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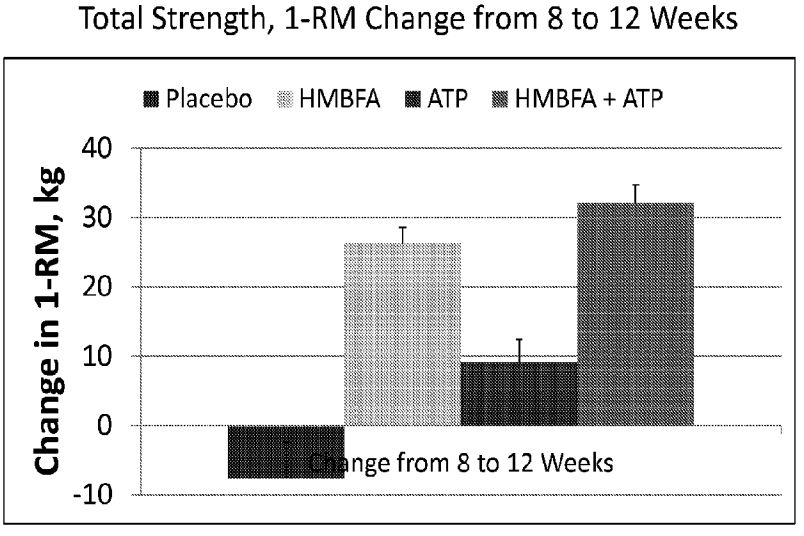
(54) Titre : COMPOSITION D'HMB ET D'ATP ET PROCEDES D'UTILISATION
(54) Title: COMPOSITION OF HMB AND ATP AND METHODS OF USE



Main Effects: HMB p=0.0001; ATP, p=0.0009; HMB*ATP, p=0.11

(57) Abrégé/Abstract:
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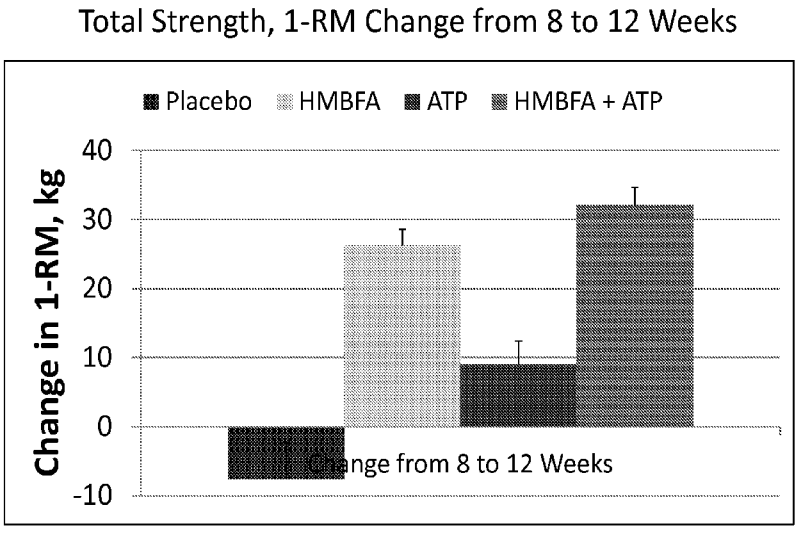
(54) Title: COMPOSITION OF HMB AND ATP AND METHODS OF USE

Fig. 2



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Composition of HMB and ATP and Methods of Use

[1] *This paragraph removed intentionally.*

Background of the Invention

Field

[2] The present invention relates to a composition comprising β-hydroxy-β-methylbutyrate (HMB) and adenosine-5'-triphosphate (ATP), and methods of using a combination of HMB and ATP to improve strength and power, improve muscle mass and prevent or lessen typical declines in performance characteristic of overreaching.

Background

HMB

[3] The only product of leucine metabolism is ketoisocaproate (KIC). A minor product of KIC metabolism is β-hydroxy-β-methylbutyrate (HMB). HMB has been found to be useful within the context of a variety of applications. Specifically, in U.S. Patent No. 5,360,613 (Nissen), HMB is described as useful for reducing blood levels of total cholesterol and low-density lipoprotein cholesterol. In U.S. Patent No. 5,348,979 (Nissen et al.), HMB is described as useful for promoting nitrogen retention in humans. U.S. Patent No. 5,028,440 (Nissen) discusses the usefulness of HMB to increase lean tissue development in animals. Also, in U.S. Patent No. 4,992,470 (Nissen), HMB is described as effective in enhancing the immune response of mammals. U.S. Patent No. 6,031,000 (Nissen et al.) describes use of HMB and at least one amino acid to treat disease-associated wasting.

[4] HMB is an active metabolite of the amino acid leucine. The use of HMB to suppress proteolysis originates from the observations that leucine has protein-sparing characteristics. The

essential amino acid leucine can either be used for protein synthesis or transaminated to the α-ketoacid (α-ketoisocaproate, KIC). In one pathway, KIC can be oxidized to HMB. Approximately 5% of leucine oxidation proceeds via the second pathway. HMB is superior to leucine in enhancing muscle mass and strength. The optimal effects of HMB can be achieved at 3.0 grams per day, or 0.038g/kg of body weight per day, while those of leucine require over 30.0 grams per day.

[5] Once produced or ingested, HMB appears to have two fates. The first fate is simple excretion in urine. After HMB is fed, urine concentrations increase, resulting in an approximate 20-50% loss of HMB to urine. Another fate relates to the activation of HMB to HMB-CoA. Once converted to HMB-CoA, further metabolism may occur, either dehydration of HMB-CoA to MC-CoA, or a direct conversion of HMB-CoA to HMG-CoA, which provides substrates for intracellular cholesterol synthesis. Several studies have shown that HMB is incorporated into the cholesterol synthetic pathway and could be a source for new cell membranes that are used for the regeneration of damaged cell membranes. Human studies have shown that muscle damage following intense exercise, measured by elevated plasma CPK (creatine phosphokinase), is reduced with HMB supplementation within the first 48 hrs. The protective effect of HMB lasts up to three weeks with continued daily use. Numerous studies have shown an effective dose of HMB to be 3.0 grams per day as CaHMB (calcium HMB) (~38 mg/kg body weight-day^{-1}). This dosage increases muscle mass and strength gains associated with resistance training, while minimizing muscle damage associated with strenuous exercise (34) (4, 23, 26). HMB has been tested for safety, showing no side effects in healthy young or old adults. HMB in combination with L-arginine and L-glutamine has also been shown to be safe when supplemented to AIDS and cancer patients.

[6] Recently, HMB free acid, a new delivery form of HMB, has been developed. This new delivery form has been shown to be absorbed quicker and have greater tissue clearance than CaHMB. The new delivery form is described in U.S. Patent Publication Serial No. 20120053240.

ATP

[7] Adenosine-5'-triphosphate (ATP) has long been known as the chemical energy source for tissues including muscle (19). Intracellular ATP concentrations (1- 10 m*M*) are quite high in contrast to extracellular concentrations (10-100nM) and therefore release of ATP from cells such as erythrocytes and muscle is strictly controlled. More recently extracellular effects of ATP, acting through purinergic receptors found in most cell types, have been elicited (20). Several extracellular physiological functions of ATP have been described including vasodilation (21), reduced pain perception (22), and as a neurotransmission cotransmitter (23, 24). Importantly, small and transient increases in vascular ATP in muscle can cause vasodilation and an increase in blood flow to the muscle (25). Therefore, if ATP increases blood flow to muscle, especially during periods of strenuous resistance training, substrate availability would be improved and removal of metabolic waste products would be better facilitated. Ellis et al recently reviewed the studies supporting the role of ATP in increasing muscle blood flow through purinergic signaling and neurotransmission (25).

[8] ATP has been shown to have an inotrophic effect ATP on cardiac muscle (26, 27). Another study supporting systemic effects of ATP demonstrated that oral administration of ATP to rabbits for 14 days resulted in a reduction in peripheral vascular resistance, improvement of cardiac output, reduction of lung resistance, and increased arterial PaO_2 (28).

[9] Adenosine, resulting from the degradation of ATP, may also act as a signaling agent through purinergic receptors (29) or may be degraded by adenosine deaminase (30). Adenosine acting through purinergic receptors can essentially mimic the effects of ATP (29). Adenosine infusion into muscle results in increased nitric oxide formation and similar vascular effects as seen with ATP infusion (31).

[10] Fatigue resistance in repeated high intensity bouts of exercise is a much sought after attribute in athletics. This is true for both augmentation of training volume, as well as sustained force and power output in intermittent sports such as hockey. During fatiguing contractions acute adaptations in blood flow occur to stave off declines in force generating capacity (40, 45). There is a tight coupling between oxygen demand in skeletal muscle and increases in blood flow (45). Research suggests that it is red blood cells that regulate this response by acting as "oxygen sensors" (45). ATP is carried in red blood cells and when oxygen is low in a working muscle region, the red blood cell deforms resulting in a cascade of events which lead to ATP release and binding to endothelial cells in smooth muscle (43). Binding results in smooth muscle relaxation and subsequent increases in blood flow, nutrient and oxygen delivery (43). Specifically, extracellular ATP directly promotes the increased synthesis and release of nitric oxide (NO) and prostacyclin (PGI_2) within skeletal muscle and therefore directly affects tissue vasodilation and blood flow (31). This is supported by research suggesting increased vasodilation and blood flow in response to intra-arterial infusion (47) and exogenous administration of ATP. These changes in blood flow likely lead to an increased substrate pool for skeletal muscle by virtue of increased glucose and O_2 uptake (42) . The outcome is maintenance of energy status in the cell under fatiguing contractions. (54, 56)

[11] The physiological effects of ATP have led researchers to investigate the efficacy of oral supplementation of ATP (24). Jordan et al. (32) demonstrated that 225 mg per day of enteric-coated ATP supplementation for 15 days resulted in increased total bench press lifting volume (i.e. sets•repetitions•load) as well as within-group set-one repetitions to failure. More recently, Rathmacher et al. (52) found that 15 days of 400 mg per day of ATP supplementation increased minimum peak torque in set two of a knee extensor bout. Collectively the results discussed indicate that ATP supplementation maintains performance and increases training volume under high fatiguing conditions. However, greater fatigue increases recovery demands between training sessions.

[12] Current evidence suggests that HMB acts by speeding regenerative capacity of skeletal muscle following high intensity or prolonged exercise (3). When training and/or diet are controlled, HMB can lower indices of skeletal muscle damage and protein breakdown in a dose-dependent fashion (50, 3, 2). Recently, HMB in a free acid form (HMB-FA) has been developed with improved bioavailability (18). Initial studies have shown that this form of HMB supplementation results in approximately double the plasma levels of HMB in about one-quarter the time after administration when compared with the presently available form, calcium HMB.

Further, HMB-FA given 30 minutes prior to an acute bout of high volume resistance training was able to attenuate indices of muscle damage and improve perceived recovery in resistance trained athletes (61). Moreover acute ingestion of 2.4 grams of HMB-FA increases skeletal muscle protein synthesis and decreases protein breakdown by +70 % and – 56 % respectively (58).

[13] A need exists for a composition and methods to increase strength and power and improve muscle mass. In addition, a need exists for a composition that prevents or lessens the typical

decay seen in performance following an overreaching cycle. The present invention comprises a composition and methods of using a combination of ATP and HMB that results in these improvements.

