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Research article

Eight Weeks of Phosphatidic Acid Supplementation in Conjunction with Resistance Training Does Not Differentially Affect Body Composition and Muscle Strength in Resistance-Trained Men

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Abstract

This study attempted to determine the effects of eight weeks of resistance training (RT) combined with phosphatidic acid (PA) supplementation at a dose of either 250 mg or 375 mg on body composition and muscle size and strength. Twenty-eight resistance-trained men were randomly assigned to ingest 375 mg [PA375 (n = 9)] or 250 mg [PA250 (n = 9)] of PA or 375 mg of placebo [PLC (n = 10)] daily for eight weeks with RT. Supplements were ingested 60 minutes prior to RT and in the morning on non-RT days. Participants' body composition, muscle size, and lower-body muscle strength were determined before and after training/supplementation. Separate group x time ANOVAs for each criterion variable were used employing an alpha level of ≤ 0.05. Magnitude- based inferences were utilized to determine the likely or unlikely impact of PA on each criterion variable. A significant main effect for time was observed for improvements in total body mass ($p = 0.003$), lean mass ($p = 0.008$), rectus femoris cross-sectional area [RF CSA ($p = 0.011$)], and lower-body strength ($p < 0.001$), but no significant interactions were present ($p > 0.05$). Collectively, magnitude-based inferences determined both doses of PA to have a likely impact of increasing body mass (74.2%), lean mass (71.3%), RF CSA (92.2%), and very likely impact on increasing lower-body strength (98.1% beneficial). When combined with RT, it appears that PA has a more than likely impact on improving lower-body strength, whereas a likely impact exists for increasing muscle size and lean mass.

Key words: Phosphatidic acid, resistance training, body composition, muscle strength.

Introduction

E Resistance training (RT) produces muscle hypertrophy due to the accretion of muscle protein over the course of the training period. The progressive accrual of myofibrillar protein is the net result of an overall increase in muscle protein synthesis (MPS) that occurs with each bout of resistance exercise. The process of MPS is governed by an integrated network of intracellular events that can be regulated by both systemic and local effects. Resistance exercise is known to induce systemic effects such as the release of hormones such as testosterone, growth hormone (GH), and insulin-like growth factor 1 (IGF-1) that can affect MPS by up-regulating intracellular signaling pathways. One such pathway involves the orchestration of phosphatidylinositol-3 kinase (PI3K), protein kinase B (Akt), and mechanistic target of rapamycin (mTOR) and is known as the PI3K/Akt-mTOR pathway. This intracellular signaling pathway has been recognized as a stimulus for skeletal muscle protein synthesis (MPS), and the cumulative effects of increased MPS over time can lead to muscular hypertrophic adaptations (Bodine et al., 2001; Koopman et al., 2006; Sandri et al., 2008). Activity of the PI3K/Akt-mTOR pathway has been shown to be sensitive to various substances such as L-leucine (Dennis et al., 2011), ursolic acid (Ogasawara et al., 2013), and phosphatidic acid (PA) (Joy et al., 2014).

Regarding PA, it is an acid form of phosphatidate, a part of common phospholipids which are major components of cell membranes. PA is a simplistic form of diacyl-glycerophospholipids, and is a vital cell lipid acting as a biosynthetic precursor for the materialization (directly or indirectly) of all acylglycerol lipids in the cell (Foster et al. 2014). PA is formed from the hydrolysis of phosphatidylcholine by the enzyme, phospholipase-D (PLD). PLD is a prime regulator in the activation of mTOR signaling by a variety of stimuli (Yoon et al., 2015). PA binding to mTOR consequently results in the stimulation of mTORC1 kinase activity and exogenous PA has been shown to directly activate mTORC1 signaling, possibly due to its association with the FKBP12-rapamycin-binding (FRB) binding domain of mTOR (Joy et al., 2014). An additional stimuli for mTOR up-regulation is muscle contractions which are associated with damage to the sarcolemma resulting in phospholipase D (PLD) to be dislodged from the z-line of muscle tissue and hydrolyzes phosphatidylcholine to yield PA, a lipid second messenger, and choline (Yamada et al., 2012). More specifically, eccentric contractions have demonstrated the ability to result in a significant elevation of intracellular PA which inhibited the synthesis of PA by PLD and blocked the eccentric contraction-induced increase in S6K1 phosphorylation (O'Neil et al., 2009). Yoon et al. (2015) provided evidence that the mTOR inhibitor domain-containing mTOR-interacting protein (DEPTOR) is displaced from mTORC1 by PA when generated by PLD. This leads to activation of mTORC1 and when taken together, this data provides indication that the increase in PA promotes the plausible activation of mTOR signaling.

