NUTRITION RESEARCH XX (2013) XXX–XXX

Available online at www.sciencedirect.com

SciVerse 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 17β-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-β-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,

Please cite this article as: Willoughby DS, Leutholtz B, D-Aspartic acid supplementation combined with 28 days of heavy resistance training has no effect on body composition..., Nutr Res (2013), http://dx.doi.org/10.1016/j.nutres.2013.07.010

an estimated 50% 1-RM was used to complete 5 to 10 repetitions. After a 2-minute rest period, a load of 70% of estimated 1-RM was used to perform 3 to 5 repetitions. Weight was gradually increased until a 1-RM was reached with each following lift, with a 2-minute rest period in between each successful lift.

2.5. Body composition assessment

Total body mass (in kilograms) was determined on a standard dual-beam balance scale (Detecto, Bridgeview, IL, USA). Percent body fat, fat mass, and fat-free mass were determined using dual-energy x-ray absorptiometry (Hologic Discovery Series W, Waltham, MA, USA). 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 before each testing session [9,10]. Total body water was determined by bioelectric impedance analysis (Xitron Technologies Inc, San Diego, CA, USA) [9,10]. Based on previous studies in our laboratory, the accuracy of the dual-energy x-ray absorptiometry for body composition assessment is ±3.7%, as assessed by direct comparison with hydrodensitometry and scale weight.

2.6. Venous blood sampling

Venous blood samples were obtained at days 0 and 29 from the antecubital vein into a 10-mL collection tube using a standard vacutainer apparatus. The blood sample on day 29 was obtained 24 hours after the final dose of supplement ingested on day 28. Blood samples were allowed to stand at room temperature for 10 minutes and then centrifuged. The serum was removed and frozen at –80°C for later analysis.

2.7. Supplementation protocol

Participants were randomly assigned a 28-day supplementation protocol, in a double-blind fashion, to either a PLC or a D-ASP (DAA) group. The PLC group consisted of the oral ingestion of 4 capsules daily containing 3 g of guar gum, whereas the DAA group involved 4 daily capsules containing 3 g of D-ASP (Better Body Sports, Ventura, CA, USA), based on company guidelines for D-ASP and because the dosage was previously shown effective at increasing endogenous testosterone [2]. Capsules for the PLC and DAA groups were identical in color, shape, and size. For each supplement, the entire dosage was ingested in the morning upon waking. Supplementation compliance was monitored by having participants complete a daily supplement compliance questionnaire that was returned, along with containers of their supplement, ate the testing session on day 29.

2.8. Dietary monitoring

In line with our previous studies [9,10], to monitor dietary intake, participants were required to record their food and drink intake for 4 consecutive days before each of the 2 testing sessions at days 0 and 29. For standardization purposes, participants' diets were not controlled and participants were asked not to change their dietary habits during the course of the study. The 4-day dietary recalls were evaluated with the Food Processor IV Nutrition Software (ESHA, Salem, OR, USA) to determine the average daily macronutrient intake.

2.9. Resistance training protocol

Based on our previous studies [9,10], participants completed a periodized 28-day resistance training program split into 2 upper-extremity and 2 lower-extremity exercise sessions each week. This constituted a total of 16 exercise sessions, with 8 upper-body and 8 lower-body exercise sessions. Each exercise session was supervised by study personnel, and before each exercise session, participants performed a standardized series of stretching exercises. The participants then performed an upper-extremity resistance training program consisting of 9 exercises (bench press, lat pull, shoulder press, seated rows, shoulder shrugs, chest flies, biceps curl, triceps press down, and abdominal curls) twice per week and a program consisting of 7 lower-extremity exercises (leg press or squat, back extension, step ups, leg curls, leg extension, heel raises, and abdominal crunches). Participants performed 3 sets of 10 repetitions at 70% to 80% 1-RM. Rest periods were 2 minutes between exercises and between sets.

