Progress in Nutrition 2019; Vol. 21, Supplement 2: 157-164 DOI: 10.23751/pn.v21i2-S.6919

The effects of eight weeks of phosphatidic acid, leucine, beta-hydroxy-beta-methylbutyrate, and vitamin D3 supplementation on blood pressure, triglycerides, and total cholesterol in resistance trained males

Guillermo Escalante[1], Michelle Alencar[2], Bryan Haddock[1], Phillip Harvey[3]

[1]California State University- San Bernardino,University Parkway, San Bernardino - E-mail: gescalan@csusb.edu; [2]California State University- Long Beach, Long Beach; [3]Max Muscle Nutrition, Orange; University of Phoenix, San Diego Campus, San Diego

Summary. *Objective:* MaxxTOR® (MT) is a multi-ingredient supplement that contains phosphatidic acid (PA) as the main active ingredient as well as leucine, beta-hydroxy-beta-methylbutyrate, and vitamin D3. The effects of MT on blood pressure, total cholesterol (TC), and triglycerides (TG) were examined. *Methods:* Eighteen healthy strength-trained males were randomly assigned to a group that consumed MT (n = 8, 22.0 +/- 2.5 yrs; 175.8 +/- 11.5 cm; 80.3 +/- 15.1 kg) or a placebo (PLA) (n = 10, 25.6 +/- 4.2 yrs; 174.8 +/- 9.0 cm; 88.6 +/- 16.6 kg) as part of a double-blind, placebo controlled pre/post experimental investigation. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and blood samples were taken from the participants at week 0 and week 9 of the study to determine the effects of MT on cardiovascular risk factors. Blood serum was analyzed for TC and TG. Subjects were placed and monitored on a eucaloric diet consisting of 25% protein, 50% carbohydrates, and 25% fat by a registered dietitian. Separate two-way mixed factorial repeated measures ANOVA's (time (Pre, Post) x group (MT and PLA) were used to investigate SBP, DBP, TC, and TG changes. Analysis were performed via SPSS (version 22) with significance at $p \leq 0.05$. *Results:* No significant differences were noted between MT and PLA groups for SBP, DBP, TC, or TG. *Conclusion:* Results suggest that the addition of MT to a 3-day per week resistance training program do not significantly affect SBP, DBP, TC, or TG after eight weeks.

Key words: phospholipid, phosphatidic acid, leucine, HMB, vitamin D3, cardiovascular health, muscle protein synthesis

List of Abbreviations

ANOVA- analysis of variance
cGMP- current good manufacturing practices
DBP- diastolic blood pressure
HMB- beta-hydroxy-beta-methylbutyrate
HPLC- high pressure liquid chromatography
MT- MaxxTOR
mTOR- mammalian target of rapamycin
PA- phosphatidic acid
PLA- placebo
SBP- systolic blood pressure
TC- total cholesterol
TG- triglycerides

Background

Multi-ingredient sport supplements are often marketed to improve athletic performance or body composition; however, the effects on targeted metabolic markers are not always reported (1-2). Maxx-TOR® (MT) is a supplement that contains 750 mg phosphatidic acid (PA) as the main active ingredient but also contains other potentially beneficial compounds including 900 mg of leucine, 900 mg of beta-hydroxy-beta-methylbutyrate (HMB), and 1000 IU of vitamin D3. Studies investigating the effectiveness of PA, Leucine, HMB, and vitamin D3 on markers of strength, lean body mass, fat mass, or protein synthesis have reported mixed results (3-15). In comparison, research on the safety of leucine, HMB, and vitamin D3 supplementation have generally been reported to be safe (16-19). The effects of PA or the combination of PA, Leucine, HMB, and vitamin D3 supplementation on cardiovascular risk factors, to the authors' knowledge, have not been reported.

Although MT contains a dosage of PA that has been proven to be effective at increasing strength and lean body mass (3, 4), the dosage of leucine and HMB in MT is lower than has been reported as effective in previous studies (8, 10). The manufacturers of MT had limited capsule space and focused on utilizing PA as the active ingredient. Leucine, HMB, and vitamin D3 were added to the PA to act as potentially synergistic compounds to the PA. Hence, the primary active ingredient of MT was intended to be PA.

