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Acute D-Aspartic Acid Supplementation does not have an Effect on Serum Testosterone but does have an Effect on Strength Measures in College Aged Male Athletes

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ABSTRACT

Background: *D-aspartic acid may enhance athletic performance by regulating the hypothalamus-pituitary-gonadal axis to increase plasma testosterone. Increasing testosterone via D-aspartic acid, may lead to improved muscle function and concurrent improvements in athletic performance.*

Purpose: *To determine the effect of D-aspartic acid supplementation on athletic performance in young male athletes.*

Methods: *After screening for ACSM low risk, 15 healthy male athletes (average age=20.9 years, body weight=79.1 kg and body fat=9.2%) were randomized to two groups for supplementation using a double blinded parallel arm experimental design. They ingested either 3 grams of d-aspartic acid (DAA, n=9) or a Placebo (PL, n=6) for 14 days supplied in capsule form. Subjects recorded and replicated previous 3 days diets prior to testing. Blood was collected for testosterone determination before and after the supplementation. Physical assessments were performed prior to and after supplementation and included a peak VO_2 test by cycle ergometer, 1 maximal repetition bench press and 1 maximal repetition squat (average values ± SEM before supplementation were 41.7 ± 6.4 ml/kg/min, 108.1 ± 3.5 kg and 138.7 ± 5.4 kg, respectively).*

Results: *The DAA group had a significant increase in the 1 maximal repetition squat by 8.5 ± 10.5 kg (p=0.013) and a positive trend in performance during the 1 maximal repetition bench press by 3.5 ± 6.8 kg (p=0.06). DAA improved performance in VO_2 peak test increasing maximum workload by 48.8 ± 15.9 W (p<0.01) and time to peak 1.4 ± 0.5 min (p<0.05) following supplementation. No change in performance measures were observed in the Placebo group. There was no change in testosterone levels in the control group: 2.5 ± 70.4 ng/dL or in the experimental group: -0.76 ± 72.8 ng/dL. Body composition did not change for either group.*

Conclusion: *D-aspartic acid supplementation leads to improved upper and lower body muscle performance but does not increase testosterone levels after 14 days of supplementation.*

Keywords: Aspartate, Testosterone, Supplementation, Bench press, Squat press

INTRODUCTION

Background

Dietary supplements are currently used to increase athletic performance for many athletes. Many new supplements come to the market every year with little research done on their efficacy in improving athletic performance [1]. Dietary protein supplements have become a large portion of the type of supplements used and even singling out specific types of amino acids alone for improvement of athletic performance. D-aspartic acid (DAA) is one such amino acid that is being sold as a supplement used to improve muscle function in athletes [1-3].

Previous studies have shown that DAA has a role in the activation of the hypothalamo-pituitary-gonadal axis by increasing the production of Gonadotropin Releasing Hormone (GnRH), Luteinizing Hormone (LH) and Testosterone in rats as well as in humans [3,4]. This has led to the current movement of supplement companies advertising the assumed effect of DAA as a hormonal enhancing compound [5,6].

DAA is found in neuroendocrine tissue, specifically synaptic terminals and synaptic vesicles, indicating its possible role in neurotransmission [1,7]. Studies have shown the D-Aspartate racemase, the enzyme that converts DAA into its active form, is found in the highest concentrations in the pituitary gland and the testis. DAA also has been shown to have an effect on testosterone production by Leydig cells in the testes in rats [4]. At the level of the gonads in the hypothalamal-pituitary-gonadal (HPG) axis, LH is the hormone responsible for stimulating Leydig cells to produce testosterone. A study has reported that Leydig cells incubated with DAA lead to an increase in testosterone production [8]. Specifically, the group showed that increased DAA in Leydig cells lead to an upregulation of Steroid Acute Regulatory protein (STAR) which is a protein responsible for cholesterol transport in the mitochondria in the initial steps of steroidogenesis [8].

It is known that testosterone has an anabolic effect, especially in the musculoskeletal system. Administration of exogenous testosterone increases protein synthesis thus increasing strength measures and accelerating the rate of muscle recovery. Testosterone also mediates the IGF-1 system, in muscle cells, which leads to increased protein synthesis [9,10]. With testosterone's proven benefits in muscle synthesis and recovery, increasing testosterone levels is of interest for athletes trying to improving muscle performance. With the legal constraints of hormonal therapy, attention has turned to supplements that could enhance the natural production of testosterone.

