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Research article

Heavy Resistance Training and Supplementation with the Alleged Testosterone Booster NMDA Has No Effect on Body Composition, Muscle Performance, and Serum Hormones Associated with the Hypothalamo-Pituitary-Gonadal Axis in Resistance-Trained Males

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Abstract
The effects of 28 days of heavy resistance training while ingesting the alleged testosterone-boosting supplement, NMDA, were determined on body composition, muscle strength, serum cortisol, prolactin, and hormones associated with the hypothalamo-pituitary-gonadal (HPG) axis. Twenty resistance-trained males engaged in 28 days of resistance training 4 times/wk while orally ingesting daily either 1.78 g of placebo (PLAC) or NMDA. Data were analyzed with separate 2 x 2 ANOVA ($p < 0.05$). Criterion measures involved body composition, muscle strength, serum cortisol, prolactin, and gonadal hormone levels [free and total testosterone, luteininzing hormome (LH), gonadotrophin releasing hormone (GnRH), estradiol], and were assessed before (Day 0) and after (Day 29) resistance training and supplementation. No changes were noted for total body water and fat mass in response to resistance training ($p > 0.05$) or supplementation ($p > 0.05$). In regard to total body mass and fat-free mass, however, each was significantly increased in both groups in response to resistance training ($p < 0.05$), but were not affected by supplementation ($p > 0.05$). In both groups, lower-body muscle strength was significantly increased in response to resistance training ($p < 0.05$); however, supplementation had no effect ($p > 0.05$). All serum hormones (total and free testosterone, LH, GnRH, estradiol, cortisol, prolactin) were unaffected by resistance training ($p > 0.05$) or supplementation ($p > 0.05$). The gonadal hormones and cortisol and prolactin were unaffected by 28 days of NMDA supplementation and not associated with the observed increases in muscle strength and mass. At the dose provided, NMDA had no effect on HPG axis activity or ergogenic effects in skeletal muscle.

Key words: D-Aspartic Acid, N-Methyl-D-Aspartic Acid, testosterone, resistance training.

Introduction

Within the nutritional/sport supplement industry there are a vast amount of products that are marketed as "testosterone boosters." Many of these products contain a proprietary blend of various ingredients alleged to increase endogenous testosterone levels. However, in most cases there is little data, either on the complete product itself or the various active ingredients typically contained within a proprietary blend, to substantiate manufacturers' claims. In many cases, the studies that are available which are used to substantiate product claims involve animal or cell culture models; therefore, the results may not be germane to humans. Nevertheless, the nutrition/sport supplement industry often attempts to take advantage of this, often times irrelevant, information by manufacturing products with the intent of them acting as "testosterone boosters" in their ability to increase endogenous testosterone levels, presumably by activation of the hypothalamo-pituitary-gonadal (HPG) axis, and subsequently augmented when combined with resistance training. Furthermore, these products usually have no data available, yet are still 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.

One such product is NMDA (Muscle Warfare, Wellington, FL), a proprietary blend nutritional product that is an alleged testosterone booster. It is advertised to be a N-methyl-D-aspartate receptor activator and testosterone releaser. Based on the manufacturers' rationale, the N-methyl-D-aspartic acid receptor would be up-regulated due to the presence in the NMDA product of D-aspartic acid (D-Asp), N-methyl-D-aspartate (NMD-Asp), trimethyl glycine, and S-adenosyl methionine (SAMe). As a result, the HPG axis would be activated, as evidenced by increases is circulating gonadotrophin releasing hormone (GnRH). Therefore, luteinizing hormone (LH) and testosterone would also be subsequently increased due to an up-regulation in the HPG axis. In addition, the herbs *Eurycoma Longfolia Jack* and *Mucuna Pruriens* contained in the product, which are alleged to be testosterone GnRH release triggers and inhibitors of prolactin and cortisol, will also help synergize the activation of the HPG axis. While there are data available in both animals and humans on various ingredients contained in the NMDA product that are associated with increases in testosterone and inhibition of prolactin and cortisol, there are no data available on the actual product itself, particularly in response to resistance training.

