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Effects of anatabine and unilateral maximal eccentric isokinetic muscle actions on serum markers of muscle damage and inflammation

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ABSTRACT

The purpose of this study was to examine the effects of anatabine supplementation in conjunction with unilateral, maximal eccentric isokinetic muscle actions on serum markers of muscle damage and pro-inflammatory cytokines in humans. Seventeen men (mean ± S.D. age = 22.4 ± 3.2 yrs) participated in this double-blinded, placebo-controlled, crossover study. Participants were randomly assigned to two 10-day conditions (anatabine and placebo) separated by a 2–4 week washout period. After seven days of supplementation, blood was sampled immediately prior to PRE, immediately following POST, and 24, 48, and 72 h after 6 sets of 10 repetitions of unilateral, maximal eccentric isokinetic forearm flexion exercise. Concentrations of serum creatine kinase, lactate dehydrogenase, myoglobin, high sensitivity c-reactive protein, and TNF-α were measured. Creatine kinase, myoglobin, and lactate dehydrogenase increased ($P < 0.05$), while high sensitivity c-reactive protein and TNF-α did not change ($P > 0.05$) after the eccentric exercise during both conditions. Lactate dehydrogenase was higher ($P < 0.05$) during the anatabine condition. The primary findings of this study were two-fold: (a) anatabine had no beneficial effects on traditional markers of muscle damage (creatine kinase, lactate dehydrogenase, and myoglobin) compared to placebo after the eccentric exercise protocol, and (b) the eccentric exercise protocol did not elicit increase in the pro-inflammatory cytokines (c-reactive protein and TNF-α). Future studies are needed to examine the effects of anatabine on naturally-occurring inflammation that is common with aging or obesity. Furthermore, additional research is needed to examine the relationship between muscle damage and inflammation after eccentric exercises of different modes, durations, and intensities.

1. Introduction

Heavy eccentric exercise induces structural damage to skeletal muscle that is characterized by *z*-line streaming and a disorganization of myofilaments (Friden et al., 1981; Newham et al., 1983), interruption of the excitation contraction coupling process (Proske and Morgan, 2001), and inflammatory responses (Clarkson et al., 1992; Houghton and Onambele, 2012). Sarcolemmal disruption (Peake et al., 2005) occurring after muscle damage facilitates the release of intercellular enzymes and muscle proteins such as creatine kinase, myoglobin, and lactate dehydrogenase into the interstitial fluid, followed by uptake into the lymphatic system and eventual release into circulation (Brancaccio et al., 2010; Nosaka et al., 2003). In fact, it is common to observe abnormally high serum creatine kinase (i.e. $> 10{,}000$ IU L^{-1}) and myoglobin (i.e. > 1000 IU L^{-1}) concentrations following eccentric muscle actions of the forearm flexors (Clarkson et al., 1992; Nosaka et al., 2003). In addition, there is production and release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and c-reactive protein, which attracts lymphocytes, monocytes, and neutrophils (Houghton and Onambele, 2012; Ostrowski et al., 1999) to the damaged tissue (Tidball, 2005). Pro-inflammatory cytokine concentrations may be related to the amount of delayed onset muscle soreness and recovery from muscle damage (Northoff and Berg, 1991; Richards and Gaulder, 1998; Smith et al., 2000). Consequently, studies have examined the efficacy of interventions such as anti-inflammatory drugs (Nieman et al., 2006), cryotherapy (Pournot et al., 2011), massage therapy (Crane et al., 2012), and dietary supplementation (Childs et al., 2001; Houghton and Onambele, 2012; Michailidis et al., 2013; Serra et al., 2012) to reduce markers of muscle damage and the pro-inflammatory cytokine responses from eccentric-induced muscle damage.

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Anatabine, a minor tobacco alkaloid with a similar chemical structure to nicotine, has demonstrated (Paris et al., 2011, 2013) anti-inflammatory properties and the ability to regulate cytokine production via inhibition of Signal Transducer and Activator of Transcription 3 (STAT3) and NFkB phosphorylation. For example, anatabine reduced the prevalence and severity of experimentally-induced autoimmune thyroiditis and limited the associated increases in interleukin-1 (IL-1) receptor type 2 and interleukin-18 in mice (Caturegli et al., 2012). Anatabine also reduced NFkB activation and suppressed amyloid beta production, which are both associated with plaque deposits in the brain during the over-production of pro-inflammatory cytokines and Alzheimer's disease (Paris et al., 2011). Anatabine has also been shown to inhibit the production of IL-1β, interleukin-6, and TNF-α induced by lipopolysaccharides in mice and in human blood (Paris et al., 2013). Thus, anatabine may minimize the pro-inflammatory cytokine responses, which in theory, may attenuate the muscle damage response after heavy eccentric exercise. To our knowledge, no previous studies have examined the effects of anatabine supplementation on humans in vivo. Therefore, the purpose of this study was to examine the effects of anatabine supplementation in conjunction with unilateral, maximal eccentric isokinetic muscle actions on serum markers of muscle damage and pro-inflammatory cytokines in humans. Based on previous animal studies (Paris et al., 2011, 2013), we hypothesized that, compared to a placebo, anatabine would attenuate the increases in serum markers of muscle damage and pro-inflammatory cytokines over a 72-h period after eccentric exercise.

