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Original article

Short-term L-arginine supplementation attenuates elevation of interleukin 6 level after resistance exercise in overweight men

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SUMMARY

Background and aim: L-Arginine (L-arg) supplementation and resistance exercise can induce changes in inflammatory and anti-inflammatory cytokines; however, it has not been investigated in obese hypertensive men. This study examines the effects of short-term L-arg supplementation and acute resistance exercise (AREX) on cytokine levels in obese hypertensive men.

Methods: Eight obese hypertensive men aged 46 ± 6 yrs. with an average body weight of 92.56 ± 9.9 kg and a BMI of 31.68 ± 2.18 kg/m^2 participated in a randomized, double-blinded, crossover study. The patients were distributed into exercise groups based on the type of supplementation (6 g/day of placebo or L-arg for 7 days). Supplementation periods were separated by a seven-day washout period. The AREX regimen consisted of eight exercises with an exercise intensity of 60% of 1 repetition maximum. The interleukins IL-1ra, IL-6, and IL-10 and the IL-6/IL10 ratio were determined at rest, immediately after exercise and 1 h after exercise sessions.

Results: IL-1ra levels exhibited a significant difference both immediately and 1 h after exercise when the L-arg and placebo groups were compared ($P < 0.05$). IL-6 levels increased significantly after exercise in the placebo group compared with the L-arg group ($P < 0.05$). The placebo group showed a decrease in the IL-10 levels 1 h after exercise compared with resting levels ($P < 0.05$). The IL-6/IL-10 ratio showed a statistically significant increase in the placebo group after exercise compared to the L-arg group ($P < 0.05$).

Conclusions: LARG supplementation attenuates the cytokine increase after AREX, in particular peak IL-6 levels decrease and exercise induced decreases in IL-10 levels are attenuated.

1. Introduction

Chronic diseases, such as overweight/obesity, hypertension, dyslipidemia, diabetes, aging, cancer cachexia, sleep disruption and others, are characterized by the presence of low grade inflammation and increased pro-inflammatory cytokine levels, principally interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) [1–5]. This low grade inflammation can aggravate the disease and reduce the quality of life of the individual.

L-Arginine (L-arg) is a conditionally essential amino acid in the human diet that functions as the substrate for nitric oxide synthases (NOS) to produce nitric oxide (NO) [6]. Recent studies have demonstrated that L-arginine supplementation (9 g of L-arginine, over 6 months) affects zinc status and reduces plasminogen activator inhibitor type 1 levels in obese patients, due, at least in part, to the improvements in insulin sensitivity [7,8]. We recently showed that L-Arginine supplementation (6 g over 7 days) reduced

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LDL cholesterol and non-ester fatty acid levels after acute exercise in overweight men [9]. In addition, both acute and chronic exercise can also modify the immune system through increased levels of cytokines, such as IL-6, IL-Ira, IL-10 and TNF-α. This increase in cytokine level is dependent on the intensity, duration and type of exercise [10]. This exercise-induced regulation of the immune system is partly explained by an increase in IL-6 that induces IL-1 and TNFα receptor antagonists (IL-1ra and TNF-R, respectively) and other anti-inflammatory cytokines [10]. This condition favors an anti-inflammatory status after exercise and, when individuals continue in an exercise training program, leads to an anti-inflammatory condition with increases in IL-1ra and IL-10 levels.

The effects of 7 days L-arg supplementation combined with acute resistance exercise (AREX) on cytokines remain unclear. Given the paucity of studies investigating the effects of combined L-arg supplementation and AREX on cytokines, we hypothesized that 7 days L-arg supplementation combined with AREX may have effects on pro and anti-inflammatory cytokines in obese hypertensive men.

2. Materials and methods

2.1. Subjects

Eight obese, hypertensive men who were sedentary non-smokers with a mean age of 46 ± 6 yrs., body weight of 92.56 ± 9.9 kg and body mass index (BMI) of 28.6–35 kg/m^2, participated in this study. Inclusion criteria for the study were non-smokers with controlled use of antihypertensive medications who did not have a comorbidity that compromised their participation in the study. Co-morbidities, such as disorders of the locomotor system or chronic ischemia, excluded subjects from participating in the study. Volunteers were recruited through newspaper and internet advertisements. Benefits and risks were explained to participants before written consent was obtained according to an established protocol. The study procedures were previously approved by the Ethics Committee of the Universidade Federal de São Paulo – CEP #001/10. All subjects completed the study.