The current research on the effect of DAA in humans is limited and inconclusive. Willoughby et al. [11] and Melville et al. [2] both showed that DAA did not have an effect on testosterone levels which contradicted the findings of Topo et al. [5]. These groups used commercially available DAA, which leads to the question of how pure the product was that was used in their studies. This study used pharmaceutical grade DAA to isolate the effects it may have on testosterone status and strength measures following supplementation [11].

METHODS

Subjects

Fifteen male subjects, between the ages of 18-28, in the Buffalo, NY area were recruited and enrolled in the study after signing informed consents. Each subject was screened to ensure they were be free of signs and symptoms of cardiovascular and pulmonary disease and met the criteria for the American College of Sports Medicine (ACSM) low risk stratification for coronary artery disease for participating in low intensity exercise programs. Subjects enrolled in the study met the ACSM low risk for CVD, were a conditioned athlete as determined by their performance in the VO_2 peak test (42-46 ml/kg/min) and were able to perform the athletic performance exercises. Subjects were excluded from the study if they failed to meet the ACSM criteria, were unable to perform the exercises or if they were taking any performance enhancing supplements. The State University of New York University at Buffalo's Institutional Review Board for Human Subjects approved all procedures conducted prior to the start of the study.

Experimental design

This study utilized a double blinded, placebo-controlled, parallel arm experimental design. After a successful screening visit, subjects were randomized into either the experimental group (DAA) or the placebo group (PL). The course of the study consisted of three visits, a screening (visit 1), pre-supplementation (visit 2), and post-supplementation (visit 3). Body composition measurements were taken at each visit using a BodPod (BodPod Indianapolis, IN). Physical assessments were performed pre and post-supplementation to evaluate strength changes.

Venous blood collection was performed by a certified phlebotomist at the beginning of each visit. Approximately 8 ml of blood was taken from the median cubital vein and centrifuged for 20 min at 3000 RPMs. Aliquots of serum were collected and stored at -80°C until they were ready to be analyzed. Blood serum samples were analyzed for total testosterone by Kaleida Health's Department of Pathology and Laboratory Sciences in Buffalo, NY.

Supplementation

Supplementation was based on the current recommended dose of 3 g/day by popular supplement companies [1,2]. The DAA group (n=9) consumed 3.0 g of D-Aspartic Acid (DAA) a day (Sigma-Aldrich St. Louis, MO.) while the PL group (n=6) was given microcrystalline cellulose in capsulated form (Sigma-Aldrich St. Louis, MO) Subjects consumed 4 capsules per day with a meal. Two capsules contained 800mg of treatment each and the remaining two capsules contained 700 mg of treatment each, giving a total of 3.0 g of treatment. The subjects received a one week supply of either DAA or microcrystalline cellulose after completion of visit 2. Subjects were instructed to begin supplementation the following morning, and were required to fill out a daily log of when the pills were consumed to assure compliance. After one week of supplementation, pill consumption logs were submitted and another week supply of the pills was administered. Immediately after two weeks of supplementation, subjects completed visit 3.

Exercise testing

All subjects completed their lab visits in the morning, refrained from strenuous activity for 24 h prior to their visit, and arrived after a four hour fast from food. The same sequence of exercise tests were completed at each visit starting with the VO_2 max test, followed by a self-conducted warm up of 5 min. The test began at a workload of 100 W and 90 RPM that increased by 5 W every 10 s until completion. The test continually increased resistance by 5 W every 10 s until the subject could not maintain a pace of 80 RPM for 10 s.

Heart rate (HR), VO_2, and VCO_2 were continuously monitored during the test. HR and Rate of Perceived Exertion (RPE) were recorded every minute. The experimenter concluded the test if the subject was below the required cadence or by the subject when they reached their point of exhaustion. Time to peak and peak workload were collected at the conclusion of the test, and subjects were allowed to cool down on the bike.

After the VO_2 peak test, subjects began the bench press test, which was performed using a smith machine which is comprised of a barbell that is fixed within steel rails allowing only vertical or near-vertical movement. Subjects were instructed to warm up at their own pace, increasing weight in increments based on their normal routine while bench pressing. Prior to warming up the subjects were instructed on the protocol of the bench press. In order for the trial to count, the subject had to pause with the bar touching their chest. The researchers defined a paused repetition as when the momentum of the bar ceased and was touching the subject's chest at the distal part of their sternal body just proximal to their xiphoid process. The subject was given the command to finish the press when the requirements for the paused repetition were satisfied. The protocol of the bench press was designed and implemented by the researchers to ensure consistency between subjects, eliminating the possibility of an effect due to variations in form between subjects. The subjects were not limited in the amount of attempts at a maximal weight. When the subjects achieved 90% of their anticipated maximum weight. Members of the research staff were present and responsible for spotting the subject through all bench press repetition attempts.