Summary of the Invention

[14] One object of the present invention is to provide a composition for use in increasing strength and power.

[15] A further object of the present invention is to provide a composition for use in improving muscle mass.

[16] Another object of the present invention is to provide methods of administering a composition for increasing strength and power.

[17] An additional object of the present invention is to provide methods of administering a composition for improving muscle mass.

[18] Another object of the present invention is to provide a composition for use in preventing or lessening decay seen in performance following an overreaching cycle.

[19] These and other objects of the present invention will become apparent to those skilled in the art.

[20] The present invention intends to overcome the difficulties encountered heretofore. To that end, a composition comprising HMB and ATP is provided. The composition is administered to an animal in need thereof. All methods comprise administering to the animal HMB and ATP.

[20.1] A composition is described herein comprising from about 0.5 g to about 30 g of β-hydroxy-β-methylbutyric acid (HMB) and from about 10mg to about 80g of adenosine triphosphate (ATP).

[20.2] A method is described herein for providing a benefit to an animal in need thereof, which benefit is selected from the list consisting of increasing strength, increasing power, improving muscle mass, and lessening declines in performance characteristic of overreaching. The method involves administering to the animal a composition comprising an effective amount of HMB and ATP. In

certain embodiments, the amount of HMB administered is from about 0.5 g to about 30 g, and the amount of ATP administered is from about 10 mg to about 80 g of ATP. Optionally, the HMB administered may be HMB-acid or the HMB administered may be a salt.

[20.3] The composition may be provided by a route of administration selected from the group consisting of oral, parenteral, sublingual, topical, transdermal, intramuscular, and inhalation. When orally administered, the delivery form may be selected from the group consisting of tablet, capsule, powder, granule, microgranule, pellet, soft-gel, controlled-release form, liquid, solution, elixir, syrup, suspension, emulsion and magma.

[20.4] A method is described herein for increasing strength of an animal in need thereof comprising the steps of administering to said animal HMB and ATP in amounts sufficient to increase strength, wherein upon said administration of HMB and ATP to the animal, said strength is increased.

[20.5] A method for increasing power of an animal in need thereof is also described herein, which comprises the steps of administering to said animal HMB and ATP in amounts sufficient to increase power, wherein upon said administration of HMB and ATP to the animal, said power is increased.

[20.6] Further, a method for improving the muscle mass of an animal in need thereof is described herein, which comprises the steps of administering to said animal HMB and ATP in amounts sufficient to improve muscle mass, wherein upon said administration of HMB and ATP to the animal, said muscle mass is improved.

[20.7] Additionally, a method for lessening declines in a performance characteristic of overreaching for an animal in need thereof is described, comprising the steps of administering to said animal HMB and ATP in amounts sufficient to lessen said declines in performance, wherein upon said administration of HMB and ATP to the animal, said declines in performance are lessened.

Brief Description of the Figures

[21] Fig. 1 is a schemata of phases of the training program listing variables and the time points of measurement throughout the study.

[22] Fig. 2 shows total strength, 1-RM Change from 8 10 12 weeks.

[23] Figs. 3a-c show changes in squat strength and bench press strength.

[24] Figs. 4a and 4b show the percent increase in vertical jump power and Wingate peak power.

Detailed Description of the Invention

[25] It has been surprisingly and unexpectedly discovered that a combination of HMB and ATP results in greater increases in strength, power and muscle mass than use of either HMB or ATP alone. The present invention comprises a combination of HMB and ATP that has a synergistic effect and increases strength and power. The present invention also comprises a combination of HMB and ATP that has the unexpected and surprising results of improving muscle mass. The present invention also comprises a combination of HMB and ATP that has the unexpected and surprising result of preventing or lessening typical decay seen in performance following an overreaching cycle. The combination of HMB and ATP results in significant enhancements.

[26] This combination can be used on all age groups seeking increases in strength and power, increases in muscle mass, and prevention or lessening of typical decay seen in performance following an overreaching cycle.

[27] In view of the above, in one embodiment the present invention provides a composition comprising HMB and ATP.

HMB

[28] β-hydroxy-β-methylbutyric acid, or β-hydroxy-isovaleric acid, can be represented in its free acid form as $(CH_3)_2(OH)CCH_2COOH$. The term "HMB" refers to the compound having the foregoing chemical formula, in both its free acid and salt forms, and derivatives thereof. While any form of HMB can be used within the context of the present invention, preferably HMB is

selected from the group comprising a free acid, a salt, an ester, and a lactone. HMB esters include methyl and ethyl esters. HMB lactones include isovalaryl lactone. HMB salts include sodium salt, potassium salt, chromium salt, calcium salt, magnesium salt, alkali metal salts, and earth metal salts.

[29] Methods for producing HMB and its derivatives are well-known in the art. For example, HMB can be synthesized by oxidation of diacetone alcohol. One suitable procedure is described by Coffman et al., *J. Am. Chem. Soc.* 80: 2882-2887 (1958). As described therein, HMB is synthesized by an alkaline sodium hypochlorite oxidation of diacetone alcohol. The product is recovered in free acid form, which can be converted to a salt. For example, HMB can be prepared as its calcium salt by a procedure similar to that of Coffman et al. (1958) in which the free acid of HMB is neutralized with calcium hydroxide and recovered by crystallization from an aqueous ethanol solution. The calcium salt of HMB is commercially available from Metabolic Technologies, Ames, Iowa.

Calcium β-hydroxy-β-methylbutyrate (HMB) Supplementation

[30] More than 2 decades ago, the calcium salt of HMB was developed as a nutritional supplement for humans. Numerous studies have shown that CaHMB supplementation improves muscle mass and strength gains in conjunction with resistance-exercise training, and attenuates loss of muscle mass in conditions such as cancer and AIDS (1-5). Nissen and Sharp performed a meta-analysis of supplements used in conjunction with resistance training and found that HMB was one of only two supplements that had clinical studies showing significant increases in strength and lean mass with resistance training (1). Studies have shown that 38 mg of CaHMB per kg of body weight appears to be an efficacious dosage for an average person (6).

[31] In addition to strength and muscle mass gains, CaHMB supplementation also decreases indicators of muscle damage and protein degradation. Human studies have shown that muscle damage following intense exercise, measured by elevated plasma CPK (creatine phosphokinase), is reduced with HMB supplementation. The protective effect of HMB has been shown to manifest itself for at least three weeks with continued daily use (6-8) *In vitro* studies in isolated rat muscle show that HMB is a potent inhibitor of muscle proteolysis (9) especially during periods of stress. These findings have been confirmed in humans; for example, HMB inhibits muscle proteolysis in subjects engaging in resistance training (3).

[32] The molecular mechanisms by which HMB decreases protein breakdown and increases protein synthesis have been reported (10, 11). Eley et al conducted *in vitro* studies which have shown that HMB stimulates protein synthesis through mTOR phosphorylation (11, 12). Other studies have shown HMB decreases proteolysis through attenuation of the induction of the ubiquitin-proteosome proteolytic pathway when muscle protein catabolism is stimulated by proteolysis inducing factor (PIF), lipopolysaccharide (LPS), and angiotension II (10, 13, 14). Still other studies have demonstrated that HMB also attenuates the activation of caspases-3 and -8 proteases (15). Taken together these studies indicate that HMB supplementation results in increased lean mass and the accompanying strength gains through a combination of decreased proteolysis and increased protein synthesis.

HMB Free Acid form

[33] In most instances, the HMB utilized in clinical studies and marketed as an ergogenic aid has been in the calcium salt form (3, 16). Recent advances have allowed the HMB to be manufactured in a free acid form for use as a nutritional supplement. Recently, a new free acid

form of HMB was developed, which was shown to be more rapidly absorbed than CaHMB, resulting in quicker and higher peak serum HMB levels and improved serum clearance to the tissues (18).

[34] HMB free acid may therefore be a more efficacious method of administering HMB than the calcium salt form, particularly when administered directly preceding intense exercise. HMB free acid initiated 30 min prior to an acute bout of exercise was more efficacious in attenuating muscle damage and ameliorating inflammatory response than CaHMB. One of ordinary skill in the art, however, will recognize that this current invention encompasses HMB in any form.

[35] HMB in any form may be incorporated into the delivery and/or administration form in a fashion so as to result in a typical dosage range of about 0.5 grams HMB to about 30 grams HMB.

Adenosine-5'-triphosphate (ATP)

[36] Supplementation with adenosine-5'-triphosphate (ATP) has been used to elevate extracellular ATP levels. Studies have failed to show consistent positive effects of ATP to improve strength or power when combined with resistance-training exercise; however, small and transient increases in systemic ATP have been shown to increase blood flow in muscle tissue.

[37] Oral administration of ATP is usually in the form of Adenosine-5'-Triphospate Disodium. In the present invention, Adenosine-5'-Triphosphate Disodium or any form of ATP or adenosine suitable for oral administration may be combined with any of the known coatings suitable for imparting enteric properties in granular form.

[38] ATP may be incorporated into the delivery and/or administration form in a fashion so as to result in a typical dosage range of about 10mg to about 80 grams, though more or less may be desirable depending on the application and other ingredients.

[39] The composition of HMB and ATP is administered to an animal in any suitable manner. Acceptable forms include, but are not limited to, solids, such as tablets or capsules, and liquids, such as enteral or intravenous solutions. Also, the composition can be administered utilizing any pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and examples of such carriers include various starches and saline solutions. In the preferred embodiment, the composition is administered in an edible form. In addition, an effective dosage range may be administered in divided dosages, such as two to three times per day.

ATP and HMB Combination

[40] Any suitable dose of HMB can be used within the context of the present invention. Methods of calculating proper doses are well known in the art. The dosage amount of HMB can be expressed in terms of corresponding mole amount of Ca-HMB. The dosage range within which HMB may be administered orally or intravenously is within the range from 0.01 to 0.5 grams HMB (Ca-HMB) per kilogram of body weight per 24 hours. For adults, assuming body weights of from about 100 to 200 lbs., the dosage amount orally or intravenously of HMB (Ca-HMB basis) can range from 0.5 to 30 grams per subject per 24 hours.

[41] ATP is present in the composition in any form. A range of ATP in the present invention includes ATP in the amount of around 10 milligrams to around 80 grams.

[42] When the composition is administered orally in an edible form, the composition is preferably in the form of a dietary supplement, foodstuff or pharmaceutical medium, more preferably in the form of a dietary supplement or foodstuff. Any suitable dietary supplement or foodstuff comprising the composition can be utilized within the context of the present invention. One of ordinary skill in the art will understand that the composition, regardless of the form (such

as a dietary supplement, foodstuff or a pharmaceutical medium), may include amino acids, proteins, peptides, carbohydrates, fats, sugars, minerals and/or trace elements.

[43] In order to prepare the composition as a dietary supplement or foodstuff, the composition will normally be combined or mixed in such a way that the composition is substantially uniformly distributed in the dietary supplement or foodstuff. Alternatively, the composition can be dissolved in a liquid, such as water.

[44] The composition of the dietary supplement may be a powder, a gel, a liquid or may be tabulated or encapsulated.

[45] Although any suitable pharmaceutical medium comprising the composition can be utilized within the context of the present invention, preferably, the composition is combined with a suitable pharmaceutical carrier, such as dextrose or sucrose.