Elevations in circulating testosterone are known to augment muscle protein synthesis, thereby resulting in enhanced muscle mass and strength. In humans, testosterone is synthesized and released through an elaborate hormonally-orchestrated signaling cascade referred to as the HPG axis. In order for this axis to be up-regulated, D-Asp, an endogenous amino acid present in nervous tissues and endocrine glands of humans (D'Aniello et al., 2007), plays an important neuromodulating role. In males, D-

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Asp endogenously synthesizes testosterone by first converting to NMD-Asp by D-aspartate methyltransferase (NMDA synthetase). In the hypothalamus, NMD-Asp binds to its receptor, a subtype of the L-glutamate receptor, and potentiates transmission via glutaminergic neurons (Katane & Homma, 2011), which results in the release of GnRH (D'Ainello, 2007). The release of GnRH from the hypothalamus then triggers the release of prolactin, follicle stimulating hormone (FSH), and LH from the pituitary gland. The effect of the latter two hormones on the testes is that FSH stimulates spermatogenesis and LH stimulates testosterone synthesis (D'Ainello, 2007). In addition, D-Asp-induced neuromodulation induces aromatase activity, the enzyme responsible for the conversion of testosterone to 17β-estradiol (Assisi et al., 2001; Raucci et al., 2005).

Prolactin is a hormone synthesized in the adenohypophyseal lactotrophs, has no known target organ or defined role in male reproduction. Yet, expression of prolactin receptors on the choroid plexus and hypothalamus presupposes a latent role for this hormone in the regulation of male fertility (Grattan, 2001; Mangurian et al., 1992). Although the functional significance of prolactin to male reproduction has not been unequivocally established, the hormone has been associated primarily with male infertility (Gill-Sharma, 2009). Cortisol is a glucocorticoid hormone produced by the adrenal gland and its release is controlled by the hypothalamus, and its primary functions are to increase blood sugar through gluconeogenesis, suppress the immune system, and aid in fat, protein and carbohydrate metabolism. Elevated levels of cortisol, if prolonged, can lead to proteolysis and muscle wasting (Simmons et al., 1984). Prolactin and cortisol release are both governed by the hypothalamo-pituitary axis (HP axis) where prolactin is released directly from the pituitary gland; however, cortisol is released further downstream, from the adrenal gland.

Due to the lack of scientific data on the nutritional supplement, NMDA, and having to rely on manufactures' claims and anectodtal reports regarding the effectiveness of NMDA supplementation in increasing endogenous testosterone levels, it is tenuous at best to assume that the NMDA product may prove beneficial as a means in which to increase muscle performance associated with heavy resistance training. While there are data available on some of the active ingredients contained in the NMDA product, there appears to be no scientific studies, human or animal, dealing with the supplementation of NMDA. As a result, we hypothesized that NMDA 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 NMDA supplementation on body composition, muscle strength, serum cortisol, prolactin, and hormones associated with the HPG axis in resistance-trained males. It was hypothesized that NMDA would not increase endogenous testosterone, blunt the levels of cortisol and prolactin, or improve muscle mass and strength associated with resistance training.

Methods

Experimental approach

In a randomized, double-blind design, participants engaged in 28 days of heavy resistance training while also ingesting 1.78 g·day^{-1} of either placebo or NMDA. 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 NMDA supplement, muscle mass and strength may be preferentially affected compared to placebo, due to elevations in endogenous testosterone and decreases in cortisol and prolactin.

Participants

Twenty apparently healthy, resistance-trained [consistent (at least thrice weekly) resistance training for one year prior to the study] males with an average age of 21.42 ± 3.16 yr, height of 1.81 ± 0.07 m, and total body mass of 79.1 ± 16.13 kg completed the study. Enrollment was open to men of all ethnicities. All participants underwent a mandatory medical screening, and anyone with contraindications to exercise as outlined by the American College of Sports Medicine and/or who had consumed any nutritional supplements (excluding multi-vitamins) within three months, or anabolic steroids six months prior to 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. Additionally, all experimental procedures involved in this study conformed to the ethical considerations of the Helsinki Code.