2.10. Serum hormone analysis

Serum samples were analyzed in duplicate for total testosterone, free testosterone, LH, estrogen (Alpha Diagnostic International, San Antonio, TX, USA), and GnRH (Uscn Life Science, Houston, TX, USA) using commercially available enzyme-linked immunoabsorbent assay (ELISA) kits [11,12]. Absorbances for each hormone were determined at a wavelength of 450 nm using a microplate reader (iMark; Bio-Rad, Hercules, CA, USA). A set of standards of known concentrations for each hormone was used to construct standard curves, and hormone concentrations were determined using data reduction software (Microplate Manager, Bio-Rad). The overall intra-assay percent coefficients of variation were 7.5%, 6.9%, 8.3%, 9.1%, and 8.6%, respectively, for total testosterone, free testosterone, LH, estradiol, and GnRH.

2.11. Serum D-aspartate oxidase analysis

Serum samples were analyzed in duplicate for DDO using a commercially available ELISA kit (CUSABIO, Carlsbad, CA, USA). Absorbances for were determined at a wavelength of 450 nm using a microplate reader (iMark; Bio-Rad). A set of standards of known concentrations for DDO was used to a construct standard curve, and DDO concentrations were determined using data reduction software (Microplate Manager; Bio-Rad). The overall intra-assay percent coefficient of variation was 6.1% for DDO.

2.12. Serum D-ASP analysis

The concentration of serum D-ASP acid was determined by ELISA, modified from a protocol previously reported [13]. Briefly, an aggregate of D-ASP, glutaryladehyde, and bovine serum albumin (BSA) was constructed through chemical cross-linking of glutaryladehyde to D-ASP and BSA and was

Please cite this article as: Willoughby DS, Leutholtz B, D-Aspartic acid supplementation combined with 28 days of heavy resistance training has no effect on body composition..., Nutr Res (2013), http://dx.doi.org/10.1016/j.nutres.2013.07.010

then dissolved in a sodium-carbonate buffer (pH 9.6). This solution was added to the wells of a microplate, incubated for 1 hour, washed 3 times with phosphate-buffered saline containing 0.05% Tween 20 and 5% skim milk, and then incubated for another hour. The plate was washed 3 times, and then aliquots of preabsorbed serum containing an antirat polyclonal D-ASP antibody conjugated to BSA (Advanced Targeting Systems, San Diego, CA, USA) were added to each well of the microplate. The plate was incubated for 1 hour, washed 3 times, and then incubated for 1 hour with horseradish-peroxidate–conjugated goat immunoglobulin G fraction to mouse immunoglobulin G (Sigma-Aldrich, St Louis, MO, USA). After washing the plate 3 times, a substrate solution containing a sodium-citrate buffer (pH 4.0) containing 0.003% H_2O_2 and 0.4% o-phenylenediamine was added and incubated for 15 minutes in the dark. The reaction was stopped by adding 2 M HCL. Absorbances were determined in duplicate at a wavelength of 450 nm using a microplate reader (iMark; Bio-Rad). A set of standards of known concentrations using D-ASP (Advanced Targeting Systems) was used to a construct standard curve, and serum D-ASP concentrations were determined using data reduction software (Microplate Manager; Bio-Rad). The overall intra-assay percent coefficient of variation was 7.8% for D-ASP.

2.13. Statistical analyses

Data were analyzed with separate 2 (group) × 2 (time) analysis of variance using SPSS for Windows Version 19.0 software (SPSS, Chicago, IL, USA). Significant differences among groups were identified by a Tukey Honestly Significant Different post hoc test. However, to protect against type I error, the conservative Hunyh-Feldt Epsilon correction factor was used to evaluate observed within-group *F* ratios. An a priori power calculation showed that 10 participants per group was adequate to detect a significant difference between groups in the marker of total testosterone given a type I error rate of 0.05 and a power of 0.80. The index of effect size used was partial eta squared (η^2), which estimates the proportion of variance in the dependent variable that can be explained by the independent variable. Partial η^2 effect sizes were determined to be as follows: weak, 0.17; medium, 0.24; strong, 0.51; and very strong, 0.70 [14]. All statistical procedures were performed using SPSS 19.0 software, and a probability level of less than .05 was adopted throughout the study.

