 days and found that HMB supplementation reduced CRP from 111.56 ± 91.47 to 46.19 ± 45.29 mg/L while no change in CRP was observed in the placebo group. In addition, HMB supplementation reduced white blood cell numbers. However, HMB supplementation also increased total cholesterol, an effect that is different than previous studies, which have primarily shown a decrease in total cholesterol [136]. Although HMB may decrease inflammation in COPD patients, the mechanism by which this happens is not well understood. Moreover, the effects of HMB supplementation on lean mass and strength have not been investigated in COPD patients.

All of the previously discussed studies supported the use of HMB in clinical populations; however, not all studies have found beneficial effects of HMB supplementation in clinical populations. Marcora et al. [153] supplemented Rheumatoid arthritis patients with either 3g HMB, 14g arginine, and 14 g glutamine per day (n=18) or an isonitrogenous mixture of nonessential amino acids (n=18) for 12 weeks. HMB supplementation did not result in any change in total body mass, lean mass, fat mass, bone mineral density, or strength. Moreover, Clements et al. [154] supplemented the same HMB, arginine, and glutamine cocktail (n=14) or provided usual care (n=16) to gastric bypass patients after surgery for 8 weeks and also found that HMB supplementation had no effect on body weight, BMI, fat mass, lean mass, or resting

metabolic rate. Discrepancies in the results of these studies should be investigated to determine the potential reasons for differences in HMB efficacy between clinical populations.

A possible confounding factor in the previously discussed studies was that nearly all studies supplemented a cocktail of HMB along with arginine and glutamine, which were added to the cocktail for their effects on muscle protein synthesis, immune function, and wound healing [155, 156]. Therefore, it is not possible to determine if the effects observed were due to HMB supplementation alone, the addition of the amino acids, or a possible synergistic effect of HMB, arginine, and glutamine. Therefore future studies are advised to investigate this relationship and to determine the effects of HMB alone in human clinical populations.

Table 2. Summary of beta-hydroxy-beta-methybutyrate (HMB) supplementation studies in clinical populations.

Study	Population	Dosage	Study Length	Results
Clark 2000 [12]	AIDS	3g HMB 14g Glutamine 14g Arginine	8 weeks	HMB (n=22) ↑ LBM[a] by 2.6 kg measured by Bod Pod Placebo group (n=21) ↓ LBM by 0.7 kg measured by Bod Pod HMB ↑ CD3+, CD4+, and CD8+ cell numbers
May 2002 [11]	Cancer	3g HMB 14g Glutamine 14g Arginine	24 weeks	HMB (n=18) ↑ LBM by approximately 1 kg measured by Bod Pod Placebo group (n=14) had no change in LBM
Marcora 2005 [153]	Rheumatoid Arthritis	3g HMB 14g Glutamine 14g Arginine	12 weeks	No effects on LBM, fat mass, bone mineral density, or strength in either HMB or placebo group (n=18 in each group)
Hsieh 2006 [152]	Chronic Obstructive Pulmonary Disease	3g HMB	1 week	HMB (n=18) ↓ CRP[b] and WBC[c] count No change in CRP in control group (n=16)
Kuhls 2007 [151]	Trauma Patients	3g HMB 14g Glutamine 14g Arginine	4 weeks	HMB (n=28) and HMB/Arginine/Glutamine (n=22) groups but not placebo (n=22), ↓ nitrogen excretion No change in CRP, IL-6[d], or pre-albumin in any group
Clements 2011 [154]	Gastric Bypass	3g HMB 14g Glutamine 14g Arginine	8 weeks	No differences total bodyweight, BMI[e], fat mass, LBM, or resting metabolic rate between HMB (n=14) and placebo (n=16) groups

[a]LBM = lean body mass, [b]CRP = C reactive protein, [c]WBC = white blood cell, [d]IL-6 = interleukin-6, [e]BMI = body mass index

Efficacy of HMB in animal models of disease

The previously discussed studies are the only published investigations on the efficacy of HMB supplementation in human clinical populations. However, HMB supplementation has also been investigated in animal models of muscular unloading, sepsis and muscular dystrophy. Hao et al. [157] investigated the effects of HMB using an animal model of unloading and reloading. They found that rats supplemented with HMB had significantly greater force production and increased plantaris and soleus cross sectional area after reloading. Moreover, HMB reduced the number of apoptotic nuclei after unloading. Kovarik et al. [158] induced mice with sepsis and injected either HMB or saline. HMB reduced protein degradation, leucine oxidation, and proteosome activity indicating that HMB may attenuate muscle loss during sepsis. Additionally, Payne et al. [159] gave a combination therapy of HMB, creatine monohydrate, conjugated linolenic acid and alpha linoleic acid to MDX mice (an animal model of muscular dystrophy) and found that the cocktail increased grip strength, decreased grip strength fatigue, and decreased the amount of internalized myonuclei. These results indicate that HMB may be able to decrease muscle damage in muscular dystrophy and result in an increase in physical function. However, to date no human studies of HMB supplementation in models of injury rehabilitation and unloading, muscular dystrophy or sepsis have been performed.

While HMB supplementation has shown positive results in clinical populations, more work is needed to determine the long-term efficacy and safety of HMB in the clinical populations discussed. Furthermore, research on the efficacy of HMB supplementation should be extended to additional clinical populations, such as congestive heart failure, diabetes mellitus, chronic kidney disease, neurodegenerative diseases, or patients recovering from injuries.

HMB mechanism of action

Muscle tissue mass represents the net balance between muscle protein synthesis and degradation. The continuous process of building and replacing muscle protein allows muscle to repair and adapt to environmental conditions. Net protein balance is positive during growth when protein synthesis exceeds degradation, while net balance is negative during weight loss, aging, and in clinical populations when degradation exceeds synthesis. Accordingly, numerous studies have investigated the effects on HMB on muscle protein balance. A summary of the potential mechanisms by which this occurs is found in Figure 2. Results of studies using cultured muscle cells have found that HMB reduces muscle protein degradation [147, 160]. Moreover, HMB has been shown to decrease whole body proteolysis in vivo [161]. However, the effects of HMB on muscle protein synthesis have shown mixed results with studies finding that HMB supplementation results in increases [147] or no change [162] in muscle protein synthesis. Despite the discrepancies in the results of turnover studies, HMB supplementation has been shown to result in increases in phosphorylation of the mammalian target of rapamycin (mTOR) and its downstream signaling targets, indicating an increase in skeletal muscle protein translation [149, 160]. Additionally, increased muscle insulin like growth factor (IGF-1) expression has been observed after culture of myoblasts with HMB, which may contribute to an increase in protein synthesis [163]. Overall, it appears that HMB inhibits protein degradation and may also stimulate protein synthesis which may be due at least in part to an upregulation of muscle IGF-1, and increased mTOR activation.

HMB may attenuate protein degradation through inhibition of multiple catabolic pathways. The ubiquitin-proteosome pathway is upregulated in catabolic states [164], and results in increased degradation of proteins. However, HMB has been shown to decrease

ubiquitin-proteosome expression [147] and activity [147, 158, 161, 165] during catabolic states, thereby attenuating ubiquitin-proteosome induced protein degradation.

Caspases are commonly upregulated in catabolic states and induce muscle proteolysis by apoptosis of myonuclei, and are also involved in the initial cleavage of the actomyosin complex [166]. However, HMB has been shown to attenuate increases in activated caspases in catabolic states such as skeletal muscle unloading [157] and in skeletal muscle cells cultured with large concentrations of TNF alpha and angiotensin II [167]. In these studies, the decrease in caspase activation was correlated with decreased myonuclear apoptosis [157, 167]. Thus, it appears that HMB attenuates apoptosis in catabolic states through attenuation of caspase activation.

Muscle regenerative capacity is impaired in the elderly and cachexic states, which may further contribute to muscle protein catabolism [168]. Recently, HMB has been investigated for its effects on muscle regenerative capacity. Kornasio et al. [163] cultured myoblasts in a serum starved state to induce apoptosis. When myoblasts were incubated with HMB, expression of Myo D and myogenin were increased, which suggests that HMB may increase satellite cell activation and increase muscle regenerative capacity. This may have occurred though an observed increased in Akt phosphorylation which may have decreased FOX-O translocation and resulted in a reduction in atrogin-1, which may have resulted in reduced degradation of Myo D [144]. Moreover research from our lab indicated that old resistance trained rats supplemented with HMB were able to increase IGF-1 mRNA expression, while those not provided HMB did not (unpublished data). Additionally, HMB decreased apoptosis of myoblasts, which suggests that HMB may be able to attenuate the reductions in satellite cell numbers observed in the elderly and cachexic states [169, 170].

Increased inflammation is common in ageing and chronic disease and may lead to an increase in muscle protein degradation through the upregulation of the ubiquitin proteasome pathway [171]. As mentioned previously, HMB may be able to decrease systemic inflammation in clinical populations [152]; however, not all studies have found reductions in inflammatory markers in human clinical populations following HMB supplementation [151]. Recently, Nunes et al. [172] investigated the mechanism by which HMB affects inflammation by culturing human peripheral blood mononuclear cells (PBMCs) from healthy subjects in the presence of concanavalin A (ConA)to stimulate an inflammatory response, and increasing concentrations of HMB. PBMCs cultured with HMB and ConA had significantly reduced production of inflammatory cytokines including tumor necrosis factor alpha and interferon gamma compared with PBMCs cultured with ConA alone. These results suggest that the decrease in muscle protein catabolism by HMB may be due in part to the effect of HMB on inflammatory cells; however, future studies are needed to confirm this potential mechanism.

Overall, HMB appears to exert its effects on skeletal muscle protein metabolism through numerous mechanisms, including improved muscle protein balance. HMB may increase skeletal muscle protein synthesis through activation of the mTOR pathway and by increasing skeletal muscle IGF-1 expression. In addition, HMB may decrease protein degradation by decreasing activity and expression of the ubiquitin-proteosome pathway and caspases. Moreover, HMB may reduce inflammation and have beneficial effects on muscle regeneration. However, it should be noted that many of the mechanistic studies were performed in animal or cell culture models where dosages of HMB were much larger than the dosages commonly used in human studies. Thus, future studies are needed to investigate the mechanisms of HMB action using more physiological doses.