Potentially, the combination of resistance training (RT) with exogenous PA supplementation could further stimulate an up-regulation of mTOR, thereby augmenting increases in MPS. Although, to date there are no known

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published studies examining the effectiveness of PA supplementation and RT on mTORC1 activity in human skeletal muscle. However, there are three known studies investigating PA supplementation (750 mg daily) in combination with RT and the subsequent effects on body composition and muscle mass and strength in humans (Escalante et al., 2016; Hoffman et al., 2012; Joy et al., 2014). As with many studies, however, discrepancies exist in various aspects of these three investigations and can likely be attributed to such issues as differences in the experimental designs of the studies such as RT program structure and RT session supervision, to name a few. In regards to lean mass, muscle cross sectional area (CSA), and strength, Hoffman found only significant increases with RT whereas Joy et al. (2014) and Escalante et al. (2016) founds significant increase with RT that were a result of daily PA supplementation. Interestingly, Escalante et al. (2016) found the most robust impact on strength and lean body mass improvements utilizing a similar design to the study of Joy et al. (2014), but in addition to 750 mg PA the supplement included L-leucine, HMB, and vitamin D which make it difficult to clearly discern if these results were primarily attributable to PA.

The purpose of this study was to compare the effects of an eight week resistance training (RT) program in conjunction with daily, orally-delivered PA supplementation on body composition and muscle mass and strength at doses of 375 mg and 250 mg, compared to placebo.

Methods

Participants

Using a double-blind, randomized, placebo-controlled design, thirty-two resistance trained males (thrice weekly >1 year prior to study) volunteered as participants. Participants were initially screened via email and following an explanation of all procedures, risks and benefits, each participant signed a university-approved informed consent document prior to starting the study. Approval to conduct the study was granted by the Institutional Review Board for the Protection of Human Subjects in Research of Baylor University. Additionally, all experimental procedures involved in the study conformed to the ethical consideration of the Declaration of Helsinki.

Participants were instructed to not use any anabolic dietary supplements or drugs known to increase muscle mass and/or performance. Screening for dietary supplements and anabolic steroids was accomplished by a health questionnaire, completed during participant screening. Participants reported to the Exercise and Biochemical Nutrition Laboratory (EBNL) on two separate occasions, at baseline (day 0) and after eight weeks of RT and supplementation (Day 57). Testing procedures included collection of dietary logs and testing for body composition testing, rectus femoris CSA (RF CSA) for muscle mass, and muscle strength. The participants' diets were not standardized and they were instructed not to change their normal dietary habits during the course of the study. Participants were required to record their dietary intake for 4 consecutive days prior to each of the two testing sessions at visits 1 (Day 0) and 2 (Day 57) to confirm adherence to their typical daily dietary regimen.

Participants were randomly-assigned to one of three treatment groups, 375 mg PA (PA375), 250 mg PA (PA250), or 375 mg of rice flour placebo (PLC). The PA supplement (Mediator™) was obtained from Chemi Nutra (Austin, TX). Both PA and PL were in capsule form and identical in size, shape, color, and texture. Participants were provided the entire allotment of supplement capsules in which daily doses were individually bagged. Participants were required to consume three capsules of either the placebo or PA once per day 60 minutes prior to RT and with dinner on non-RT days. Supplementation compliance was monitored by participants returning empty containers and individual bags of their supplement on Day 57, and also by completing a weekly supplement compliance questionnaire.

Body composition testing

At each of the 2 testing sessions at visit 1 (Day 0) and 2 (Day 57), total body mass (kg) was determined on a standard dual beam balance scale (Detecto Bridgeview, IL). Fat mass and fat-free mass were determined using DEXA (Hologic Discovery Series W, Waltham, MA). Quality control calibration procedures were performed on a spine phantom (Hologic X-CALIBER Model DPA/QDR-1 anthropometric spine phantom) and a density step calibration phantom prior to each testing session. For the variable of lean mass using DEXA, the intraclass correlation coefficient (ICC), standard error of measurement (SEM), and minimal differences needed to be considered real (MD) were 0.95, 1.69, and 4.74, respectively. Total body water was determined with bioelectrical spectroscopy [(BIS) ImpediMed Ltd., Australia] using a low energy, high frequency current (500 micro amps at a frequency of 50 kHz).