3. Results

3.1. Participant demographics

Twenty-four participants began the study; however, 4 were withdrawn because of reasons unrelated to the study. One participant became ill, 1 sustained an injury unrelated to the study, and 2 became too busy with their schedule; therefore, none of the 4 were able to remain compliant with the resistance training program. As a result, 20 participants completed the study. The PLC group (n = 10) had an average (±SD) age of 21.25 ± 1.03 years, height of 179.68 ± 6.42 cm, total body mass of 80.99 ± 18.43 kg, and body mass index of 24.76 ± 5.38 kg/m^2. The DAA group (n = 10) had an average (±SD) age of 20.11 ± 1.36 years, height of 177.93 ± 4.81 cm, total body mass of 74.03 ± 8.06 kg, and body mass index of 23.36 ± 2.33 kg/m^2.

3.2. Dietary analyses, supplement and exercise compliance, and reported adverse effects

The diet logs were used to analyze the average daily caloric and macronutrient consumption (Table 1). Neither group significantly increased their caloric intake during the course of the study ($P > .05$). Furthermore, there were no significant differences between groups for total calories ($P = .11$; effect size, 0.45) or for the intake of protein ($P = .25$; effect size, 0.32), carbohydrate ($P = .12$; effect size, 0.46), and fat ($P = .60$; effect size, 0.23).

Relative to compliance, PLC and DAA were 89.9% ± 10.92% and 91.4% ± 10.23% compliant to the resistance training program, respectively. For supplementation compliance, PLC and DAA were 99.25% ± 2.25% and 98.55% ± 2.65% compliant to the supplementation protocol.

Regarding adverse effects from supplementation, over the course of the 28 days, 2 participants in DAA and 1 in PLC reported adverse effects. All 3 participants reported feelings of irritability, nervousness, rapid heart rate, and headache.

3.3. Body composition

Total body mass was significantly increased in both groups with training ($P = .02$; effect size, 0.15), but there were no differences between groups ($P = .98$; effect size, 0.001). In addition, there were no significant changes occurring in total body water as a result of training ($P = .75$; effect size, 0.002) or supplementation ($P = .91$; effect size, 0.004). Fat mass was unchanged with resistance training ($P = .71$; effect size, 0.03)

Table 1 – Dietary caloric and macronutrient intake for the PLC and DAA groups

Variable	PLC	DAA	Test	Group × Test
Total calories (kcal/kg)			.46	.11
Day 0	31.35 ± 9.34	32.87 ± 8.49		
Day 29	34.82 ± 10.21	36.18 ± 10.36		
ΔTotal calories	3.47 ± 0.73	3.31 ± 0.83		
Protein (g/kg)			.53	.25
Day 0	1.17 ± 0.41	1.19 ± 0.44		
Day 29	1.22 ± 0.53	1.27 ± 0.63		
ΔProtein	0.05 ± 0.01	0.08 ± 0.02		
Carbohydrate (g/kg)			.81	.12
Day 0	3.78 ± 2.03	3.89 ± 1.32		
Day 29	3.84 ± 2.78	4.13 ± 2.67		
ΔCarbohydrate	0.06 ± 0.02	0.24 ± 0.04		
Fat (g/kg)			.43	.60
Day 0	1.28 ± 0.41	1.26 ± 0.46		
Day 29	1.32 ± 0.44	1.31 ± 0.59		
ΔFat	0.04 ± 0.01	0.05 ± 0.01		

Dietary caloric and macronutrient intake for the PLC (n = 10) and DAA (n = 10) groups. Values are means ± SD. No significant differences were detected for any of the dietary variables ($P > .05$).

Please cite this article as: Willoughby DS, Leutholtz B, D-Aspartic acid supplementation combined with 28 days of heavy resistance training has no effect on body composition..., Nutr Res (2013), http://dx.doi.org/10.1016/j.nutres.2013.07.010

and supplementation (P = .95; effect size, 0.02). However, fat-free mass was significantly increased in both groups in response to training (P = .04; effect size = 0.19), but not preferentially affected in the DAA group (P = .96; effect size, 0.002) (Table 2).

