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Pharmacokinetic effect of Astragalus *membranaceus* and *Panax notoginseng* saponins on arginine absorption and nitric oxide production in healthy subjects

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ABSTRACT

Background: To the best of our knowledge, there are no clinical trials conducted with Astragalus and ginseng extracts on nitric oxide (NO) levels in humans. Therefore, this study aimed to examine whether the standardized intake of Astragalus *membranaceus and Panax notoginseng saponins* (APS) could increase NO production by enhancing arginine absorption and reducing levels of asymmetric dimethylarginine (AMDA).

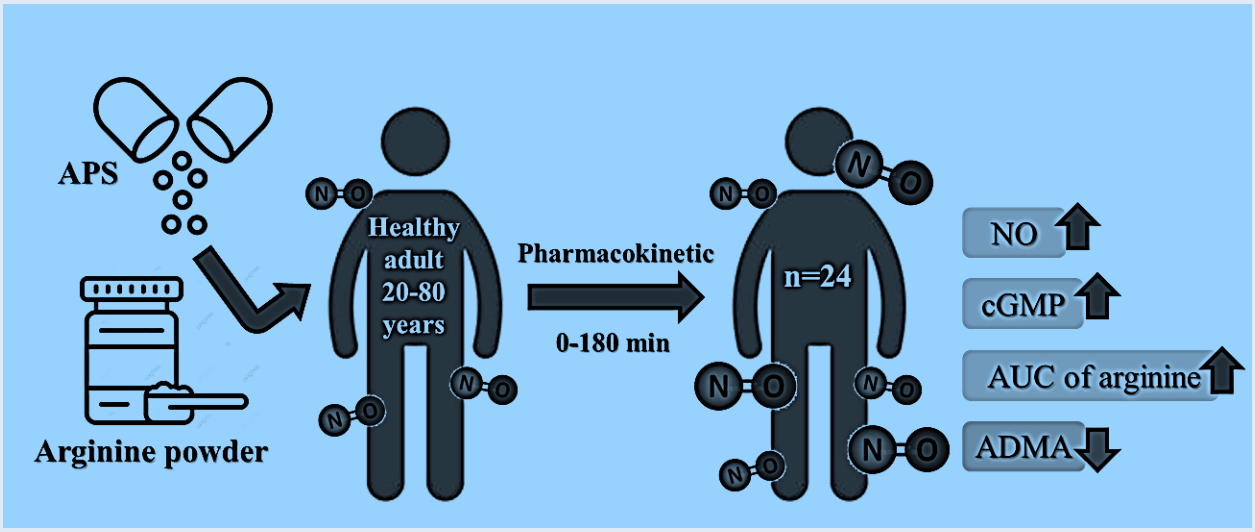
Methods: A clinical trial involving healthy adult participants aged between 20 to 80 years was conducted as a randomized, double-blind, cross-over study. The participants received 5 g of arginine powder and one capsule of APS or placebo twice, with a wash-out period between each administration. Plasma and urine were collected for testing, and 24 subjects were included for analysis after excluding six subjects with great individual differences.

Results: It was found that after APS supplementation, the area under the curve (AUC) of arginine significantly increased by 17.3% (p = 0.041), the maximum concentration (Cmax) increased by 11.1%, and the Arg/ADMA ratio significantly increased by 167.1% (p = 0.007). Moreover, urinary nitrate and cGMP levels increased by 20.8% and 18.9%, respectively.

Conclusions: APS showed increases in arginine absorption, decrease ADMA levels, and enhance NO production. With these findings, the addition of APS to arginine supplements could be advantageous for pre-workout and cardiovascular health.