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, dietary intake and body composition assessed, and muscle strength tests performed.

Strength assessment

Upper- and lower-body one repetition maximum (1-RM) strength tests were performed using the free weight bench press and angled leg press exercises (Nebula, Versailles, OH), respectively, based on our previous studies (Shelmadine et al., 2009; Spillane et al., 2011; Willoughby and Leutholtz, 2013). As a warm-up, an estimated 50% 1-RM was utilized to complete 10 repetitions. After a two-min rest period, a load of 70% of estimated 1-RM was utilized to perform five repetitions. At this point, the weight was gradually increased until a 1-RM was reached with each following lift, with a two-min rest period in between each successful lift. Test-retest reliability of performing these strength assessments on subjects within our laboratory has demonstrated low mean coefficients of variation and high reliability for the bench press (1.9%, intraclass r = 0.91) and leg press (0.79%, intraclass r = 0.92), respectively.

Body composition assessment

Total body mass (kg) was determined on a standard dual beam balance scale (Detecto Bridgeview, IL). Percent body fat, fat mass, and fat-free mass were determined

using DEXA (Hologic Discovery Series W, Waltham, MA). 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 prior to each testing session [9,10,DAA]. Total body water was determined by bioelectric impedance analysis (Xitron Technologies Inc., San Diego, CA) (Shelmadine et al., 2009; Spillane et al., 2011; Willoughby & Leutholtz, 2013). Based on previous studies in our laboratory, the accuracy of the DEXA for body composition assessment is ± 3.7% as assessed by direct comparison with hydrodensitometry and scale weight.

Venous blood sampling

At Day 0 and 29, venous blood samples were obtained from the antecubital vein into a 10 ml collection tube using a standard vacutainer apparatus. The blood sample on Day 29 was obtained 24 hr after the final dose of supplement ingested on Day 28. At each time point, an aliquot of whole blood was used to determine hematocrit (Symex XS 1000i, Lincolnshire, IL) and then subsequently used to determine any changes in plasma volume between Day 0 and Day 29 for each participant (van Beaumont, 1972). The remaining blood for each sample was allowed to stand at room temperature for 10 min and then centrifuged at 2,000 rpm. The serum was removed and frozen at -80°C for later analysis.

Supplementation protocol

In a randomized, double-blind manner, participants were assigned a 28-day supplementation protocol, consisting of the oral ingestion of 4 capsules daily of either 1.78 g of cellulose placebo [PLAC (Nutricology, Alameda, CA) or 1.78 g of NMDA (Muscle Warfare, Wellington, FL), based on company guidelines for NMDA. A certificate of analysis (COA) for NMDA performed by an independent lab (Nutricap Labs, Farmingdale, NY) was obtained from Muscle Warfare. From the COA, the content of each ingredient was accurately depicted on the product label and the purity of each ingredient was 100%. Capsules for the PLAC and NMDA groups were identical in color, shape, and size. Two capsules were ingested in the morning upon waking and the final two capsules were ingested mid-afternoon. Supplementation compliance was monitored by having each participant return empty containers of their supplement at the testing session on day 29. In addition, participants completed a daily supplement compliance questionnaire.

Dietary monitoring

Dietary intake was determined by having participants record their food and drink intake for four consecutive days prior to each of the two testing sessions at Day 0 and Day 29 (Shelmadine et al., 2009; Spillane et al., 2011; Willoughby and Leutholtz, 2013). For standardization purposes, diets were not controlled and participants were asked not to change their typical dietary habits during the course of the study. In an attempt to determine the average daily macronutrient intake of fat, carbohydrate, and protein prior to each assessment point, the four-day dietary recalls were evaluated with the Food Processor IV Nutrition Software (ESHA, Salem OR).