[46] Furthermore, the composition of the pharmaceutical medium can be intravenously administered in any suitable manner. For administration via intravenous infusion, the composition is preferably in a water-soluble non-toxic form. Intravenous administration is particularly suitable for hospitalized patients that are undergoing intravenous (IV) therapy. For example, the composition can be dissolved in an IV solution (e.g., a saline or glucose solution) being administered to the patient. Also, the composition can be added to nutritional IV solutions, which may include amino acids, peptides, proteins and/or lipids. The amounts of the composition to be administered intravenously can be similar to levels used in oral administration. Intravenous infusion may be more controlled and accurate than oral administration.

[47] Methods of calculating the frequency by which the composition is administered are well-known in the art and any suitable frequency of administration can be used within the context of the present invention (e.g., one 6 g dose per day or two 3 g doses per day) and over any suitable

time period (e.g., a single dose can be administered over a five minute time period or over a one hour time period, or, alternatively, multiple doses can be administered over an extended time period). The combination of HMB and ATP can be administered over an extended period of time, such as weeks, months or years.

[48] It will be understood by one of ordinary skill in the art that HMB and ATP do not have to be administered in the same composition to perform the claimed methods. Stated another way, separate capsules, pills, mixtures, etc. of ATP and of HMB may be administered to a subject to carry out the claimed methods.

[49] Any suitable dose of HMB can be used within the context of the present invention. Methods of calculating proper doses are well known in the art. Likewise, any suitable dose of ATP can be used within the context of the present invention. Methods of calculating proper doses are well known in the art.

[50] In general, an amount of HMB and ATP in the levels sufficient to increase strength and power is described. Both HMB free acid alone and HMB free acid plus ATP supplementation increased strength and power gains greater than those observed with placebo supplementation ($p < 0.001$, treatment*time). Surprisingly, post hoc analysis showed that HMB plus ATP supplementation significantly further improved strength and power gains over those for HMB supplementation alone ($p < 0.05$). The following experimental examples indicate that HMB does have a positive effect on strength, power, and muscle mass and reduces muscle damage while aiding in recovery. Surprisingly, the combination of HMB plus ATP resulted in even greater improvement in strength and power compared to HMB alone and these effects are synergistic. Additionally, the HMB-ATP combination also demonstrated surprising and unexpected effects on muscle mass and declines in performance that are characteristic of overreaching.

Experimental Examples

[51] The following examples will illustrate the invention in further detail. It will be readily understood that the composition of the present invention, as generally described and illustrated in the Examples herein, could be synthesized in a variety of formulations and dosage forms. Thus, the following more detailed description of the presently preferred embodiments of the methods, formulations and compositions of the present invention are not intended to limit the scope of the invention, as claimed, but it is merely representative of the presently preferred embodiments of the invention.

[52] In the examples, overreaching is an increase in training volume and/or intensity of exercise resulting in performance decrements. Recovery from this condition often requires a few days to a week or more. Many structured training programs utilize phases of overreaching to induce an adaptive response.

[53] Lean body mass (LBM) and hypertrophy increases are used as indicators of improving muscle mass.

[54] *Study Design*

[55] The current study was a randomized, double-blind, placebo- and diet-controlled experiment consisting of 12 weeks of periodized resistance training. The training protocol was divided into 3 phases (Tables 1, 2, and 3). Phase 1 consisted of a non-linear periodized resistance training program (3 times per week) modified from Kraemer et al. (36) (Table 1).

Table 1. Phase 1 of the training cycle (Daily Undulating Periodization).

Monday	Wednesday	Friday
Squat	Squat	Squat (5 sets)
Barbell Bench Press	Bench	Barbell Bench Press (5 sets)
Deadlifts Pull Ups/ Dips (Superset)	Deadlift	Deadlifts Pull Ups/Dumbbell Shoulder Press (Superset)
Bent Over Row		Bent Over Row
Dumbbell Shoulder Press		Dumbbell Shoulder Press
Barbell Curl/ Triceps Extension (Superset)		Barbell Curls/ Triceps Extension (Superset)
Repetition, Set Schema	**Repetition, Set Schema**	**Repetition, Set Schema**
3 sets	5 sets	3 sets *(Squat and Bench= 5 sets)
8-12 RM loads	5 maximal intended velocity repetitions	3-5 RM loads
60 seconds timed rest	180 seconds timed rest	240 seconds timed rest

Phase 2 (Table 2) consisted of a two week overreaching cycle.

Table 2. Phase 2 of the training cycle (Overreaching).

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Squat	Leg Press	Squat	Leg Press	Squat 1 RM	Wingate and Maximal Power Testing
Barbell Bench Press	Barbell Bench Press	Barbell Bench Press	Barbell Bench Press	Bench Press 1 RM	
Deadlifts	Military Press	Deadlifts	Military Press	Deadlift 1 RM	
Pull Ups/ Dips (Superset)	Supinated Pull Ups/ Dips (Superset)	Pull Ups/ Dips (Superset)	Supinated Pull Ups/ Dips (Superset)		
Bent Over Row	Bent Over Row	Bent Over Row	Bent Over Row		
Dumbbell Shoulder Press	Hammer Curls/ Close Grip Bench (Superset)	Dumbbell Shoulder Press	Hammer Curls/ Close Grip Bench (Superset)		
Barbell Curl/ Triceps Extension (Superset)		Barbell Curl/ Triceps Extension (Superset)			
Repetition, Set Schema	**Repetition, Set Schema**	**Repetition, Set Schema**	**Repetition, Set Schema**	**Repetition, Set Schema**	
3 sets	3 sets	3 sets	3 sets	3 Maximal Attempts (Highest Counted as 1RM)	
8 RM loads	8 RM loads	12 RM loads	12 RM loads	1 RM load	
60 seconds timed rest	60 seconds timed rest	60 seconds timed rest	60 seconds timed rest	5 minutes timed rest	
75% 1 RM	75% 1 RM	65% 1 RM	65% 1 RM		

Finally, phase 3 consisted of a tapering of the training volume for weeks 11 and 12 (Table 3).

Table 3. Phase 3 of the training cycle (Taper).

Week 11			Week 12		
Monday	**Wednesday**	**Friday**	**Monday**	**Wednesday**	**Friday**
Squat	Squat (3 sets)	Squat	Squat (3 sets)	Squat	Squat 1 RM
Barbell Bench Press	Barbell Bench Press (3 sets)	Barbell Bench Press	Barbell Bench Press (3 sets)	Barbell Bench Press	Bench Press 1 RM
Deadlifts	Deadlifts	Deadlifts	Deadlifts	Deadlifts	Deadlift 1 RM
	Pull Ups/Dumbbell Shoulder Press (Superset) Bent Over Row		Pull Ups/Dumbbell Shoulder Press (Superset) Bent Over Row		
	Dumbbell Shoulder Press		Dumbbell Shoulder Press		
	Barbell Curls/ Triceps Extension (Superset)		Barbell Curls/ Triceps Extension (Superset)		
Repetition, Set Schema	**Repetition, Set Schema**	**Repetition, Set Schema**	**Repetition, Set Schema**	**Repetition, Set Schema**	**Repetition, Set Schema**
5 sets	1 set *(Squat and Bench= 3 sets)	5 sets	1 set *(Squat and Bench= 3 sets)	5 sets	3 Maximal Attempts (Highest Counted as 1RM)
5 maximal intended velocity repetitions	3-5 RM loads	5 maximal intended velocity repetitions	3-5 RM loads	5 maximal intended velocity repetitions	1 RM load
180 seconds timed rest	240 seconds timed rest	180 seconds timed rest	240 seconds timed rest	180 seconds timed rest	5 minutes timed rest

40-60% 1RM	> 90 % 1 RM	40-60% 1RM	> 90 % 1 RM	40-60% 1RM	

[56] Muscle mass, body composition, strength, power, resting plasma testosterone, cortisol concentrations, and creatine kinase were examined collectively at the end of weeks 0, 4, 8, 9, 10, and 12 to assess the chronic effects of HMB-ATP; these were also assessed at the end of weeks 9 and 10, corresponding to the mid- and endpoints of the phase 2 overreaching cycle. An overview of the study design is summarized in Figure 1.

Participants

[57] Forty resistance-trained males aged 23.0 ± 0.9 years with an average squat, bench press, and deadlift of 1.7 ± 0.04, 1.3 ± 0.04 and 2.0 ± 0.05 times their bodyweight were recruited for the study. Subject characteristics are represented in Table 4. Participants could not participate if they were currently taking anti-inflammatory agents, any other performance-enhancing supplement, or if they smoked. Each participant signed an informed consent approved by the University of Tampa Institutional Review Board before participating in the study.

Table 4. Subject Descriptors.

	Treatments			
	Placebo	HMB-FA	ATP	HMB-FA plus ATP
N	10	11	11	8
Age, y	23.0±1.2	21.3±0.6	23.7±0.9	21.4±0.3
Body Weight, kg	87.4±4.3	83.1±2.8	85.7±1.7	81.9±2.1
Height, cm	180.6±2.3	179.0±2.1	179.0±1.0	177.2±1.3
Body Mass Index	26.6±0.7	25.9±0.7	26.7±0.4	26.1±0.6

Muscle Strength, Power, Body Composition and Skeletal Muscle Hypertrophy Testing

[58] After familiarization with procedures, muscle strength was assessed via 1RM testing of the back squat, bench press, and deadlift. Each lift was performed as described by the International Powerlifting Federation rules (44). Body composition (lean body mass, fat mass, and total mass) was determined by dual x-ray absorptiometry (DXA; Lunar Prodigy enCORE 2008, Madison, Wisconsin, U.S.A.). Skeletal muscle hypertrophy was determined via the combined changes in ultrasonography-determined muscle thickness of the vastus lateralis (VL) and vastus intermedius (VI) muscles. The intraclass correlation coefficient (ICC) for the test-retest of muscle thickness measurements was r=0.97.

[59] Muscle power was assessed during maximal cycling (Wingate Test) and jumping movements. During the cycling test, volunteers were instructed to cycle against a predetermined resistance (7.5% of body weight) as fast as possible for 10 seconds (36). The saddle height was adjusted to the individual's height to produce a 5–10° knee flexion while the foot was in the low position of the central void. A standardized verbal stimulus was provided to each participant. Power output was recorded in real time during the 10-second sprint test, by a computer connected to the standard cycle ergometer (Monark model 894e, Vansbro, Sweden). Peak power (PP) was recorded using Monark Anaerobic Wingate Software, Version 1.0 (Monark, Vansbro, Sweden). The ICC of muscle peak power was 0.96.

[60] Measurements of PP were also taken during a vertical jump (VJ) test performed on a multi-component AMTI force platform (Advanced Mechanical Technology, Inc., Watertown, MA), interfaced with a personal computer at a sampling rate of 1000 Hz (51). Data acquisition software (LabVIEW, version 7.1; National Instruments Corporation, Austin, TX) was used to calculate PP. Peak power was calculated as the peak combination of ground reaction force and peak velocity during the accelerated launch on the platform. The ICC of VJ power was 0.97.