Keywords: Astragalus *membranaceus* and *Panax notoginseng* saponins (APS), arginine, asymmetric dimethylarginine (ADMA), nitric oxide (NO), cyclic guanosine monophosphate (cGMP)

Clinical trial registration: NCT05024123



Graphical Abstract: Pharmacokinetic effect of Astragalus *membranaceus* and *Panax notoginseng* saponins on arginine absorption and nitric oxide production

INTRODUCTION

Arginine is a conditionally essential amino acid that plays crucial roles in a variety of biological processes. Arginine is converted into citrulline and is essential for NO production [1]. The benefits of NO include endothelial protection, vasodilation, and endogenous anti-atherogenic effects [2], thereby improving endothelial functions [3] and slowing down the onset of atherosclerosis [4]. NO is synthesized in vascular endothelial cells and rapidly oxidized to nitrites and nitrates in the body, which are then excreted through the kidneys [5]. In addition, releasing NO in endothelial cells can lead to an increase in cGMP levels, which plays a regulatory role in vasodilation. Since cGMP is excreted through urine, measuring the levels of urinary nitrates

and cGMP is a suitable and non-invasive method for assessing NO. Asymmetric dimethylarginine (ADMA) is widely recognized as the inhibitor of NO production by competing for the binding with arginine and NO synthase (NOS) [6-7]. Therefore, the ratio of arginine/ADMA (substrate/inhibitor ratio) may predict NO production better than ADMA or arginine concentrations alone [8].

The major components of Astragalus *membranaceus* (astragalus) are astragalosides. It also contains flavonoids, saponins, and alkaloids [9]. More than 40 astragalosides have been identified in astragalus, among which astragaloside IV is the main component [10]. In recent years, extensive investigations have been made on the pharmacological effects of astragalosides, including their impact on immune regulation, anti-oxidation, anti-inflammation, and metabolic syndrome [10, 11]. Studies have found that astragalosides may alleviate oxidative stress and increase the production of NO and cGMP in the myocardium, thereby improving the myocardial diastolic function in rats with metabolic syndrome [12]. Another study found that astragalosides reduced blood pressure and triglyceride levels in rats with fructose-induced metabolic syndrome, while high doses (2 mg/kg) of astragalosides reduced NG-nitro-arginine methyl ester (NAME), which blocked NOS and improved vasodilation by increasing NO and cGMP levels [13]. Lee et al. [14] found that astragalosides promoted the absorption of arginine in Caco-2 cells and mice. The major components of *Panax notoginseng* (ginseng) are ginsenosides. Ginseng is one of the most widely used Chinese herbal medicines with a variety of pharmacological properties. In addition, the active ingredients of ginseng are saponins, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids. More than 30 ginsenosides have been identified, which are divided into 20(S)-protopanaxadiol (PPD) (Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, and Rs1) and 20(S)-protopanaxatriol (PPT) (Re, Rf, Rg1, Rg2, and Rh1) [15]. Ginsenosides have been associated with various biological activities, including preventing cancer, diabetes, and dementia, neuroprotection, anti-aging, anti-inflammation, and antihypertensive effects [16-20]. Ginsenosides promoted NO release in endothelial cells to increase cGMP accumulation in the endothelium, thereby regulating vasodilation. Studies have found that ginsenosides increased NO production by inhibiting arginase activity [21-23].

The absorption of amino acids by Caco-2 cells can be increased by APS, as reported in a study [24]. To further explore the potential benefits of APS, a randomized, double-blind, cross-over trial was conducted to investigate its effect on NO production by promoting arginine absorption and reducing AMDA levels, raising Arg/ADMA ratio in healthy humans. The findings of this study provide valuable insights that could aid in the development of new natural nutritional supplements.

MATERIALS AND METHODS

Samples*:* Standardized APS samples and placebos were provided by NuLiv Science USA Inc (Brea, CA, USA). Each APS capsule contains a 50 mg mixture of equal amounts of Astragalus *membranaceus* (10:1 hydroethanolic extract) and *Panax notoginseng* (50:1 aqueous extract) that is standardized to contain ≥ 1.5% saponins. The

other component of the APS capsule is maltodextrin. The placebo contained maltodextrin, similar in color and appearance, with the same weight as the APS capsule.