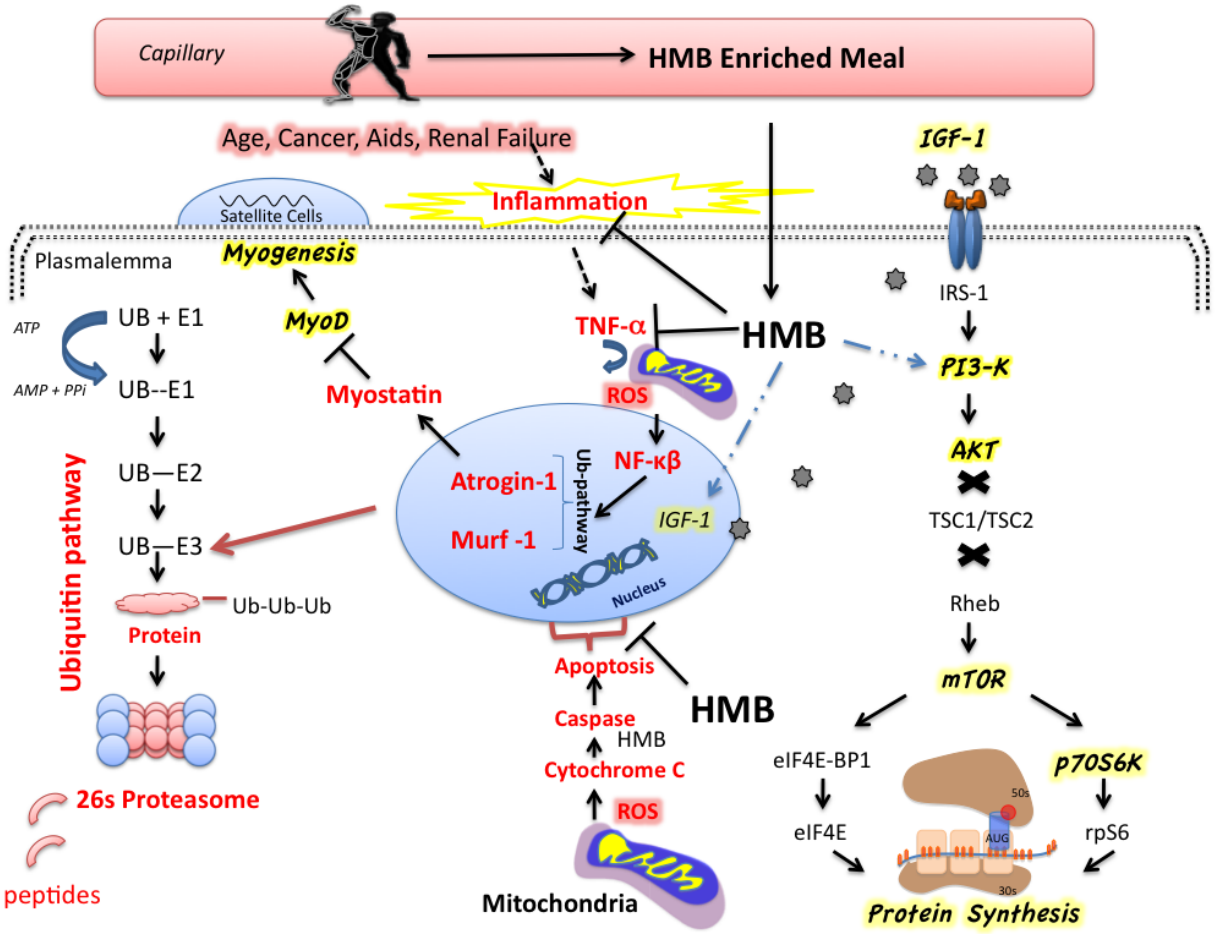

Figure 1. Potential Mechanisms by which HMB increases anabolic and decreases catabolic pathways in skeletal muscle. Italicized text refers to signaling pathways that are decreased in the skeletal muscle cells of elderly and clinical populations as compared to healthy populations, while bold text refers to signaling pathways that are increased. HMB may increase protein synthesis by decreasing inflammation, increasing IGF-1, and increasing protein translation through activation of mTOR. Additionally, HMB may decrease protein catabolism through down regulation of the ubiquitin proteasome pathway and reductions in caspase activity. Abbreviations: HMB – beta-hydroxy-beta-methylbutyrate; IGF-1 – insulin like growth factor-1; IRS-1 – insulin receptor substrate-1; mTOR – mammalian target of rapamycin; UB – ubiquitin; ROS – reactive oxygen species; TNF-α – tumor necrosis factor alpha.

Conclusions

Muscle loss is common during aging and in chronic diseases, leading to reduced physical function, a decreased quality of life, and increased mortality. Recently, HMB has been shown to attenuate muscle loss in the elderly and in clinical populations such as AIDS and cancer patients; however, a limited number of studies have investigated this question, with relatively small sample sizes. In addition, the effects of HMB in clinical populations appear to differ depending on the population investigated. Accordingly, future studies should investigate the effects of HMB in other catabolic clinical populations. Overall, several studies support the efficacy of HMB supplementation in the elderly and clinical populations as a means to increase lean mass and strength; however, the data is not entirely consistent. Larger, long-term studies are needed to clarify these promising preliminary results.

CHAPTER 4

PLASMA BETA-HYDROXY-BETA-METHYLBUTYRATE (HMB) CLEARANCE IN HEMODIALYSIS PATEINTS: A PILOT STUDY

Introduction

Beta-hydroxy-beta-menthybutyrate (HMB) is a metabolite of the amino acid leucine that has been shown to attenuate muscle mass loss in elderly and clinical populations [13]. In adults with normal renal function, approximately 20-25 percent of supplemental HMB is cleared by the kidney while the majority is cleared through conversion to either Acetyl CoA or cholesterol [131]. However, the clearance of HMB in individuals with impaired renal function is unknown.

Recently, Sipahi et al. [173] performed a retrospective analysis investigating the effects of 4 weeks of HMB, arginine, and glutamine supplementation on foot ulcer healing in diabetic maintenance hemodialysis (MHD) patients and observed a beneficial effect of supplementation. Additionally, no adverse effects of HMB supplementation were observed suggesting that HMB supplementation in this population is safe. However, to date, no other studies have been performed investigating HMB supplementation in MHD patients and the time course of supplemental HMB clearance in patients with impaired renal function is unknown. Therefore, the purpose of this small pilot study was to determine the clearance of plasma HMB in hemodialysis patients during dialysis and after supplementation.

Methods

Participant characteristics

MHD patients (n=8) receiving thrice weekly hemodialysis treatment for at least 3 months were recruited from dialysis clinics in Champaign, IL. Patients were excluded if they were not between 30-80 years of age, weighed greater than 350lbs or if they were currently taking an HMB supplement or HMB containing product. All patients were informed of the risks and benefits of the study protocol and provided written consent prior to participation. In addition, all patients received physician approval prior to participation.

Intervention protocol

On day 1, blood (5ml) was obtained at the beginning and 3hrs after the start of a hemodialysis treatment to determine the effects of hemodialysis on basal HMB concentrations. One week later, Calcium-HMB (3g) was given to MHD patients at the start of a hemodialysis treatment. Blood samples were obtained at 0, 1, 2, 3, and 48hrs after the start of the hemodialysis treatment. An additional blood sample was obtained 7 days after calcium-HMB consumption. Plasma HMB in all samples was analyzed by Metabolic Technologies Inc. (Ames, IA) using the methods described in [174].

Statistical analysis

Statistical analyses were performed using SPSS Version 22 (IBM, Armonk, NY). All data are presented as mean ± standard deviation, unless otherwise indicated. Changes in basal plasma HMB concentration were analyzed by paired t-test. Changes in plasma HMB concentration after HMB supplementation were analyzed using a repeated measure ANOVA.

When ANOVA indicated a main effect, protected LSD was used for pair-wise comparisons. For all statistical tests, significance was determined with a 5 percent chance of type 1 error.

Results

Participant characteristics are displayed in Table 1. On day 1, after 3 hours of hemodialysis treatment, basal plasma HMB levels were significantly reduced ($p<0.001$, Figure 1). After consumption of 3g supplemental calcium-HMB, plasma HMB was elevated within 1hr ($p=0.006$), peaked at 3hrs, remained significantly elevated for 48hrs ($p=0.037$), and returned to baseline by 7 days (Table 2).

Table 1. Participant Characteristics

Variable	Value
Number	8
Gender (Male/Female)	2 / 6
Age	53 (12)
Diabetes (Yes/No)	6 / 2
Smoking (Yes/No)	2 / 6
BMI	30.4 (7.4)

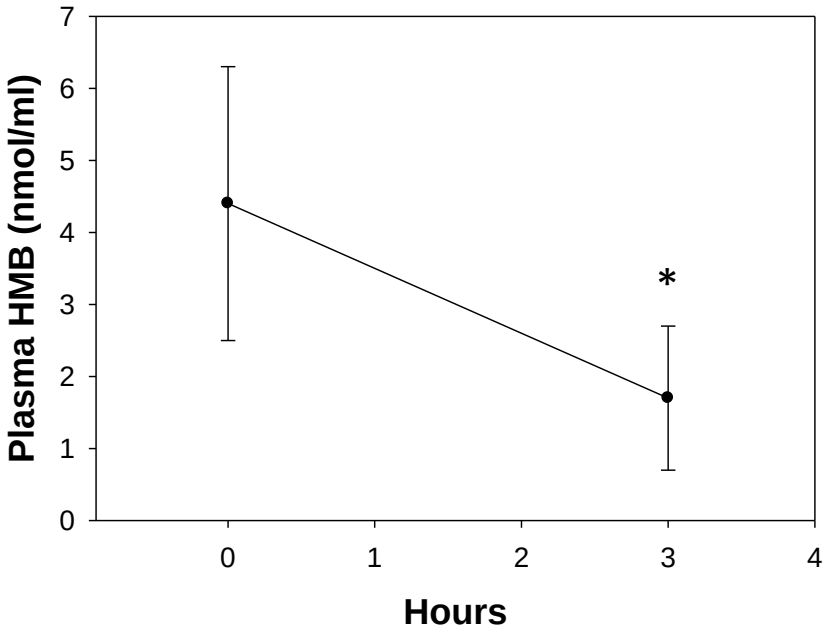

Figure 1. Plasma HMB clearance during a standard hemodialysis treatment. * indicates significant difference from time 0 hrs.

Table 2. Plasma HMB clearance following consumption of a 3g HMB supplement at the start of hemodialysis treatment.

Time	Plasma HMB Concentration (nmol/ml)
0 hr	3.8 (0.8)
1 hr	146.9 (55.2)*
2 hr	201.1 (37.8)*
3 hr	257.8 (39.8)*
48 hr	12.1 (2.2)*
7 days	4.4 (0.7)

* Significantly different than 0hr

Discussion

The primary findings from this acute pilot study were that plasma HMB concentration decreased during a standard hemodialysis session. Additionally, supplemental HMB is cleared in patients with impaired kidney function. These preliminary findings in conjunction with a previous 4 week HMB supplementation intervention in MHD patients [14] suggest that supplemental HMB at a dosage of 3g/day is not harmful in this patient population.

Hemodialysis treatment results in an increase in skeletal muscle protein catabolism contributing to reductions in lean mass up to 1-3kg annually in incident hemodialysis patients [2]. Therefore, interventions to attenuate muscle catabolism during hemodialysis treatment are needed. HMB has been shown to attenuate skeletal muscle protein catabolism through a number of mechanisms (as reviewed by [13]); however, in the present study we observed a reduction in plasma HMB concentration by approximately 50 percent during a standard hemodialysis session. This high rate of clearance is likely due to the similarity in size between HMB and amino acids, which are removed through the dialysis membrane at a rate of 6-8g per dialysis session [82]. It is also possible that the reduction in plasma HMB levels during hemodialysis may further contribute to increased rates of skeletal muscle protein catabolism at this time due to the anti-catabolic properties of HMB.