The subjects finished the visit with a squat press on the same smith machine. The machine was set up on an individual basis in which stopping pins were placed at the point where the subject made a 90º angle at the point of their knee. The stopping pins eliminated the bar descending past a desired point that may have injured the subject. The subjects were instructed prior to warming up that a successful trial was described as squatting down to level of the pins and then lifting the weight back up in a "touch-and-go" fashion. Again, this protocol was designed to standardize the movement, and eliminate possible differences in results based on variations in form between subjects. In the same manner as the bench press, subjects were to warm up at their own pace as they normally would. Members of the staff were present and spotting the subject through experimental trials.

Statistical analysis

All data was analyzed using Two Way Repeated Measures Analysis of Variance (ANOVA) with Student-Newman-Keuls (SNK) post hoc analysis. All tests were run using SigmaPlot version 13.0 software. Data was considered significant if $p<0.05$ and trending towards significance if $p<0.07$. All data reported as (Mean ± SEM).

RESULTS

Subject demographics

Sixteen subjects were enrolled in the study; however one subject withdrew from the study due to personal reasons. Nine subjects were randomized into the DAA group, while six were randomized into the PL group. Subjects had an average age of 20.9 ± 2.5 years, average weight of 79.6 ± 0.6 kg and average height of 1.75 ± 0.2 m. All subjects enrolled in the study were currently active athletes. The demographics shown in Table 1 indicate that all subjects were similar in muscle mass and exercise capacity at the start of the study.

Table 1: Subject demographics subject characteristics of body composition and exercise capacity prior to beginning the study

Subject Demographics (n=15)	
Demographic	**Mean ± SEM**
Age (y)	20.9 ± 2.5
Height (m)	1.75 ± 0.2
Weight (kg)	79.6 ± 0.6
Body Fat Percent (%)	9.2 ± 1.4
Fat Free Mass (kg)	73.9 ± 2.03
Peak Work Load (W)	288.8 ± 14.6
Maximum Bench Press (kg)	108.1 ± 3.5
Maximum Squat Press (kg)	138.7 ± 5.4

Maximum bench

The DAA group had a positive trend towards an improved 1RM bench press through an increase in 3.7 ± 6.7 kg after two weeks of supplementation ($p=0.064$). The PL group saw no improvement or trend towards improvement after supplementation ($p=1.0$). There was no difference between groups at the pre-supplementation visit ($p=0.407$) (Figure 1).

Squat press

1 RM squat performance increased 8.5 ± 10.5 kg in DAA subjects ($p<0.01$) but there was no change in placebo subjects ($p=0.5$) after two weeks of supplementation. Pre-supplementation 1RM squat performance was similar among both treatment groups ($p=0.7$) (Figure 2).

VO_2 peak

DAA subjects performed better during a VO_2 peak cycling test compared to placebo subjects after two weeks of supplementation. Maximal workload improved by 48.8 ± 15.9 Watts ($p<0.01$) (Figure 3) and time to peak increased 1.4 ± 0.5 min ($p<0.05$) (Figure 4) in the DAA subjects. There was no change in maximal work load ($p=0.6$) or time to peak ($p=0.4$) for placebo subjects.

Testosterone levels

Total testosterone levels was unchanged after supplementation in either the DAA subjects (Pre-Supplementation: 720.5 ± 70.8 ng/dL, Post Supplementation: 719.7 ± 70.8 ng/dL) ($p<0.9$. PL subjects also had similar total testosterone after supplementation (Pre-Supplementation: 731.167 ± 86.6 ng/dL, Post Supplementation: 733.667 ± 86.6 ng/dL) ($p<0.9$).

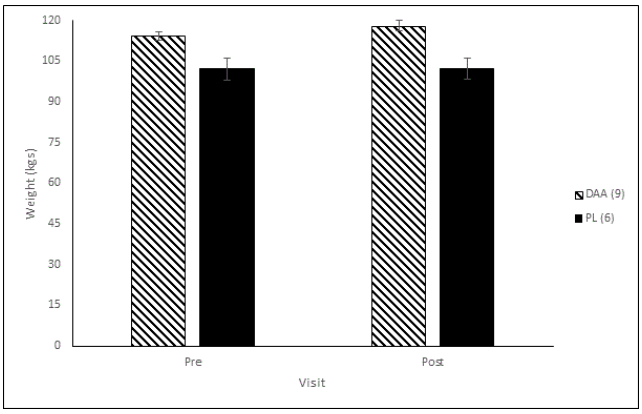

Figure 1: One rep max on bench press. Change between pre and post supplementation 1RM bench press weight in kg between DAA group (Pre-Supplementation: 114.6 ±6.7 kg, Post-Supplementation: 118.3 ± 6.7 kg; p=0.064) and PL group (Pre-Supplementation: 102.1 ± 7.5 kg, Post-Supplementation: 102.1 ± 7.5 kg; p=1.0). Results were not significant

