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A SINGLE DOSE OF ORAL ATP SUPPLEMENTATION IMPROVES PERFORMANCE AND PHYSIOLOGICAL RESPONSE DURING LOWER BODY RESISTANCE EXERCISE IN RECREATIONAL RESISTANCE TRAINED MALES

Short title: ATP supplementation and performance in resistance trained males.

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ABSTRACT

The aim of this study was to investigate the acute effect of ATP supplementation on performance and physiological responses during resistance exercise in recreationally resistance trained males. Eleven men (age= 27.5±5.5 yrs, weight= 83.4±9.8 kg, height= 182±0.04 cm) completed two randomized, double-blind trials: ATP supplement condition (ATP=400mg) or a placebo condition. Thirty minutes after supplement consumption, subjects performed four sets of half-squats until momentary muscular failure at 80% of the 1RM with two minutes of recovery between sets. The total number of repetitions, blood pressure, heart rate, blood lactate, and oxygen consumption were evaluated. The total weight lifted were higher for the ATP condition compared to placebo (Placebo= 3995.7±1137.8, ATP= 4967.4±1497.9 Kg; p= 0.005). Heart rate was higher at set-4 for ATP compared to placebo (p<0.001) and oxygen consumption during exercise was greater for ATP (p=0.021). There were no differences between conditions for lactate and blood pressure. In summary, a single oral dose of ATP supplementation improved lower body resistance training performance and energy expenditure in recreational resistance trained males.

Keywords: ATP supplementation, strength exercise, nutrition.

INTRODUCTION

Since the ability to increase lean mass and strength progressively diminishes with training experience (3), many competitive and recreational athletes supplement with nutrients and other compounds with purported ergogenic effects. The use of dietary sport supplements in conjunction with resistance training has been studied to maximize fat loss, muscle hypertrophy and performance (23,14). Adenosine-5'-triphosphate (ATP) is the primary source of energy for muscle cells, and the ability to rapidly resynthesize or maintain intramuscular ATP during

intense exercise enhances performance. As such, orally administered ATP is found in several products on the supplement market.

To our knowledge, the first study to evaluate the effects of oral ATP administration was performed by Jordan et al. (18). Subjects consumed a placebo, 150 mg or 225 mg of enterically coated ATP for 14 days. In addition to post testing, an acute test (1 hour post ingestion) was performed on day 1. Oral ATP supplementation did not increase whole blood or plasma ATP concentrations, nor did it improve any metric of performance measured via the anaerobic Wingate test either acutely or sub-chronically. There were no differences between groups sub-chronically for bench press 1 RM, however, the 1 RM of the group ingestion 225g mg ATP was significantly greater during the acute test. The group consuming 225 mg ATP performed more repetitions during 3 sets of bench press to fatigue with 70% of the 1 RM compared to the 150 mg ATP and placebo group.

Utilizing a crossover design with a 1 week washout period, Rathmacher et al. (28) reported that 14 days of 400 mg/day ATP supplementation did not enhance peak torque during 3 sets of 50 knee extension contractions, nor average power, total work, or a decrease in work fatigue in active young adults. A small improvement in torque during the final 10 repetitions of the set and a trend for lower fatigue in the third set were found. These results in addition to the improvement in total repetitions reported by Jordan et al. suggest that ATP supplementation may be most ergogenic when the exercise task is fatiguing; however, the ambiguity in findings necessitates a need for further research.

Several studies have investigated the chronic effects of ATP supplementation on strength and hypertrophic adaptations. Wilson et al. (35) reported that oral supplementation of ATP (400 mg/day) combined with resistance training for 12 weeks increased muscle mass and strength in resistance-trained males. Lowery et al. (22) reported a synergistic effect between ATP and beta-hydroxy-beta-methylbutyrate on lean body mass and strength during 12 weeks of resistance

training. Although these studies suggest chronic ATP supplementation may enhance hypertrophic adaptations, discrepancies in the methods and inconsistencies in the reporting of the group characteristics (27) call into question the results of the aforementioned studies, and no other studies have evaluated hypertrophic adaptations associated with chronic ATP supplementation.