In adults with normal renal function, approximately 20-25 percent of supplemental HMB is cleared by the kidney and the remainder is converted to acetyl CoA and/or cholesterol. However, the rate at which supplemental HMB is cleared in patients with impaired renal function was previously unknown. In the present study, supplementation of 3g HMB at the start of hemodialysis treatment resulted in a peak in plasma HMB at approximately 3hrs after supplementation, significantly longer than previously reported in healthy adults [131]. This

discrepancy may have been due to a reduction in splanchnic perfusion during hemodialysis [175]. Additionally, participants in the previous study were fasted while we did not control for food intake in the present study due to the high prevalence of diabetes in our patient population.

Plasma HMB concentration was nearly reduced to baseline levels after 48hrs. Importantly, the majority of this clearance occurred during the interdialytic period and independently of kidney function and/or hemodialysis suggesting that plasma HMB was likely cleared though conversion to cholesterol to either Acetyl CoA or cholesterol [131]. Moreover, at 7 days, plasma HMB returned to baseline levels in all patients. Importantly, these results suggest that supplementation of HMB at the start of hemodialysis, a period in which HMB is rapidly cleared, results in a significant and sustained elevation in plasma HMB levels.

The present pilot study has a number of limitations. We did not control nutritional intake prior to our intervention due to the number of diabetics in our patient population. We also did not measure dialysate HMB concentrations so it is unclear what percentage of supplemental HMB is cleared through the dialysis membrane. We only measured plasma HMB at a limited number of time points so we are only able to provide a rough time course of HMB clearance kinetics in patients with impaired renal function. In addition, we included a small sample size. Despite this, we were able to observe statistically significant changes in plasma HMB concentrations. Based on the findings of this study and previous work [14] we conclude that supplemental HMB results in meaningful increases and clearance of plasma HMB in MHD patients. Furthermore, HMB supplementation does not appear to adversely affect patients with impaired renal function based upon these preliminary findings.

CHAPTER 5

EFFECTS OF BETA-HYDROXY-BETA-METHYLBUTYRATE (HMB) SUPPLEMENTATION ON LEAN MASS, STRENGTH, AND PHYSICAL FUNCTION IN MAINTENANCE HEMODIALYSIS PATIENTS

Abstract

Patients with renal failure undergoing maintenance hemodialysis (MHD) therapy suffer from a number of co-morbidities including skeletal muscle loss, reduced physical function, a significantly increased fall risk, and reduced quality of life (QOL). Therefore, interventions to combat these co-morbidities are needed. Beta-hydroxy-beta-methylbutyrate (HMB) is a metabolite of the amino acid leucine that has been shown to improve lean mass and physical function in the elderly and clinical populations, but had not previously been studied in MHD patients. We performed a double blind, placebo controlled, randomized trial to assess the effects of daily HMB supplementation on co-morbidities in MHD patients. MHD patients were recruited and assigned to either daily supplementation with HMB (n=16) or placebo (n=17) for 6 months. No significant effects of HMB on lean mass, strength, physical function, fall risk, or quality of life were found using an intent-to-treat analysis. However, upon analysis of plasma HMB concentrations, 5 of 16 patients (31%) who completed the study in the HMB group were found to be non-compliant at 3 or 6 months. Therefore, we performed a per-protocol analysis with compliant participants only. Although this analysis was underpowered, we observed a trend for improvements in several physical function tests with HMB supplementation. However, no effects of HMB were observed for lean mass, strength, fall risk, or quality of life. As a whole, these results do not support the efficacy of HMB to attenuate muscle loss and declines in

physical function in MHD patients. However, the observed low-compliance with study pills may have affected the results. Moreover, it highlights the need for future interventions targeted at reducing pill burden and improving pill compliance in this population.

Introduction

Patients with renal failure undergoing maintenance hemodialysis (MHD) therapy experience a number of co-morbidities, including skeletal muscle loss, which occurs for several reasons including: metabolic acidosis, inflammation, insulin resistance, decreased nutrient intake, hormonal abnormalities, an elevated metabolic rate, and loss of amino acids into the dialysate (as reviewed by [30, 31]). Moreover, the dialysis process has been shown to up-regulate expression of genes involved in skeletal muscle protein degradation and result in a breakdown of skeletal muscle proteins [32]. In addition, this decline in skeletal muscle mass contributes to reductions in physical function [5], a significantly increased fall risk [6], and reduced quality of life (QOL) [7]. Pharmacological agents have been investigated to treat muscle loss in MHD patients; however, many of these treatments are expensive and have undesirable side effects [8, 9]. As a result, low-cost interventions designed to attenuate declines in muscle mass are needed in this population.

Beta-hydroxy beta-methylbutyrate (HMB) represents a potential low-cost nutritional intervention to attenuate muscle loss in MHD patients. HMB is a metabolite of the amino acid leucine that has been shown to safely attenuate muscle mass loss in other clinical populations with accelerated muscle loss, such as the elderly [10], cancer [11], and AIDS patients [12], primarily through reductions in skeletal muscle protein catabolism (as reviewed by [13]). A recent study examining the effects of 4 weeks of HMB supplementation in diabetic MHD patients observed beneficial effects on wound healing [14]; however, lean mass, strength, physical function, and quality of life were not measured. Therefore, the primary purpose of this trial was to determine if oral supplementation with HMB attenuates muscle loss and declines in muscle strength and physical function in MHD patients.

Methods

Study overview

MHD patients (n=41) were recruited from dialysis clinics in Champaign, IL and Chicago, IL and randomly assigned to daily supplementation with HMB (n=20) or placebo (n=21) for 6 months. Patients in the HMB group ingested three 1000mg capsules of Calcium-HMB (Optimum Nutrition, Aurora, IL) 7 days a week for 6 months. Patients in the CON group ingested a non-nutritive placebo capsule daily. At baseline and 6 months following the start of the supplementation period, patients came to the lab for measurement of clinical outcomes.

Study participants

Patients who had been receiving at least twice weekly hemodialysis for greater than 3 months were recruited. Patients were excluded if they were not between 30-80 years of age, weighed greater than 350lbs due to weight restrictions of the DXA machine, were not willing to be randomized to HMB or placebo, or if they were currently taking an HMB supplement or HMB containing product. All patients were informed of the risks and benefits of the study protocol and provided written consent prior to participation. In addition, all patients received written physical approval prior to participation.

Randomization and intervention protocol

Following baseline testing, patients were randomly assigned to either the HMB or placebo group. Patients in the HMB group ingested three 1000mg capsules of Calcium-HMB (Metabolic Technologies Inc, Ames, IA) 7 days a week for 6 months. These pills do not contain significant amounts of nitrogen, potassium, or phosphorous. We chose a dose of 3g HMB/day

because previous research has shown that 3g/day HMB significantly increased lean mass in other clinical populations such as the elderly [10], cancer [11], and AIDS [12] patients. Patients in the placebo group consumed non-nutritive pills containing cellulose, caramel, beet root, magnesium stearate, and silicon. These placebo pills have been used in a previously published study in our laboratory [176]. Patients in both groups received a pill bottle containing a month's supply of capsules (HMB or placebo) prior to the beginning of each month.

Compliance

Blood was obtained at baseline, 3, and 6 months for plasma HMB analysis (as described by [174]) to assess compliance. All plasma HMB analysis was performed by Metabolic Technologies Inc. (Ames, IA) by blinded assessors. All patients who completed the study were included in intent-to-treat analysis; however, patients with an absence of elevated plasma HMB levels at 3 and 6 months in the HMB group (defined as at least a 3-fold increase in plasma HMB from baseline), were excluded from per-protocol analysis.

Outcome testing

At baseline and after 6 months of intervention, patients came to the lab for measurement of clinical outcomes. The details of these tests are described below.

Anthropometric measures

Barefoot standing height was measured to the nearest 0.1 cm with a stadiometer (Seca, Chino, CA) and body weight was measured on a balance scale (Tanita Corporation, Arlington Heights, IL) with shoes and superfluous outer garments (jackets, etc) removed.

Body composition and bone density

Whole body fat, lean and bone mass was measured by dual emission x-ray absorptiometry (DXA) (Hologic QDR 4500A, Bedford, Massachusetts). Precision for DXA measurements of interest are ~1.0 – 2.0% in our laboratories. Appropriate calibrations were performed prior to each test. DXA scans were analyzed by an experienced technician blinded to treatment status.

Muscle strength

Unilateral quadriceps femoris and hamstring muscle strength was evaluated using a Biodex System 3 dynamometer (Shirley, NY). Knee extension and flexion isokinetic muscle torque was evaluated at a speed of 60 degrees per second. The axis of rotation of the machine was aligned with the lateral epicondyle of the femur. The calf pad was positioned halfway between the lateral malleolus of the fibula and lateral epicondyle of the femur, and securely attached to the subject using straps. Straps were placed over the thighs, pelvis, and torso regions to minimize movement during the test. Participants performed two sets of 6 repetitions, with a 3-minute rest between sets, and the best effort was used for analysis. For all tests, participants were verbally encouraged to perform as vigorously as possible.

Shuttle walk test

Each subject underwent a shuttle walk test to assess physical performance. This is a progressive test in which patients walk over a 10 m course paced by programmed beeps with progressively increasing speeds. The shuttle walk test has been correlated with VO2max and

may be more beneficial than the VO2max test in this population due to functional limitations such as muscle weakness [177].

Objective physical performance test

A five item functional fitness test was used as a second objective evaluation of physical function [178]. Five physical function tasks were included: 1) Chair Stand Test where subjects were asked to stand from a seated position as many times as possible in 30 seconds, 2) Arm Curl Test in which subjects were asked to complete as many arm curls as possible during 30 seconds using either a 5 pound dumbbell for females, an 8 pound dumbbell for males, 3) Chair Sit and Reach Test that asked subjects to reach forward with both arms to try and touch their extended leg to assess flexibility, 4) Shoulder flexibility test where subjects were instructed to try to touch their middle fingers behind their back to assess upper body flexibility, and 5) 8 Foot Up-and-Go test in which subjects were asked to walk around a cone placed 8 feet away from their chair and back again during a timed trial.

Fall risk

Measurement of standing and balance and gait characteristics occurred in a sub-section of participants. In order to assess balance, subjects were asked to stand on a specialized force platform (Bertec Inc, Columbus, OH) for 6 trials (2 with eyes open, 2 with eyes closed, and 2 while verbally answering unrelated questions). To assess gait characteristics, subjects were asked to walk across a specialized gait mat (Gaitrite, Sparta, NJ) for 4 trials (2 while not talking and 2 while verbally answering unrelated questions).

Quality of life (QOL)

QOL was assessed using a validated Kidney Disease Quality of Life (KDQOL^{TM}) questionnaire [179].