3.4. Muscle strength

For muscle strength, bench press strength was unchanged with resistance training (P = .11; effect size, 0.05) and supplementation (P = .96; effect size, 0.001). However, for leg press strength, both groups underwent significant increases with training (P = .01; effect size, 0.13); however, these increases were not preferentially affected by DAA supplementation (P = .91; effect size, 0.004) (Table 3).

3.5. Serum hormones

In response to resistance training, total testosterone (P = .63; effect size, 0.005), free testosterone (P = .57; effect size, 0.007), LH (P = .12; effect size, 0.0.04), GnRH (P = .79; effect size, 0.001), and estradiol (P = .49; effect size, 0.01) were not significantly changed. Similarly in response to D-ASP supplementation, total testosterone (P = .98; effect size, 0.001), free testosterone (P = .91; effect size, 0.003), LH (P = .29; effect size, 0.04), GnRH (P = .47; effect size, 0.03), and estradiol (P = .96; effect size, 0.001) were not significantly changed in the DAA group (Table 4).

3.6. Serum D-ASP and DDO

In the DAA group, there was an increase in the serum levels of D-ASP that was not significantly greater than the levels of day 0 (P = .31; effect size, 0.05) or significantly different from PLC (P = .56; effect size, 0.007). For serum DDO, when compared with PLC, there was a significant increase in the levels of DDO at day 29 in the DAA group (P = .042; effect size, 0.19) (Table 5).

Table 2 – Body composition variables for the PLC and DAA groups

Variable	PLC	DAA	Test	Group × Test
Body mass (kg)				
Day 0	80.99 ± 18.43	74.03 ± 8.06		
Day 29	82.30 ± 17.53	75.60 ± 7.90		
ΔBody mass	1.31 ± 1.32 *	1.57 ± 1.14 *	P = .03	P = .98
Body water (kg)				
Day 0	46.52 ± 5.76	45.04 ± 3.52		
Day 29	46.00 ± 5.48	45.36 ± 3.66		
ΔBody water	−0.51 ± 0.69	0.31 ± 2.22	P = .75	P = .91
Fat mass (kg)				
Day 0	15.35 ± 10.34	9.59 ± 3.66		
Day 29	13.43 ± 8.10	8.72 ± 3.13		
ΔFat mass	−1.91 ± 3.12	−0.86 ± 1.22	P = .71	P = .95
Fat-free mass (kg)				
Day 0	58.88 ± 7.71	56.43 ± 7.04		
Day 29	60.32 ± 8.91	57.74 ± 7.05		
ΔFat-free mass	1.44 ± 2.41 *	1.31 ± 1.37 *	P = .04	P = .96

Body composition for the PLC (n = 10) and DAA (n = 10) groups. Values are means ± SD.

* Denotes a significant increase at day 29. Resistance training increased total body mass (P = .03) and fat-free mass (P = .04) for both groups; however, there were no differences associated with D-ASP supplementation (P > .05).

Table 3 – Muscle strength variables for the PLC and DAA groups

Variable	PLC	DAA	Test	Group × Test
BP strength (kg/kg)				
Day 0	1.07 ± 0.35	1.14 ± 0.18		
Day 29	1.19 ± 0.39	1.29 ± 0.16		
ΔBP strength	0.12 ± 0.05	0.15 ± 0.04	P = .11	P = .96
LP strength (kg/kg)				
Day 0	3.85 ± 1.22	4.71 ± 0.77		
Day 29	4.38 ± 1.42	5.53 ± 0.69		
ΔLP strength	0.53 ± 0.33 *	0.81 ± 0.23 *	P = .01	P = .91

Bench press (BP) and leg press (LP) strength for the PLC (n = 10) and DAA (n = 10) groups. Values are means ± SD.

* Denotes a significant increase at day 29. Resistance training increased leg press strength (P = .01) for both groups; however, there was no difference associated with D-ASP supplementation (P > .05).