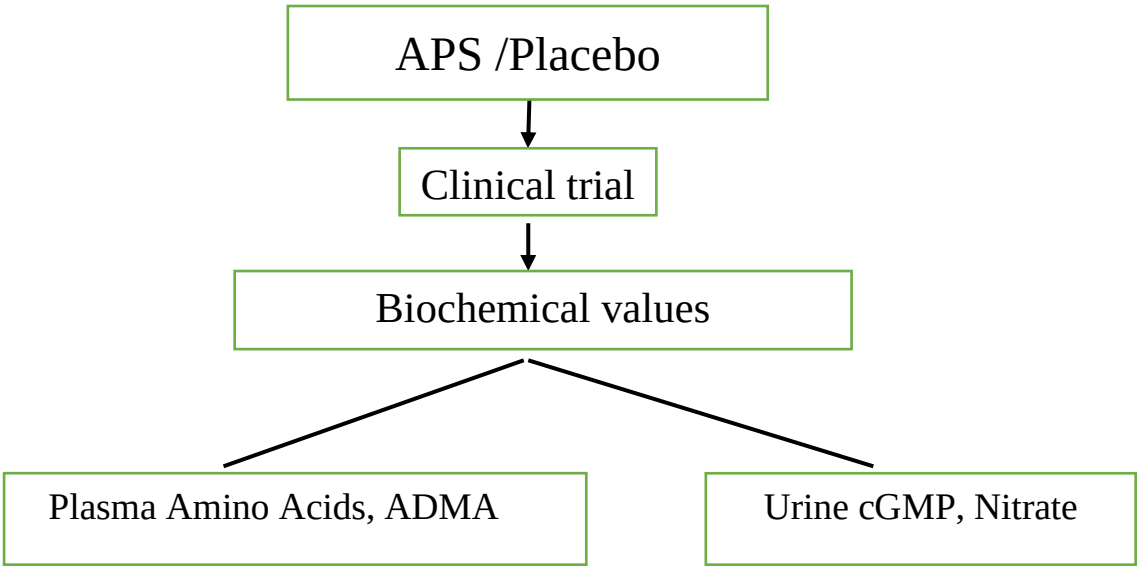


Figure 1. The schematic representation of the present study.

Ethics: This study was conducted at Chung Shan Medical University in Taiwan, and 30 volunteers between the ages of 20 and 80 were recruited openly. This trial was designed as a randomized, double-blind, cross-over study. This study was approved by the institutional review board of Chung Shan Medical University Hospital (Protocol No. CS2-20203) (see Appendix 1) and was registered at *ClinicalTrials.gov* under reference number NCT05024123. Prior to enrollment, the investigator or an assistant provided a clear explanation of the experimental procedures and all related information to the subjects, who were required to sign an informed consent form. Eligible subjects were then randomized into two groups (see Figure 1).

Inclusion criteria: Subjects between the ages of 20 and 80 were included in the study. Prior to each testing day, subjects were instructed to adhere to a diet avoiding foods that are high in nitrates, such as garlic, nuts and seeds, beets, and dark chocolate, as well as processed meats like hot dogs, bacon, and ham.

Exclusion criteria: The exclusion criteria included individuals with chronic diseases (such as cardiovascular, kidney, or liver disease, cancer, uncontrolled diabetes mellitus, or major medical or operation history). Individuals who smoked, consumed alcohol regularly, were pregnant or lactating, were participating in another human trial during the first 30 days of the study, or were taking any health products or drugs that might interfere with the research (such as health foods or amino acid supplements) were excluded from the study. A subject was considered to have reached the clinical endpoint when any of the following conditions were met: completion of the protocol and required follow-up, the occurrence of an adverse event, loss of contact, non-compliance, use of medication, medical contraindication, withdrawal of consent, death, or other reasons.

Withdrawal criteria: Subjects were free to decide whether or not to participate in this study. They may withdraw their consent at any time during the study and

discontinue the trial without any reason and without causing any unpleasantness. In case of any discomfort, the subjects may withdraw from the trial at any time without penalty or damage to their rights and interests.