Cardiovascular measurements

Blood pressure was measured in duplicate using an automated cuff following a 10min quiet rest (Omron IntelliSense HEM-907XL, Lake Forest, IL). Central pressures were estimated from the radial wave form using a validated transfer function by tonometry (SphygmoCor, AtCor Medical, Sydney, Australia).

Aortic PWV was determined by tonometry (SphygmoCor, AtCor Medical, Sydney, Australia). In short, Aortic PWV was calculated as the time delay (Δt) between the R-wave of the ECG and the foot of the forward pressure wave form (Intersecting Tangent) between the carotid and femoral arteries using the equation: $PWV = D/\Delta t$ (m/sec); where D is distance in meters and Δt is the time interval in seconds [180]. D was calculated by subtracting the distance between the sternal notch and the location the carotid pressure was measured from the distance between the sternal notch and the location the femoral pressure was measured.

Dietary recall

Subjects completed a 24-hour food recall interview with a registered dietitian using the USDA 5-pass method at baseline and at the end of the 6-month intervention period [181]. Two interviews were conducted at each time point, a dialysis day and a non-dialysis day, to account for variations in eating patterns associated with days of the week.

Physical activity recall

A seven-day physical activity recall was used to estimate average daily energy expenditure [182].

Blood Markers

Blood was collected from each subject at baseline and 6 months for measurement standard clinical lab parameters and of inflammatory markers. Plasma and serum were collected by centrifugation and stored at -80°C until analyzed. Standard clinical lab parameters were analyzed by Spectra Laboratories, a renal specific laboratory service provider (Rockleigh, NJ). Plasma C-reactive protein (CRP) was measured in duplicate using a commercially available ELISA kit (ALPCO Diagnostics, Salem, NH).

Statistical analysis

Statistical analysis was performed using SPSS Version 22 (IBM, Armonk, NY). All data are presented as mean ± standard deviation, unless otherwise indicated. Differences in baseline characteristics for continuous variables were analyzed with a T-Test. A Chi-square test was used to analyze baseline differences in gender, diabetic and smoking status between groups. An intent-to-treat analysis was performed with repeated measure ANOVA was used to determine time by treatment interactions for measurements of interest using all participants who completed the 6 month intervention (HMB n=16, Placebo n=17). Differences in plasma HMB concentrations between groups were analyzed using repeated measures ANOVA. After analysis of plasma HMB levels, a per-protocol analysis was performed by repeated measures ANOVA using only participants in the HMB group with elevated levels of HMB (n=11). In both

analyses, when ANOVA indicated a main effect, protected LSD was used for pair-wise comparisons. For all statistical tests, significance was determined with a 5 percent chance of type 1 error.

Sample Size Determination

No previous studies have examined the effect of HMB supplementation on lean mass or physical function in hemodialysis patients. In addition, many previous studies in clinical populations used less precise measurements of lean mass, such as BIA or skin folds. Therefore, we performed a power analysis based on changes in lean mass, as measured by Bod Pod, from a published study in AIDS patients. Clark et al. [12] supplemented AIDS patients with either 3g HMB or a placebo for 8 weeks and observed a 2.55 ± 0.75 kg (mean ± SEM) increase in lean mass in the HMB group and a 0.70 ± 0.69 kg loss in lean mass in the placebo group, d = 1.02. Based on this study, we have determined that with α=0.05 and β=0.2, we will need approximately 15 patients per group to observe significant changes in lean mass between groups over time. We anticipated patient dropout due to extended illness or significant hospitalization based on previous studies in our lab. To accommodate a 20 - 25% drop-out rate, we aimed to recruit approximately 20 subjects to each group with the expectation that at least 15 subjects in each group would complete all testing.

Results:

Study population

MHD patients were recruited from hemodialysis clinics in Champaign and Chicago, IL. Details of study recruitment and enrollment can be found in Figure 1. In total, 33 participants (n=17 placebo, n=16 HMB) completed the 6 month intervention. The causes of end-stage renal disease in these patients were type 2 diabetes (n=14), hypertension (n=16), glomerular nephritis (n=2), and unknown (n=1). There were no significant differences between groups in participant demographics at baseline (Table 1). In addition, patient perception of their assigned group did not differ between groups (p=0.895), suggesting that participant blinding was adequate.

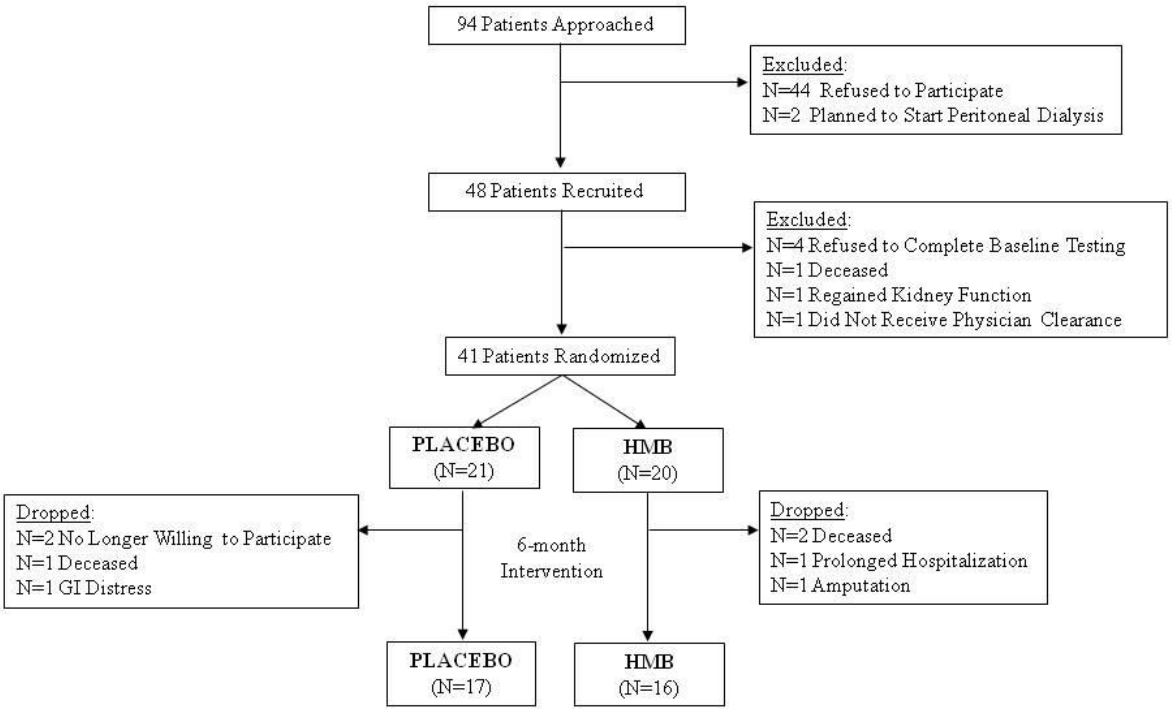

Figure 1. Study enrollment tree.

Table 1. Participant characteristics

Demographics	Placebo (n=17)	HMB (n=16)	P-value
Age (years)	53 (13)	57 (8)	0.262
Gender (male/female)	8 / 9	11 / 5	0.208
Smoking (%)	35.2 %	43.8 %	0.619
Diabetes (%)	70.6 %	43.8 %	0.119
Dialysis Vintage (months)	58 (35)	43 (44)	0.315
BMI (kg/m^2)	30.8 (6.4)	31.9 (7.0)	0.664
Albumin (g/dl)	4.0 (0.3)	3.8 (0.5)	0.134

Intent to treat analysis:

No group x time interaction was observed in any measure of body composition, bone density, physical function, or balance and gait parameters (Tables 2-4). However, a trend for improvement with the placebo was observed for sway velocity during standing balance in the dual task condition ($p=0.074$) and a trend for an increase in stride length during the normal walk condition with HMB supplementation was observed ($p=0.054$). There was a main effect of time for an improvement in sway velocity during the eyes closed condition and 95 percent ellipse area during the eyes open and eyes closed conditions, independent of group ($p<0.05$ for all).

No significant group x time interactions were observed in any domain of the quality of life questionnaire; however, satisfaction with care significantly declined over time, independent of group ($p<0.001$, Table 5). Central pulse wave velocity was 9.9 ± 2.2 m/s and 9.8 ± 2.3 m/s in the placebo and HMB groups, respectively, at baseline and did not significantly change throughout the intervention ($p=0.241$ for group x time interaction). No significant differences in standard blood lab values were observed (Table 6). However, blood urea nitrogen significantly increased over time independent of group ($p=0.016$). Dietary intake and physical activity did not differ between groups at baseline; however, there was a significant group x time interaction in energy and fat intake throughout the intervention indicating that the HMB group consumed fewer calories and protein at the end of the intervention while the placebo group did not change ($p<0.05$ for each, Table 7). Carbohydrate intake significantly declined over time independent of group ($p=0.002$).

Table 2. No significant differences in bone density or body composition were observed.

Measure	Placebo (n=17)		HMB (n=16)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
BMI (g/kg^2)	30.8 (6.4)	30.9 (6.9)	31.9 (7.0)	31.3 (6.8)	0.398	0.447
Arm Lean Mass (kg)	6.79 (1.76)	6.81 (1.30)	7.18 (2.05)	7.29 (2.07)	0.717	0.623
Leg Lean Mass (kg)	18.27 (4.61)	17.96 (4.02)	19.02 (5.02)	18.82 (4.82)	0.745	0.153
Appendicular Lean Mass (kg)	25.06 (5.97)	24.78 (5.18)	26.19 (6.93)	26.11 (6.77)	0.657	0.421
Whole Body Lean Mass (kg)	57.78 (11.65)	57.58 (10.04)	62.04 (13.24)	62.32 (13.27)	0.610	0.928
Whole Body Fat Mass (kg)	26.61 (13.40)	26.50 (13.26)	30.33 (12.67)	30.76 (13.01)	0.602	0.760
Whole Body Percent Fat (%)	30.13 (10.74)	30.00 (9.84)	31.84 (6.78)	32.08 (6.75)	0.673	0.902
Bone Mineral Content (kg)	2.37 (0.62)	2.37 (0.62)	2.38 (0.45)	2.36 (0.43)	0.520	0.167
Bone Mineral Density (g/cm^2)	1.14 (0.15)	1.14 (0.15)	1.10 (0.12)	1.10 (0.11)	0.468	0.229

A: time*treatment interaction; B: main effect of time

Table 3. No significant changes in physical function were observed with supplementation.