Because extracellular ATP is tightly regulated at very low concentrations (15), it is unlikely that ATP is absorbed intact in humans (4), which would explain why despite increases in performance have been observed with ATP supplementation, increases in plasma ATP following supplementation have not (Jordan et al.). The ergogenic effects of ATP supplementation may therefore be the result of extracellular ATP carried by red blood cells signaling purinergic receptors, which control blood flow and muscle excitability (7). During exercise extracellular ATP is released from blood erythrocytes (7, 25). When ATP binds the P2Y receptor in endothelial tissue, the synthesis of vasodilator substances occurs (nitric oxide, prostaglandin and endothelial-derived hyperpolarization factors), thereby potentially enhancing the supply of oxygen and nutrients to the active muscles during exercise (13). Although oral ATP supplementation likely does not increase the bioavailability of extracellular ATP, Kichenin et al. (19) demonstrated that chronic ATP administration in rats enhanced ATP uptake and synthesis in the erythrocyte. These findings suggest that greater ATP levels in erythrocyte via ATP supplementation may enhance blood flow and exercise performance in humans.

Another physiological mechanism that may explain these ergogenic effects is that extracellular ATP can modulate the contractility of skeletal muscle during exercise via the purinergic receptor P2X4. The binding of extracellular ATP at P2X4 receptor increases calcium intracellular influx (30). During intense exercise, the decrease in rate of intracellular calcium influx contributes to reduction of force generation by skeletal muscle (11). Activation of P2X4 by extracellular ATP in muscle cell increases the release of calcium by the sarcoplasmic reticulum (30), enhancing muscle production via greater interactions of actin and myosin filaments (16,21).

The purpose of this study was to investigate the effect of a single dose of ATP supplementation on lower-body resistance exercise performance and the physiological responses in recreationally resistance trained males. We hypothesize that acute ATP supplementation will attenuate fatigue and result in greater resistance training volume and oxygen consumption.

MATERIALS AND METHODS

Experimental Approach to the Problem

This study utilized a randomized, double-blind design. Subjects completed 3 experimental trials at the laboratory separated by a minimum of 3 days. All trials were performed at the same time (morning) to ensure chronobiological control and were separated by a week. A schematic illustration of the experimental design can be seen in Figure 1A. The first visit aimed to determine anthropometric measurements and 1RM test for half-squat. On the following two visits, each subject consumed randomly either the placebo or ATP supplement. We used a simple randomization techniques for allocation, which ensured that trial participants had an equal chance of being allocated to a given treatment group (10). After that, the participants completed 4 sets of half-squat until momentary muscular failure with a load corresponding to 80% of the 1RM and 2 minutes of rest between sets. The total number of repetitions performed was recorded for each set and was used to analyze performance. Blood lactate and oxygen consumption were analyzed during and after resistance exercise to determine metabolic costs. Heart rate and blood pressure also were collected (**Figure 1**).

Insert Figure 1

Subjects

Eleven recreationally resistance trained males with at least 1 year of resistance training experience (experience, 3.4±1.5 yr) at a frequency of 3 days per week and 60 minutes per day were recruited for this study. Written informed consent was obtained from all subjects after they had been informed about the purpose and risks of the study. All participants reported that they had not used any dietary supplements for at least six months prior to the study. Subjects were instructed not to use any other ergogenic substances or supplements during the study. The project was approved by the Ethics Research Group of the University of São Judas, São Paulo-SP, Brazil (Protocol number: 66523717.2.0000.0089) and the research was conducted according to the 2008 Revision of the Declaration of Helsinki.

****Insert table 1****

Procedure

Anthropometric Measurements, Supplementation Protocol and Dietary Intake.

Height was measured on a fixed stadiometer of the Sanny brand, with an accuracy of 0.1 cm and a length of 2.20 m. Body weight was measured using an electronic scale (Filizola PL 50, Filizola Ltda., Brazil), with a precision of 0.1 kg.

A previous pilot study was performed to match the supplements to taste. Approximately 35 ml of each sample was served in 200 mL glass beakers codified with random two digit numbers (1 or 2), under white light at refrigeration temperature (10 to 15ºC). The samples were evaluated regarding color, smell and taste via the nine-point hedonic scale (1 = disliked extremely, 9 = liked extremely) according to Villanueva et al (33).

Fifteen subjects were escorted to individual booths and the participants received instructions about the use of the specific scale to be used in that session tested each glass. Participants identified similar color, smell and taste between placebo and ATP. Each participant randomly consumed either the placebo (Clight Juice Kraft Foods Brazil - 200 mL: energy 21 kj, 5 kcal, sodium 4,7 mg) or 400mg of oral ATP supplementation (ProMera Health LLC, Norwell, MA, USA - 200ml: 400mg adenosine-5'-triphosphate disodium). The supplement or placebo was ingested 30 minutes prior to the resistance exercise tests supplied in identical bottles (35).