Resistance-training protocol

Participants completed a periodized 28-day resistance-training program, split into two upper-extremity and two lower-extremity exercise sessions each wk, based on our previous studies (Shelmadine et al., 2009; Spillane et al., 2011; Willoughby and Leutholtz, 2013). As a result, this constituted a total of 16 exercise sessions, with eight upper-body and eight lower-body exercise sessions. Each exercise session was supervised and documented by study personnel, and prior to each exercise session participants performed a standardized series of stretching exercises as a warm-up. The upper-body resistance-training program consisted of nine exercises (bench press, lat pull, shoulder press, seated row, shoulder shrug, chest fly, biceps curl, triceps press down, and abdominal curl) twice per week. The lower-body resistance-training program consisted of seven exercises (leg press or squat, back extension, step up leg curl, leg extension, heel raise, and abdominal crunch) twice per week. Participants performed 3 sets of 10 repetitions at 70 – 80% 1-RM. Rest periods were two min between exercises and sets.

Serum hormone analysis

Serum samples were analyzed in duplicate for total testosterone, free testosterone, LH, estrogen (Alpha Diagnostic International, San Antonio, TX), GnRH (Uscn Life Science, Houston, TX), and cortisol and prolactin (Cayman Chemical, Ann Arbor, MI) using commercially-available ELISA kits (Rohle et al., 2007; Willoughby et al., 2007; Willoughby and Leutholtz, 2013). Absorbances for each hormone were determined at a wavelength of 450 nm using a microplate reader (iMark, Bio-Rad, Hercules, CA). A set of standards of known concentrations for each hormone was utilized to construct standard curves and hormone concentrations were determined using data reduction software (Microplate Manager, Bio-Rad, Hercules, CA). The overall intra-assay percent coefficients of variation were 7.5%, 6.9%, 8.3%, 9.1%, 8.6%, 8.2%, and 3.7%, respectively, for total testosterone, free testosterone, LH, estradiol, GnRH, cortisol, and prolactin.

Statistical analysis

Data were analyzed with separate 2 (group) x 2 (time) analysis of variance (ANOVA) using SPSS for Windows Version 20.0 software (SPSS, Chicago, IL). Significant differences among groups were identified by a Tukey HSD 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 criterion variable of total testosterone, given a type I error rate of 0.05 and a power of 0.80. The index of effect size utilized was partial Eta squared ($\eta 2$), which estimates the proportion of variance in the dependent variable that can be explained by the independent variable. Partial Eta squared effect sizes were determined to be: weak = 0.17, medium = 0.24, strong =

Table 1. Dietary caloric and macronutrient intake for the PLAC and NMDA groups. Values are means (± SD).

Variable	Day	PLAC	NMDA	Test	Group x Test
Total Calories (kcal ·kg^{-1})	0	32.56 (8.49)	33.16 (9.85)	.44	.18
	29	35.48 (10.04)	38.65 (11.61)		
Protein (g·kg^{-1})	0	1.27 (.48)	1.21 (.48)	.53	.21
	29	1.32 (.56)	1.34 (.58)		
Carbohydrate (g·kg^{-1})	0	3.92 (2.76)	4.08 (1.45)	.88	.15
	29	4.0 2 (2.34)	4.34 (2.15)		
Fat (g·kg^{-1})	0	1.32 (.48)	1.35 (.54)	.88	.15
	29	1.39 (.51)	1.42 (.72)		

Dietary caloric and macronutrient intake for the PLAC (n = 10) and NMDA (n = 10) groups. No significant differences were detected for any of the dietary variables ($p > 0.05$).

0.51, very strong = 0.70 (O'Connor et al., 2007). For statistical procedures, a probability level of ≤ 0.05 was adopted throughout the study.