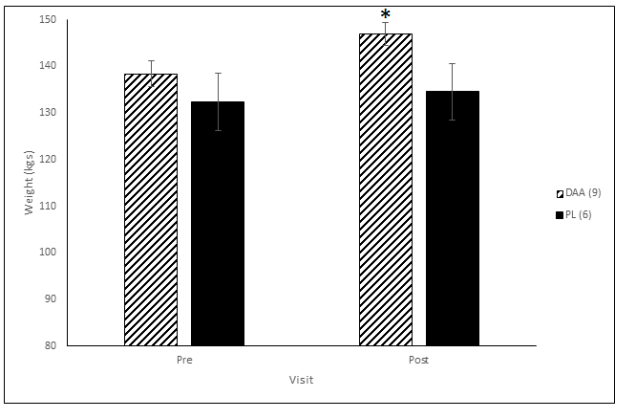

Figure 2: One rep max on squat press. Change between pre and post supplementation 1RM squat press weight in kg between DAA group (Pre-Supplementation: 138.4 ± 10.5 kg, Post-Supplementation: 146.9 ± 10.5; p<0.01), and PL group (Pre-Supplementation: 132.3 ± 12.1 kg, Post-Supplementation: 134.6 ± 12.1 kg; p<0.5). Two Way Repeated Measures ANOVA with SNK post hoc analysis; *p<0.05 compared to Pre values

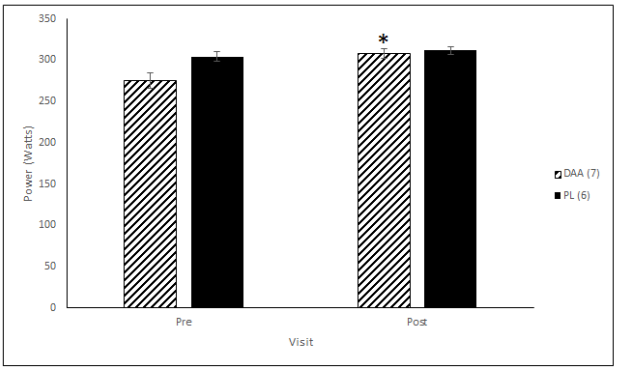

Figure 3: Change in peak work load. Change between pre and post supplementation peak work load during VO_2 peak in DAA group (Pre-Supplementation: 277.8 ± 15.9 W, Post-Supplementation: 325.8 ± 15.9 W, p<0.01) and PL group (Pre-Supplementation: 304.1 ± 17.3 W, Post-Supplementation: 311.6 ± 17.3 W, p=0.6). Two Way Repeated Measures ANOVA with SNK post hoc analysis; p<0.05 * compared to Pre values

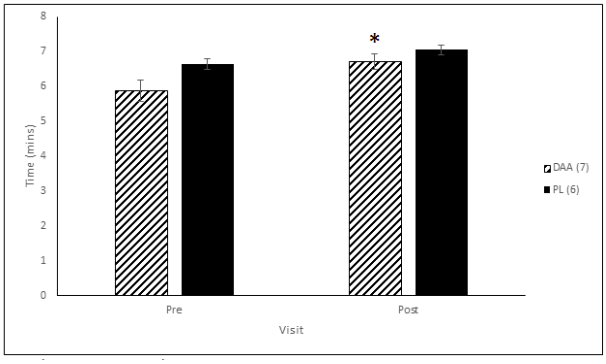

Figure 4: Change in time to peak. Change between pre and post supplementation time to peak during VO_2 peak in DAA group (Pre-Supplementation: 5.9 ± 0.5 min, Post-Supplementation: 7.3 ± 0.5 min, $p<0.05$) and PL group (Pre-Supplementation: 6.6 ± 0.05 min, Post-Supplementation: 7.0 ± 0.5 min, $p=0.4$). Two Way Repeated Measures ANOVA with SNK post hoc analysis; $p<0.05$* compared to pre values

DISCUSSION

The studies aim was to determine if supplementing pharmaceutical grade D-Aspartic Acid (DAA) would be able to increase testosterone production and subsequently increase maximal strength measures in college age males. Our results indicate that supplementing 3 g of DAA a day for 2 weeks does not increase serum total testosterone. However, DAA supplementation increased performance in a VO_2 peak test and resulted in a higher 1RM squat press. DAA supplementation had no effect on 1RM bench press.