Measure	Placebo (n=17)		HMB (n=16)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Gait Speed (m/s)	0.91 (0.30)	0.92 (0.28)	0.93 (0.23)	0.98 (0.17)	0.282	0.161
Shuttle Walk Test (s)	272 (119)	255 (113)	228 (85)	232 (94)	0.085	0.306
Chair Stand (n)	11.6 (4.0)	10.9 (4.2)	10.2 (3.6)	10.2 (3.1)	0.426	0.426
Arm Curl (n)	16.9 (10.4)	17.4 (8.8)	17.3 (5.5)	18.4 (3.7)	0.681	0.293
Sit and Reach (in)	-2.3 (3.6)	-2.7 (3.7)	-2.5 (3.5)	-2.6 (4.7)	0.707	0.655
Shoulder Flexibility (in)	-3.7 (4.8)	-5.5 (5.6)	-7.3 (5.8)	-6.2 (7.0)	0.193	0.761
Up and Go (s)	6.6 (2.9)	7.0 (3.1)	7.1 (2.1)	6.7 (4.9)	0.434	0.989
Peak Knee Extension (Ft/lb)	79.7 (36.6)	75.9 (32.5)	83.6 (26.7)	84.4 (32.2)	0.335	0.521
Peak Knee Flexion (Ft/lb)	40.9 (22.0)	38.8 (19.1)	37.9 (12.2)	40.2 (15.4)	0.169	0.948

A: time*treatment interaction; B: main effect of time

Table 4. There were no significant differences between groups in standing balance or gait parameters throughout the intervention.

Measure	Placebo[C]		HMB[D]		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Force Plate Variables						
Ellipse Area – Eyes Open (cm^2)	12.59 (18.70)	10.10 (6.35)	6.24 (9.12)	3.02 (1.44)	0.849	0.149
Ellipse Area – Eyes Closed (cm^2)	7.45 (3.84)	5.17 (4.67)	5.12 (3.16)	4.25 (3.03)	0.345	**0.048**
Ellipse Area – Dual Task (cm^2)	3.64 (3.15)	4.26 (3.58)	5.27 (5.22)	5.26 (4.76)	0.663	0.673
Velocity – Eye Open (cm/s)	3.41 (1.80)	2.59 (1.70)	2.94 (0.95)	2.42 (0.77)	0.347	**<0.001**
Velocity – Eyes Closed (cm/s)	3.24 (0.65)	2.50 (1.06)	3.81 (1.49)	3.29 (1.15)	0.550	**0.003**
Velocity – Dual Task (cm/s)	2.68 (0.43)	2.18 (1.07)	2.81 (0.82)	2.83 (0.95)	0.074	0.103
Gait Mat Variables						
Stride Length – Normal Walk (cm)	126.76 (18.26)	121.89 (14.88)	121.50 (22.22)	124.10 (20.46)	0.054	0.545
Stride Length – Dual Task (cm)	118.94 (18.95)	122.08 (27.23)	111.70 (20.39)	114.38 (19.21)	0.902	0.130
Single Support – Normal Walk (%)	34.12 (2.27)	34.14 (2.42)	33.80 (2.76)	33.58 (4.12)	0.782	0.820
Single Support – Dual Task (%)	33.07 (2.41)	33.31 (4.11)	32.53 (3.28)	32.72 (3.06)	0.958	0.627
Double Support – Normal Walk (%)	31.73 (4.44)	31.89 (5.30)	32.56 (5.49)	33.27 (8.26)	0.769	0.643
Double Support – Dual Task (%)	34.50 (5.76)	34.56 (7.30)	35.18 (7.95)	34.94 (7.05)	0.831	0.901
Velocity – Normal Walk (cm/s)	110.00 (18.38)	103.43 (14.68)	104.01 (26.93)	107.13 (27.27)	0.093	0.537
Velocity – Dual Task (cm/s)	93.68 (15.54)	90.41 (19.14)	89.56 (24.07)	91.50 (25.46)	0.225	0.753
Cadence – Normal Walk (steps/min)	104.27 (10.40)	101.70 (7.52)	102.03 (10.52)	102.75 (9.55)	0.321	0.575
Cadence – Dual Task (steps/min)	95.10 (8.64)	90.30 (11.51)	95.69 (14.96)	94.84 (13.18)	0.353	0.189
Swing Time – Normal Walk (%CV)	3.57 (1.43)	4.27 (2.25)	9.11 (9.04)	11.30 (11.65)	0.478	0.179
Swing Time – Dual Task (%CV)	6.38 (5.14)	10.62 (13.41)	12.93 (10.70)	11.51 (11.04)	0.199	0.516

A: time*treatment interaction; B: main effect of time; C: force plate variables (n=8), gait mat variables (n=10); D: force plate variables (n=10), gait mat variables (n=13); CV: coefficient of variance

Table 5. No significant differences in quality of life were observed between groups, but patient satisfaction with care was reduced over time, independent of group.

Measure	Placebo (n=17)		HMB (n=16)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Burden	57.7 (27.6)	64.0 (28.8)	58.2 (30.9)	64.1 (27.9)	0.962	0.150
Cognitive	88.2 (16.6)	85.1 (18.9)	88.3 (13.9)	82.5 (23.1)	0.633	0.119
Social	82.4 (16.7)	81.2 (20.3)	82.5 (19.3)	79.6 (23.7)	0.784	0.521
Symptom	78.2 (16.8)	78.4 (16.2)	85.7 (9.4)	83.5 (10.7)	0.572	0.650
CKD Effect	73.5 (23.4)	72.1 (21.9)	74.6 (22.5)	74.4 (19.0)	0.860	0.818
Sleep	64.0 (24.4)	64.9 (23.4)	59.8 (21.7)	63.8 (23.7)	0.649	0.472
Support	82.4 (19.1)	78.4 (24.8)	77.1 (26.4)	84.4 (17.7)	0.205	0.700
Work Status	6.8 (16.4)	6.9(16.6)	32.1 (35.7)	32.1 (40.0)	0.994	0.994
Satisfaction[C]	69.6 (17.9)	56.9 (22.9)	81.3 (19.1)	62.5 (25.5)	0.458	**< 0.001**
Encouragement[D]	77.9 (22.3)	67.6 (36.5)	73.4 (35.9)	86.7 (15.5)	0.058	0.805

A: time*treatment interaction; B: main effect of time; C: satisfaction with care; D: staff encouragement

Table 6. No significant differences were observed in concentrations of standard lab values.

Measure	Placebo (n=17)		HMB (n=16)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Potassium (mEq/L)	4.5 (0.6)	4.8 (0.6)	4.9 (0.6)	5.1 (0.6)	0.587	0.064
Calcium (mg/dL)	9.3 (1.1)	9.1 (0.8)	8.9 (1.3)	9.9 (3.6)	0.304	0.498
Phosphorous (mg/dL)	5.2 (1.5)	4.9 (1.0)	6.0 (1.9)	5.3 (1.8)	0.558	0.119
Albumin (g/dL)	4.0 (0.3)	4.0 (0.4)	3.8 (0.5)	3.9 (0.4)	0.231	0.381
BUN (mg/dL)	49.9 (16.0)	53.3 (15.9)	56.7 (20.8)	68.5 (21.7)	0.180	**0.019**
Creatinine (mg/dL)	9.3 (3.8)	10.0 (4.0)	8.9 (2.7)	9.6 (2.6)	0.961	0.084
Hemoglobin (g/dL)	10.9 (1.0)	11.1 (1.2)	11.3 (1.1)	11.1 (1.1)	0.623	0.997
Hematocrit (%)	34.5 (3.1)	34.3 (3.9)	34.9 (3.5)	34.6 (3.1)	0.943	0.784
WBC (1000/μL)	5.5 (1.5)	5.4 (1.2)	5.7 (2.2)	5.9 (2.4)	0.622	0.983
CRP (mg/dl)	8.9 (9.0)	11.2 (10.8)	11.2 (15.4)	10.4 (10.9)	0.409	0.675

A: time*treatment interaction; B: main effect of time; BUN: blood urea nitrogen; WBC: White Blood Cell; CRP: C-reactive protein

Table 7. Changes in energy and fat consumption throughout the intervention differed between groups.

Measure	Placebo (n=17)		HMB (n=16)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Energy Consumed (kcal/day)	1795 (482)	1843 (850)	2154 (1069)	1578 (625)	**0.044**	0.087
Energy Consumed (kcal/kg/day)	21.7 (4.8)	22.9 (10.8)	24.4 (12.7)	17.7 (9.7)	**0.025**	0.118
Protein Consumed (g/day)	71.5 (23.8)	79.6 (42.6)	94.3 (46.1)	79.5 (31.8)	0.146	0.666
Protein Consumed (g/kg/day)	0.85 (0.22)	1.00 (0.56)	1.07 (0.55)	0.90 (0.51)	0.099	0.872
Carbohydrates Consumed (g/day)	233.3 (95.3)	201.9 (72.0)	235.7 (106.5)	179.9 (81.4)	0.357	**0.002**
Fat Consumed (g/day)	66.2 (16.8)	87.7 (53.9)	96.2 (73.3)	63.80 (25.33)	**0.024**	0.634
Energy Expended (kcal/kg/day)	34.47 (3.75)	34.18 (4.68)	32.47 (1.08)	32.15 (1.26)	0.970	0.426

A: time*treatment interaction; B: main effect of time

Treatment Compliance

Plasma HMB was analyzed through GC-MS, as previously described [174], to assess treatment compliance. Changes in plasma HMB levels of participants in the placebo group during the intervention can be seen in Figure 2. In the HMB group, 11 patients had elevated plasma HMB concentrations at 3 and 6 months and were labeled as compliant (Figure 3) while 5 patients did not have elevated plasma HMB concentrations at 3 and/or 6 months and were labeled to be non-compliant (Figure 4). Based on this data, a per-protocol analysis was performed using the 17 participants in the placebo group and 11 compliant HMB participants. Mean plasma HMB concentrations significantly differed between groups at 3 and 6 months in participants used in the per-protocol analysis ($p<0.001$ for each, Figure 5).

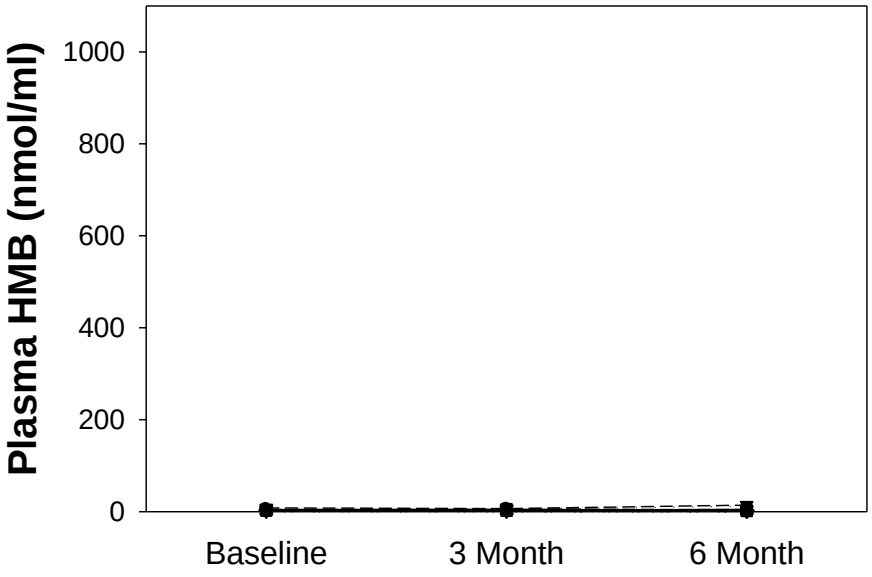

Figure 2. Plasma HMB concentrations in participants in the placebo group throughout the intervention.