Results

Subject demographics

Twenty-three participants began the study; however, three were withdrawn due to reasons unrelated to the study. Two participants sustained injuries unrelated to the study, and one became too busy with their schedule. As a result, they were not able to remain compliant with the resistance training program. As a result, 20 participants completed the study. The PLAC group (n = 10) had an average (±SD) age of 21.25 ± 1.03 yr, height of 1.80 ± 0.06 m, and total body mass of 84.25 ± 17.37 kg. The NMDA group (n = 10) had an age of 20.11 ±1.36 yr, height of 1.80 ± 0.05 m, total body mass of 89.46 ± 17.55 kg.

Dietary analysis, supplement and exercise compliance, and reported side 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 ($p > 0.05$). Furthermore, there were no significant differences between groups for total calories ($p = 0.18$, effect size = 0.43) or for the intake of protein ($p = 0.21$, effect size = 0.34), carbohydrate ($p = 0.15$, effect size = 0.47), and fat ($p = 0.56$, effect size = 0.22).

In regard to compliance, PLAC and NMDA were 89.9 ±10.92 % and 92.7 ±8.61 % compliant to the resistance training program, respectively. For supplementation compliance, PLAC and NMDA were 99.25 ±2.25 % and 99.30 ±2.21 % compliant to the supplementation protocol. Regarding side effects from supplementation, over the course of the 28 days, one participant in PLAC and two in NMDA reported side effects. All three participants reported feelings of irritability, nervousness, rapid heart rate, and headache.

Body composition

Total body mass was significantly increased in both groups with training ($p = 0.04$, effect size = 0.14), but there were no differences between groups ($p = 0.63$, effect size = 0.05). In addition, there were no significant changes occurring in total body water as a result of training ($p = 0.67$, effect size = 0.006) or supplementation ($p = 0.87$, effect size = 0.001). Fat mass was unchanged with resistance training ($p = 0.79$, effect size = 0.002) and supplementation ($p = 0.75$, effect size = 0.002). However, fat-free mass was significantly increased in both groups in response to training ($p = 0.03$; effect size = 0.13), but not preferentially affected in the NMDA group ($p = 0.61$, effect size = 0.05) (Table 2).

Muscle strength

For muscle strength, bench press strength was unchanged with resistance training ($p = 0.30$, effect size = 0.03) and supplementation ($p = 0.91$, effect size = 0.001). However, for leg press strength both groups underwent significant increases with training ($p = 0.04$, effect size = 0.13); however, these increases were not preferentially affected by NMDA supplementation ($p = 0.79$, effect size = 0.002) (Table 3).

Serum hormones

Plasma volume was not changed ($p = 0.65$, effect size = 0.006) from Day 0 to Day 29; therefore, serum hormone concentrations did not need to be adjusted for changes in plasma volume. In response to resistance training, total testosterone ($p = 0.77$, effect size = 0.003), free testosterone ($p = 0.76$, effect size = 0.003), LH ($p = 0.67$, effect size = 0.006), GnRH ($p = 0.74$, effect size = 0.003),

Table 2. Body composition variables for the PLAC and NMDA groups. Values are means (±SD).

Variable	Day	PLAC	NMDA	Test	Group x Test
Body Mass (kg)	0	84.25 (17.37)	86.46 (17.55)	p = 0.04	p = 0.63
	29	86.03 (16.81)*	88.47 (11.90)*		
Body Water (kg)	0	45.25 (5.37)	46.67 (2.55)	p = 0.67	p = 0.87
	29	45.86 (5.88)	46.06 (2.46)		
Fat Mass (kg)	0	16.72 (9.85)	18.12 (11.92)	p = 0.79	p = 0.75
	29	16.58 (9.62)	18.68 (11.46)		
Fat-Free Mass (kg)	0	70.17 (7.22)	71.45 (7.93)	p = 0.03	p = 0.61
	29	71.75 (8.85)*	72.93 (7.61)*		

Body composition for the PLAC (n = 10) and NMDA (n = 10) groups. * denotes a significant increase at Day 29. Resistance training increased total body mass ($p = 0.04$) and fat-free mass ($p = 0.03$) for both groups; however, there were no differences associated with NMDA supplementation ($p > 0.05$).