The safety of some of the ingredients present in MT have been previously investigated. Bauer et al. (19) reported that a 13-week vitamin D3 and a leucine-enriched whey protein nutritional supplement with 800 IU of vitamin D3 and 3 grams of leucine given to older adults improved their strength and muscle mass with no serious adverse events including hemodynamic changes, blood lipid changes, gastrointestinal issues, changes in vital signs, liver, or renal lab parameters. Although the researchers concluded that the combination of vitamin D3 with a leucine enriched whey protein appears to be safe with no cause of concern, they did not measure TC or TG in their investigation.

Nissen et al. (16) summarized the safety data collected in nine studies in which humans were fed 3 g HMB/day. The studies were from 3 to 8 weeks in duration and included various demographics (males, females, young, old, exercisers, non-exercisers). The authors reported a net decrease in TC (5.8%, $p < 0.03$), a decrease in LDL cholesterol (7.3%, $p < 0.01$) and a decrease in SBP pressure (4.4 mm Hg, $p < 0.05$). Nissen et al. (16) concluded that the effects of HMB on surrogate markers of cardiovascular health could result in a decrease in the risk of heart attack and stroke. In this study, the dosage of HMB in the MT group was 900 mg/day for 8 weeks and the effects of HMB in combination with the other ingredients found in MT were tested for its effect on TC, TG, DBP, and SBP.

PA is a phospholipid that contains a glycerol backbone, a phosphate group, and typically one saturated and one unsaturated fatty acid (20). Researchers have reported that the fatty acid chemical make-up of PA appears critical in its ability to activate the mammalian target of rapamycin (mTOR) (21); in turn, mTOR activation stimulates muscle protein synthesis (22, 23). Bond stated that in addition to increasing muscle protein synthesis, PA might also attenuate muscle protein breakdown through regulation of the FoxO family of transcription factors, atrogin-1, and muscle ring finger-1 (24).

In terms of lipid metabolism, the physiological roles PA plays is two-fold. PA plays a major role in the biosynthesis of phospholipid membranes and triacylglycerols (25). The question surrounding PA within a multi-ingredient supplement lies with the physiological conversion of PA into diacylglycerols. PA serves as a second messenger acting as a mediator of vesicle formation (25, 26), where the enzyme which catalyzes the hydrolysis of PA is involved in the regulation and influence of overall lipid metabolism. Since PA influences lipid metabolism, TC and TG may be affected with PA supplementation; however, to the author's knowledge this has not been previously investigated. Furthermore, it has been suggested that research investigating the safety of PA supplementation is lacking and safety data has not been reported (24). In order to help fill the gap in the literature with regards to the effects of cardiovascular risk factors of PA supplementation, the purpose of this investigation was to study the effects of a supplement containing PA in combination with Leucine, HMB, and vitamin D3 on blood pressure, TC, and TG.

Methods

Subjects

The participants in this study were the same as those previously reported (5) where the data analyzed was on the effects of MT on strength, body composition, muscular endurance, power, agility, and vertical jump in resistance trained men. Initially, nineteen healthy, strength-trained male volunteers signed an informed consent form approved by the Institutional Review Board at California State University, San Bernardino and agreed to participate in this randomized, double-blind, placebo-controlled study. One subject voluntarily withdrew from the study due to time constraints. Eighteen participants completed the trial, where eight (22.0 +/- 2.5 yrs; 175.8 +/- 11.5 cm; 80.3 +/- 15.1 kg) were randomly assigned to the MT group and ten (25.6 ± 4.2 yrs, 174.8 ± 9.0 cm, 88.6 ± 16.6 kg) were randomly assigned to the PLA group. The protocol and subject inclusion/exclusion criteria were similar to the study performed by Joy et al. (4).

All participants were required to abstain from consuming any muscle-building supplements (e.g., creatine) for at least 1 month prior to pretest measures, abstain from training outside of the prescribed protocol during the study, be non-smokers, have resistance training experience of at least one year, and have participated in resistance training at least three days per week for the past six months to be included in this study. Additionally, participants had to be free of any injuries or medical conditions (metabolic, cardiovascular, orthopedic, or neurological) that would prohibit them from participating in a resistance training program. The training program implemented is described in the authors' previous investigation on the effects of MT on strength and lean body mass (5).