Anthropometric examination and blood collection: During the study period, subjects underwent two assessments of anthropometric parameters and blood tests. Body mass index (BMI) was measured by a body fat analyzer (Karada Scan 371, OMRON, Japan). Blood samples were collected in the morning from subjects who had fasted for 12 hours. Each blood sample had a volume of 45 mL and was collected using vacuum blood collection tubes. The tubes contained lithium heparin and were centrifuged within 4 hours of collection at 3000 rpm, 4°C for 10 minutes to obtain plasma.

L-arginine absorption analysis: Subjects in the study were instructed to take either an APS or placebo (maltodextrin) capsule at 9 p.m. and then fast for 12 hours, except for consuming a small amount of water the night before the experiment. At 9 a.m. the following morning, the first blood sample was collected from the median cubital vein using the indwelling catheter method. Immediately after the first blood collection, all subjects ingested the APS/placebo capsule along with 5 g of arginine mixed with 250 mL of water [24]. Additional blood samples were collected at 15, 30, 45, 60, 90, 120, 150, and 180 minutes for the purpose of analyzing the plasma concentration of arginine. After a one-week washout period, the test product was replaced with the placebo (or vice versa) to repeat the experiment. The amino acids analysis employed a 96-well plate (MSRLN0450, Millipore) to extract and purify the blood samples, thereby reducing the complexity of the matrix background. This involved deproteinizing the blood sample (typically cold ethanol or acetic acid) and centrifuging to collect the supernatant [25, 26]. An ACQUITY Premier BEH C18 Column with VanGuard FIT (Waters) was used to separate amino acids—the mobile phase blended organic solvents (HPLC grade acetonitrile, methanol, and water, containing acetic acid). Gradient elution was achieved by adjusting the proportion of the organic solvent to separate the amino acids effectively. This procedure was executed on an Agilent 290 Infinity UHPLC. The separated samples were subsequently examined using an API 4000 Triple Quadrupole Mass Spectrometer (AB SCIEX, USA).

Plasma ADMA analysis: Plasma ADMA was analyzed by enzyme-linked immunosorbent assay (ELISA) (Elabscience Biotechnology Inc. USA), as previously described [27].

Urinary Nitrate and cGMP Analysis: The levels of urinary nitrate and cGMP were assessed using ELISA [28]. The Nitrate Kit (Systems, Inc. USA) was used to measure nitrate levels, while the cGMP Kit (Elabscience Biotechnology Inc. USA) was used to measure cGMP levels. The urinary excretion rates of nitrate and cGMP were adjusted for creatinine excretion.

Statistical Methods: The results obtained in this study were analyzed using the Student's t-test and paired t-test in the social science statistical software (SPSS version 20.0 for Windows; SPSS Inc., Chicago). Results with a *p*-value less than 0.05 were considered statistically different.

RESULTS AND DISCUSSIONS

Subject characteristics: A total of 31 subjects were recruited for this study. One subject withdrew during the trial, and 30 subjects completed the study. Six subjects were excluded due to incomplete data or large individual differences, and the data of 24 subjects were ultimately presented. A randomized, double-blind cross-over design

was adopted, with two interventions before and after as well as a one-week wash-out period (Figure 2). The test team reminded the subjects of the precautions via text messages and phone calls.