Rectus femoris cross sectional area

Prior to muscle strength assessment, determination of muscle size involved measuring RF CSA using ultrasonography (Sonosite M-Turbo, Milwaukee, WI, USA) based on previously-established guidelines (Seymour et al., 2009; Menon et al., 2012). Participants were placed in a supine position with a rolled-up towel placed in the popliteal fossa to relax the upper-thigh. Imaging was conducted after participants had rested in this position for five minutes to allow for the normalization of fluid shifts (Gibson et al., 2015). Excess conducting gel was applied to minimize underlying soft tissue distortion and optimize image clarity. The scanning site was identified as the midpoint of the distance from the greater trochanter to the knee joint line. The scanning depth was set to where the femur could be discerned for orientation. A 13.5 MHz linear array transducer was placed perpendicular to the long axis of the thigh to obtain a frozen real-time cross-sectional image of the rectus femoris muscle in order to determine RF CSA. At each time point, duplicate measures were performed and the average reported and images for all participants were obtained by the same investigator. The ICC, SEM, and MD were 0.93, 0.23, and 0.64, respectively.

Lower-body muscle strength

Participants performed 1-repetition maximum (1-RM) tests on the same angled leg press machine (Church et al., 2016) that was used during RT. Muscle strength testing occurred prior to the first dose of supplement and beginning of the resistance-training program at visit 1 (Day 0) and visit 2 (Day 57), after 56 days of supplementation and RT. Participants completed a standardized warm up consisting of 5 to 10 repetitions at approximately 150% of their total body mass for the angled leg press. The participant rested for 1 minute, and then completed 3 to 5 repetitions at approximately 175% of their total body mass. The weight was then increased conservatively, and the participant attempted to lift the weight for one repetition. If the lift was successful, the participant rested for 2 min before attempting the next weight increment. This procedure continued until the participant failed to complete the lift. The 1-RM was recorded as the maximum weight that the participant was able to lift for one repetition.

Resistance training protocol

Participants engaged in a periodized 4-day per week resistance-training program split into two upper- and two lower-extremity workouts per week for a total of eight weeks. This training protocol has been used previously (Spillane et al., 2012; 2014; 2016). Prior to the workout, participants performed a standardized series of stretching exercises. The participants then performed an upper body resistance-training program consisting of such exercises as bench press, lat pull, shoulder press, seated row, shoulder shrug, chest fly, biceps curl, triceps press down, and abdominal curl twice per week, and a lower-body program consisting of such exercise as leg press, back extension, step up, leg curl, leg extension, heel raise, and abdominal crunch, also performed twice per week. Participants performed 3 sets of 12, 10, 8 repetitions with as much weight as they can lift per set for weeks 1-4 and 3 sets of 8, 6, 4 repetitions for weeks 5-8 (typically 70 – 80% of 1RM). Rest periods between exercises and sets lasted no longer than 2 minutes. Resistance training sessions were supervised by study personnel, and each session was monitored and the exercises, number of repetitions and sets, and amount of weight per set was documented via weekly training logs.

Reported side effects from supplements

At visit 2 (Day 57), participants reported by questionnaire whether they tolerated the supplement, supplementation protocol, as well as report any medical problems/symptoms they may have encountered throughout the protocol of the study (Spillane et al., 2016).

Statistical analysis

Statistical analysis was performed by utilizing separate repeated-measure 2-factor [treatment groups (3) x time point (2)] analysis of variance (ANOVA) for each criterion variable. In addition, for all statistical analyses not meeting the sphericity assumption for the within-subjects analyses, a Huynh-Feldt correction factor will be applied to the degrees of freedom in order to adjust the critical F-value to a level that would prevent the likelihood of committing a type I error. An a-priori power calculation showed that 10 participants per group was adequate to detect a significant difference between groups in the dependent variable of muscle strength and the independent variable of resistance training, given a type I error rate of 0.05 and a power of 0.80. The index of effect size utilized was partial Eta squared ($\eta 2$), which estimates the proportion of variance in the dependent variable that can be explained by the independent variable. Partial Eta squared effect sizes were determined to be: weak = 0.17, medium = 0.24, strong = 0.51, very strong = 0.70 (O'Connor et al., 2007).