Anthropometric and Blood Pressure Assessment

The participant's height and weight was measured prior to measuring blood pressure. Resting SBP and DBP were taken from the participants at week 0 and 9 of the study. Blood pressure was measured by the same trained member of the research team that was blinded as to the group in which the participants were assigned. Each participant sat in a quiet office with their feet on the ground and their arms at their lap for 5 minutes. The researcher then had the participant rest their arm on a table at approximately the level of the heart, fitting the participant with a properly sized sphygmomanometer (Welch Allyn Inc, Skaneateles Falls, NY). Using standard blood pressure measuring practices, two measurements were taken for the blood pressure interspersed by 2 minutes. The average of the two SBP and DBP readings measured at week 0 and week 9 were used in the data analysis. Intra-rater reliability for the two blood pressure measurements in the pre and the post testing was excellent since all blood pressure measurements were within 6 mmHg.

Blood Sampling and Analysis

Blood samples were obtained after a 12-hour fast one week prior to beginning the supplement/exercise protocol and within one week of completing the supplement/exercise protocol. Approximately 20 ml of blood were drawn from the antecubital vein with the participants in a seated position using standard sterile venipuncture procedures. Blood samples were then centrifuged at 3,000 rpm and the serum aliquoted to separate tubes that were frozen for future analyses. Serum samples were then assayed in duplicate for TC and TG using a cholesterol fluorometric assay kit (Cayman Chemical Company, Ann Arbor, MI) and a triglyceride colorimetric assay kit (Cayman Chemical Company, Ann Arbor, MI), respectively. All samples were analyzed as per manufacturer guidelines in the kinesiology hemodynamics and chemistry labs at California State University- San Bernardino. The coefficient of variation of duplicate samples was less than 5%.

Dietary/Supplement Supervision

The diet/supplementation supervision was the same previously reported (5). Prior to the study, participants were required to watch a video made by a registered dietitian specializing in sports nutrition discussing their diet protocols, the diet recording/reporting protocol, and emphasizing the importance of adherence to the diet plan. Two weeks prior to the start of training each participant was provided with a standardized meal plan, designed by the dietitian. This meal plan was to be followed throughout the study. Daily caloric need for each participant was estimated via the Harris Benedict equation and was designed to

be eucaloric in nature by adding 55% more calories to their resting metabolic rate estimation in order to compensate for their moderate activity level of strength training three days per week. The diet consisted of 25% protein, 50% carbohydrates, and 25% fat. Although a sample meal plan was provided for each subject, the dietitian explained in the video that participants could choose any foods they desired as long as the final calorie count and macronutrient breakdown was within the guidelines provided. After the video was watched by the participants, the registered dietitian and principal investigator oversaw the diet logs of the participants throughout the study. All participants were instructed to use the smartphone app MyFitnessPal® to record their nutritional intake and to submit a weekly summary of their diet logs from the MyFitnessPal® website via an email to ensure compliance. Subjects not familiar with the mobile app were instructed by the research team on how to utilize it, but twelve of the eighteen subjects had used this app prior to this study. The use of mobile apps for dietary self-reporting has been previously used in research (27).

The MT group received 5 x 150 mg capsules of MT per day while the PLA group received 5 x 150 mg of rice flour; all capsules were 5 visually identical. A single production lot of both MT and PLA supplements were manufactured in a facility compliant with current Good Manufacturing Practices (cGMP) for dietary supplements (21 CFR 111). PA purity and potency were analyzed by an ultra-performance liquid chromatograph with triple quadrupole mass spectrometry (LC/MS/MS) methods (Avanti Polar Lipids, Inc., Alabaster, AL). Purity and potency of L-Leucine, HMB and vitamin D3 were analyzed by High Pressure Liquid Chromatography (HPLC) at Micro Quality Laboratories, Inc, Burbank, CA. On resistance training days, participants consumed 3 capsules of their respective supplement 30 minutes prior to resistance training and 2 capsules immediately following resistance training along with 24 g of hydrolyzed collagen protein powder from beef skin (Peptiplus XB agglomerated, Gelita AG, Eberbach, Germany) mixed with 500 ml water. The protein supplement was provided by the researchers only to ensure control for post-exercise meals between groups and not to intervene with the effects of MT. The hydrolyzed collagen protein was purposely selected because it is an incomplete protein source low in leucine and would therefore potentially minimize the impact on the supplement being studied. On non-resistance training days, participants consumed 3 of their respective supplement pills with breakfast and 2 pills with dinner. In order to ensure compliance, participants were required to return to the laboratory with their empty containers 3 weeks after starting the study in order to receive their next bottle of supplements. The dietitian and/or principal investigator had weekly interaction with the participants via email to monitor compliance throughout the study. Each subject turned in empty bottles at designated times and reported taking the supplement as directed.