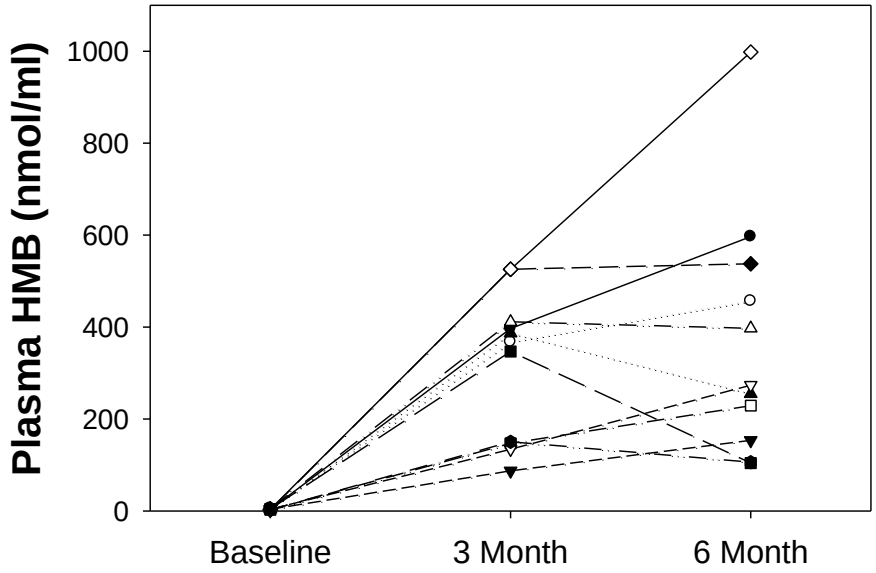

Figure 3. Plasma HMB concentrations in compliant individuals in the HMB group throughout the intervention.

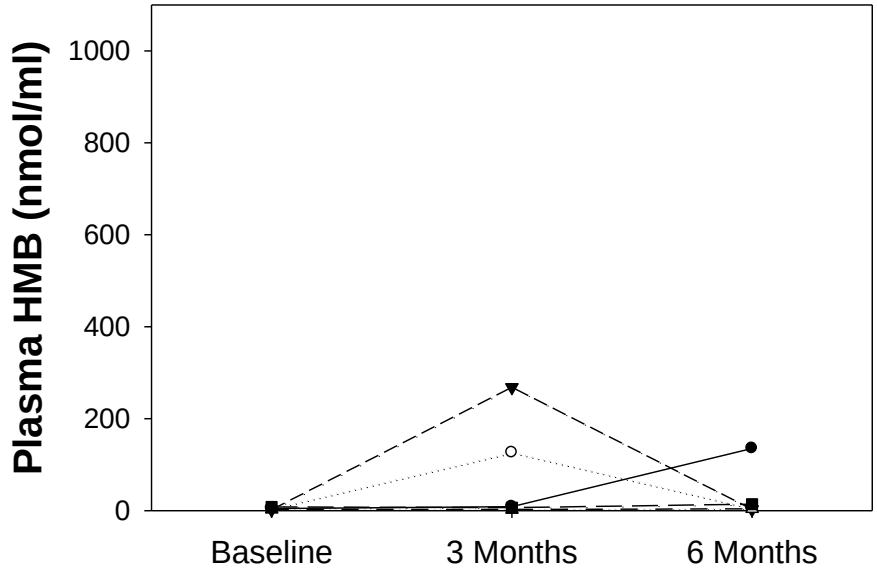

Figure 4. Plasma HMB concentrations in non-compliant individuals in the HMB group throughout the intervention.

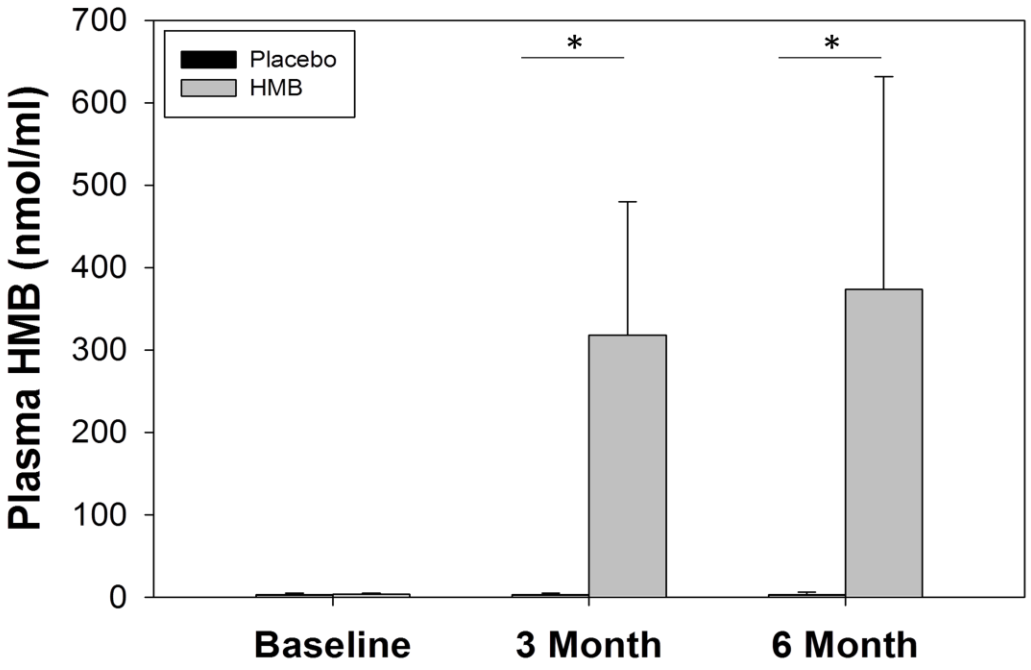

Figure 5. Mean plasma HMB concentrations significantly differed between groups at 3 and 6 months in participants used in per-protocol analysis (* $p<0.001$).

Per protocol analysis

In total, 28 patients were included in the per-protocol analysis (Placebo n=17, HMB n=11). The primary causes of end stage renal disease in these patients were type 2 diabetes (n=12), hypertension (n=15), and glomerular nephritis (n=1). Dialysis vintage was significantly lower in the HMB group than the placebo group (p=0.010); however, there were no other significant differences in patient characteristics between groups (Table 8). Perception of group assignment was not different between groups (p=0.954) suggesting that participant blinding was adequate.

Table 8. Participant characteristics in per-protocol analysis

Demographics	**Placebo (n=17)**	**HMB (n=11)**	**P value**
Age (years)	53 (13)	58 (9)	0.253
Gender (male/female)	8 / 9	7 / 4	0.390
Smoking (%)	35.2 %	45.5 %	0.591
Diabetes (%)	70.6 %	63.6 %	0.700
Dialysis Vintage (months)	58 (35)	30 (16)	**0.010**
BMI (kg/m^2)	30.8 (6.4)	32.1 (7.0)	0.634
Albumin (g/dl)	4.0 (0.3)	3.8 (0.5)	0.159

No group x time interaction was observed in any measure of body composition, bone density, physical function, or balance and gait parameters (Tables 9-11). However, a trend for an improvement in the chair stand ($p=0.094$), up-and-go (0.096), and shoulder flexibility tests (0.064) were observed with HMB supplementation. In addition, a trend for an increase in stride length during the normal walk condition with HMB supplementation was observed ($p=0.052$). Conversely, a trend for improvement with the placebo was observed for sway velocity during standing balance in the dual task condition ($p=0.074$). Sway velocity during the eyes closed condition and 95 percent ellipse area during the eyes open and eyes closed conditions significantly improved over time, independent of group.

No significant group x time interactions were observed in any domain of the quality of life questionnaire; however, satisfaction with care significantly declined over time, independent of group ($p<0.001$, Table 12). Aortic pulse wave velocity was 9.9 ± 2.2 m/s and 10.6 ± 2.2 m/s in the placebo and HMB groups, respectively, at baseline and did not significantly change throughout the intervention ($p=0.390$ for group x time interaction). No significant differences between groups over time were observed in standard lab values (Table 13). However, creatinine significantly increased independent of group ($p=0.046$). Dietary intake and physical activity did not differ between groups at baseline and no significant group x time interaction was observed in any dietary or physical activity measure (Table 14). However, carbohydrate intake significantly declined over time independent of group ($p=0.02$).

Table 9. No significant differences in bone density or body composition were observed.

Measure	Placebo (n=17)		HMB (n=11)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
BMI (g/kg^2)	30.8 (6.4)	30.9 (6.9)	32.1 (7.0)	31.1 (6.1)	0.200	0.226
Arm Lean Mass (kg)	6.79 (1.76)	6.81 (1.30)	6.75 (1.89)	6.89 (1.93)	0.695	0.614
Leg Lean Mass (kg)	18.27 (4.61)	17.96 (4.02)	18.41 (4.85)	18.39 (4.70)	0.492	0.412
Appendicular Lean Mass (kg)	25.06 (5.97)	24.78 (5.18)	25.17 (6.56)	25.28 (6.48)	0.463	0.740
Whole Body Lean Mass (kg)	57.78 (11.65)	57.58 (10.04)	61.03 (12.54)	61.86 (13.11)	0.340	0.554
Whole Body Fat Mass (kg)	26.61 (13.40)	26.50 (13.26)	31.89 (12.23)	31.20 (11.83)	0.576	0.439
Whole Body Percent Fat (%)	30.13 (10.74)	30.00 (9.84)	33.49 (5.94)	32.92 (5.57)	0.638	0.452
Bone Mineral Content (kg)	2.37 (0.62)	2.37 (0.62)	2.43 (0.47)	2.42 (0.46)	0.736	0.308
Bone Mineral Density (g/cm^2)	1.14 (0.15)	1.14 (0.15)	1.13 (0.12)	1.12 (0.11)	0.327	0.167

A: time*treatment interaction; B: main effect of time

Table 10. No significant changes in physical function were observed.