Table 3. Muscle strength variables for the PLAC and NMDA groups. Values are means (± SD).

Variable	Day	PLAC	NMDA	Test	Group x Test
BP Strength (kg·kg^{-1})	0	1.18 (.39)	1.27 (.27)	p = 0.30	p = 0.91
	29	1.29 (.37)	1.38 (.29)		
LP Strength (kg·kg^{-1})	0	4.15 (1.55)	4.49 (1.75)	p = 0.04	p = 0.79
	29	4.98 (1.78)	5.25 (1.67)		

Bench press (BP) and leg press (LP) strength for the PLAC (n = 10) and NMDA (n = 10) groups. * denotes a significant increase at Day 29. Resistance training increased leg press strength (p = 0.04) for both groups; however, there was no difference associated with NMDA supplementation (p > 0.05).

estradiol (p = 0.53, effect size = 0.01), cortisol (p = 0.41, effect size = 0.021), and prolactin (p = 0.77, effect size = 0.003) were not significantly changed. Similarly in response to NMDA supplementation, total testosterone (p = 0.86, effect size = 0.001), free testosterone (p = 0.85, effect size = 0.003), LH (p = 0.67, effect size = 0.006), GnRH (p = 0.24, effect size = 0.04), estradiol (p = 0.83, effect size = 0.001),), cortisol (p = 0.76, effect size = 0.03), and prolactin (p = 0.93, effect size = 0.013) were not significantly changed when compared to PLAC (Table 4).

Discussion

We sought to determine the effects of 28 days of heavy resistance training and NMDA supplementation on body composition, muscle strength, serum cortisol and prolactin, and hormones associated with the HPG axis in resistance-trained males. Our results demonstrate similar increases in muscle mass and strength in both groups associated with 28 days of resistance training as in our previous studies, which used the identical training protocol (Shelmadine et al., 2009; Spillane et al., 2011; Willoughby and Leutholtz, 2013). Moreover, as with our previous study which involved D-Asp supplementation (Willoughby and Leutholtz, 2013), in the present study we also found that resistance training had no effects on the serum levels of testosterone and estradiol, along with no effects on cortisol and prolactin. Furthermore, we also found that NMDA supplementation had no preferential effect on augmenting testosterone or decreasing estrogen, cortisol, and prolactin. Consequently, while resistance training was effective in increasing muscle mass and strength, it was not preferentially due to NMDA supplementation. Therefore, we accept our hypothesis that NMDA would not increase endogenous testosterone, blunt the levels of cortisol and prolactin, or improve muscle mass and strength associated with resistance training. In light of our findings, this study refutes alleged marketing claims that NMDA increases muscle mass and strength due to its ability to elevate endogenous testosterone levels and lower cortisol and prolactin.

Much of the rationale for the alleged effectiveness of the NMDA product is based on the premise that endogenous testosterone synthesis will occur due to localized hypothalamic NMD-Asp receptor-mediated up-regulation of the HPG axis. D-Asp is an endogenous precursor for NMD-Asp synthesis as D-Asp serves as the substrate and SAMe as the donor of the methyl group; therefore, D-Asp and NMD-Asp have both been implicated in the hormonal regulation of the HPG axis (D'Aniello et al., 2000). This has been further substantiated as synthetic NMD-Asp has been shown to be involved in the adenohypophysial hormone secretion (Wilson and Knobil, 1982; Pinilla et al., 1999). Even though there appears to be no previously-published data, human or otherwise, addressing the effectiveness of NMDA on the HPG axis, our results show that the inclusion of NMD-Asp and D-Asp in the NMDA product had no effect on the activity of the HPG axis, evidenced by a lack of effect on GnRH, LH, and testosterone. In addition, it also had no effect on the levels of estradiol. However, a previous study with humans (Topo et al., 2009) showed 12 days of D-Asp supplementation at a daily dose of 3 grams to be effective at increasing LH and testosterone; however, this study did not involve resistance training and evaluate muscle strength and mass. Consequently, the results of our present study are corroborated by a recent study from our laboratory showing that 3 gr·day^{-1} of D-Asp for 28 days while combined with a resistance training program was ineffective at up-regulating the HPG axis and increasing

Table 4. Serum hormone variables for the PLAC and NMDA groups. Values are means (± SD).