4. Discussion

In this study, we sought to determine the effects of 28 days of heavy resistance training and D-ASP supplementation on body composition, muscle strength, and serum hormones associated with the HPG axis in resistance-trained men. Herein we report similar increases in muscle mass and strength in both groups associated with resistance training similar to our previous studies, which used the identical training protocol [9,10]. We also found that D-ASP supplementation had no effects on serum testosterone, and although resistance training was effective in increasing muscle mass and strength, it was not preferentially caused by D-ASP supplementation. As a result, we accept our hypothesis that D-ASP would not increase endogenous testosterone levels or improve muscular performance associated with resistance training. In light of our findings, this study refutes alleged marketing claims that D-ASP increases muscle mass and strength owing to its ability to elevate endogenous testosterone levels.

Because D-ASP has been shown to activate the HPG axis in rats, by facilitating the release of GnRH from the hypothalamus, LH from the pituitary gland, and testosterone from the testes [1], our approach with the present study was to determine if D-ASP supplementation was indeed effective at increasing endogenous testosterone levels in humans and whether it was caused by an activation of the HPG axis. A previous study with humans [2] showed 12 days of D-ASP supplementation at a daily dose of 3 g to be effective at increasing LH and testosterone but did not use resistance training. Therefore, we chose to assess the effects of several hormones associated with the HPG axis. We first assessed the effects on GnRH and found the levels of this hormone to be unchanged after 28 days of D-ASP supplementation and resistance training. Furthermore, we observed the same

Please cite this article as: Willoughby DS, Leutholtz B, D-Aspartic acid supplementation combined with 28 days of heavy resistance training has no effect on body composition..., Nutr Res (2013), http://dx.doi.org/10.1016/j.nutres.2013.07.010

Table 4 – Serum hormone variables for the PLC and DAA groups

Variable	PLC	DAA	Test	Group × Test
Total test (ng/mL)				
Day 0	7.84 ± 1.78	8.08 ± 0.69		
Day 29	8.06 ± 1.59	8.88 ± 0.55		
ΔTotal test	0.22 ± 0.88	0.82 ± 0.52	$P = .99$	$P = .98$
Free test (pg/mL)				
Day 0	98.62 ± 7.77	89.64 ± 9.87		
Day 29	99.68 ± 7.93	91.30 ± 9.69		
ΔFree test	1.06 ± 2.73	2.16 ± 6.68	$P = .75$	$P = .91$
LH (mIU/mL)				
Day 0	2.67 ± 1.76	2.51 ± 3.10		
Day 29	3.45 ± 3.79	5.25 ± 3.68		
ΔLH	0.77 ± 3.40	2.88 ± 4.35	$P = .71$	$P = .95$
GnRH (pg/mL)				
Day 0	291.75 ± 116.05	257.31 ± 126.45		
Day 29	256.64 ± 117.69	256.33 ± 107.55		
ΔGnRH	−35.10 ± 122.66	−0.97 ± 119.86	$P = .77$	$P = .96$
Estradiol (pg/mL)				
Day 0	1284.88 ± 958.35	1429.01 ± 700.34		
Day 29	1156.43 ± 835.66	1362.85 ± 819.34		
ΔEstradiol	−128.45 ± 360.28	−119.67 ± 388.36	$P = .77$	$P = .96$

Serum hormones representing the HPG axis for the PLC (n = 10) and DAA (n = 10) groups. Values are means ± SD. No significant differences were detected for any of the serum hormones ($P > .05$).

results with LH and free and total testosterone. Therefore, our results indicate that 28 days of D-ASP supplementation at a daily dose of 3 g, in conjunction with heavy resistance training, had no apparent activating effect on the HPG axis.

In lieu of our results, a previous study using humans showed that 12 days of D-ASP supplementation at the same daily dose used in the present study was effective at increasing total testosterone from 4.5 ng/mL at baseline to 6.4 ng/mL after day 12, which corresponded to a 42% increase in total testosterone levels [2]. In that study, the age range of the participants range was 27 to 37 years, which corresponds to a reference clinical range of serum total testosterone, which is 3 to 10 ng/mL [15]. However, the mean baseline testosterone value in the previously mentioned study [2] was within 25% of the lower clinical range, and D-ASP supplementation only elevated testosterone levels to approximately 50% of the clinical range. Interestingly, in the present study, the mean baseline testosterone value for participants supplemented with D-ASP was approximately 8 ng/mL, which is within 25% of the upper clinical range. The discrepancy in baseline testosterone values between that study [2] and ours could be age related because the age range of participants in the present study was 18 to 23 years. Another factor could be the training status of the participants. In the present study, we used resistance-trained participants, and it has been shown that prolonged intensive strength training may influence the pituitary and possibly hypothalamic levels, leading to increased serum levels of testosterone [16]. In light of these age- and training-related possibilities, our results indicate that D-ASP supplementation may only be effective with lower testosterone levels, which are at approximately 25% of the lower clinical range.