Measure	Placebo (n=17)		HMB (n=11)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Gait Speed (m/s)	0.91 (0.30)	0.92 (0.28)	0.93 (0.21)	1.00 (0.15)	0.257	0.144
Shuttle Walk Test (s)	272 (119)	255 (113)	215 (53)	210 (63)	0.358	0.105
Chair Stand (n)	11.6 (4.0)	10.9 (4.2)	8.9 (2.0)	9.7 (2.0)	0.094	0.851
Arm Curl (n)	16.9 (10.4)	17.4 (8.8)	17.3 (6.7)	18.0 (3.9)	0.891	0.488
Sit and Reach (in)	-2.3 (3.6)	-2.7 (3.7)	-2.9 (4.2)	-2.8 (5.1)	0.641	0.784
Shoulder Flexibility (in)	-3.7 (4.8)	-5.5 (5.6)	-8.7 (5.1)	-5.7 (8.3)	0.064	0.637
Up and Go (s)	6.6 (2.9)	7.0 (3.1)	7.3 (2.2)	5.8 (4.7)	0.096	0.351
Peak Knee Extension (Ft/lb)	79.7 (36.6)	75.9 (32.5)	83.4 (28.4)	86.4 (33.7)	0.212	0.872
Peak Knee Flexion (Ft/lb)	40.9 (22.0)	38.8 (19.1)	38.0 (12.8)	40.8 (16.0)	0.194	0.834

A: time*treatment interaction; B: main effect of time

Table 11. There were no significant differences between groups in standing balance or gait parameters throughout the intervention.

Measure	Placebo[C]		HMB[D]		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Force Plate Variables						
Ellipse Area – Eyes Open (cm^2)	12.59 (18.70)	10.10 (6.35)	3.62 (1.60)	3.30 (1.38)	0.450	0.333
Ellipse Area – Eyes Closed (cm^2)	7.45 (3.84)	5.17 (4.67)	5.23 (2.79)	3.49 (1.06)	0.747	**0.038**
Ellipse Area – Dual Task (cm^2)	3.64 (3.15)	4.26 (3.58)	6.77 (5.94)	5.99 (5.56)	0.395	0.923
Velocity – Eye Open (cm/s)	3.41 (1.80)	2.59 (1.70)	2.85 (1.18)	2.41 (0.97)	0.337	**0.005**
Velocity – Eyes Closed (cm/s)	3.24 (0.65)	2.50 (1.06)	3.90 (1.63)	3.37 (1.36)	0.612	**0.007**
Velocity – Dual Task (cm/s)	2.68 (0.43)	2.18 (1.07)	2.85 (1.00)	2.99 (1.14)	0.072	0.282
Gait Mat Variables						
Stride Length – Normal Walk (cm)	126.76 (18.26)	121.89 (14.88)	115.31 (17.73)	119.02 (15.51)	0.052	0.783
Stride Length – Dual Task (cm)	118.94 (18.95)	122.08 (27.23)	105.65 (16.75)	109.85 (15.97)	0.799	0.088
Single Support – Normal Walk (%)	34.12 (2.27)	34.14 (2.42)	33.07 (2.25)	32.77 (4.23)	0.747	0.780
Single Support – Dual Task (%)	33.07 (2.41)	33.31 (4.11)	31.73 (3.14)	32.00 (2.97)	0.982	0.623
Double Support – Normal Walk (%)	31.73 (4.44)	31.89 (5.30)	34.08 (4.40)	35.07 (8.32)	0.699	0.592
Double Support – Dual Task (%)	34.50 (5.76)	34.56 (7.30)	36.85 (8.07)	36.55 (7.02)	0.823	0.884
Velocity – Normal Walk (cm/s)	110.00 (18.38)	103.43 (14.68)	96.57 (21.25)	100.20 (17.89)	0.111	0.635
Velocity – Dual Task (cm/s)	93.68 (15.54)	90.41 (19.14)	82.45 (21.46)	85.35 (23.12)	0.193	0.936
Cadence – Normal Walk (steps/min)	104.27 (10.40)	101.70 (7.52)	100.20 (10.30)	100.89 (5.97)	0.371	0.603
Cadence – Dual Task (steps/min)	95.10 (8.64)	90.30 (11.51)	93.36 (16.40)	92.27 (13.65)	0.443	0.228
Swing Time – Normal Walk (%CV)	3.57 (1.43)	4.27 (2.25)	10.86 (9.68)	13.85 (12.23)	0.334	0.129
Swing Time – Dual Task (%CV)	6.38 (5.14)	10.62 (13.41)	15.84 (10.54)	14.11 (11.41)	0.238	0.615

A: time*treatment interaction; B: main effect of time; C: force plate variables (n=8), gait mat variables (n=10); D: force plate variables (n=6), gait mat variables (n=10); CV: coefficient of variance

Table 12. Patient satisfaction with care was significantly reduced over time, but no significant differences in quality of life were observed between groups.

Measure	Placebo (n=17)		HMB (n=11)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Burden	57.7 (27.6)	64.0 (28.8)	58.0 (25.6)	67.0 (26.5)	0.765	0.115
Cognitive	88.2 (16.6)	85.1 (18.9)	90.9 (12.0)	81.8 (24.4)	0.334	0.053
Social	82.4 (16.7)	81.2 (20.3)	87.3 (16.5)	77.0 (26.6)	0.187	0.100
Symptom	78.2 (16.8)	78.4 (16.2)	85.6 (9.9)	83.1 (10.8)	0.607	0.673
CKD Effect	73.5 (23.4)	72.1 (21.9)	78.1 (12.3)	74.7 (19.1)	0.813	0.552
Sleep	64.0 (24.4)	64.9 (23.4)	60.7 (23.4)	63.2 (28.4)	0.832	0.658
Support	82.4 (19.1)	78.4 (24.8)	84.8 (18.9)	87.9 (16.8)	0.472	0.926
Work Status	6.8 (16.4)	6.9(16.6)	32.5 (40.1)	37.1 (44.8)	0.595	0.586
Satisfaction[C]	69.6 (17.9)	56.9 (22.9)	84.8 (15.7)	60.6 (27.2)	0.209	**<0.001**
Encouragement[D]	77.9 (22.3)	67.6 (36.5)	81.8 (25.2)	87.5 (12.5)	0.153	0.675

A: time*treatment interaction; B: main effect of time; C: satisfaction with care; D: staff encouragement

Table 13. No significant differences were observed in concentrations of standard lab values.

Measure	Placebo (n=17)		HMB (n=11)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Potassium (mEq/L)	4.5 (0.6)	4.8 (0.6)	4.9 (0.6)	5.1 (0.7)	0.760	0.079
Calcium (mg/dL)	9.3 (1.1)	9.1 (0.8)	9.3 (0.6)	9.2 (1.0)	0.850	0.573
Phosphorous (mg/dL)	5.2 (1.5)	4.9 (1.0)	5.8 (2.1)	5.4 (1.9)	0.921	0.307
Albumin (g/dL)	4.0 (0.3)	4.0 (0.4)	3.8 (0.6)	3.9 (0.4)	0.334	0.501
BUN (mg/dL)	49.9 (16.0)	53.3 (15.9)	61.8 (18.4)	68.0 (25.3)	0.622	0.105
Creatinine (mg/dL)	9.3 (3.8)	10.0 (4.0)	8.4 (2.4)	9.5 (2.7)	0.578	**0.046**
Hemoglobin (g/dL)	10.9 (1.0)	11.1 (1.2)	11.1 (0.7)	10.7 (1.0)	0.389	0.679
Hematocrit (%)	34.5 (3.1)	34.3 (3.9)	34.4 (2.9)	33.5 (3.0)	0.735	0.611
WBC (1000/μL)	5.5 (1.5)	5.4 (1.2)	6.4 (1.6)	6.5 (2.5)	0.676	0.965
CRP (mg/dl)	8.9 (9.0)	11.2 (10.8)	14.1 (17.6)	13.2 (11.8)	0.484	0.738

A: time*treatment interaction; B: main effect of time; BUN: blood urea nitrogen; WBC: white blood cells; CRP: C-reactive protein

Table 14. Changes in diet and physical activity levels during the intervention did not differ between groups.

Measure	Placebo (n=17)		HMB (n=11)		P-Value[A]	P-Value[B]
	Baseline	6 Month	Baseline	6 Month		
Energy Consumed (kcal/day)	1795 (482)	1843 (850)	1713 (754)	1370 (566)	0.197	0.215
Energy Consumed (kcal/kg/day)	21.7 (4.8)	22.9 (10.8)	19.2 (9.0)	14.5 (5.5)	0.099	0.221
Protein Consumed (g/day)	71.5 (23.8)	79.6 (42.6)	78.3 (32.3)	65.6 (25.2)	0.337	0.566
Protein Consumed (g/kg/day)	0.85 (0.22)	1.00 (0.56)	0.90 (0.44)	0.70 (0.24)	0.145	0.581
Carbohydrates Consumed (g/day)	233.3 (95.3)	201.9 (72.0)	196.0 (92.3)	156.1 (80.2)	0.681	**0.020**
Fat Consumed (g/day)	66.2 (16.8)	87.7 (53.9)	69.1 (40.9)	57.8 (22.1)	0.112	0.724
Energy Expended (kcal/kg/day)	34.47 (3.75)	34.18 (4.68)	32.3 (1.25)	31.9 (1.26)	0.869	0.448

A: time*treatment interaction; B: main effect of time

Discussion:

Supplementation of beta-hydroxy-beta-methylbutyrate (HMB) has been previously shown to attenuate muscle loss in catabolic populations such as cancer patients [11], AIDS patients [12], and the elderly [10]. Maintenance hemodialysis (MHD) patients represent another catabolic population that could benefit from HMB supplementation; however, the present study does not support its efficacy. Supplementation with HMB for 6 months did not improve any marker of body composition, strength, physical function, balance and gait parameters, quality of life, or inflammation in either intent-to-treat or per-protocol analysis. However, it should be noted that the per-protocol analysis was underpowered and trends for improvement of markers of physical function (chair stand, up-and-go test, and shoulder flexibility) were observed with HMB supplementation in this analysis.

MHD patients are a catabolic population reported to lose as much as 1-3 kg lean mass annually [2]. Previous nutritional interventions aimed at attenuating this rapid decline in muscle mass have been largely unsuccessful [112, 113, 116]. HMB has been shown to increase muscle mass in other catabolic populations. Increases of 0.5kg and 0.55kg after six months [183] and one year [10], respectively, in the elderly, 2.6kg in 8 weeks in AIDS patients [12], and 1kg in 24 wks in cancer patients [11] have been observed. Moreover, HMB attenuated muscle loss during 10 days of bed rest in the elderly [184]. However, in the present study, no significant changes in whole body or regional lean mass were observed after 6 months of HMB supplementation in either intent-to-treat or per-protocol analysis. This finding is similar to previous studies in gastric bypass [154] and rheumatoid arthritis patients [153] which also did not observe changes in lean mass with HMB supplementation. A potential limitation lean body mass assessment in the present study is that interdialytic fluid shifts in MHD patients may have affected the accuracy

of DXA measurements [185]. However, all patients were tested 18-24hrs following hemodialysis treatment. In addition, all baseline and 6 month testings were performed on the same day of the week at the same time of day to account for fluid changes.