During the study all participants were instructed not to use any other supplement or ergogenic substance, as well change their regular diet and exercise behaviors. They were also instructed not to consume caffeine 12 h prior to each experimental test. Food questionnaires were distributed to all participants to record their food and fluid intake for 24 h and pre-exercise meal (breakfast) prior to each trial. Participants were instructed to replicate the first trial's dietary intake for the subsequent trial. All food intakes were analyzed for total kilocalorie and macronutrient intakes ((Software - Dietpro version 5.8) to ensure that dietary intake was similar between experimental trials. Database of Brazilian food composition table (TACO) was utilized to calculate dietary intake.

Blood pressure and heart rate measurements

Subjects were instructed to remain resting awake in silence for 30 minutes, breathing spontaneously in the supine position before and after aerobic exercise. After the procedures had been explained, an elastic strap was placed at the height of the xiphoid process of the subject and a heart rate receptor on the wrist (Polar Electro, model S810i or RS800, Finland).

Blood pressure and heart rate (HR) were recorded at baseline, 30 minutes post-ingestion, during exercise (post set-1, 2, 3 and 4), post-10, post-20, post-30, post-40,

post-50 and post-60 minutes after exercise. The systolic and diastolic blood pressure were measurement using automatic blood pressure monitor (Omron Healthcare brand, Inc., Intellisense, Model HEM 742 INT, Bannockburn, Illinois, USA).

Oxygen Consumption, Energy Expenditure and Blood Lactate

To estimate the energy expenditure of all exercise bouts, the sum of the contribution of the three energy systems (aerobic, anaerobic lactic and alactic) was used. The aerobic metabolism was estimated using the oxygen uptake integral during the exercise, the anaerobic alactic was assessed using the fast phase of excess of oxygen uptake as presented by Bertuzzi et al. (6) and the lactic anaerobic contribution using net blood lactate accumulation as proposed by Di Prampero and Ferretti (9).

Oxygen uptake was measured continuously for 10 min after the exercise protocols using a breathe-by-breath Quark PFT (COSMED, Rome, Italy) system. The blood samples collected from the ear lobe were utilized to analyze the lactate concentration [La-]. This measurement was obtained at rest, between each set and 3, 5, 30 and 60 minutes after trials. The analyses were performed using the lactate analyzer Yellow Spring 1500 Sport (Yellow Springs, USA). For the anaerobic alactic contribution, the fast component of excess post-exercise oxygen consumption (EPOC) was determined using a modified bi-exponential decay equation. The anaerobic alactic contribution corresponded to the product of bi-exponential fast component amplitude and tau (4, 36). The aerobic metabolism was estimated by subtracting rest oxygen consumption from exercise oxygen consumption. To estimate the total energy expenditure and oxygen consumption during each protocol, the energy expenditure were summed and converted to kJ, assuming that 1 L of oxygen consumed was equivalent to 20.9 kJ (12).

193 **Resistance exercise protocol**

194 Before their first trial, subjects completed two familiarization sessions to become
195 acquainted with the 1 RM test procedures and training equipment. Prior to 1RM testing,
196 subjects completed a warm-up protocol, which consisted 5 minutes of walking and subsequent 1
197 set of 10 repetitions at approximately 50% of the 1RM. The load was increased gradually (10–
198 15%) during the test until the participants were no longer able to perform the entire movement,
199 and 3–5 attempts were allowed as previously described (29).
200
201 Prior to the experimental trials subjects performed a warm-up with walking for 5
202 minutes on a treadmill and a subsequent 1 set of 15 repetitions at 30% of 1RM. After 3 minutes
203 of recovery, each participant completed 4 sets until movement failure at 80% of 1RM with
204 normal speed (1-s eccentric and 1-s concentric) and 2 minutes of rest intervals between sets.
205 Two fitness professionals supervised all testing sessions. For better control of the strength test
206 procedures and resistance exercise protocol, a wooden seat with adjustable heights was placed
207 behind the participant in order to keep the bar displacement and knee angle constant on each
208 repetition.
209
210 **Statistical analysis**
211 The data normality was verified using the Shapiro-Wilk test. The comparison of the total
212 weight lifted under the different conditions was analyzed via a repeated measured *t* test. A 2 x 4
213 repeated measures analysis of variance (RMANOVA) with the Bonferroni adjustment for
214 multiple comparisons was used to compare the maximum number of repetitions performed in
215 each set across conditions and time, respectively. A 2 x 12 RMANOVA was used to compare
216 heart rate and blood pressure across condition and time and 2 x 10, 2 x 5 RMANOVA was used
217 to compare lactate, respectively across condition and time. For all measured variables, the
218 estimated sphericity was verified according to Mauchly's W test and the Greenhouse–Geisser
219 correction was used when necessary. Statistical significance was set at $p<0.05$. The effect size
220 for total repetitions performed and workload was calculated via Cohen´s *d* ([treatment mean -