Supplementation, Diet Control, and Exercise Protocol

[61] Prior to the study, participants were randomly assigned to receive either 3 g per day of HMB Free Acid (HMB) (combined with food-grade orange flavors and sweeteners), 400 mg per day of ATP (PEAK ATP®; TSI, Inc.), a combination of both 3g of HMB and 400mg per day of ATP, or a placebo (food-grade orange flavors and sweeteners) divided equally into three servings daily with the first serving given 30 minutes prior to exercise and the remaining two servings daily with the mid-day and evening meals. On the non-training days participants were instructed to consume one serving with each of three separate meals. The supplementation was continued daily throughout the training and testing protocols. Each serving was formulated with 1 gram of HMB free acid to account for fill and emptying variation and achieve a minimum effective dosage of 0.800 grams. This dosage would be equivalent to a 1 gram Ca-HMB dosage.

[62] The participants must not have taken any nutritional supplements for at least three months prior to the start of data collection. Two weeks prior to and throughout the study, participants were placed on a diet consisting of 25% protein, 50% carbohydrates, and 25% fat by a registered dietician who specialized in sports nutrition. The participants met as a group with the dietitian, and they were given individual meal plans at the beginning of the study. Diet counseling was continued on an individual basis throughout the study.

[63] All participants performed a high volume resistance training protocol during the 12-week study. The phases of the study and measurements taken are shown in Figure 1, and the exercise protocols for each phase of the study are shown in Tables 1 to 3. The training was divided into 3 phases, with Phase 1 consisting of daily undulating periodization (weeks 1 to 8), Phase 2 consisting of the overreaching cycle (weeks 9 and 10), and Phase 3 consisting of the taper cycle (weeks 11 and 12).

Resting Blood Draws

[64] All blood draws throughout the study were obtained via venipuncture after a 12-hour fast by a trained phlebotomist. Whole blood was collected and transferred into appropriate tubes for obtaining serum and plasma and centrifuged at 1,500 g for 15 min at 4°C. Resulting serum and plasma were then aliquoted and stored at -80°C until subsequent analyses.

Biochemical Analysis

[65] Samples were thawed one time and analyzed in duplicate for each analyte. All blood draws were scheduled at the same time of day to negate confounding influences of diurnal hormonal variations. Serum total and free testosterone, cortisol, and C-reactive protein (CRP) were assayed via ELISA kits obtained from Diagnostic Systems Laboratories (Webster, TX). All hormones were measured in the same assay on the same day to avoid compounded interassay variance. Intra-assay variance was less than 3% for all analytes. Serum creatine kinase (CK) was measured using colorimetric procedures at 340 nm (Diagnostics Chemicals, Oxford, CT). Twenty-four hour urine collections were made and 3-methylhistine was determined by previously described methods (Rathmacher et al 1992 and Wilson et al, 2013).

Perceived Recovery Status Scale

[66] Perceived Recovery Status (PRS) scale was measured at weeks 0, 4, 8, 9, 10, and 12 to assess subjective recovery during the training phases. The PRS scale consists of values between 0-10, with 0-2 being very poorly recovered and with anticipated declines in performance, 4-6 being low to moderately recovered and expected similar performance, and 8-10 representing high perceived recovery with expected increases in performance.

Statistics

[67] A one-way ANOVA model was used to analyze the baseline characteristic data using the Proc GLM procedure in SAS (Version 9.1,SAS Institute, Cary, NC)[1] (SAS Institute, Inc. (1985) SAS User's Guide: Statistics, 5th ed. Cary, NC: SAS Institute, Inc.). The main effect of treatment (Trt) was included in the model. Muscle strength and power, body composition, muscle damage, hormonal status, and perceived recovery score (PRS) changes over the 12-week study were analyzed with a 2x2 factorial, repeated measures ANOVA using the Proc Mixed procedure in SAS. The initial value, week 0, was used as a covariate with the main effects of HMB, ATP, and Time, and the interactions of HMB*time, ATP*time, and HMB*ATP*time in the model. The overreaching cycle of the study was also assessed by using the 2x2 factorial, repeated measures ANOVA with the Proc Mixed procedure in SAS; however, the value measured at the week 8 time point was used as a covariate with the main effects of HMB, ATP, and Time, and the interactions of HMB*time, ATP*time and HMB*ATP*time. The Least Squares Means procedure was then used to compare treatment means at each time point (post-hoc t-test). Statistical significance was determined at $p \leq 0.05$ and trends were determined between $p > 0.05$ and $p \leq 0.10$.

Results

Participant Characteristics

[68] There were no differences in age (Placebo = 23.0±1.2, ATP = 23.7±0.9 yrs., HMB= 22.3±0.6, HMB-ATP=22.4 ± 0.5), height (Placebo = 180.6±2.3, ATP = 179.0±1.0 cm, HMB= 179.3 ± 2.1, HMB-ATP=180.0 ± 1.4), or body mass (Placebo = 87.4±4.3, ATP = 85.7±1.7, HMB= 83.1 ±1.6, HMB-ATP=84.6 ± 2.2) among the treatments at the start of the study.

Muscle Strength and Power

[69] At weeks 0, 4, 8, 9, 10, and 12 during the study muscle strength (1-RM of squat, bench press, and deadlift) and muscle power (vertical jump and Wingate Peak Power, PP) were measured; both muscle strength and power increased over the 12-week study (Time, $p < 0.001$). Supplementation with HMB, ATP and the HMB-ATP combination increased total strength gains by 77.1±5.6, 55.3±6.0, and 96.0±8.2 kg, respectively, compared with the placebo-supplemented participants who gained 22.4±7.1 kg in total strength over the 12-week study (t-test, $p < 0.05$). Figures 2 and 3a-c show the synergistic effect of HMB and ATP on strength. Figure 2 shows total strength changes from weeks 8-12. Figures 3a-c show individual indicators of the synergistic combination, including squat strength and bench press strength from weeks 4-8 and 4-12.

[70] During the overreaching cycle in weeks 9 and 10, total strength declined in the placebo-supplemented participants by -4.5±0.9% from weeks 8 to 10. Total strength decreased to a lesser extent in the ATP-supplemented subjects by -2.±0.5% from week 8 to week 10 and at week 10 the ATP-supplemented participants had increased total strength compared with the placebo-supplemented participants (*t*-test, $p < 0.05$). During the overreaching cycle, HMB-supplementation attenuated the decrease in total strength (-0.5±1.2 %, *t*-test, $p < 0.05$ vs. placebo) and the HMB-ATP supplemented subjects unexpectedly continued to increase in strength (1.2±0.7 %, *t*-test, $p < 0.05$ versus placebo).

[71] Muscular power was assessed using both the vertical jump and Wingate PP tests and results are shown in Figures 4a and 4b, respectively. Both of these measures of power were significantly increased during the study with HMB (HMB*time, $p < 0.001$ for both) and with ATP supplementation (ATP*time, $p < 0.001$ and $p < 0.04$ for vertical jump power and Wingate PP, respectively, Figures 4A and 4B). Over the 12-weeks of training vertical jump power

increased 614±52, 991±51, 796±75, and 1076±40 watts in placebo, HMB, ATP, and HMB-ATP supplemented groups, respectively (t-test $p < 0.05$). The percentage increases in vertical jump power were synergistic with HMB and ATP supplemented in combination (HMB*ATP*time, $p < 0.004$, Figure 4a). Vertical jump power during the overreaching cycle decreased more in the placebo group, 5.0±0.4%, compared with the smaller decreases in vertical jump power for the HMB, ATP, and HMB-ATP supplemented groups, 1.4±0.4, 2.2±0.4, and 2.2±0.5%, respectively, over weeks 9 and 10 (t-test, $p < 0.05$, Figure 4A). During the 2-week overreaching cycle, Wingate PP decreased by 4.7±1.5, 0.3±0.9, 2.9±0.7, and 2.0±0.9% in the placebo, HMB, ATP, and HMB-ATP supplemented groups, respectively (Figure 4B). After the first week of the increased training, HMB, ATP, and HMB-ATP supplementation resulted in the participants maintaining greater Wingate PP power than the placebo supplemented group with gains in power of 10.2±1.6, 9.0±1.6, and 14.5±1.2% from baseline, respectively (t-test, $p < 0.05$). However, after the second week of the overreaching cycle only the HMB-ATP supplemented group had maintained significantly greater Wingate PP than the placebo-supplemented group, 1022±21 and 940±66 watts, respectively (t-test, $p < 0.05$, Figure 4B).

Body Composition and Muscle Hypertrophy

[72] Resistance training resulted in increased lean body mass (LBM) and quadriceps thickness (Time, $p < 0.001$) whereas, fat percentage was decreased with the training, (Time, $p < 0.001$) at weeks 0, 4, 8, and 12. Supplementation with HMB increased body weight, LBM, and quadriceps thickness and decreased body fat (HMB*time, $p < 0.03$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively) whereas ATP supplementation increased LBM and quadriceps thickness (ATP*time, $p < 0.01$ and 0.04, respectively). Lean body mass was increased in an additive manner by 2.1±0.5, 7.4±0.4, 4.0±0.4, and 8.5±0.8 kg in placebo, HMB, ATP, and HMB-ATP

supplemented participants, respectively (*t*-test, $p < 0.05$, Table 5), and fat percentage decreased by 7.0±0.6 and 8.5±0.9% in HMB and HMB-ATP supplemented participants, respectively (*t*-test $p < 0.05$). Only the HMB supplementation was shown to have a significant effect on fat percentage (HMB*time $p < 0.001$). There was no main effect of ATP*time during the study on body weight; however, the ATP alone supplemented group did have a greater body weight by week 12 of the study than the placebo-supplemented group (*t*-test, $p < 0.05$). The 12-week increases in quadriceps thicknesses were 2.5±0.6, 7.1±1.2, 4.9±1.0, and 7.8±0.4 mm in placebo, HMB, ATP, and HMB-ATP supplemented participants, respectively, and HMB, ATP, and HMB-ATP supplementation resulted in a greater 12 week quadriceps thickness compared with placebo supplementation (*t*-test, $p < 0.05$, Table 5).