Similar to the approach utilized by Hoffman et al. (2012) and Outlaw et al. (2014), an analysis of magnitude-based inferences of differences in means was utilized in attempt to make inferences on true effects of PA on body composition and muscle mass and strength. A published spreadsheet using the unequal variances t-statistic was used (Batterham and Hopkins, 2006), and the overall impact of PA supplementation was determined as the change score by calculating the difference between the pre and post-supplementation scores for both the PA and PL groups. Confidence limits for the magnitude based inference were established at 90% using the p-value analogous to the t-statistic. The published spreadsheet calculated inferences whether the true population effect was considerably beneficial, harmful, or inconsequential based on the confidence interval range comparative to the value for the smallest clinical meaningful effect. An effect was stated to be unclear if the confidence interval overlapped the thresholds for positive and negative substantiveness (>5% chance that the value was both substantially positive and negative). If the value was positive or negative it was gauged by: <1%, almost certainly not; 1-5%, very unlikely; 5-25%, unlikely; 25-75%, possible; 75-95%, likely; 95-99% very likely; and > 99% almost certain. Results were interpreted using magnitude-based statistics, using Cohen's thresholds (< 0.1, trivial; 0.1-0.3, small; 0.3-0.5, moderate; > 0.5 large). All statistical procedures will be performed using SPSS 21.0 software (Chicago, IL, USA) and a probability level of ≤ 0.05 adopted throughout.

Results

Participant retention, reported side effects, and baseline measures

During the course of the study, four participants were withdrawn from the study due to injuries sustained during recreational activity not related to the study. As a result, 28 completed the study. The mean ±SD age, height, total body mass, and years of RT were: 19.7 ± 1.7 years, 1.74 ± 0.06 m, 75.5 ± 10.2 kg, and 3.2 ± 1.3 years for PA375; 20.2 ± 1.8 years, 1.79 ± 0.06 m, 84.6 ± 14.5 kg, and 2.8 ± 1.7 years for PA375; and 20.9 ± 2.8 years, 1.79 ± 0.08 m, 77.9 ± 12.7 kg, and 3.6 ± 2.6 years for PLC. Both PA groups and PLC were tolerated well and no adverse side effects were reported.

Body composition, muscle mass, and muscle strength

The means ±SD for each criterion variable at Day 0 and

Table 1. **Means (±standard deviations) for the criterion variables for each group.**

Variable	Variables	PA375	PA250	PLC	Time, p ≤	Group x Time, p ≤
Day 0	Body Mass (kg)	75.5 (10.2)	84.6 (14.5)	77.9 (12.7)		
	Body Water (kg)	44.2 (3.3)	47.4 (4.8)	45.5 (5.2)		
	Lean Mass (kg)	56.2 (5.8)	62.5 (8.3)	58.9 (9.7)		
	Fat Mass (kg)	11.4 (4.1)	13.8 (7.6)	10.9 (4.5)		
	RF CSA (mm^2)	2.84 (.9)	2.67 (.4)	2.87 (.8)		
	LP Strength (kg)	362.8 (76.1)	348.3 (62.5)	342.5 (86.0)		
Day 57	Body Mass (kg)	77.5 (9.8)	85.3 (14.5)	78.8 (12.6)	.003	.28
	Body Water (kg)	44.1 (3.7)	47.7 (4.8)	46.1 (5.6)	.25	.31
	Lean Mass (kg)	57.5 (5.9)	63.0 (7.1)	60.5 (9.1)	.008	.55
	Fat Mass (kg)	11.0 (4.6)	13.3 (7.4)	10.1 (5.0)	.04	.96
	RF CSA (mm^2)	3.22 (1.01)	3.41 (1.0)	3.21 (.8)	.01	.61
	LP Strength (kg)	411 (89.2)	418.4 (83.0)	370.2 (67.1)	< .001	.58

57 are displayed in Table 1 for all groups. No significant group x time interactions were noted for total body mass ($p = 0.28$, effect size = 0.11), total body water ($p = 0.31$, effect size = 0.11), lean mass ($p = 0.55$, effect size = 0.12), fat mass ($p = 0.96$, effect size = 0.10), RF CSA ($p = 0.70$, effect size = 0.10), and lower-body strength ($p = 0.58$, effect size = 0.13). However, there was a significant main effect for time for total body mass ($p = 0.03$; effect size = 0.66), lean mass ($p = 0.04$, effect size = 0.62), RF CSA ($p = 0.03$, effect size = 0.68), and lower-body strength ($p = 0.01$, effect size = 0.71).