Product formulations were blinded and coded to both the investigators and the participants so that neither knew which formulation was consumed during the study as previously reported (5). Each participant randomly selected a 4-digit participant code that corresponded to a code on their respective bottles. The research team recorded the code on each bottle for each participant; however, the key for each code that determined whether the bottle contained MT or PLA was revealed to the researchers after all the data was collected at the end of the study. Each participant was provided with their supplement bottle (PLA or MT) for 3 weeks on the first Monday and every third Monday thereafter.

Statistical Analysis

Data are presented as mean +/- SD throughout this manuscript. Separate two-way mixed factorial Repeated Measures Analysis of Variance (time (Pre, Post) x group (MT and PLA) were used to investigate changes in SBP, DBP, TC, and TG. All analyses were performed using SPSS version 22 (SPSS, Inc., Chicago, IL). An alpha level was set at $p \leq 0.05$.

Results

The subjects' baseline height, weight, body mass index (BMI), and age are presented in Table 1. There were no significant differences between the MT or the PLA group for any baseline cardiovascular measurement (Table 2). The weekly nutrition logs turned in

Table 1. Height, Weight, BMI, and Age Baseline Characteristics (Mean +/- SD)

Variable	MT Group	PLA Group
Height	175.8 +/- 11.5 cm	174.8 +/- 9.0 cm
Weight	80.3 +/-15.1 kg	88.6 +/- 16.6 kg
BMI	25.8 +/- 2.0	29.1 +/- 5.8
Age	22.0 +/- 2.5 yrs	25.6 +/- 4.2 yrs

by the participants to the principal investigator on a weekly basis throughout the study also showed no significant differences in calories consumed (MT: 2709.0 +/- 357.0 Cal vs PLA: 2681.7 +/- 277.8 Cal), carbohydrates consumed (MT: 320.4 +/- 47.8 g vs PLA: 325.9 +/- 34.3 g), protein consumed (MT: 166.5 +/- 22.9 g vs PLA: 158.0 +/- 27.8 g), and fat consumed (MT: 84.7 +/- 26.5 g vs PLA: 82.9 +/- 14.3 g). There were also no significant group x time interactions for SB, DB, TC, or TG (Table 2).

Discussion

The current study investigates the effects of a multi-ingredient supplement (MT) containing 750 mg of PA, 900 mg of L-Leucine, 900 mg of HMB, and 1000 IU of vitamin D3 on SBP, DBP, TC, and TG after the completion of an eight-week training/supplement intervention. Previous research on these ingredients have focused on their effects on strength, body composition, and other physiological variables (3-15). While research on the safety of leucine, HMB, and vitamin D3 has been performed (16-19), it has been suggested that research investigating the safety of PA supplementation, inclusive of any cardiovascular risk factors, is lacking (24). Overall, the potentially synergistic compounds within MT including HMB (16) and leucine (17) have been reported to be safe and effective without known changes in blood pressure and blood lipids. One review on the effects of 3 g HMB/day reported that 3 g HMB /day could result in a decrease in the risk of heart attack and stroke via a decrease in SBP and TC (16). Additionally, Vitamin D3 supplementation has been reported to be safe and may be effective at reducing potential arterial stiffness (18). To the authors' knowledge, this is the first investigation that has examined changes in blood pressure and blood lipids as a result of resistance training when combined with a supplement containing 750 mg of PA, 900 mg of Leucine, 900 mg of HMB, and 1000 IU vitamin D3.