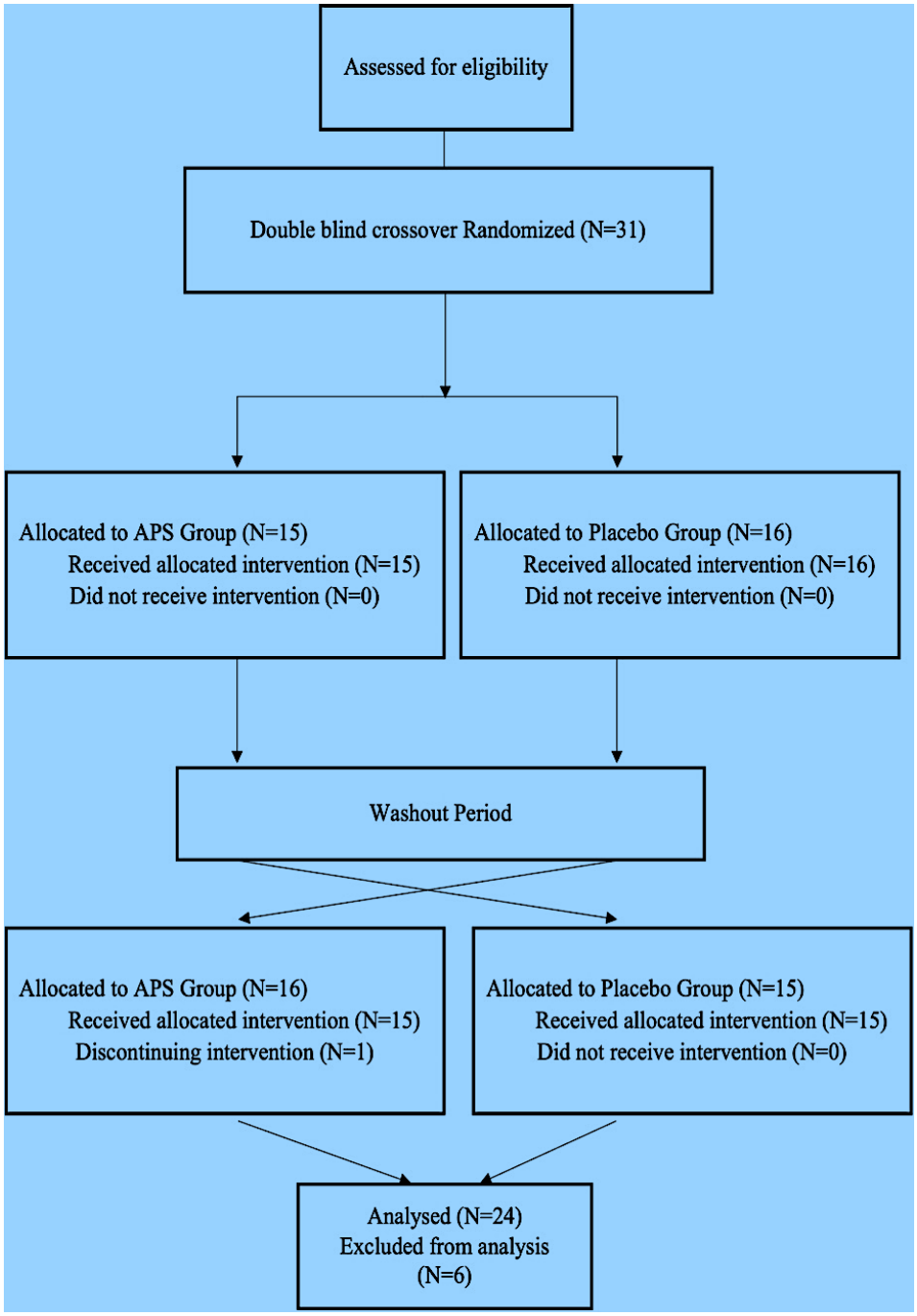


Figure 2. Flow chart of present study.

The characteristics of the 24 subjects who completed the study are shown in Table 1. There were 7 males and 17 females aged 28 to 74 years, with a mean age of 50.50 years, all of which were healthy. The BMI of the subjects ranged from 22.84 to 23.93, which were all within the normal range.