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Table 5: Effect of Beta-hydroxy-Beta-methylbutyrate free acid (HMB-FA) and adenosine-5'-triphosphate (ATP) supplementation on weight, lean body mass (LBM), percent body fat, and quadriceps muscle thickness in subjects performing a 12 week weight training regimen.[a]

	Week of Study				Main Effects[b]		
	0	4	8	12	HMB-FA*Time	ATP*Time	HMB-FA*ATP*Time
Weight, kg							
Placebo	87.4±4.3	88.3±4.6	88.7±4.8	87.7±4.7			
HMB-FA	83.1±2.8	83.9±2.8	84.8±2.9	85.0±3.0#	0.03	0.63	0.42
ATP	85.7±1.7	86.9±2.0	87.0±2.0	87.0±2.1#			
HMB Plus ATP	81.9±2.1	82.9±1.9	83.4±1.9	83.6±1.9#			
DXA LBM, kg							
Placebo	68.5±2.6	70.0±2.3	71.2±2.4	70.5±2.4			
HMB-FA	66.2±2.6	69.2±2.7#	71.3±2.7#	73.5±2.7#	0.001	0.01	0.80
ATP	67.7±2.0	70.1±1.9	71.4±2.0	71.7±1.9#			
HMB Plus ATP	67.0±1.2	70.5±1.3#	72.5±1.6#	75.4±1.5#			
DXA Fat, %							
Placebo	21.0±1.1	19.8±1.6	18.6±1.9	18.6±1.7			
HMB-FA	20.4±1.4	17.6±1.4	15.9±1.5#	13.5±1.5#	0.001	0.28	0.99
ATP	19.5±1.8	18.1±1.8	16.6±1.6	16.0±1.5			
HMB Plus ATP	18.0±1.9	14.7±2.2#	12.7±2.5#	9.5±2.2#			
Quad, mm							
Placebo	50.2±2.1	52.2±2.3	52.6±2.4	52.7±2.4			
HMB-FA	50.7±1.5	53.6±1.4	56.0±1.4#	57.8±1.6#	0.001	0.04	0.45
ATP	50.9±0.9	53.4±1.3	54.8±1.7	55.8±1.8#			
HMB Plus ATP	50.5±1.2	53.9±1.2	57.0±1.2#	58.3±1.1#			

Muscle Damage, Hormonal Status and Performance Recovery Scale

[73] Muscle damage was assessed by blood CK, which was increased by training, particularly after the changes in training volume at the initiation of the study and at weeks 9 and 10 during the overreaching cycle (Table 6; Time, $p < 0.001$). The initial training resulted in a 342±64% increase and the two-week overreaching cycle resulted in a 159±55% increase in CK levels in the placebo-supplemented group. Supplementation with HMB significantly attenuated the increase in CK at both the initiation of training (weeks 0 to 1) and during the overreaching cycle (weeks 9 and 10) (HMB*time, $p < 0.001$). Supplementation with ATP alone did not attenuate the increases in CK compared with the placebo supplementation; however, HMB-ATP supplementation resulted in a significant attenuation in CK increase compared with placebo at weeks 1, 4, 9, and 10 that was similar to the effect of HMB supplementation alone (*t*-test, $p < 0.05$).

[74] The rate of muscle protein degradation was evaluated by measuring urinary 3-MH:Cr ratio (Table 6).

Table 6. Effect of Beta-hydroxy-Beta-methylbutyrate free acid (HMB-FA) and adenosine-5'-triphosphate (ATP) supplementation blood creatine kinase (CK), C-reactive protein (CRP), cortisol, free and total testosterone, lactate dehydrogenase (LDH) and perceived recovery score (PRS) in subjects performing a 12 week weight training regimen [a]

	Week of Study							Main Effects[b]		
	0	1	4	8	9	10	12	HMB-FA*Time	ATP*Time	HMB-FA*ATP*Time
CK, IU/L										
Placebo	141±12	582±77	373±13	246±29	484±52	528±72	187±21			
HMB-FA	158±16	322±35#	280±22#	255±28	288±18#	250±14#	147±15	0.001	0.89	0.85
ATP	145±8	500±71	324±14	234±32	426±44	449±62	160±20			
HMB/ATP	162±31	310±42#	232±30#	212±22	262±23#	269±31#	169±15			
24 h 3MH:Cr, µmol:mg										
Placebo	0.127±0.007	0.130±0.003		0.123±0.004	0.134±0.005	0.152±0.005				
HMB-FA	0.127±0.004	0.122±0.002		0.124±0.008	0.120±0.003	0.141±0.004		0.05	0.009	0.005
ATP	0.136±0.008	0.127±0.007		0.143±0.007#	0.143±0.008	0.131±0.012#				
HMB/ATP	0.121±0.007	0.153±0.009#		0.131±0.008	0.131±0.009	0.142±0.005				
CRP, mg/L										
Placebo	1.9±0.7	1.1±0.1	1.3±0.3	2.0±0.7	1.6±0.7	1.2±0.2	1.6±0.4			
HMB-FA	1.0±0.1	1.3±0.3	1.0±0.1	0.9±0.01	1.0±0.1	1.8±0.8#	1.1±0.1	0.08	0.95	0.92
ATP	1.4±0.4	1.1±0.1	1.2±0.2	1.9±0.6	1.7±0.6	1.1±0.1	1.2±0.2			
HMB/ATP	1.2±0.2	1.6±0.6	1.1±0.2	1.1±0.2	0.9±0.06	1.1±0.1	1.0±0.1			
Cortisol, µg/dL										
Placebo	19.7±1.1	20.8±1.3	19.0±1.2	19.2±0.4	22.0±0.4	23.6±0.3	20.3±0.6			
HMB-FA	21.5±1.4	20.3±1.2	20.9±1.0	18.8±1.5	19.6±1.1#	18.6±1.2#	17.4±1.2#	0.001	0.78	0.77
ATP	20.9±1.2	20.5±1.3	18.4±1.4	19.0±0.4	21.5±0.4	22.6±0.2	19.7±0.6			
HMB/ATP	20.4±1.2	18.2±2.2#	17.7±1.6	17.2±1.5	19.1±1.0#	17.8±1.9#	16.8±1.4#			
Free Testosterone, ng/dL										
Placebo	103±13	112±10	119±6	111±9	98±6	100±9	113±12			
HMB-FA	109±10	104±8	116±11	115±9	118±8	116±8	127±8	0.21	0.96	0.76
ATP	112±13	114±9	118±6	117±11	108±7	110±10	125±13			
HMB/ATP	90±5	98±5	116±10	103±14	102±11	115±12	118±9			
Total Testosterone, ng/dL										
Placebo	591±73	620±58	625±55	585±58	551±46	536±88	605±72			
HMB-FA	708±35	708±48	730±63	652±35	752±61#	701±34	728±39	0.18	0.93	0.93
ATP	660±67	645±54	695±60	645±60	621±49	592±84	673±69			
HMB/ATP	568±39	583±34	636±49	533±51	581±71	617±37	655±27			
PRS[c]										

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Placebo	9.1±0.3	4.7±0.4	7.0±0.3	7.6±0.2	4.8±0.3	4.4±0.3	7.6±0.2	0.001	0.79	0.06
HMB-FA	9.1±0.3	6.3±0.3[#]	7.6±0.3	8.5±0.3[#]	8.0±0.2[#]	7.7±0.2[#]	9.5±0.2[#]			
ATP	9.6±0.2	4.9±0.4	7.5±0.3	8.2±0.3	5.5±0.4	5.5±0.4[#]	8.6±0.4[#]			
HMB/ATP	9.6±0.2	6.6±0.3[#]	8.4±0.2[#]	8.5±0.3	7.6±0.2[#]	7.4±0.2[#]	9.6±0.2[#]			

[a]Mean±SEM for n=10 placebo, n=11 HMB (3 g HMB free acid/d in three 1 g doses daily), n=11 ATP (one 400 mg dose of ATP in the morning), and n=8 for HMB plus ATP (3 g HMB free acid/d in three 1 g doses daily and one 400 mg dose of ATP in the morning) supplemented subjects.

[b]Probability of treatment by time difference between the treatments over the 12-week study. The mixed model 2x2 Factorial Repeat ANOVA (SAS®) was used, with the value for week 0 used as a covariate.

[c]Perceived recovery score is rated on the participants feeling of recovery from the last workout on a scale of 0-10.

[#]Significantly different than corresponding placebo, t-test ($p < 0.05$).

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[75] C-Reactive protein levels were not significantly affected by any of the treatments during the study. A trend was observed for an HMB effect (HMB*time, $p < 0.08$) and HMB supplementation resulted in a greater mean CRP value at week 10 than did placebo supplementation (t-test, $p < 0.05$). Supplementation with ATP did not affect cortisol levels, while HMB supplementation decreased cortisol levels during the study (HMB*time, $p < 0.001$, Table 6). Supplementation with HMB alone resulted in decreased cortisol levels at weeks 9, 10, and 12 during the overreaching and taper cycles (t-test, $p < 0.05$) and supplementation with HMB-ATP resulted in decreased cortisol levels after both the initiation of training, week 1, and the overreaching and taper cycles, weeks 9, 10, and 12 (t-test $p < 0.05$). There were no main effect differences of either HMB or ATP on either free or total testosterone.

[76] Muscle recovery and readiness to train in the next training session were measured by perceived recovery score (PRS, Table 6). Supplementation with HMB and HMB-ATP resulted in improved PRS over the 12-week study (HMB*time, $p < 0.001$). While no main effect of ATP supplementation was observed, ATP-supplemented participants had improved PRS scores after the overreaching cycle at weeks 10 and 12 compared with placebo-supplemented participants (t-test, $p < 0.05$). At week 4, the HMB/ATP-supplemented group was the only group with a significantly improved PRS compared with the placebo-supplementation (t-test, $p < 0.05$). A trend for an HMB and ATP interaction, indicating a synergistic effect of the combined supplementation on PRS, was also observed (HMB*ATP*time, $p < 0.06$).

[77] The experimental examples demonstrate that HMB-ATP supplementation results in increased strength and power adaptations compared to just HMB or ATP supplementation alone, and this increase is synergistic.

[78] Further, the results indicated greater increases in LBM and muscle thickness in the HMB-ATP, HMB, and ATP groups as compared to the placebo and the administration of HMB-ATP has greater effects on muscle hypertrophy and lean body mass compared to just HMB or ATP supplementation alone.

[79] The administration of HMB-ATP results in increases in LBM, muscle hypertrophy, strength, and power. These increases are, in the instances of strength and power, synergistic, and in the instances of lean body mass and muscle hypertrophy, additive. Moreover, when faced with greater training frequencies, as demonstrated with the overreaching cycle of training, HMB-ATP prevents typical declines in performance that are characteristic of overreaching. All of these results were unexpected and surprising.

[80] The foregoing description and drawings comprise illustrative embodiments of the present inventions. The foregoing embodiments and the methods described herein may vary based on the ability, experience, and preference of those skilled in the art. Merely listing the steps of the method in a certain order does not constitute any limitation on the order of the steps of the method. The foregoing description and drawings merely explain and illustrate the invention, and the invention is not limited thereto, except insofar as the claims are so limited. Those skilled in the art who have the disclosure before them will be able to make modifications and variations therein without departing from the scope of the invention. The terms subject and animal are used interchangeably throughout this application and are in no way limited to one term or the other.

Literature Cited

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Methylbutyrate Free Acid Reduces Markers of Exercise Induced Muscle Damage and Improves Recovery in Resistance Trained Men. *British Journal of Nutrition* In Press.

CLAIMS:

1. A composition comprising a combination of from about 0.5 g to about 30 g of β-hydroxy-β-methylbutyric acid (HMB) and from about 10 mg to about 80 g of adenosine triphosphate (ATP) for use in providing a benefit to an animal in need thereof selected from the group consisting of increasing strength, increasing power, improving muscle mass, and lessening declines in performance characteristic of overreaching.

2. The composition of claim 1, wherein the combination of HMB and ATP is a synergistic combination in providing said benefit to the animal in need thereof.

3. The composition of claim 1 or 2, wherein the HMB is a salt, HMB-acid, a lactone or an ester.

4. Use, for providing a benefit to an animal in need thereof selected from the group consisting of increasing strength, increasing power, improving muscle mass, and lessening declines in performance characteristic of overreaching, of a composition comprising a synergistic combination of an effective amount of HMB and ATP,

wherein the amount of HMB is from about 0.5 to about 30 g and the amount of ATP is from about 10 mg to about 80 g of ATP.

5. Use, for increasing strength of an animal in need thereof, of a synergistic combination of from about 0.5 to about 30 g HMB and from about 10 mg to about 80 g ATP in amounts sufficient to increase the strength of the animal upon use.

6. Use, for increasing power of an animal in need thereof, of a synergistic combination of from about 0.5 to about 30 g HMB and from about 10 mg to about 80 g ATP in amounts sufficient to increase power of the animal upon use.