In addition to loss of skeletal muscle mass, MHD patients have been shown to have reduced strength compared to age-matched healthy controls [5, 23, 99]. HMB has been previously shown to improve muscle strength in the elderly in 12 weeks [142, 183]; however, in the current study no changes in quadriceps or hamstring muscle strength were observed with HMB supplementation in either intent-to-treat or per-protocol analysis. One previous study suggested that changes in muscle strength with HMB supplementation may be vitamin D dependent [143]. Vitamin D deficiency is prevalent in as many as 92 percent of MHD patients and as a result a majority of patients receive supplemental vitamin D as part of standard care [186]. However, in the present study, we were unable to control for changes in vitamin D prescription throughout the intervention which may have effected changes in muscle strength observed in this intervention. In addition, it should also be noted that increases in strength have not been observed in all previous studies with HMB in elderly and clinical populations. Marcora et al. [153] supplemented rheumatoid arthritis patients with either HMB or placebo for 12 weeks and did not observe an increase in handgrip, elbow flexor, or knee extensor strength suggesting that HMB may not increase muscle strength in all clinical populations.

The effects of HMB supplementation on physical function in elderly and clinical populations have provided mixed results. Flakoll et al. [142] observed an increase in up-and-go test time after 12 weeks of HMB supplementation in the elderly; however, Marcora et al. [153] did not observe any change in sit-to-stand test following 12 weeks of HMB supplementation in rheumatoid arthritis patients. In MHD patients, physical function is reduced compared to healthy

controls, even in high functioning MHD patients [99]; therefore, interventions to improve physical function are needed in this population. After 6 months of HMB supplementation in MHD patients, no significant change was observed in any measurement of physical function in the intent-to-treat analysis. However, a trend for improvements in chair stand, up-and-go, and shoulder flexibility tests were observed with HMB supplementation in the per-protocol analysis. It should be noted that although the intent-to-treat analysis was adequately powered, the per-protocol analysis was slightly underpowered. Therefore, it is possible these trends may have been significant given adequate statistical power. Future studies are needed to conclusively determine the effects of HMB supplementation on physical function in MHD patients.

MHD patients have a greater postural sway [187], worse gait [188], and an increased dual task cost [100] compared to age, gender, and BMI matched healthy controls. This results in a significantly elevated fall risk and contributes to a hip fracture risk of around 4-fold higher than the general population [189, 190]. Previous interventions with HMB in elderly and clinical populations have not examined the effects of HMB supplementation on fall risk. In the present study, we did not observe any significant differences in any balance or gait parameter measured. However, a limitation of this analysis is that it was only performed on a subsection of participants limiting statistical power. In addition, several force plate variables significantly improved over time in both the intent-to-treat and per-protocol analysis, regardless of group, suggesting a learning effect. Although the present study does not support the use of HMB for fall risk prevention in MHD patients, future studies are needed examine interventions to decrease falls in this high-risk population.

Quality of Life is significantly lower in hemodialysis patients than in the general population [105]. Previous studies examining the effects of HMB on quality of life in clinical

and elderly populations have primarily shown no effect [10, 11, 153]. Similarly, using a quality of life questionnaire previously validated in kidney disease patients [179], we did not observe a group x time interaction in any domain of the quality of life questionnaire. However, we observed a significant decrease in satisfaction with care over time, independent of group, in both intent-to-treat and per-protocol analysis suggesting that patient dislike for hemodialysis treatment increases the longer they are on dialysis.

Although a previous short-term study did not observe any adverse effects of HMB supplementation [14], the long-term safety of HMB supplementation has previously not been determined in MHD patients. In the present study, we did not observe any significant differences in standard plasma laboratory measures between patients supplemented with HMB or placebo. However, it is possible that the additional calcium in the calcium-HMB supplement may result in negative effects on arterial health. Use of calcium based phosphate binders (approximately 1.5g calcium/day from binders) has been shown to result in increased vascular calcification [191]. However, it is unclear if the approximately 400 mg calcium present in the calcium HMB supplement exhibits similar effects on the vasculature. In addition, it should be noted that large scale clinical trials have not observed a survival benefit in patients taking non-calcium based phosphate binders compared with those receiving calcium based phosphate binders (as reviewed by [192]). To address these concerns we measured aortic pulse wave velocity, a measure of arterial stiffness, and standard blood laboratory measures of calcium and phosphorous at baseline and after 6 months of the intervention. No significant differences in arterial stiffness or standard lab parameters were observed throughout the intervention suggesting that HMB supplementation did not result in increased vascular calcification and arterial stiffness in MHD patients.

There are several potential reasons for the discrepancies in results between the beneficial effects of HMB observed in many of the previous studies in elderly and clinical populations and the lack of effect of HMB in the current study. First, many of the studies the observed beneficial effects of HMB supplementation on lean mass, strength, and physical function supplemented with a cocktail of HMB along with other amino acids [10-12, 142, 143] while we supplemented with HMB alone. The absence of additional amino acids in our supplementation protocol may have contributed to differences in outcomes. However, not all studies supplementing a cocktail of HMB and amino acids in elderly and clinical populations have observed beneficial effects of supplementation [153, 154]. In addition, supplementation of HMB alone has been found to increase lean mass and strength in 24 weeks in the elderly [183], preserve muscle mass during 10 days of bed rest in the elderly [184], reduce inflammation in patients with chronic obstructive pulmonary disease [152], reduce nitrogen loss in elderly patients in the intensive care unit receiving tube feeding [140], attenuate lean mass loss and strength loss in animal models of aging [193] and unloading [157], and activate anabolic pathways while inhibiting catabolic pathways in skeletal muscle (as reviewed by [13]). Therefore, it is unlikely that the absence of amino acids in the supplementation cocktail in the current study affected study results.

MHD patients in the present study were not as catabolic as previously reported in this population. Pupim et al. observed a mean reduction of lean mass of 1.1 kg in non-diabetic and 3.4 kg in malnourished (average albumin 3.4 g/dl) MHD patients during the first year of dialysis [2]. However, in the placebo group we observed a 0.2 kg reduction in lean mass in 6 months with a mean albumin of 4.0 g/dl. Therefore, it is possible that better nutritional status in our population did not allow us to observe significance in outcomes of interest over the study period.

Patients in this study were instructed to maintain dietary and physical activity patterns throughout the study. However, in the intent-to-treat analysis there was a group x time interaction for a reduction in energy and fat intake with a trend for a reduction in protein intake in the HMB group. There was also a significant reduction in carbohydrate intake over time regardless of group. In the per-protocol analysis, there were no significant group x time interactions in nutrient intake or energy expenditure; however, the significant reduction in carbohydrate intake over time, independent of group remained. It is possible that the reductions in calorie and protein intake in the HMB group over the course of the intervention blunted potential benefits from HMB supplementation. In addition, we were unable to control for changes in medication or dialysis prescription during the study period which may have affected outcomes.

Although no effect of HMB supplementation on lean mass was observed in the present study, single nutrient interventions to combat lean mass in MHD patients have primarily shown no effect [112, 113, 116, 176]. Moreover, recent randomized clinical trials assessing the effects of either resistance and/or endurance exercise training on metrics related to physical function, body composition, and CVD risk have been somewhat equivocal [8, 84, 194-197]. This is likely due to the complex nature of muscle loss in this population resulting from a number of causes such as: metabolic acidosis, inflammation, insulin resistance, decreased nutrient intake, hormonal abnormalities, an elevated metabolic rate, and loss of amino acids into the dialysate (as reviewed by [30, 31]). Future interventions in this population likely need to provide a more comprehensive approach in order to successfully attenuate muscle loss in MHD patients.

A limitation of this study is the small sample size in this intervention. Initial power analysis indicated a sample size of at least 15 patients per group would be needed in order to

have appropriate power to detect changes in lean mass. Therefore, the intent-to-treat protocol in this study was adequately powered with n=16 HMB and n=17 placebo. However, it was discovered that 5 participants in the HMB group were no compliant. Therefore a per-protocol analysis with n=11 HMB and n=17 placebo was run; however, this analysis was underpowered. Despite this, we observed trends for improvements in chair stand, up and go test, and shoulder flexibility tests in the per-protocol analysis which require further investigation in a study appropriately powered with compliant participants.

Pill compliance is a major hurdle for clinicians when treating MHD patients. On average, MHD patients take 10-12 different types of medication per day, with a median pill intake approximately 19 pills/day [198, 199]. Moreover, 25 percent of patients take over 25 pills daily [199]. This high level of pill burden leads to rates of non-compliance with prescription medication as high as 50-80 percent, depending upon the method used to measure compliance [200]. The non-compliance observed in the HMB group in this study occurred in 31 percent of patients which is likely lower than previously reported for prescription drugs due to patients being reminded 2-3 times weekly to consume pills and/or selection bias of more compliant patients who volunteered for study participation.

A number of factors have been reported to lead to the high rates of non-compliance observed in this population including: water intake restrictions, pill taste, pill size, frequency of pill consumption, number of pills consumed, low income, low education, depression, cognitive impairment, recreational drug use, a poor social support system, poor satisfaction with care, and cultural beliefs [201, 202]. In addition, the more pills patients consume daily, the lower the compliance [203]. Therefore, attempts to reduce pill burden are needed to increase pill compliance in this population. Phosphorus binders represent approximately one-half of total

daily pill intake in MHD patients [199] and could be reduced through dietary modification. Dietary phosphorus is classified as organic, found animal and plant sources, or inorganic, added to foods to increase shelf-life and palatability [204]. Organic phosphorus from animal and plant sources is absorbed at a rate of 40-60 and 20-40 percent, respectively, while inorganic phosphorus is absorbed at a rate of 100 percent. Therefore, inorganic phosphorus has a significantly larger effect of blood phosphorus levels and is a potential target for dietary intervention. Future studies are needed to determine if dietary interventions to reduce inorganic phosphorus intake reduce pill burden and increase pill compliance in MHD patients.

In summary, 6 months of HMB supplementation did not result in any significant changes in body composition, strength, physical function, fall risk, or quality of life in this population in the intent-to-treat analysis. A trend for an improvement in chair stand, up-and-go, and shoulder flexibility tests was observed in the per-protocol analysis. However, the per-protocol analysis was underpowered due to low pill compliance in this population. Future studies are needed to improve pill compliance and attenuate muscle and strength losses to improve quality of life in this critically ill patient population.

Conclusion

Six month of beta-hydroxy-beta-methylbutyrate (HMB) supplementation did not result in any significant changes in body composition, bone density, strength, physical function, fall risk, or quality of life in maintenance hemodialysis (MHD) patients in an adequately-powered intent-to-treat analysis. Upon analysis of plasma HMB concentrations, 31 percent of participants in the HMB group were found to be non-compliant, likely due to the large number of oral prescription medications MHD patients consume daily. Therefore we performed a per-protocol analysis using only participants who were compliant throughout the study. Similarly to intent-to-treat analysis, no significant effects of HMB supplementation on outcomes of interest were observed in the per-protocol analysis. However, a trend for improvement in several markers of physical function was observed with HMB supplementation in per-protocol analysis, despite being underpowered. Taken together, these results do not support the use of HMB attenuation of co-morbid disease conditions in MHD patients; however, future studies are needed to reduce pill burden and improve pill compliance in this population.

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