Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

# D-Aspartic acid supplementation combined with 28 days of heavy resistance training has no effect on body composition, muscle strength, and serum hormones associated with the hypothalamo-pituitary-gonadal axis in resistance-trained men

*Darryn S. Willoughby*, Brian Leutholtz*

*Department of Health, Exercise and Biochemical Nutrition Lab, Human Performance, and Recreation, Baylor University, Waco, TX, USA*

ARTICLE INFO

*Article history:*
Received 6 June 2013
Revised 11 July 2013
Accepted 13 July 2013

*Keywords:*
D-Aspartic acid
Resistance training
Luteinizing hormone
Testosterone
Estradiol

ABSTRACT

It was hypothesized that D-aspartic acid (D-ASP) supplementation would not increase endogenous testosterone levels or improve muscular performance associated with resistance training. Therefore, body composition, muscle strength, and serum hormone levels associated with the hypothalamo-pituitary-gonadal axis were studied after 28 days of resistance training and D-ASP supplementation. Resistance-trained men resistance trained 4 times/wk for 28 days while orally ingesting either 3 g of placebo or 3 g of D-ASP. Data were analyzed with 2 × 2 analysis of variance ($P < .05$). Before and after resistance training and supplementation, body composition and muscle strength, serum gonadal hormones, and serum D-ASP and D-aspartate oxidase (DDO) were determined. Body composition and muscle strength were significantly increased in both groups in response to resistance training ($P < .05$) but not different from one another ($P > .05$). Total and free testosterone, luteinizing hormone, gonadotropin-releasing hormone, and estradiol were unchanged with resistance training and D-ASP supplementation ($P > .05$). For serum D-ASP and DDO, D-ASP resulted in a slight increase compared with baseline levels ($P > .05$). For the D-ASP group, the levels of serum DDO were significantly increased compared with placebo ($P < .05$). The gonadal hormones were unaffected by 28 days of D-ASP supplementation and not associated with the observed increases in muscle strength and mass. Therefore, at the dose provided, D-ASP supplementation is ineffective in up-regulating the activity of the hypothalamo-pituitary-gonadal axis and has no anabolic or ergogenic effects in skeletal muscle.



*Abbreviations:* 1-RM, 1 repetition maximum; BSA, bovine serum albumin; D-ASP, D-aspartic acid; DDO, D-aspartate oxidase; ELISA, enzyme-linked immunoabsorbent assay; GnRH, gonadotropin-releasing hormone; $H_2O_2$, hydrogen peroxide; HCL, hydrochloric acid; HPG axis, hypothalamo-pituitary-gonadal axis; LH, luteinizing hormone; NMDA, N-methyl D-aspartic acid.

* *Corresponding author*. Department of Health, Human Performance, and Recreation, Baylor University, Waco, TX 76798, USA. Tel.: +1 254 710 3504; fax: +1 254 710 3527.

E-mail address: darryn_willoughby@baylor.edu (D.S. Willoughby).

0271-5317/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nutres.2013.07.010

## 1. Introduction

In men desiring to increase their muscle mass and strength and enhance their exercise/sport performance, the androgenic hormone testosterone can undoubtedly play a beneficial role. D-Aspartic acid (D-ASP) has recently emerged on the exercise/sports supplement market and is being touted as a means of increasing muscle mass and strength owing to its ability to increase endogenous levels of testosterone. In rats, D-ASP has been shown to activate the hypothalamo-pituitary-gonadal axis (HPG axis) by facilitating the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, luteinizing hormone (LH) from the pituitary gland, and testosterone from the testes [1]. In addition, a more recent study involved D-ASP supplementation in rats and men that resulted in significant increases in LH and testosterone for both species [2]. As a result of these studies, the nutrition/sport supplement industry has attempted to take advantage of this information by manufacturing D-ASP–containing products with the intent of these products increasing endogenous testosterone levels, presumably by activation of the HPG axis. Furthermore, these products are being marketed on the premise that increases in endogenous testosterone will result in increases in muscle mass, especially when ingested in conjunction with a resistance training program.