Moreover, compared with the study of Topo et al [2], our results suggest another potential mechanistic reason why the HPG axis was not affected by D-ASP supplementation. In the present study, D-ASP supplementation may have been ineffective because the participants' baseline levels of total testosterone were already close to the maximum clinical range. As a result, the higher level of testosterone, compared with the lower level observed in the study of Topo et al [2], was likely being maintained by androgen negative feedback. The HPG axis is a prototypical autoregulatory control system that operates by opposing feedback (inhibitory) and feedforward (stimulatory) mechanisms, which, together, maintain the physiological level of endogenous testosterone [17]. Oftentimes, the deviation of endogenous testosterone production beyond the reference clinical range is prevented by inhibitory feedback on the hypothalamus, thereby maintaining a normal, physiological level of endogenous testosterone. For example, in males [18] and experimental animals [17,19],

Table 5 – Serum D-ASP and D-aspartate oxidase levels for the PLC and DAA groups

Variable	PLC	DAA	Test	Group × Test
Serum D-ASP (ng/mL)				
Day 0	40.62 ± 16.16	43.11 ± 18.56		
Day 29	42.05 ± 19.64	52.51 ± 25.24		
ΔDAA	1.38 ± 0.65	9.40 ± 6.68	$P = .31$	$P = .56$
Serum DDO (pg/mL)				
Day 0	24.63 ± 12.69	22.75 ± 9.87		
Day 29	29.94 ± 9.97	44.63 ± 9.69		
ΔDDO	5.31 ± 9.59	21.88 ± 20.79 *,**	$P = .03$	$P = .04$

Serum D-ASP and D-aspartate oxidase for the PLC (n = 10) and DAA (n = 10) groups. Values are means ± SD.
* Denotes a significant increase at day 29.
** Denotes a significant increase compared with PLC.
Supplementation produced a significant increase in serum DDO in the DAA group ($P = .03$) compared with the PLC group.

testosterone inhibits hypothalamic GnRH outflow (secretions and actions) to modulate the activity of the HPG axis.

The provision of D-ASP has been shown to increase estrogen levels. There are in vitro data from boar [20] and lizard [8] testes demonstrating that endogenous testicular D-ASP enhances gonad aromatase activity. Therefore, based on the possibility that D-ASP supplementation may increase endogenous estrogen levels, thereby altering the testosterone/estrogen ratio, we assessed the levels of estrogen. However, our results showed estrogen levels to be unchanged by D-ASP supplementation, thereby indicating no effectiveness of D-ASP supplementation on up-regulating aromatase activity.

Although there are pharmacokinetic and pharmacodynamic data available for D-ASP in mammals, the data in humans appear to be lacking. Nevertheless, the ingestion of D-ASP by rats increases the D-ASP levels in blood, and the levels peak within 1 to 2 hours, after which they decrease progressively to baseline levels within 24 to 48 hours [21]. Approximately 10% to 20% of ingested D-ASP is excreted in urine and feces within a 48-hour period, and the remainder absorbed by the intestine where it is then transferred through the blood to the liver and kidneys and then metabolized by DDO [4]. As with a previous study [2], we showed increases in serum D-ASP levels in the DAA group. In our study, we obtained the final blood sample 24 hours after the final supplement dose and found serum D-ASP to be moderately increased. Therefore, there was an enhanced level of D-ASP from 28 days of supplementation that could have potentially up-regulated the HPG axis. Based on our data presented herein, this may indicate another potential mechanistic reason why the HPG axis was not affected by D-ASP supplementation. Although we observed nonsignificant increases in serum D-ASP in the DAA group, we showed significant increases in DDO levels in response to D-ASP supplementation. The degradative role of DDO is to catalyze the oxidative deamination of D-amino acids to generate the corresponding 2-oxo acids, along with hydrogen peroxide and ammonia (or methylamine). In rodents, the administration of D-ASP was shown to increase DDO activity [22,23], suggesting that DDO activity is induced by increased levels of D-ASP. Based on this information, in the present study, it is possible that because of the higher baseline levels of testosterone, as a means of androgen-regulated feedback of the HPG axis, the level of serum D-ASP induced by supplementation was conceivably being degraded by DDO at a rate that rendered it unable to effectively activate the HPG axis.