Magnitude-based inferences on changes in anthropometric measures and muscle strength are described in Table 2. Magnitude-based inferences comparing differences between PA375 and PA250, and also PA375 and PLC, determined there to be a possible benefit in lean mass improvements, whereas increases in total body mass, RF CSA, and muscle strength are likely. When comparing differences between PA250 and PLC, there was a possible benefit in lean mass, a likely increase for lean mass and RF CSA, and a very likely increase in muscle strength. Compared to placebo, magnitude-based inferences determined both doses of PA to have a likely impact of increasing body mass, lean mass, and RF CSA, and very likely impact on increasing lower-body strength.

Discussion

In the present study, we investigated the effects of PA at a daily dose of 375 mg and 250 mg on body composition and muscle strength while participants engaged in eight weeks of supervised RT. Herein, we demonstrate neither dose of PA supplementation to have a differential effect, compared to each other and placebo, on increasing lean mass, RF CSA, or lower-body strength.

In rodent models which utilized muscle overstretch or resistance exercise, the literature to date indicates that direct binding of PA to mTOR activates mTORC1 (Hornberger 2006; Lehman 2007). Resistance exercise is a known stimulus for skeletal MPS via the PI3K/Akt-mTOR signaling pathway (Bodine et al., 2001; Koopman et al., 2006; Sandri et al., 2008); therefore, it is conceivable that exogenous PA supplementation combined with resistance exercise could further stimulate the mTOR pathway during RT. However, this was not investigated in our current study, nor has it been investigated in any of the previous human studies involving RT and PA supplementation (Escalante et al., 2016; Hoffman et al., 2012; Joy et al., 2014). In the human trial portion of the study by Joy et al. (2014), PA supplementation at a dose of 750 mg was shown to be effective at increasing muscle

Table 2. **Magnitude-based inferences between groups for each criterion variable.**

		Mean Difference	Clinical Inference	% Beneficial/ Positive	% Negligible/ Trivial	% Harmful/ Negative
Group PA375 vs. PA250	Body Mass	1.33	Likely	86.8	12.2	1.0
	Body Water	.065	Most Unlikely	0.0	100	0.0
	Lean Mass	.995	Possibly	74.4	22.7	2.9
	Fat Mass	-4.85	Unlikely	19.2	4.7	76.1
	RF CSA	19.77	Likely	76.5	1.1	22.4
	Leg Press 1-RM	161.4	Likely	87.1	.1	12.8
Group PA375 vs. PLC	Body Mass	1.41	Likely	87.8	11.2	1.0
	Body Water	.19	Most Unlikely	0.4	99.6	0.0
	Lean Mass	1.47	Possibly	67.3	14.4	18.3
	Fat Mass	-5.05	Unlikely	16.2	4.6	79.1
	RF CSA	22.95	Likely	92.5	0.8	6.7
	Leg Press 1-RM	135	Likely	92.3	0.1	7.6
Group PA250 vs. PLC	Body Mass	.755	Possibly	60.6	29.6	9.8
	Body Water	.415	Possibly	41.5	57	1.5
	Lean Mass	.995	Likely	75.3	22.3	2.4
	Fat Mass	-.56	Unlikely	9.3	37.6	53
	RF CSA	23.18	Likely	91.9	0.8	7.2
	Leg Press 1-RM	146.6	Very Likely	98.1	0.1	1.8

mass and strength. In addition, the *in vitro*, cell culture portion of their study, performed by co-authors portion of their study determined PA is capable of increasing mTOR signaling. However, direct implications from the *in vitro* portion of their study cannot be used to substantiate the role of mTOR up-regulation *in vivo* during the eight weeks of PA supplementation and RT that was also employed in humans. Furthermore, while the *in vitro* results from Joy et al. (2014) are noteworthy they provide little, if any, substantiation to the role that PA supplementation may have on mTOR-induced increases in MPS in humans in response to RT. Therefore, a study employing an *in vivo* design in humans would be much more meaningful in attempting to elucidate the impact of PA supplementation may have on the up-regulation of mTOR when combined with RT.