placebo mean] / pooled standard deviation) whereby a value of > 0.20 was considered small, > 0.50 moderate, and > 0.80 large (8). The data were analyzed using the Statistical Package for Social Sciences 17.0 (SPSS Inc. Chicago. IL.USA).

RESULTS

Table 1 presents the mean and standard deviation values for age, body weight, height, experience, resistance training frequency, body composition and dietary intake.

Figure 2 shows the differences in performance for placebo and ATP condition. There was a main effect of time (F= 29.959, p< 0.001) and statistically significant differences between condition (F= 12.208, p= 0.006) but no interaction was observed (F= 2.145, p= 0.115). Figure 2A shows that the volume decreased during the sets of squat in both conditions and there were statistically significant differences between conditions for total repetitions (Fig.2B: Placebo= 40± 11 vs ATP= 49.4± 11.5 Kg; p= 0.006) and total weight lifted (Fig.2C: Placebo= 3995.7± 1137.8 vs ATP= 4967.4± 1497.9 Kg; p= 0.005). Effect sizes were large for number of repetitions (d= 0.83) and ranged from moderate to large in the total weight lifted (d= 0.73).

Insert Figure 2

Figure 3 presents the differences in heart rate and blood pressure in placebo and ATP condition. For Heart rate (bpm) there was a main effect of time (F= 251.3, p<0.001) and significant interaction (F= 3.120, p=0.001). The Post-hoc test identified a higher HR for ATP condition at set-4 compared to placebo (p< 0.001). For SBP (mmHg) there was a main effect of time (F= 35.106, p< 0.001) and significant interaction (F= 2.073, p= 0.028), but no difference between conditions (F= 2.070, p= 0.181). For DBP (mmHg) there was a main effect of time (F= 4.399, p< 0.001) but no differences between conditions or interaction (p> 0.05).

Insert figure 3

Figure 4 presents the differences in lactate concentrations in placebo and ATP condition. For lactate, there was a main effect of time (F= 6.019, p< 0.001), and a tendency for a significant difference between condition (F= 4.766, p= 0.05) but no interactions were observed (F= 0.964, p= 0.474).

Insert figure 4

Figure 5 presents the differences in oxygen uptake during exercise, EPOC and total oxygen consumption. For relative and absolute oxygen uptake during exercise, there were statistically significant differences between conditions (p<0.005). For EPOC, there were no differences between condition when expressed relative to body weight but a significant difference was observed in Kcalories (p= 0.041). For total oxygen consumption there was difference between conditions in Kcalories (p= 0.041).

Insert figure 5

DISCUSSION

To our knowledge, this was the first study to investigate the effects of acute oral ATP supplementation on performance and physiological responses during lower-body resistance exercise in recreationally trained men. The main findings of the present study were that ATP supplementation increased performance and oxygen consumption during lower body resistance exercise.

Our results are in agreement with previous studies that have investigated the ergogenic effect of ATP supplementation. Jordan et al. (18) showed that 14 days of 225 mg of ATP supplementation was effective to increase total weight lifted and number of repetitions to failure

(first set) in the bench press exercise, but they found that a lower dosage (150 mg) did not have benefits on performance. Rathmacher et al. (28) reported that 14 days of 400 mg/day ATP supplementation led to small improvements in torque during the final 10 repetitions of a 50 repetition set. Given that ATP supplementation did not improve 1 RM (18) nor peak force (28), it is plausible that the ergogenic effects of ATP supplementation are limited to resistance training activities that are of a high volume and carried out with a high degree of muscular fatigue (i.e.: several sets with many repetitions to failure).