However, our study does possess 2 noteworthy limitations. One limitation is that we relied on participant self-report for dietary intake and supplement compliance. As a result, it is possible that the information reported for both dietary intake and supplement ingestion does not accurately reflect what was actually consumed. The other limitation of our study is the sample size. Although a sample size of 20 is somewhat small, it is larger than many other studies in the literature using a very similar experimental design. We did perform a power analysis a priori; therefore, our study should be adequately powered. However, in lieu of the small sample size, this limitation cannot be overlooked.

In light of the limitations of the present study, it is clear that more research needs to be conducted on D-ASP supplementation in humans regarding its ability to increase endogenous levels of testosterone, along with its potential ability to increase muscle mass and strength. Based on the results of the current study, we conclude that 28 days of D-ASP of supplementation at a daily dose of 3 g is ineffective in up-regulating the activity of the HPG axis and has no preferential effects in which to increase skeletal muscle mass and strength in resistance-trained men.

Acknowledgment

We would like to thank the individuals who participated in this study. This study was partially supported by a D-ASP donation from Better Body Sports. Funding from this study came from the Exercise and Biochemical Nutrition Laboratory at Baylor University. The authors declare that they have no competing interests.

REFERENCES

[1] D'Aniello A, Di Fiore MM, Fisher GH, Milone A, Seleni A, D'Aniello S, et al. Occurrence of D-aspartic acid and N-methyl-D-aspartic acid in rat neuroendocrine tissues and their role in the modulation of luteinizing hormone and growth hormone release. FASEB J 2000;14:699–714.

[2] Topo E, Soricelli A, D'Aniello A, Ronsini S, D'Aniello G. The role and molecular mechanism of D-aspartic acid in the release and synthesis of LH and testosterone in humans and rats. Reprod Biol Endocrinol 2009;7:120.

[3] D'Aniello G, Grieco N, Di Filippo MA, Cappiello F, Topo E, D'Aniello E, et al. Reproductive implication of D-aspartic acid in human pre-ovulatory follicular fluid. Hum Reprod 2007;22:3178–83.

[4] Katane M, Homma H. D-Aspartate—an important bioactive substance in mammals: a review from an analytical and biological point of view. J Chromatogr B Analyt Technol Biomed Life Sci 2011;879:3108–21.

[5] D'Aniello A. D-Aspartic acid: an endogenous amino acid with an important neuroendocrine role. Brain Res Rev 2007;53:215–34.

[6] Errico F, Pirro MT, Affuso A, Spinelli P, De Felice M, D'Aniello A, et al. A physiological mechanism to regulate D-aspartic acid and NMDA levels in mammals revealed by D-aspartate oxidase deficient mice. Gene 2006;374:50–7.

[7] Assisi L, Botte V, D'Aniello A, Di Fiore MM. Enhancement of aromatase activity by D-aspartic acid in the ovary of the lizard *Podarcis s. sicula*. Reproduction 2001;121:803–8.

[8] Raucci F, D'Aniello S, Di Fiore MM. Endocrine roles of D-aspartic acid in the testis of lizard *Podarcis s. sicula*. J Endocrinol 2005;187:347–59.

[9] Shelmadine B, Cooke M, Buford T, Hudson G, Redd L, Leutholtz B, et al. Effects of 28 days of resistance exercise and consuming a commercially available pre-workout supplement, NO-shotgun, on body composition, muscle strength and mass, markers of satellite cell activation, and clinical safety markers in males. J Int Soc Sports Nutr 2009;6:16.