In the current study, we showed increases in body mass of 2.65%, 0.82%, and 1.15% for PA375, PA250, and PLC, respectively. For lean mass, increases of 2.31%, 0.8%, and 2.71% were observed for PA375, PA250, and PLC, respectively. Increases in RF CSA of 13.33%, 27.71%, and 11.85%, respectively, were observed for PA375, PA250, and PLC. Respective increases in lower-body strength of 13.36%, 20.12%, and 8.89% were observed for PA375, PA250, and PLC. In regard to our observed increases in lean mass and RF CSA, there were no significant differences, either due to PA or RT, in total body water. The results of all four studies compare very similarly, yet our current study and that of Hoffman et al. (2012) only observed significant increases in response to RT that were not due to the PA supplement. However, despite the overall similarity in results, the studies of Joy et al., (2014) and Escalante et al., (2016) showed significant increases that favored the PA group.

There are a number of potential discrepancies between the current investigation and the previous investigations that could help explain the incongruence in the outcomes of the four studies which include: 1) differences in RT program design, 2) resistance training experience of participants 3) supervised/monitored exercise sessions, 4) use of an energy-controlled diet, 5) provision of a collagen protein drink following each workout, 6) timing of supplement ingestion, 7) different exercises used to assess lower-body strength, and 8) different methods to assess thigh muscle size.

The current investigation utilized a similar resistance training program as Hoffman et al. (2012), eight weeks of RT 4 day/week with two upper-body and two lower-body training days, however, using identical designs Joy et al. (2014) and Escalante et al. (2016) examined eight weeks of supervised RT, but utilized an undulating single-set resistance training periodization program 3 day/week (with each muscle group being trained 1-2 days/week). Hoffman et al. (2012) utilized an identical PA dose as Joy and Escalante (750 mg), yet produced similar results in regards to strength and lean mass as seen in our current investigation with 375 mg and 250 mg that did not favor PA supplementation. Therefore, the discrepancy in results among the four studies could be the differences between RT programs and overall training volumes. A meta-analysis by Wolf et al. (2004) examined single set vs. multiple set resistance training studies in trained individuals and determined a multiple set approach produces greater adaptations. Comparatively, there appears to be no difference in set variation in untrained individuals during a short RT period. Based on the results of the meta-analysis of Wolf et al. (2004), given the reduced RT stimulus it is unclear how similar PA doses (750 mg) utilized in the Joy et al. (2014) and Escalante et al. (2016) studies produced significant increases in muscle size and strength compared to Hoffman et al. (2012), which also used 750 mg PA, and the current study which used 375 mg and 250 mg PA. Although, it is interesting to note that even in untrained males a multiple set approach has demonstrated a superior ability to improve strength and lean mass accumulation in the lower-body (Ronnestad 2007).

Some of the potential discrepancies between the current investigation and the previous investigations could be the time point at which the supplements were ingested. Hoffman et al. (2012) did not control the time of supplement ingestion. Joy et al. (2014) had participants ingest the supplements 30 minutes prior to exercise, whereas Escalante et al. (2016) had participants ingest supplements 30 minutes prior to exercise and immediately following exercise. It is obvious that there is no standardized time in which to ingest the PA supplement. As a result, we chose for our participants to ingest the supplements 60 minutes prior to exercise.

Another discrepancy could be the lower dosages of PA ingested and the lack of the provision of a post-workout collagen protein supplement or an energy-controlled diet. Previous investigations utilizing daily PA supplementation in conjunction with RT are limited to date and have all utilized 750 mg of PA in resistance-trained males while also providing a collagen protein post workout (Hoffman et al., 2012; Joy et al., 2014; Escalante et al., 2016). In the current investigation, we utilized resistance-trained males and a RT program more similar Hoffman et al. (2012); however, it involved lower doses of PA, did not employ an energy-controlled diet, nor did it provide a collagen protein post workout. Joy et al. (2014) examined eight weeks of supervised RT combined with 750 mg of PA (7day/week), but utilized an undulating single-set resistance training periodization program 3 day/week. PA significantly improved skeletal muscle size (determined by RF CSA using ultrasound), lean body mass, and leg press strength. Escalante et al. (2016) utilized the same experimental design and also observed significant improvements in lean body mass and strength that favored the PA group. However, in the Escalante et al. (2016) study thigh muscle mass was assessed by DEXA rather than ultrasound. In addition to 750 mg PA, the experimental supplement provided in their study also contained L-leucine, hydroxyl-methyl butyrate (HMB) and vitamin D3. Obviously, this is a major limitation to their study as there is no way to discern the actual impact of PA on lean mass and muscle mass and strength.