7. Use, for improving the muscle mass of an animal in need thereof, of a synergistic combination of from about 0.5 to about 30 g HMB and from about 10 mg to about 80 g ATP in amounts sufficient to improve muscle mass of the animal upon use.

Date Reçue/Date Received 2020-04-22

8. Use, for lessening declines in performance characteristic of overreaching for an animal in need thereof, of a synergistic combination of from about 0.5 to about 30 g HMB and from about 10 mg to about 80 g ATP in amounts sufficient to lessen said declines in performance of the animal upon use.

9. Use, for providing a benefit to an animal in need thereof selected from the group consisting of increasing strength, increasing power, improving muscle mass, and lessening declines in performance characteristic of overreaching, of a composition comprising a combination from about 0.5 grams to about 30 grams of HMB and from about 10 mg to about 80 mg of ATP.

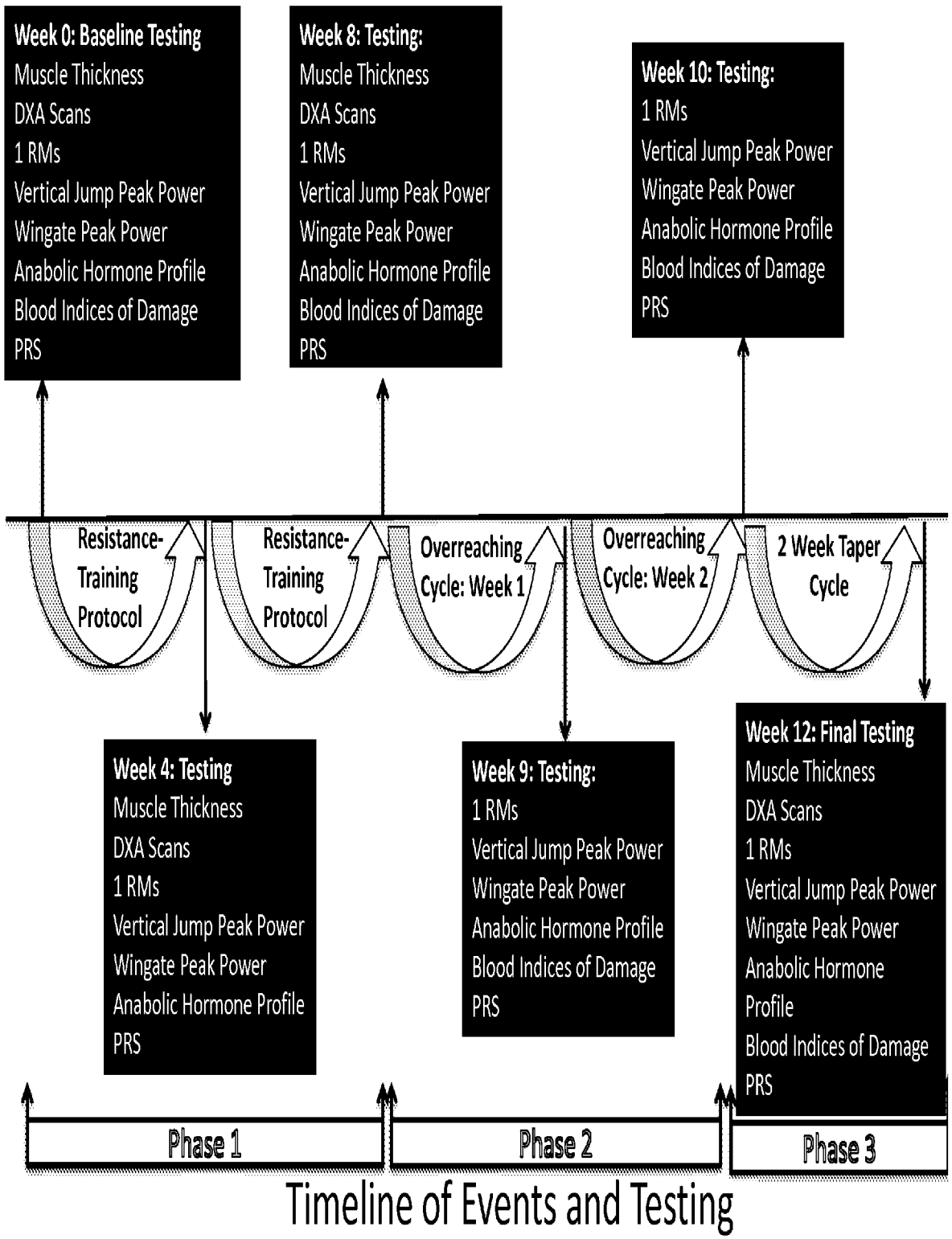
10. The use of any one of claims 4 to 9, wherein the HMB is a salt, HMB-acid, a lactone or an ester.

11. The use of any one of claims 4 to 10, wherein the composition is for oral use, parenteral use, sublingual use, topical use, transdermal use, intramuscular use, or inhalation.

12. The use of claim 11, wherein the composition is for oral use in a delivery form selected from the group consisting of a tablet, capsule, powder, granule, microgranule, pellet, soft-gel, controlled-release form, liquid, solution, elixir, syrup, suspension, emulsion and magma.

Date Reçue/Date Received 2020-04-22

Fig. 1


Week 0: Baseline Testing
Muscle Thickness
DXA Scans
1 RMs
Vertical Jump Peak Power
Wingate Peak Power
Anabolic Hormone Profile
Blood Indices of Damage
PRS
Week 8: Testing:
Muscle Thickness
DXA Scans
1 RMs
Vertical Jump Peak Power
Wingate Peak Power
Anabolic Hormone Profile
Blood Indices of Damage
PRS
Week 10: Testing:
1 RMs
Vertical Jump Peak Power
Wingate Peak Power
Anabolic Hormone Profile
Blood Indices of Damage
PRS
Resistance-Training Protocol
Resistance-Training Protocol
Overreaching Cycle: Week 1
Overreaching Cycle: Week 2
2 Week Taper Cycle
Week 4: Testing
Muscle Thickness
DXA Scans
1 RMs
Vertical Jump Peak Power
Wingate Peak Power
Anabolic Hormone Profile
PRS
Week 9: Testing:
1 RMs
Vertical Jump Peak Power
Wingate Peak Power
Anabolic Hormone Profile
Blood Indices of Damage
PRS
Week 12: Final Testing
Muscle Thickness
DXA Scans
1 RMs
Vertical Jump Peak Power
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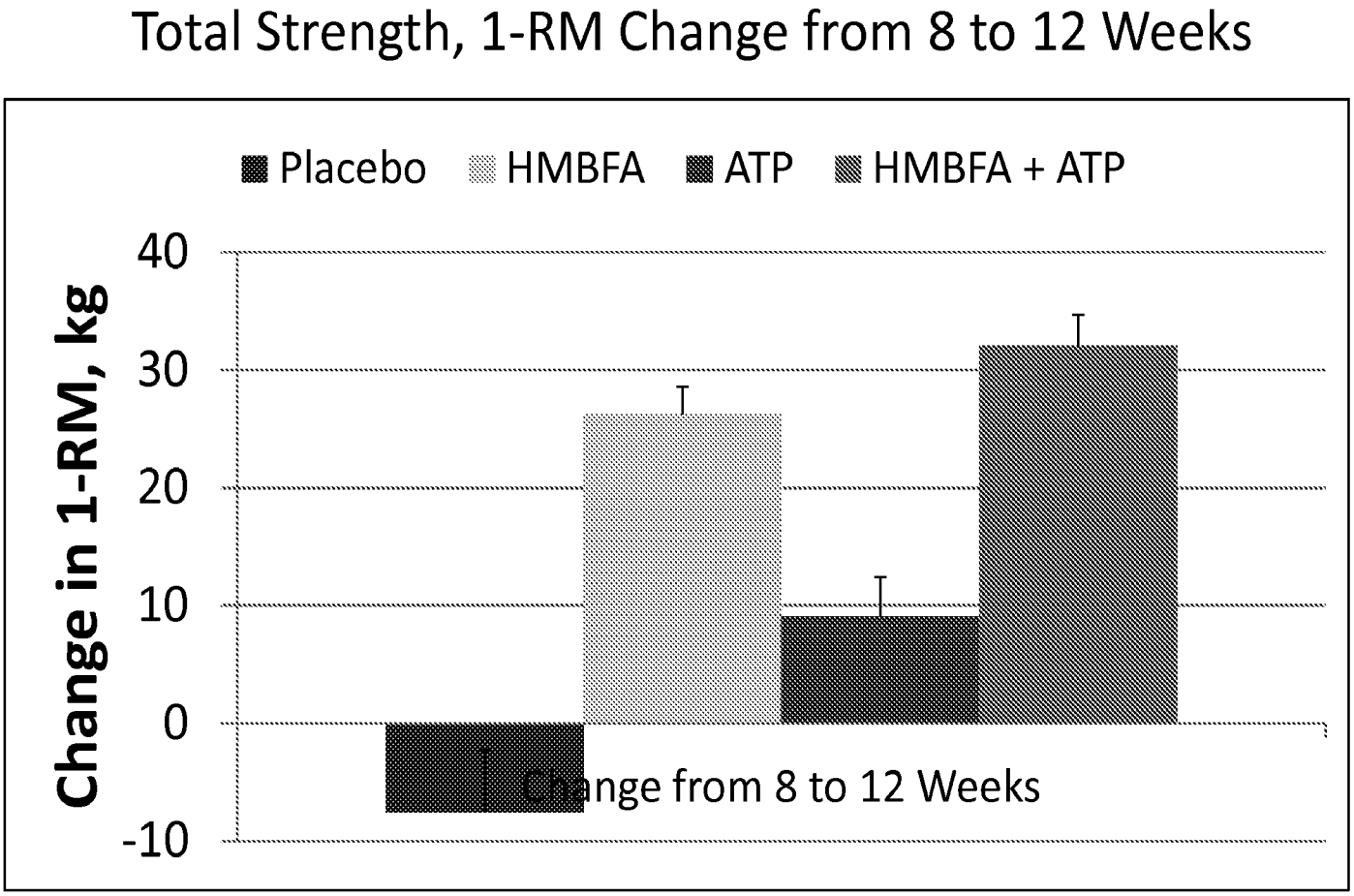
Anabolic Hormone Profile
Blood Indices of Damage
PRS
Phase 1
Phase 2
Phase 3
Timeline of Events and Testing