An endogenous amino acid present in nervous tissues and endocrine glands of humans [3], D-ASP is considered to play an important neuromodulating role in activating the HPG axis. For example, in males, this axis is responsible for synthesizing endogenous testosterone and occurs due to D-ASP converting to *N*-methyl D-aspartic acid (NMDA) by D-aspartate methyltransferase (NMDA synthetase). In the hypothalamus, NMDA binds to its receptor, a subtype of the L-glutamate receptor, and potentiates glutaminergic neurotransmission [4], which results in the release of GnRH [5]. The release of GnRH from the hypothalamus then triggers the release of both follicle-stimulating hormone and LH from the pituitary gland. The effect of these 2 hormones on the testes is that follicle-stimulating hormone stimulates spermatogenesis and LH stimulates testosterone synthesis [5].

In an attempt to provide a feedback mechanism for the HPG axis, in which to maintain normal, physiological levels of endogenous circulating testosterone, the enzyme D-asparate oxidase (DDO) is capable of degrading D-ASP by way of deaminative oxidation [6]. In addition, D-ASP is also capable of inducing an increase in the activity of aromatase, the enzyme responsible for the conversion of testosterone to 17$\beta$-estradiol (estrogen) [7]. Additional data support this and help confirm that D-ASP is involved in the local production of estrogen [8].

Because there are data supporting the role of D-ASP supplementation in increasing endogenous testosterone levels, this amino acid product may prove beneficial as a means in which to increase muscle performance associated with heavy resistance training. However, because there appears to be a paucity of human studies dealing with D-ASP supplementation, and apparently none when D-ASP is ingested in conjunction with resistance training, we hypothesized that D-ASP would not increase endogenous testosterone levels or improve muscular performance associated with resistance training. Therefore, the purpose of this study was to determine the effects of resistance exercise and D-ASP supplementation on body composition, muscle strength, and serum hormones associated with the HPG axis in resistance-trained men.

## 2. Methods and materials

### 2.1. *Experimental approach*

In a randomized, double-blind manner, participants engaged in 28 days of heavy resistance training while also ingesting 3 g/d of either placebo (PLC) or D-ASP. Testing and evaluation occurred before (day 0) and after (day 29) and involved assessments of body composition, muscle strength, and serum hormones associated with the HPG axis. This approach was based on the premise that after ingesting the D-ASP supplement, muscle mass and strength may be preferentially affected compared with PLC owing to elevations in endogenous testosterone.

### 2.2. *Participants*

Twenty apparently healthy, recreationally active, resistance-trained (consistent [at least thrice weekly] resistance training for 1 year before the study) men with an average age of 22.8 ± 4.67 years, height of 179.5 ± 6.38 cm, and total body mass of 79.1 ± 16.13 kg completed the study. Enrollment was open to men of all ethnicities. All participants passed a mandatory medical screening. Participants with contraindications to exercise as outlined by the American College of Sports Medicine and/or who had consumed any nutritional supplements (excluding multivitamins) such as creatine monohydrate, nitric oxide–stimulating, hydroxy-$\beta$-methylbutyrate, or pharmacologic agents such as anabolic steroids 3 months before the study were not allowed to participate. All eligible participants signed a university-approved informed consent document based on the guidelines set forth by the Institutional Review Board for the Protection of Human Subjects of Baylor University. In addition, all experimental procedures involved in this study conformed to the ethical considerations of the Helsinki Code.

### 2.3. *Testing sessions*

The study included baseline testing at day 0, followed by a follow-up testing session at day 29 in which blood samples were obtained, body composition was assessed, muscle strength tests were performed, dietary intake was determined, and any noted adverse effects from supplements were reported.

### 2.4. *Strength assessment*

Based on our previous studies [9,10], upper- and lower-body 1 repetition maximum (1-RM) strength tests were performed using the free weight bench press and angled leg press exercises (Nebula, Versailles, OH, USA), respectively. Initially,