As with our current study, Hoffman et al. (2012) observed no statistical interactions between groups for lower-body strength and thigh muscle mass, and even though the current study used lower doses of PA and did not provide collagen protein post-exercise, both studies

yielded very similar increases over the course of RT. Further analysis using magnitude-based inferences revealed PA to have a likely benefit for improvements in lower-body strength and lean body mass. This led the authors to suggest a combination of daily PA supplementation combined with resistance training to have a likely benefit on strength improvement for lower-body, and very likely benefit of lean tissue accruement in young, resistance trained males. Incidentally, our current results are very similar to those of Hoffman et al. (2012). Both studies also utilized magnitude-based inferences, and both were in agreement that PA produced a more than likely benefit for lower-body strength and muscle mass increases.

Another difference between the previous PA studies is the utilization of direct supervision of RT sessions in Joy et al. (2014) and Escalante et al. (2016) study, but not in the study of Hoffman et al. (2012). However, in the current investigation we utilized supervised RT sessions and our results are very similar to Hoffman et al. (2012). Even though directly supervised RT in resistance-trained males has been shown to produce a greater rate of training load increase and strength gains compared with unsupervised training over 12 weeks (Mazzetti 2000), this does not appear to confound the results between these two studies.

A notable limitation with the current study, and the three previous studies, is that supplementation compliance was only monitored through compliance logs, thus the potential for misreports and non-compliance does exist. Although, in agreement with Hoffman et al. (2012), based on magnitude-based inferences, the results of our current study do provide evidence that a 4-day/week split routine RT program for eight weeks, combined with daily ingestion PA, appears to have a likely benefit on strength and may have a role in lean tissue accruement. However, additional research is necessary to complement these results including: 1) bioavailability studies to determine the absorption profile of orally administered PA and at different doses, and 2) studies utilizing humans and RT supplemented with PA, and perhaps at different doses of PA, while obtaining muscle biopsies to determine the role of PA-induced mTOR activation on MPS. Further, given the limited amount of current literature and the incongruence among results of the studies, the justification for additional investigations remains present. If PA has a positive impact on lean mass accruement and strength measures, based on the studies performed thus far there is undoubtedly a dose response relationship that warrants additional investigations. While human RT studies can indicate possible effectiveness of PA in muscle mass and performance, until muscle biopsy samples are collected during a training study it is difficult to infer that any beneficial effects of PA supplementation that have occurred with the studies of Joy et al. (2014) and Escalante et al. (2016) are due to PA-induced activation of mTOR.

Conclusion

In conclusion, PA supplementation at 375 mg and 250 mg for eight weeks in conjunction with RT did not produce a differential effect for significant gains in lean mass, rectus femoris cross-sectional area, and lower-body strength. However, when using magnitude-based inferences PA has a more than likely impact on improving lower-body strength, whereas a likely impact for increasing muscle size and lean mass when combined with resistance training.

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Key points

- In response to eight weeks resistance training and PLC and PA (375 mg and 250 mg) supplementation, similar increases in lower-body muscle strength occurred in all three groups; however, the increases were not different between supplement groups.
- In response to eight weeks resistance training and PLC and PA (375 mg and 250 mg) supplementation, similar increases in lean mass occurred in all three groups; however, the increases were not different between supplement groups.
- In response to eight weeks resistance training and PLC and PA (375 mg and 250 mg) supplementation, similar increases in muscle mass (RF CSA) occurred in all three groups; however, the increases were not different between supplement groups.
- Supplementation of PA in conjunction with RT does not impose a differential benefit; however, regarding trends in the data magnitude-based inferences indicate that PA has a more than likely impact on improving lower-body strength, whereas a likely impact for increasing muscle mass when combined with resistance training.

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