Fig. 2



Main Effects: HMB p=0.0001; ATP, p=0.0009; HMB*ATP, p=0.11

Fig. 3a

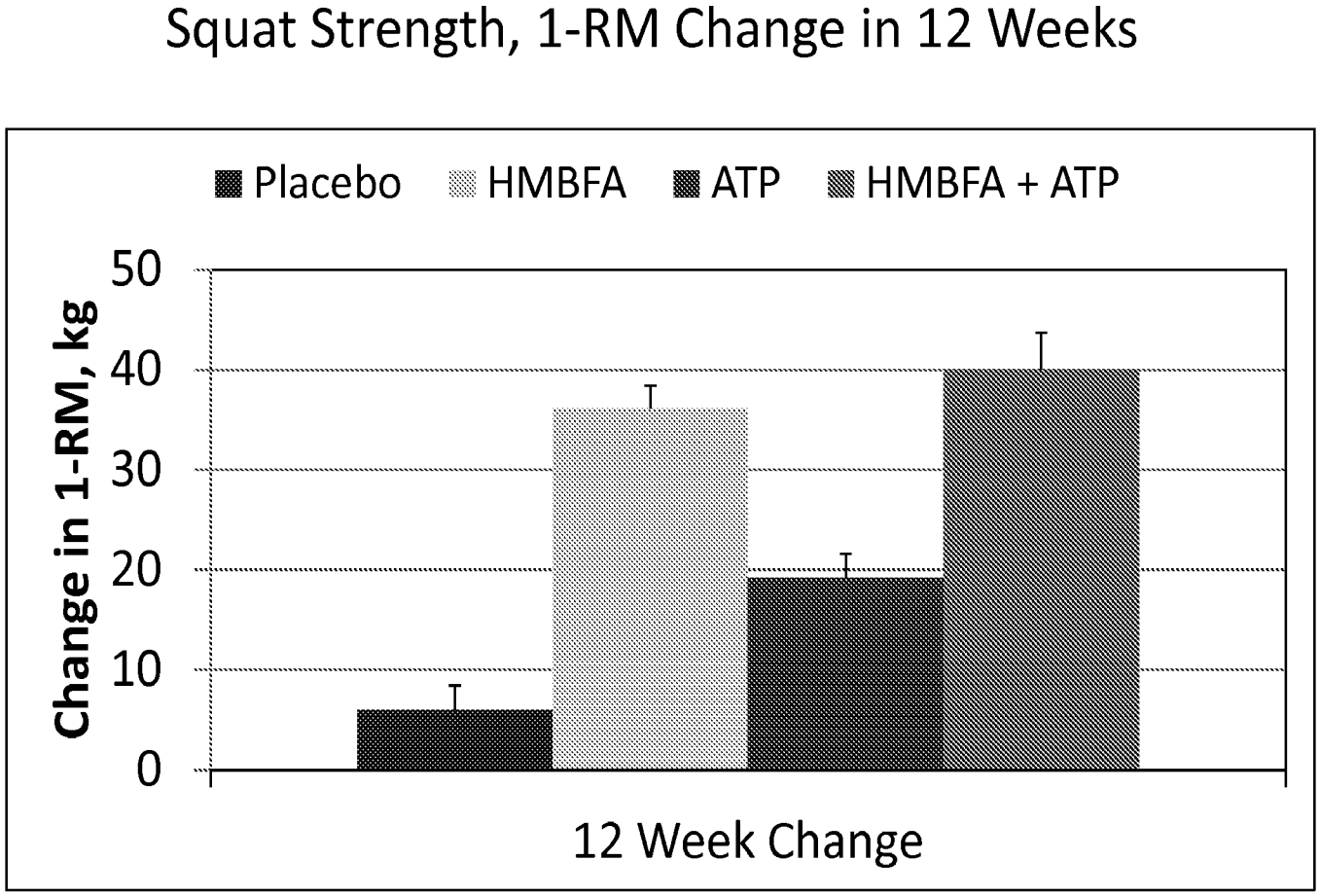


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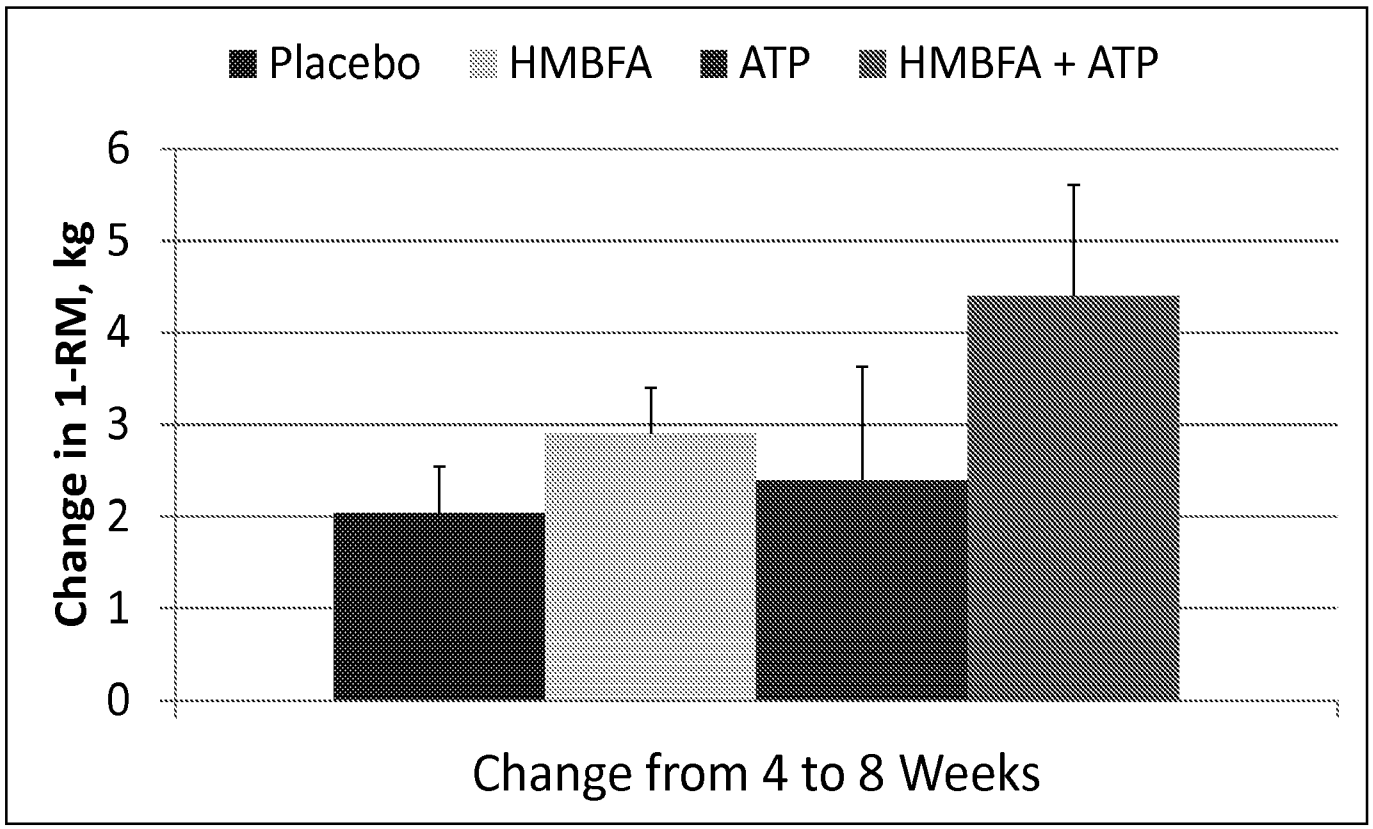
Fig. 3b

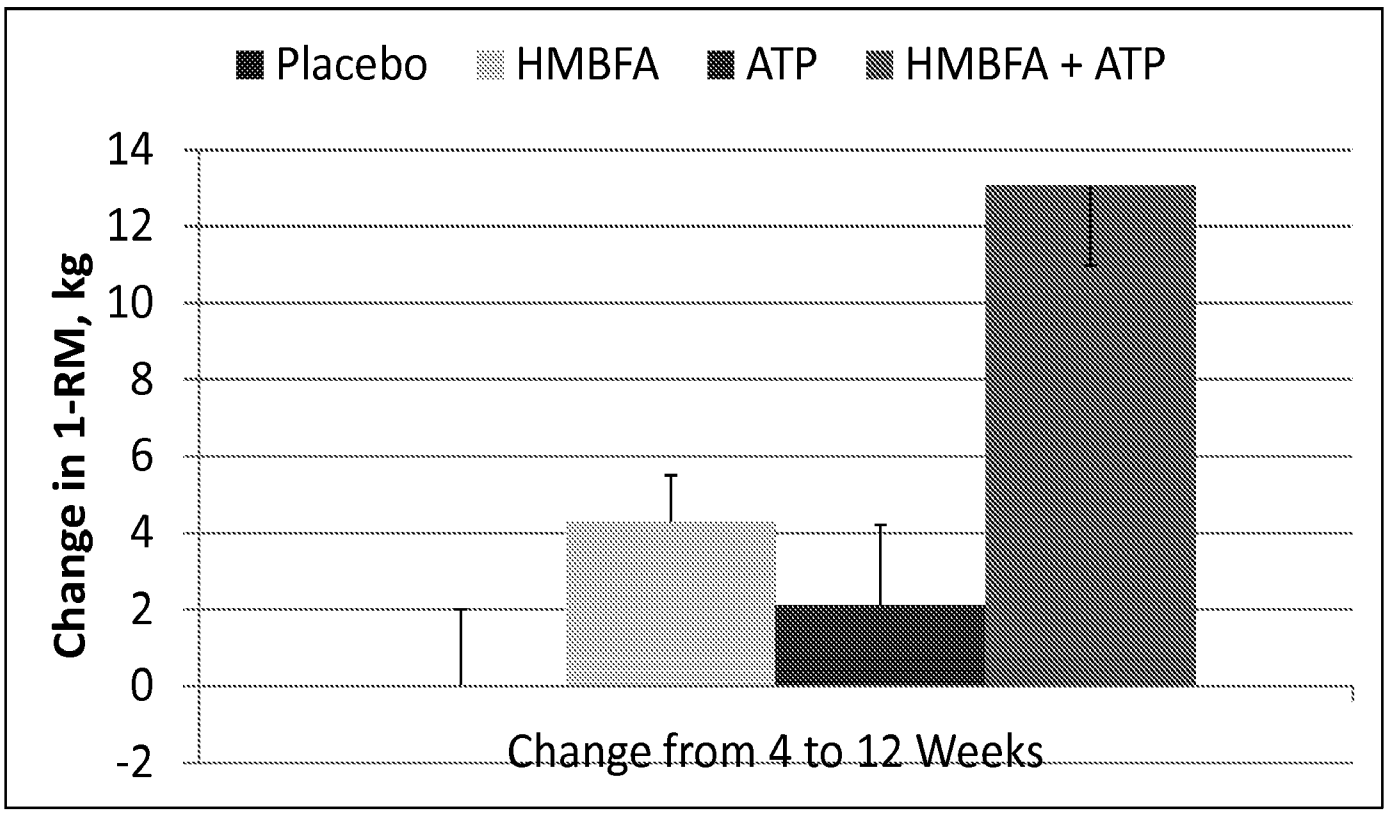
Bench Press Strength, 1-RM Change from 4 to 8 Weeks