The possible mechanisms by which ATP supplementation enhances resistance exercise performance may be attributed to the effects of extracellular ATP on muscle excitability. Previous studies demonstrated that muscle fatigue and the reduction in force production induced by high intensity contractions is due impairment of calcium release by the sarcoplasmatic reticulum (2, 32, 34). Extracellular ATP can modulate the contractility of skeletal muscle by binding to P2X4 receptor and increasing intracellular calcium influx (30). Therefore, it is possible that ATP supplementation may delay the reduction of calcium release during muscle contractions, maximizing muscle strength production by greater interactions of actin and myosin filaments (16, 21); however, further research is needed to investigate this hypothesis.

Another possible mechanism whereby ATP supplementation may enhance performance during acute resistance exercise is through the effects of extracellular ATP on vascular tissue. During muscle contractions under hypoxic conditions blood erythrocytes releases ATP which binds to the P2Y receptor in the endothelial tissue. This induces production of endothelium-derived hyperpolarizing factor, prostacyclin, and nitric oxide by endothelial cells, relaxing the smooth muscle of the vasculature (7). We found that ATP supplementation increased oxygen uptake during resistance exercise. It is possible that the vasodilatory effects of ATP may have enhanced oxygen delivery to the working tissues thereby resulting in the improved performance observed in this study. In support of this hypothesis, Jager at al. (17) verified the effects of ATP

supplementation on blood flow after resistance exercise in rats and humans and reported higher blood flow following resistance exercise with ATP supplementation.

Previous studies demonstrated that total weight lifted can influence energy expenditure during a resistance training session (1, 24). In this study we found an increased energy expenditure during the ATP condition. This suggests that ATP supplementation may increase aerobic energy expenditure, possibly due to a greater volume of work performed. Some studies have demonstrated that total volume has important implications for muscle hypertrophy and strength gains (20, 26, 31). Our findings demonstrate that acute ATP supplementation increased total weight lifted in recreationally trained males during lower body resistance exercise, however, more research is needed to investigate chronic ATP supplementation and resistance training in populations of different training status on body composition and the immunemetabolic response to resistance exercise.

In conclusion, a single dose of ATP supplementation improved performance, oxygen consumption and energy expenditure during lower body resistance exercise in recreational resistance trained males.

PRACTICAL APPLICATIONS

This study suggests that resistance-training practitioners can supplement ATP prior to a fatiguing, high volume lower body resistance exercise session to enhance total weight lifted and energy expenditure. Additionally, the dose and form of ATP supplementation (400 mg) was well tolerated in this study.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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TABLES AND FIGURES CAPTIONS

Figure 1

Experimental Design

Table 1: General characteristics of the sample, dietary intake and macronutrient distribution.

Figure 2: Comparison between placebo and ATP condition on the performance.

Legend: 2-A= total weight lifted (Kg); 2-B= maximum number of repetitions in each series; a= main effect of time with Bonferroni's test and p-value < 0.05 compared to set-1. b= main effect of time with Bonferroni's test and p-value < 0.05 compared to set-2.

Figure 3: Comparison between placebo and ATP condition on the heart rate and blood pressure.

Legend: HR= heart rate (bpm); SBP= systolic blood pressur (mmHg); DBP= diastolic

Figure 4: Comparison between placebo and ATP condition on the lactate concentration.

Figure 5: Differences on the oxygen uptake during exercise, excess post-exercise oxygen consumption and total oxygen consumption in placebo and ATP condition.

Legend: EPOC= excess post-exercise oxygen consumption.

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494 **Table 1**. General characteristics of the sample, dietary intake

495 and macronutrient distribution.

Age (y)	27.5±5.5
Height (cm)	182.0±0.04
Weight (kg)	83.5 ±9.8
Squat 1 RM (kg)	127.8±19.7
Dietary Intake 24 hours	
Diet CHO (g)	211,3± 55.8
Diet PRO (g)	149,7±81.1
Diet FAT (g)	54,13±21.7
Total Intake (kcal)	1931±562.1
Dietary Intake Pre-training	
CHO (g)	44,21±18.1
PRO (g)	20,69±9.1
FAT (g)	12,54±6.6
Total Intake (kcal)	372,4±109.8

496 CHO= carbohydrate (g); PRO= protein (g); FAT= lipids (g);

497 1RM= one maximum repetition.

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