Table 1. Age and BMI of subject characteristics

	Male (7)	Female (17)	Total (24)
Age	47.00±17.64	51.94±14.66	50.50±15.36
BMI	23.24±1.71	23.32±1.81	23.29±1.75

* Data were presented as mean ± standard deviation

Pharmacokinetics of nutrient absorption: The study has shown changes in plasma amino acid levels at various time points (0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes) during the pharmacokinetic analysis of nutrient absorption and calculated the AUC. The liver is responsible for the urea cycle, which converts arginine to ornithine and citrulline. The study observed a significant increase in the AUC of arginine by 17.3% (p = 0.041), citrulline by 20.2%, and ornithine by 4% following APS supplementation, compared to the placebo group. Moreover, the Cmax of arginine, ornithine, and citrulline increased by 11.1%, 8.7%, and 1.1%, respectively. Furthermore, the time to reach the maximum concentration (Tmax) of citrulline and ornithine was found to be earlier by 7.9% and 7.1%, respectively (as shown in Table 2).

The observed increase in the AUC and Cmax of citrulline and ornithine can be attributed to the conversion of arginine into these amino acids via the urea cycle, which was stimulated by APS supplementation. This finding is similar to a previous study by Moinard et al. [29], which also showed increases in arginine, ornithine, and citrulline levels after supplementation with arginine, with no significant changes in the rest of the amino acids (Appendix I). These results suggest that APS supplementation can enhance the absorption and utilization of amino acids [28], particularly in individuals with nutritional requirements.

Table 2. Pharmacokinetics of Arginine, Citrulline, and Ornithine (N=24)

		AUC (µmol*hr/L)	Tmax (min)	Cmax (µmol/L)
Arginine	Placebo	259.30±76.02	60.00±26.54	236.21±41.80
	APS	304.24±91.95	68.75±31.87	262.50±49.78
	p-value	0.041	0.352	0.062
Ornithine	Placebo	241.24±109.96	96.88±38.56	209.92±67.65
	APS	250.82±118.18	90.00±45.11	228.08±70.83
	p-value	0.666	0.332	0.323
Citrulline	Placebo	12.01±8.62	103.13±51.07	42.15±11.38
	APS	14.44±10.57	95.00±49.78	42.63±10.92
	p-value	0.268	0.544	0.720

* Data were expressed as mean ± standard deviation and a *p*-value less than 0.05 was considered statistically different

Changes in ADMA level and Arg/ADMA ratio: ADMA and arginine compete for binding to NOS, which can lead to the inhibition of NO production. The present study observed a 42.5% reduction in ADMA levels following the APS group compared to the placebo group (Table 3). The ratio of arginine to ADMA can be used as an indicator to assess NO production [3]. The study results show that the APS supplementation significantly increased the

Arg/ADMA ratio, which indicates NO production, by 167.0% (p = 0.007) compared to the placebo group (Table 3). The production of NO is an important process in maintaining cardiovascular health, as it helps to regulate blood flow and blood pressure. ADMA, a molecule that competes with arginine to bind to NOS and inhibit NO production, has been shown to be a predictor of cardiovascular disease risk. Therefore, the Arg/ADMA ratio has been proposed as a potential indicator of NO production. This study found that APS supplementation significantly increased the Arg/ADMA ratio by 167.0% and reduced ADMA levels by 42.5%, suggesting an increase in NO production. Based on previous research demonstrating the ability of astragalus and ginseng to reduce ADMA levels [29, 30], it is hypothesized that APS increases NO production by reducing ADMA levels. Moreover, this finding is consistent with previous research on the benefits of arginine supplementation for cardiovascular health [31, 32]. Overall, these findings suggest that APS supplementation may be a promising way to enhance NO production and improve cardiovascular health.

Table 3. Plasma ADMA and Arg/ADMA ratio of subjects (N = 24)

	Placebo	APS	p-value
ADMA	1.53±1.24	0.88±1.02	0.081
Arg /ADMA ratio	77.91±93.40	208.01±239.47	0.007

* Data were expressed as mean ± standard deviation and a *p*-value less than 0.05 was considered statistically different

Urinary nitrate and cGMP contents: The production of NO plays a crucial role in regulating blood pressure and maintaining cardiovascular health. After being synthesized in the vascular endothelial cells, NO is quickly oxidized into nitrites and nitrates in the body, which are then excreted through the kidneys, thus increasing the urinary concentrations of nitrates and cGMP [3]. This study found that APS supplementation increased nitrate and cGMP levels by 20.8% and 18.9%, respectively, compared to the placebo group (Table 4). These findings suggest that APS supplementation increases NO production and may support blood pressure regulation. Interestingly, previous research on ginsenosides also showed a dose-dependent reduction in blood pressure in rats [21]. These findings suggest that natural compounds such as APS and ginsenosides may have potential as alternative treatments for hypertension. Overall, the results of this study provide promising evidence for the potential benefits of APS supplementation in regulating blood pressure and improving cardiovascular health.