The lack of testosterone change with DAA supplementation is consistent with Willoughby et al. [11] who used the same DAA dosage and timing but Topo et al. did show it was possible after only 12 days of supplementation [5]. The discrepancy in DAA supplementation and testosterone production could be due to multiple factors. First, the baseline testosterone levels in subjects were much higher in this study than the baseline testosterone levels observed by Topo et al. [5] which is likely due to a combination of the younger age of this study's population. In addition, the current studies subjects were athletically trained rather than sedentary as in Topo et al. [5]. The difference in basal testosterone is not surprising as resistance training can alter the neuroendocrine system such that steroidogenic hormone production is increased [13-16]. Taken together, the contradictory results may be due to a difference in subject demographics and resistance training history.

To our knowledge the only other study to report strength measure changes due to DAA supplementation was Willoughby and Leutholtz in 2013 [12], who showed that supplementing DAA for 28 days in addition to a resistance training protocol does not change 1RM bench performance, but saw significant increases in leg press strength in both their DAA and PL groups, which would eliminate the possibility that any effects seen were due to DAA supplementation [12]. When comparing our results on leg strength, we found a significant increase in 1RM with in the DAA groups, but not in the PL group. The differences between our findings and the findings from Willoughby and Luetholtz [12] may be due to differences in our experimental design. First, our study used a squat 1RM to assess leg strength whereas Willoughby and Leutholtz [12] used an angled leg press. The literature shows that when comparing the squat and leg press, that the squat test is more indicative of quadricep and hamstring strength than the leg press [17]. Differences could also be due to the length of supplementation, and the utilization of a standardized training protocol. Due to the variability of the athletes work out schedules, we opted to not implement a standardized training protocol. This eliminated the possibility of acute strength gains due to a new workout program, so that any findings could be directly attributed to DAA supplementation.

Our study also used pharmaceutical grade DAA rather than a commercialized DAA supplement. In the initial review of the literature on DAA and athletic performance, our lab noted that the DAA being used was a commercial product produced by a supplement company. It has been shown that commercial grade products may not always be as pure as advertised, so we wanted to conduct our study with pharmaceutical grade DAA and compare our results to the current literature [18]. Interestingly, the results of our hormone analysis are consistent with Willoughby and Leutholtz [11,12], but we did see improved performance in the strength measures tested whereas other studies did not. These results could reveal that DAA may have a mechanism of increasing performance measures, other than the widely hypothesized function to increase testosterone.

Limiting factors for this study include the small number of subjects and duration of supplementation. Two weeks of DAA supplementation may not be a sufficient amount time to see a change in hormone production. We also only compared a pre-supplementation and post-supplementation blood sample. The hypothalamal-pituitary-gonadal axis operates as a feedback loop, so we may have missed any spikes in hormone production that may have occurred. Future studies should look at the effect of pharmaceutical grade DAA supplementation over a longer period with and increased number of blood collection time points. Research should also be done on the effect of DAA supplementation on metabolic pathways associated with muscle function and endurance.

CONCLUSION

Supplement companies often advertise the testosterone enhancing abilities of DAA, but there is a lack of conclusive evidence in human subjects to support that claim. In the present study, we report that supplementing 3 g of DAA a day may lead to improved performance in strength and endurance tests without a concurrent increase in testosterone levels.

DECLARATIONS

Acknowledgement

We would like to thank all of the subjects who participated in the study.

Funding

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Availability of data and materials

Considering subjects enrolled in the study were student athletes at the University at Buffalo, it has been requested that results of individuals be kept confidential. To maintain integrity, we will not present data beyond what has been reported in the manuscript.

Authors' contributions

ZML designed the study, oversaw data collection, data analysis and manuscript preparation. BTW assisted with study design, data analysis and manuscript preparation. TJF assisted with data collection, and manuscript preparation. PJH assisted with study design, data analysis and manuscript preparation. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The research was approved by the Human Subject Institutional Review Board of the University at Buffalo. Each participant gave their written informed consent after explanation of the study purpose, experimental procedures, possible risks and benefits. All the volunteers signed an informed consent term before participation.

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