Variable	Day	PLAC	NMDA	Test	Group x Test
Total Test (ng·ml^{-1})	0	11.04 (1.58)	11.96 (1.26)	.77	.86
	29	11.96 (1.39)	12.02 (1.18)		
Free Test (pg·ml^{-1})	0	103.26 (6.27)	106.54 (5.85)	.76	.85
	29	101.81 (7.03)	106.85 (4.85)		
LH (mIU·ml^{-1})	0	2.97 (2.16)	3.27 (2.55)	.53	.83
	29	3.25 (2.94)	3.30 (2.71)		
GnRH (pg·ml^{-1})	0	319.57 (126.55)	333.39 (135.01)	.74	.24
	29	335.44 (137.06)	396.23 (122.49)		
Estradiol (pg·ml^{-1})	0	1332.84 (912.58)	1376.25 (885.15)	.53	.83
	29	1254.36 (805.83)	1284.39 (932.17)		
Cortisol (pg·ml^{-1})	0	124.56 (32.58)	134.35 (31.14)	.41	.76
	29	122.83 (35.58)	129.56 (33.23)		
Prolactin (ng·ml^{-1})	0	7.59 (8.23)	7.96 (9.21)	.77	.93
	29	6.81 (7.45)	7.24 (8.67)		

Serum hormones representing the HPG axis for the PLAC (n = 10) and NMDA (n = 10) groups. No significant differences were detected for any of the serum hormones (p > 0.05).

endogenous testosterone and estradiol, and had no anabolic or ergogenic effects in skeletal muscle (Willoughby and Leutholtz, 2013).

Although D-Asp and its subsequent SAMe-induced conversion to NMD-Asp is a primary ligand for the NMD-Asp receptor, this receptor is also an excitatory ionotropic receptor that is permeable to sodium, calcium, and potassium, following their concentration gradient. The most characteristic feature of the NMD-Asp receptor is its voltage-dependent regulation by magnesium (Mayer et al., 1984; Nowak et al., 1984). Furthermore, a multitude of different binding sites, besides the glutamate site, such as the glycine, zinc, and magnesium sites, allow for a vast number of allosteric interactions (Low et al., 2000). In addition to D-Asp, NMD-Asp, and SAMe, the NMDA product contains zinc, magnesium, and glycine. Even with the inclusion of the latter three allosteric modulators of NMD-Asp receptor activity, our results presented herein show no increased activity of the HPG axis as a result of NMDA supplementation.

The NMDA product also contains *Eurycoma Longifolia Jack*, also known as *Tongkat Ali* (TA), which is an herbal medicinal plant traditionally used to attenuate age-related decrements in energy, mood, and libido. However, TA can be found in a variety of products intended to improve libido and energy, increase testosterone, decrease cortisol, and enhance body composition and exercise performance. It has been speculated that TA induces an increase in testosterone by potentiating the release of testosterone from SHBG, thereby increasing the amount of free, bioavailable testosterone (Chaing et al., 1994). A recent study using older men and women involved TA supplementation at 400 mg daily for 5 weeks showed that TA resulted in significant increases in total and free testosterone and muscular force. The increase in free testosterone in women is thought to be due to the significant decline in SHBG concentrations, yet affirms the ergogenic benefit of TA through enhanced muscle strength (Henkel et al., 2013). In older, hypogonadal men, four weeks of TA supplementation at 200 mg·day^{-1} increased serum testosterone levels (Tambi et al., 2012). A study of young men involved in a five-week weight training program found that TA 100 mg·day^{-1} of TA supplementation increase muscle strength and mass to a greater extent than placebo (Hamzah and Yusof, 2003). While there are studies suggesting the potential effectiveness of TA supplementation on increases in serum testosterone and subsequent improvements in muscle performance, our results suggest the contrary as we showed no preferential effect on the serum levels of testosterone and cortisol or muscle strength by NMDA supplementation.