2.2. Anthropometric

Anthropometric measurements included height, body weight, hip and waist circumference. Body composition, fat and lean body mass were taken using the BOD POD® (Body Composition System, v2.14; Life Measurement Instruments, CA).

2.3. Supplementation

Supplements of L-arg (Sigma®) or placebo (starch) gelatin capsules (6 g/day, 3 times per day and 2 g each time) were administered orally for one week [11]. The study used a randomized, double blinded, crossover design. Supplementation periods were separated by a seven-day washout period. The placebo capsules were the same size, color and flavor as the L-arg capsules.

2.4. Dietary assessment

Dietary assessments were conducted using three-day food records (two weekdays and one weekend day), which participants completed following a dietitian's instructions. Participants reported their dietary intake during each of the three days, including the type, amount and quality of foods they ate. From these records, we quantified and analyzed the intake of macronutrients, arginine and energy (Nutrisurvey® software, São Paulo, Brazil).

2.5. Acute resistance exercise session

After medical evaluation, the participants underwent three exercise adaptation sessions to learn correct techniques for executing the movements. The exercises were conducted at the Centro de Estudos em Psicobiologia e Exercício (CEPE). After each adaptation session, we performed a one repetition maximum (1RM) test to determine the percentage of the workload for all exercises. The maximum weight lifted in a single repetition was identified as the 1RM.

The participants performed four AREX sessions, in three sets of 12 repetitions, as shown in the study design (Fig. 1). The intensity of the exercise session was 60% of 1RM and exercises alternated muscle groups, beginning with exercises that required larger muscle groups and continuing to exercises that required smaller muscle groups. The following exercises required the use of weight machines (Technogym®, Italy) and comprised the AREX: chest presses, leg presses, handle backs, leg extensions, shoulder press, leg curls, bicep curls, and triceps pulleys. An execution speed of 2:2 was used with recovery intervals of 60 s between sets and 2 min between exercises. Before each exercise session, participants performed stretching exercises for the major muscle groups.

2.6. Blood sampling and analysis

Blood samples were collected at baseline (after fasting for 12 h), immediately after AREX sessions and 1 h after AREX sessions. Blood samples (10 mL) were immediately allocated into two 5-mL vacutainer tubes (Becton Dickinson, BD, Brazil) containing EDTA for plasma separation. The tubes were centrifuged at 2500 g for 12 min at 4 °C. Plasma samples were stored at −20 °C until analysis. Cytokines (IL-1ra, IL-6, IL-10) were analyzed using commercial ELISA kits (R&D Systems, 614 McKinley Place NE, Minneapolis, MN 55413, USA).

2.7. Statistical analysis

The data distribution was checked with the Shapiro–Wilk's test. The data are reported as the mean ± standard error of the mean. The differences for the blood parameters were evaluated by a 2 × 2 factorial design with two supplements (placebo or arginine) and three AREX sessions (before, immediately after), and 1 h after exercise using a two-way ANOVA with repeated measures and a post hoc Tukey test. The analysis was conducted using GraphPad Prism (version 5.0) software, and the significance level was set at $p < 0.05$.

3. Results

The baseline characteristics of the subjects are described in Table 1. The high fat mass (%), BMI and waist circumference/Hip circumference, which were greater than the normal-to-healthy range and characterized the subjects as overweight 31.68 ± 2.18, could be attributed to several risk factors.

Between periods of supplementation volunteers showed no changes in measurements of macronutrients and energy (Table 2). However, food intake was irregular and imbalanced, exhibited a high intake of lipids and energy, and may have contributed to the maintenance of excess weight in these volunteers and influenced their health. IL-1ra levels (pg/mL) decreased immediately after an AREX session, but only 1 h later had statistical significance in the L-arg when compared to the resting level (518.7 ± 42.3 vs 323.7 ± 31.2; $p < 0.05$; Fig. 2). There was little difference in IL-1ra levels immediately after exercise and 1 h later.

IL-6 levels (pg/mL) increased after *Exe* in the placebo vs L-arg (2.32 ± 0.25 vs 1.34 ± 0.21; Fig. 3). The increase in IL-6 levels after