Sports supplement companies sometimes test multi-ingredient products for their effectiveness and safety, especially in regards to blood lipid changes (28, 29). Since PA is involved in the biosynthesis of phospholipid membranes and triacylglycerol's (25) and the enzyme which catalyzes the hydrolysis of PA plays a role in the regulation of overall lipid metabolism (25, 26), it was hypothesized that PA supplementation may affect blood lipid levels. However, the results of the present study indicate that MT supplementation, which has PA as the primary active ingredient, did not significantly affect TC or TG after 8 weeks of supplementation and resistance training. The presence of sufficient lipid precursors is required for membrane biosynthesis, cell growth, and proliferation to produce known physiological changes when combined with resistance training including improved body composition and performance (30). It has been acknowledged that mTOR serves as a sensor in the body to indicate the presence of essential nutrients needed for the synthesis of muscle proteins and that cells require lipids for membrane biosynthesis (30). Within the present study design the multi-component product does not affect TC or TG levels. In contrast, other multi-ingredient

Table 2. Changes in Blood Pressure, Total Cholesterol, and Triglycerides (Mean +/- SD) ($p < 0.05$)

	Systolic Blood Pressure (mmHg)		Diastolic Blood Pressure (mmHg)		Total Cholesterol (mg/dL)		Triglycerides (mg/dL)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
MT	122.5 +/- 9.4	121.0 +/- 9.3	65.5 +/- 2.6	65.5 +/- 4.0	127.8 +/- 29.0	122.6 +/- 28.2	124.2 +/- 75.1	91.1 +/- 44.0
PLA	121.8 +/- 6.2	121.2 +/- 6.5	70.0 +/- 6.3	68.6 +/- 5.5	122.7 +/- 25.3	128.8 +/- 25.7	139.2 +/- 72.6	174.8 +/- 127.9

No significant differences were noted between the MT vs PA group at baseline for any of the variables
No significant differences were noted between any variables Pre vs Post for the MT or PLA groups

products containing PA only report the effectiveness of the product on performance or body composition (3-7).

A limitation to this investigation is that the multi-ingredient make-up of MT makes it difficult to isolate the individual effects of PA, leucine, HMB, and vitamin D3 in the proprietary blend of the supplement. Despite this limitation, no significant changes in SBP, DBP, TC, or TG were noted over the course of the eight-week study. Therefore, the combination of 750 mg of PA, 900 mg of leucine, 900 mg of HMB, and 1000 IU of vitamin D3 does not appear to affect the cardiovascular risk factors investigated. Furthermore, since MT has been reported to be effective at improving LBM and strength in resistance trained men (5), MT appears to have a favorable risk to benefit ratio. Future research is indicated to further confirm these findings and the effectiveness of PA.

Conclusions

The results of this investigation suggest that the addition of MT to a 3-day per week resistance training program do not affect SBP, DBP, TC, or TG. MT appears to be a safe and effective supplement for resistance trained men without affecting the cardiovascular risk factors measured after eight weeks.

Competing Interests and Funding

The investigators disclose that Dr. Phil Harvey is currently the Chief Science Officer for Max Muscle Nutrition and that he developed the MaxxTOR® (MT) supplement while being employed by Max Muscle Nutrition. Furthermore, the funds for this study were provided by Max Muscle Nutrition (Orange, CA) as well as from Chemi Nutra (Austin, TX), which is the patent holder of PA. These funds were used to purchase the necessary supplies, pay research assistants, and pay the investigators a small stipend approved by the CSUSB IRB to conduct their research.

The investigators further disclose that the principal investigator won the Max Muscle MaxxTOR® body transformation challenge in June of 2014 and was awarded $5,000 for winning the contest. The principal investigator currently receives no further compensation for the sale of the product as the money paid in June 2014 was merely for winning the contest. The principal investigator is a sponsored Max Muscle athlete and receives a personal allotment of Max Muscle supplements as part of his contract. Aside from the stipends reported in the budget approved by the IRB, no investigator will receive any additional compensation from either Max Muscle Nutrition or Chemi Nutra for their involvement in this study. The results of this study are independent and are not biased despite the relationships mentioned in this section. This product and the use of these supplements is not endorsed by California State University- San Bernardino, California State University- Long Beach, or the University of Phoenix.

Author's contributions

GE was the primary investigator and supervised all study recruitment. All authors worked collectively to design the study. GE, MA, and BH supervised and/or performed the data collection, GE and MA analyzed the data, and GE performed the statistical analysis. GE was the primary author and supervised the manuscript preparation. MA, BH, and PH helped in drafting the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank all of the California State University- San Bernardino volunteers that helped to make this study possible. Furthermore, the authors would like to acknowledge Max Muscle Nutrition (Orange, CA) and Chemi Nutra (Austin, TX) for their support in this investigation.