The release of prolactin is caused by a direct action of D-Asp on the pituitary gland and also mediated by the indirect action of NMD-Asp on the hypothalamus. It has been shown that D-Asp possesses the capacity to induce the release of prolactin in rat blood (D-Aniello et al., 2000). Synthetic NMD-Asp has also been shown to stimulate hypothalamic GnRH neurons involved in prolactin release (Gay and Plant, 1987). Acute hyperprolactemia is known to suppress testosterone synthesis and male fertility through prolactin-induced hypersecretion of adrenal corticoids or by inhibiting the secretion of GnRH thorough prolactin receptors on hypothalmic dopaminergic neurons (Albertson et al., 1987; Bartke, 1986). Our results show that resistance training had no effect on prolactin, and that the inclusion of D-Asp and NMD-Asp in the NMDA product apparently had no effect on inhibiting the levels of serum prolactin or cortisol.

Mucuna Pruriens (MP) is a medicinal herb traditionally used to combat emotional stress, aging, and male infertility and is a rich source of L-dihydroxyphenylalanine (L-DOPA), which is a precursor to dopamine. Dopamine is a hypothalamic neurotransmitter that has a profound effect on the release of pituitary hormones (Meites et al., 1977). Dopamine inhibits prolactin release from the anterior pituitary cells by binding to inhibitory D2 dopaminergic receptors (Xu et al., 1996). In support of this are data in humans demonstrating that MP, at an oral dose of 15 g, effectively attenuated chlorpromazine-induced increases in prolactin release (Vaiday et al., 1978). Resistance exercise is a physical stressor which activates the HP axis, thereby elevating the circulating levels of prolactin and cortisol (Stokes et al., 2013). However, in the present study we showed no change in the levels of prolactin and cortisol in response to resistance training or NMDA supplementation, and since prolactin and cortisol release are governed by the HP axis, our results suggests that this product has no effect on the inhibition prolactin and cortisol, as it is so alleged.

Limitations

In view of the results presented herein, our study does possess four 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 second limitation of our study is the sample size. While a sample size of 20 is somewhat small, it is larger than many other studies in the literature employing a very similar experimental design. We did perform a power analysis *a-priori*; therefore, our study should be adequately powered. The third limitation is the issue of bioavailability, as we did not assess the serum levels of any of the product's ingredients. Even though we did assess the levels of D-Asp in our previous study (Willoughby and Leutholtz, 2013), in the present study we did not. The fourth limitation is the COA performed for the NMDA product. Even though an actual COA was performed by an independent laboratory, we did not perform a COA in our own laboratory on the NMDA product to confirm the results of the analysis. Despite confidence in our data, in lieu of these four limitations, our results should be interpreted with some amount of caution.

Conclusion

Based on the outcomes and limitations of the present study, it is clear that more research needs to be conducted on NMDA supplementation in humans regarding its ability to increase endogenous levels of testosterone, along

with its potential ability to increase muscle mass and strength. However, based on the results of the current study we conclude that 28 days of NMDA supplementation, at a daily dose of 1.78 g, does not increase the activity of the HPG axis, nor does it preferentially increase skeletal muscle mass and strength in resistance-trained males when compared to placebo.

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Key points

- In response to 28 days of heavy resistance training and NMDA supplementation, similar increases in muscle mass and strength in both groups occurred; however, the increases were not different between supplement groups.
- The supplementation of NMDA had no preferential effect on augmenting testosterone or decreasing estrogen, cortisol, and prolactin.
- While resistance training was effective in increasing muscle mass and strength, it was not preferentially due to NMDA supplementation.
- At the dose provided, NMDA supplementation for 28 days combined with resistance training does not increases muscle mass and strength due to its ability to elevate endogenous testosterone levels and lower cortisol and prolactin when compared to placebo.

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