2. Materials and methods

2.1. Participants

Twenty-seven men (mean ± S.D. age = 21.9 ± 2.9 yrs; body mass = 82.4 ± 20.5 kg; height = 182.6 ± 5.8 cm) volunteered to participate in this investigation; however, only 17 (mean ± S.D. age = 22.4 ± 3.2 yrs; body mass = 79.9 ± 16.6 kg; height = 182.4 ± 6.3 cm) men finished the study. Ten participants did not complete the study or were considered non-compliant and were excluded from the analyses for the following reasons: a serious adverse event unrelated to the supplement, but related to the exercise protocol ($n=1$), noncompliance with supplement consumption ($n=3$), an adverse event related to the supplement, but unrelated to the exercise protocol ($n=1$), and unspecified reasons or reasons unrelated to the study ($n=5$).

Prior to any testing at visit 1, participants signed an informed consent form and completed a health history questionnaire. Each participant was free from current or ongoing neuromuscular diseases or musculoskeletal injuries involving the wrist, elbow, and shoulder joints. None of the participants had acute infections nor had they engaged in any upper-body resistance training during the 6 months prior to enrollment. In addition, none of the participants reported smoking, use of smokeless tobacco, or use of creatine within 9 weeks prior to enrollment. All of the participants were instructed to maintain their normal dietary habits and refrain from anti-inflammatory or pain medications throughout the duration of the study. This study was approved by the university Institutional Review Board for the protection of human participants.

2.2. Experimental design

This study used a randomized, double-blinded, placebo-controlled, and crossover design. At visit 1, the participants were randomly assigned to either a supplement (anatabine) or placebo condition based on their participant number and corresponding randomization code. The participants returned to the laboratory seven days (±1 day) after visit 1 and completed a bout of unilateral, maximal eccentric isokinetic forearm flexor exercise to induce muscle damage using a standard exercise protocol detailed elsewhere (Beck et al., 2007). Blood draws were performed immediately prior to (PRE), immediately following (POST), and 24, 48, and 72 h after the bout of maximal eccentric exercise in order to quantify serum concentrations of creatine kinase, lactate dehydrogenase, myoglobin, high sensitivity c-reactive protein, and TNF-α. Serum anatabine concentrations were determined at PRE, and 24, 48, and 72 h after the bout of maximal eccentric exercise (Fig. 3). Following a washout period of 2–4 weeks, participants returned for visit 6 to undergo either the anatabine or placebo condition, whichever condition was not completed during visits 1–5. The procedures during the crossover (visits 6–10) were exactly the same as during visits 1–5, except the participants completed the eccentric exercise with the opposite arm.

2.3. Supplementation

The anatabine and placebo dietary supplements were administered as mint-flavored mannitol granulation tablets. Each anatabine tablet contained 3 mg of anatabine, 834 IU vitamin A, and 66 IU vitamin D3. The placebo tablet was identical in flavor and appearance to the anatabine tablet and contained everything in the anatabine tablet except for anatabine. The participants were given a 10 day supply of study product (anatabine or placebo) at visits 1 and 6 and were instructed to self-administer the tablets with food two or three times per day beginning after visit 1 or 6. Table 1 contains the schedule for tablet consumption during each 10 day supplementation period. During the anatabine condition, the participants consumed 6 mg of anatabine during days 1 and 2, 9 mg during days 3 and 4, and 12 mg during days 5 through 10. The participants did not take any study product during the washout period of 2–4 weeks.

Compliance was assessed by counting the amount of unused tablets returned at the end of each condition. If a participant consumed less than 70% of the tablets, they were considered non-compliant ($n=2$). In addition, one participant's serum anatabine concentrations were 10 ng mL^{-1} at PRE, 24, and 48 h during the placebo condition, and this participant's data were excluded from analyses.

2.4. Eccentric exercise protocol

During visits 2 and 7 (Fig. 1), the participants completed 6 sets of 10 maximal eccentric isokinetic muscle actions of the forearm flexors at $30^{\circ} \cdot s^{-1}$ (Beck et al., 2007). The exercised arm (right or left) used during visit 2 was determined at visit 1 using a separate randomization, and the opposite arm was exercised at visit 7. It has been reported (Connolly et al., 2002) that a bout of eccentric exercise in one limb does not confer a protective effect against muscle damage in the opposite limb 2 weeks later.

2.5. Blood sampling procedures

Serum concentrations of creatine kinase, lactate dehydrogenase, myoglobin, high sensitivity c-reactive protein, TNF-α, and anatabine were measured from 7.5-mL blood samples taken from the median cubital vein of the non-exercised arm. A trained phlebotomist performed all blood draws. The samples were collected into silicon dioxide dry-coated tubes and centrifuged for 10 min at 3000 r min^{-1} to separate the serum. The separated samples were frozen and shipped on dry ice to a third party laboratory for analyses (ARUP Laboratories, Salt Lake City, Utah).