Bibliography

1. Jagim AR, Jones MT, Wright GA, Antoine CS, Kovacs A, Oliver JM. The acute effects of multi-ingredient pre-workout ingestion on strength performance, lower body power, and anaerobic capacity. Journal of the International Society of Sports Nutrition 2016; 13:11.
2. Outlaw JJ, Wilborn CD, Smith-Ryan AE, Hayward SE, Urbina SL, Taylor LW, Foster CA. Acute effects of a commercially-available pre-workout supplement on markers of training: a double-blind study. Journal of the International Society of Sports Nutrition 2014; 11:40.
3. Hoffman JR, Stout JR, Williams DR, Wells AJ, Fragala MS, Mangine GT, Gonzalez AM, Emerson NS, McCormack WP, Scanlon TC, Purpura M, Jäger R. Efficacy of phosphatidic acid ingestion on lean body mass, muscle thickness and strength gains in resistance-trained men. J Int Soc Sports Nutr 2012; 9:47.
4. Joy JM, Gundermann DM, Lowery RP, Jager R, McCleary SA, Purpura M, Roberts MD, Wilson SMC, Hornberger TA, Wilson JM. Phosphatidic acid enhance mTOR signaling and resistance exercise induced hypertrophy. Nutrition and Metabolism 2014; 11:29.
5. Escalante GE, Alencar M, Haddock B, Harvey P. The effects of phosphatidic acid supplementation on strength, body composition, muscular endurance, power, agility, and