Main Effects: HMB p=0.004; ATP, p=0.01; HMB*ATP, p=0.04

Fig. 3c

Bench Press Strength 1-RM Change from 4 to 12 Weeks



Main Effects: HMB p=0.0002; ATP, p=0.003; HMB*ATP, p=0.08

Fig. 4a

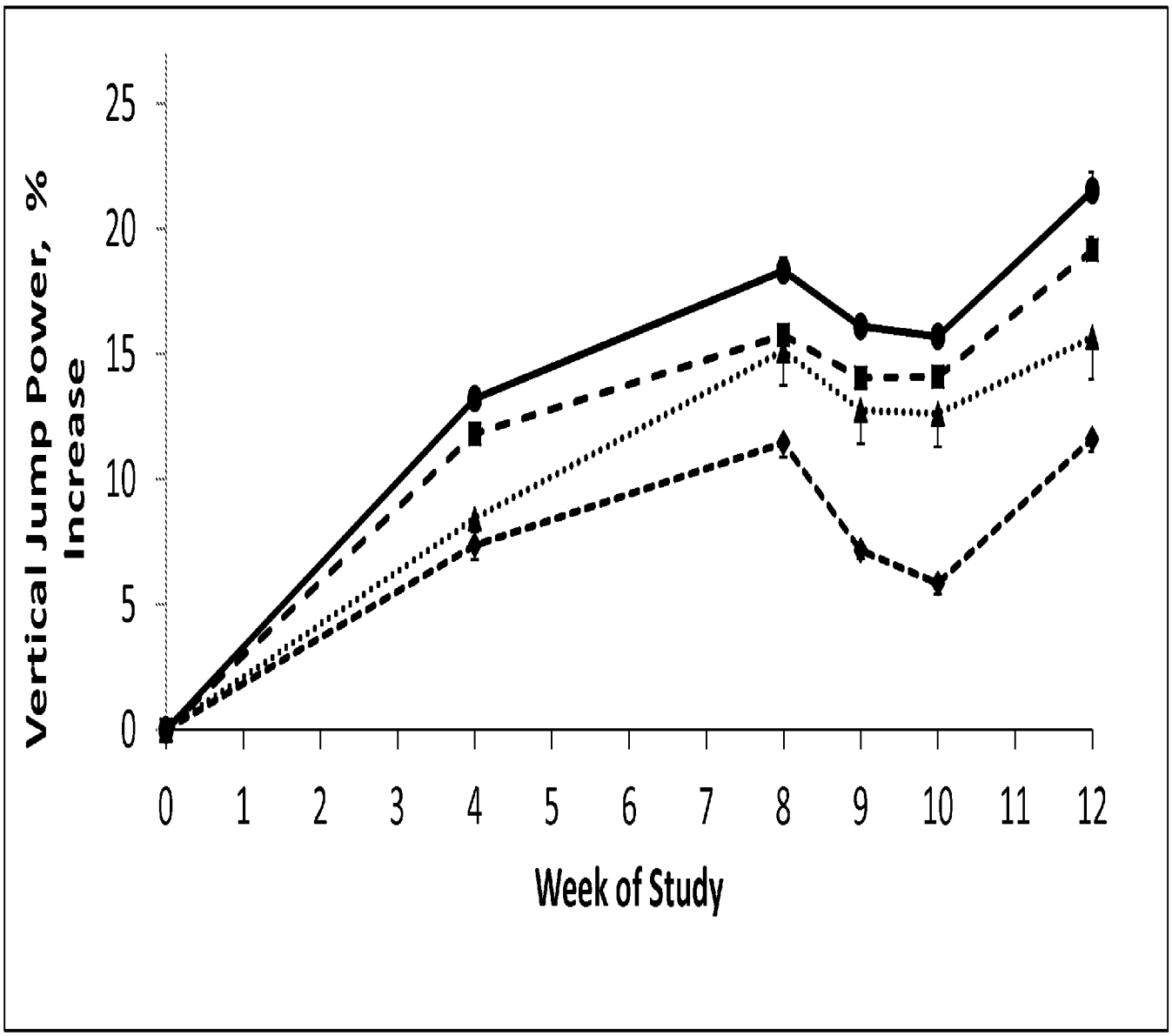
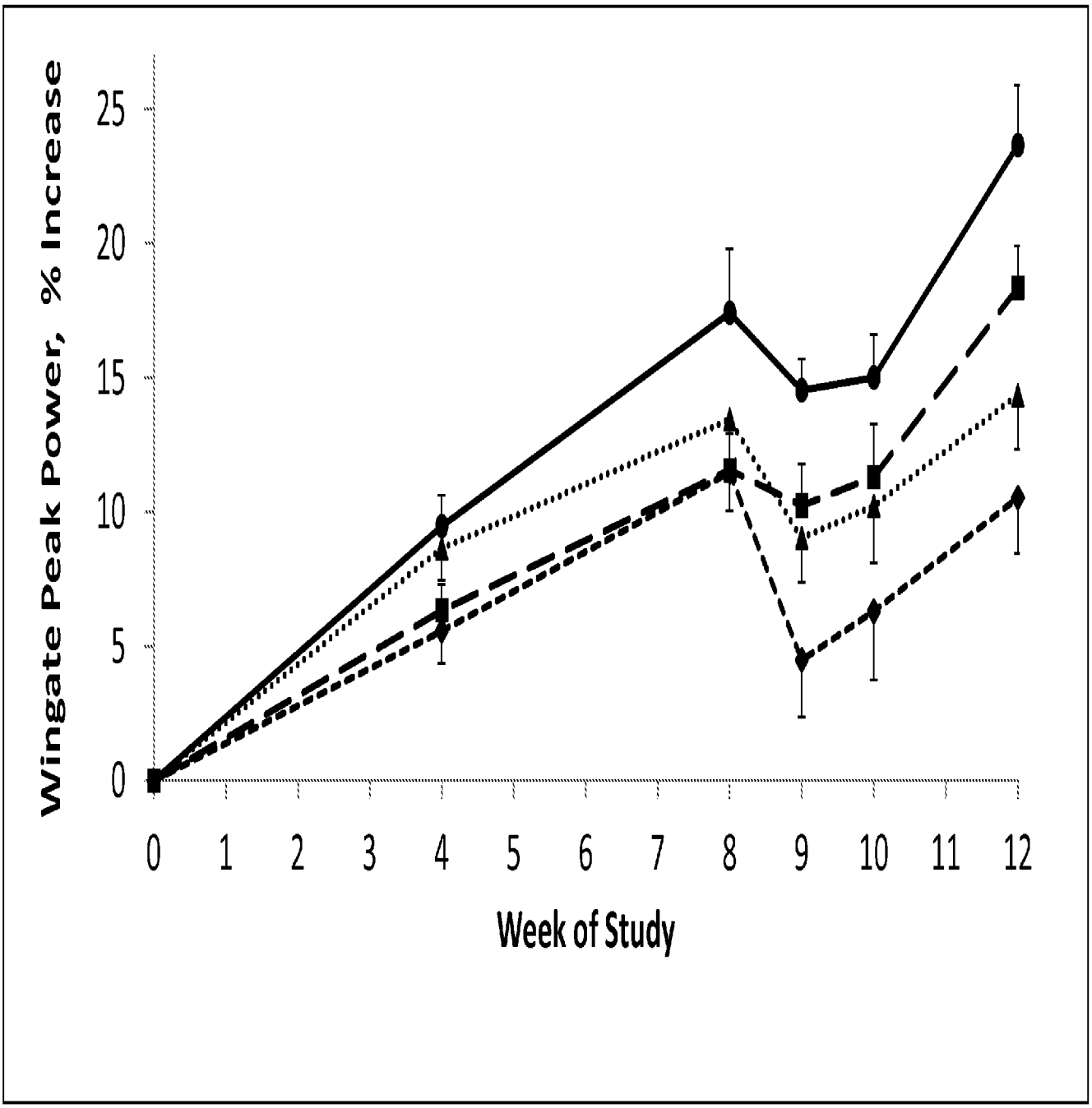
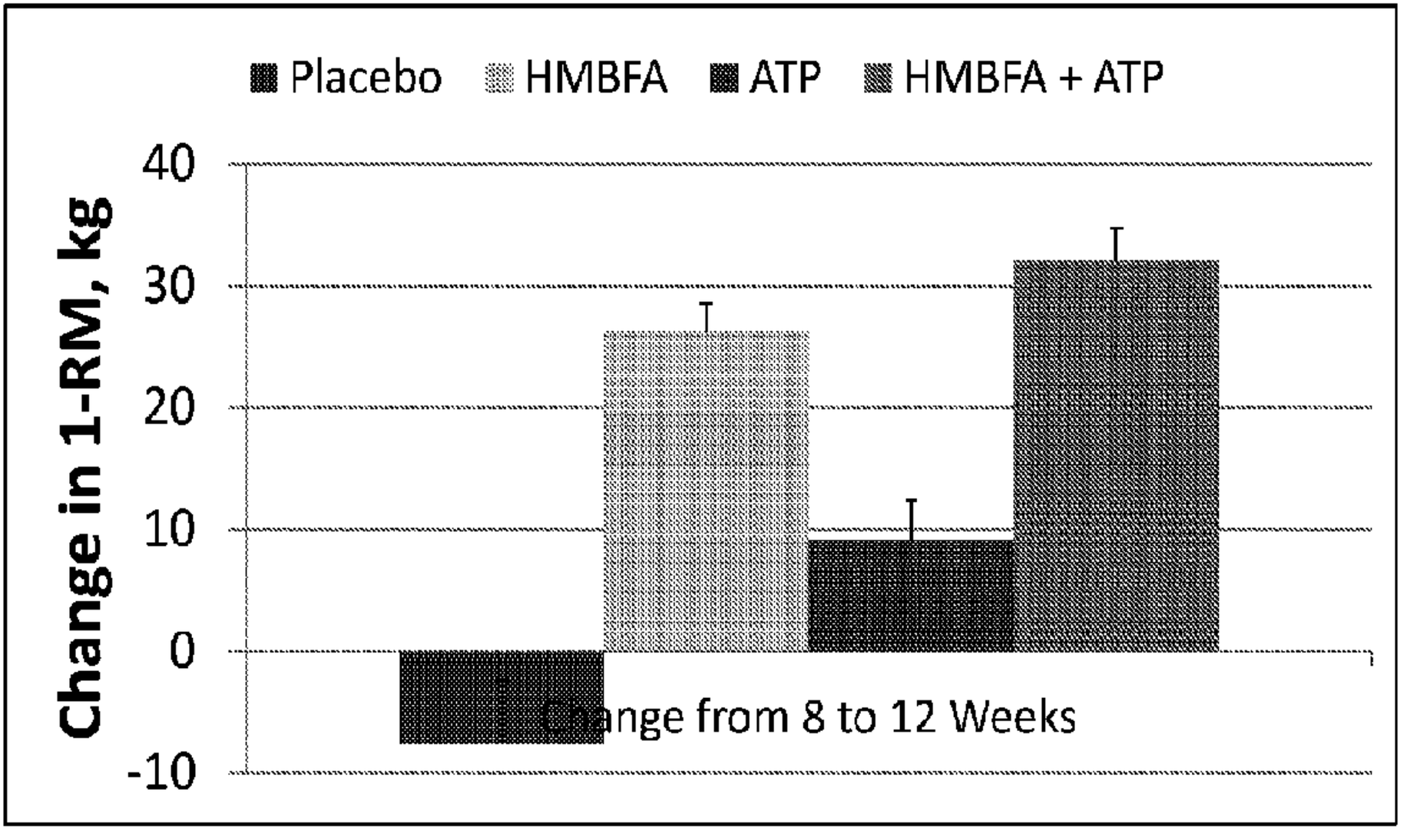

Vertical Jump Power, % Increase
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Week of Study

Fig. 4b


Wingate Peak Power, % Increase
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Week of Study

Total Strength, 1-RM Change from 8 to 12 Weeks



Main Effects: HMB p=0.0001; ATP, p=0.0009; HMB*ATP, p=0.11