[10] Spillane M, Schwarz N, Leddy S, Correa T, Minter M, Longoria V, et al. Effects of 28 days of resistance exercise while consuming commercially available pre- and post-workout supplements, NO-shotgun and NO-synthesize on body composition, muscle strength and mass, markers of protein

synthesis, and clinical safety markers in males. Nutr Metab (Lond) 2011;8:78.
[11] Willoughby D, Wilborn C, Taylor L, Campbell W. Eight weeks of aromatase inhibition using the nutritional supplement Novedex XT: effects in young, eugonadal men. Int J Sport Nutr Exerc Metab 2007;17:92–108.
[12] Rohle D, Wilborn C, Taylor L, Mulligan C, Willoughby D. Effects of eight weeks of aromatase inhibition using the nutritional supplement 6-OXO (androst-4-ene-3,6,17-trione): effects on serum hormone profiles and clinical safety markers in resistance-trained, eugonadal males. J Int Soc Sports Nutr 2007;4:13–20.
[13] Ohgusu T, Hamase K, Tanaka H, Shoyama Y, Zaitsu K. High-throughput determination of free D-aspartic acid in mammals by enzyme immunoassay using specific monoclonal antibody. Anal Biochem 2006;357:15–20.
[14] O'Connor K, Stip E, Pelissier M, Aardema F, Guay S, Gaudette G, et al. Treating delusional disorder: a comparison of cognitive-behavioural therapy and attention placebo control. Can J Psychiatry 2007;52:182–90.
[15] Morgentaler A, Dobs AS, Kaufman JM, Miner MM, Shabsigh R, Swerdloff RS, et al. Long acting testosterone undecanoate therapy in men with hypogonadism: results of a pharmacokinetic clinical study. J Urol 2008;180:2307–13.
[16] Hakkinen K, Pakarinen A, Alen M, Kauhanen H, Komi PV. Neuromuscular and hormonal adaptations in athletes to strength training in two years. J Appl Physiol 1988;65:2406–12.
[17] Veldhuis JD, Roemmich JN, Richmond EJ, Bowers CY. Somatotropic and gonadotropic axes linkages in infancy, childhood, and the puberty-adult transition. Endocr Rev 2006;27:101–40.
[18] Veldhuis JD, Urban RJ, Dufau ML. Evidence that androgen negative feedback regulates hypothalamic gonadotropin-releasing hormone impulse strength and the burst-like secretion of biologically active luteinizing hormone in men. J Clin Endocrinol Metab 1992;74:1227–35.
[19] Veldhuis JD, Keenan DM, Iranmanesh A, Takahashi PY, Nehra AX. The ensemble male hypothalamic-pituitary-gonadal axis. In: Timiras PS, editor. Physiological Basis of Aging and Geriatrics. New York: Taylor & Francis Group, Health Science Division; 2007. p. 185–203.
[20] Lamanna C, Assisi L, Botte V, Di Fiore MM. Involvement of D-Asp in P450 aromatase activity and estrogen receptors in boar testis. Amino Acids 2007;32:45–51.
[21] D'Aniello A, D'Onofrio G, Pischetola M, D'Aniello G, Vetere A, Petrucelli L, et al. Biological role of D-amino acid oxidase and D-aspartate oxidase. Effects of D-amino acids. J Biol Chem 1993;268:26941–9.
[22] Nagasaki H. Gender-related differences of mouse liver D-aspartate oxidase in the activity and response to administration of D-aspartate and peroxisome proliferators. Int J Biochem 1994;26:415–23.
[23] Yamada R, Nagasaki H, Nagata Y, Wakabayashi Y, Iwashima A. Administration of D-aspartate increases D-aspartate oxidase activity in mouse liver. Biochim Biophys Acta 1989;990:325–8.

Please cite this article as: Willoughby DS, Leutholtz B, D-Aspartic acid supplementation combined with 28 days of heavy resistance training has no effect on body composition..., Nutr Res (2013), http://dx.doi.org/10.1016/j.nutres.2013.07.010