vertical jump in resistance trained men. Journal of the International Society of Sports Nutrition 2016; 13:24.
6. Andre TL, Gann JJ, McKinely-Barnard SK, Song JJ, Willoughby DS. Eight weeks of phosphatidic acid supplementation in conjunction with resistance training does not differentially affect body composition and muscle strength in resistance trained men. Journal of Sports Science & Medicine 2016; 15(3): 532-539.
7. Gonzalez AM, Sell KM, Ghigiarelli JJ, Kelly CF, Shone EW, Accetta MR, Baum JB, Mangine GT. Effects of phosphatidic acid supplementation on muscle thickness and strength in resistance-trained men. Applied Physiology, Nutrition, and Metabolism 2017; 42(4):443-448.
8. Katsanos CS, Kobayashi J, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. American Journal of Physiology, Endocrinology, and Metabolism 2006; 291: E381 - E387.
9. Verhoeven S, Vanschoobeek K, Verdijk L, Koopman R, Wodzig WKHW, DEndale, P, van Loon LJC. Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men. American Journal of Clinical Nutrition 2009; 89(5) 1468-1475.
10. Thomson JS, Watson PE, Rowlands DS. Effects of nine weeks of beta-hydroxy-beta-methylbutyrate supplementation on strength and body composition in resistance trained men. Journal of Strength and Conditioning Research 2009; 23: 827-35.
11. Wilson JM, Fitschen PJ, Campbell B, et al. International Society of Sports Nutrition Position Stand: beta-hydroxy-betamethylbutyrate (HMB). Journal of the International Society of Sports Nutrition 2013; 10:6.
12. Slater G, Jenkins D, Logan P, Lee H, Vukovich M, Rathmacher JA, Hahn AG. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation does not affect changes in strength or body composition during resistance training in trained men. International Journal of Sports, Nutrition, Exercise, and Metabolism 2001; 11(3):384-96.
13. Kreider RB, Ferreira M, Greenwood M, Wilson M, Grindstaff P, Plisk S, Reinardy J, Cantler C, Almada AL. Effects of calcium B-HMB supplementation during training on markers of catabolism, body composition, strength and sprint performance. Journal of Exercise Physiology online 2000; 3(4):48-59.
14. Ksiazek A, Zagrodna A, Dziubek W, Pietraszewski B, Ochmann B, Slowinska-Lisowska M. 25(OH)D3 Levels Relative to Muscle Strength and Maximum Oxygen Uptake in Athletes. Journal of Human Kinetics 2016; 13(50) 71-77
15. Salles J, Chanet A, Giraudet C, Patrac V, Pierre P, Jourdan M.1,25(OH)2-vitamin D3 enhances the stimulating effect of leucine and insulin on protein synthesis rate through Akt/PKB and mTOR mediated pathways in murine C2C12 skeletal myotubes. Molecular Nutrition and Food Research 2013; 57(12):2137-46.
16. Nissen S, Sharp RL, Panton L, Vukovich M, Trappe S, Fuller JC Jr. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation is safe and may decrease cardiovascular risk factor. Journal of Nutrition 2000; 130 (8): 1937-45.
17. Elango R, Rasmussen B, Madden K. Safety and tolerability of leucine supplementation in elderly men. Journal of Nutrition 2016; 146 (12): 2630S - 2634S.
18. Forouhi NG, Menon RK, Sharp SJ, Mannan N, Timms PM, Martineau AR, Rickard AP, Boucher BJ, Chowdhury TA, Griffiths CJ, Greenwald SE, Griffin SJ, Hitman GA. Effects of Vitamin D2 or D3 supplementation on glycemic control and cardiometabolic risk among people at risk of type 2 diabetes: results of a randomized double-blind placebo-controlled trial. Diabetes, Obesity, and Metabolism 2016; (18)4: 392-400.
19. Bauer JM, VeriaanS, Bautmans I, Brandt K, Donini LM, Maggio M, McMurdo ME, Mets T, Seal C, Wijers SL, Ceda GP, DeVito G, Donders G, Drey M, Greig C, Holmback U, Narici M, McPhee J, Poggiogalle E, Power D, Scafogliery A, Schultz R, Sieber CC, Cederholm T. Effects of vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: a randomized, double blind, placebo-controlled study. Journal of the American Medical Directors Association 2015; 16(9): 740-747.
20. Castro-Gomez P, Garcia-Serrano A, Visioli F, Fontecha J. Relevance of dietary glycerophospholipids and sphingolipids to human health. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA) 2015; 101:41–51.
21. Yoon M-S, Rosenberger CL, Wu C, Truong N, Sweedler JV, Chen J. Rapid mitogenic regulation of the mtorc1 inhibitor, deptor, by phosphatidic acid. Molecular Cell. 2015; 58(3):549–56.
22. Fang Y, Vilella-Bach M, Bachmann R, Flanigan A, Chen J. Phosphatidic acid-mediated mitogenic activation of mTOR signaling. Science 2001; 294: 1942–1945.
23. You JS, Frey JW, Hornberger TA: Mechanical stimulation induces mTOR signaling via an ERK-independent mechanism: implications for a direct activation of mTOR by phosphatidic acid. PLoS One 2012; 7:e47258.
24. Bond, P. Phosphatidic acid: biosynthesis,pharmacokinetics, mechanisms of action, and effect on strength and body composition in resistance-trained individuals. Nutrition & Metabolism, 2017; 14:12.
25. Athenstaedt K, Daum G. Phosphatidic acid, a key intermediate in lipid metabolism. European Journal of Biochemistry1999; 266(1):1-16.
26. Roth MG. Lipid regulators of membrane traffic through the Golgi complex. Trends Cell Biology 1999; 9(5):174-179.
27. Turner-McGrievy GM, Beets MW, Moore JB, Kaczynski AT, Barr-Anderson DJ, Tate DF. Comparison of traditional versus mobile app self-monitoring of physical activity and dietary intake among overweight adults participating in an mHealth weight loss program. Journal of the American Medical Informatics Association 2013; 20:513 – 8.

28. Ormsbee, et al. The effects of a multi-ingredient dietary supplement on body composition, adipokines, blood lipids, and metabolic health, Journal of the International Society of Sports Nutrition 2014; 11:37.
29. Shelmadine et al. Effects of 28 days of resistance exercise and consuming a commercially available pre-workout supplement, NO Shotgun, on body composition, muscle strength and mass, markers of satellite cell activation, and clinical safety markers in males. Journal of the International Society of Sports Nutrition 2009; 6:16.
30. Foster DA, Salloum D, Menon D, Frias MA. Phospholipase D and the maintenance of phosphatidic acid levels for regulation of mammalian target of rapamycin (mTOR). Journal of Biological Chemistry 2014; 289(33):22583-22588.

Correspondence:
Guillermo Escalante (Corresponding Author)
California State University- San Bernardino
5500 University Parkway
San Bernardino, CA 92407
E-mail: gescalan@csusb.edu