Table 4. Urine nitrate and cGMP (N=24)

	Placebo	APS	p-value
Nitrate (µmol)	132.63±76.25	160.28±124.91	0.135
cGMP (pmol)	0.37±0.19	0.44±0.30	0.199

* Data were expressed as mean ± standard deviation and a *p*-value less than 0.05 was considered statistically different

One potential limitation of the study is the need for detailed dietary records. This limitation arises from the possibility of inter-individual differences in adherence to dietary instructions and other uncontrolled dietary variables. Future studies should consider incorporating detailed dietary records to better control and analyze the influences of diet on NO production.

CONCLUSIONS

After supplementation with APS, the study observed that the AUC of arginine increased significantly by 17.3% ($p = 0.041$), and the Cmax increased by 11.1%, compared to the placebo group. Additionally, the Arg/ADMA ratio increased significantly by 167.1% ($p = 0.007$). These findings suggest that APS supplementation could enhance NO production and promote arginine absorption while decreasing ADMA levels. Therefore, APS could be a beneficial addition to supplements with arginine for pre-workout and cardiovascular health.

Conflicts of Interest: All authors declare that there is no conflict of interest with respect to the publication of this research article.

List of Abbreviations: APS: Astragalus membranaceus and Panax notoginseng saponins, ADMA: asymmetric dimethylarginine, NO: nitric oxide, cGMP: cyclic guanosine monophosphate, AUC: the area under the curveCmax: the maximum concentration

Authors' Contribution: Concept and design: Y.C.S. Analysis and interpretation: C.P.L., C,T,L., and I.C.W. Data collection: I.C.W and T.Y.P. Writing the article: Y.C.S. Critical revision of the article: C.T.L. Final approval of the article: all authors. Statistical analysis: C.P.L. Had primary responsibility for final content: Y.C.S.

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Supplementary Materials

Appendix I. Pharmacokinetics of the other 19 amino acids (N=24)

		AUC (μmol*hr/L)	Tmax (min)	Cmax (μmol/L)
Asparagine	Placebo	319.01±420.83	95.63±61.58	68.18±9.30
	APS	220.51±452.12	63.75±62.44	65.01±13.94
	p-value	0.392	0.066	0.265
Glutamine	Placebo	5556.36±6297.48	100.63±61.05	717.33±96.14
	APS	3163.74±3025.60	106.88±57.39	688±46.113.65
	p-value	0.112	0.711	0.257
Serine	Placebo	807.69±978.66	55.63±60.56	153.88±40.51
	APS	601.76±750.14	81.88±70.49	158.12±37.45
	p-value	0.387	0.117	0.591
Threonine	Placebo	321.23±833.34	63.13±63.17	137.60±31.61
	APS	123.99±282.21	35.00±62.35	143.75±30.51
	p-value	0.306	0.111	0.328
Glycine	Placebo	1594.18±3159.54	70.00±64.50	318.63±82.10
	APS	1248.59±1580.23	78.75±49.30	310.21±79.21
	p-value	0.654	0.600	0.389
Alanine	Placebo	895.06±1591.28	58.13±58.73	424.17±100.74
	APS	576.55±1087.60	34.38±46.91	455.54±89.29
	p-value	0.355	0.106	0.085
Hydroproline	Placebo	23.58±47.71	79.38±61.53	13.93±6.52
	APS	22.29±47.05	50.00±63.13	11.85±5.46
	p-value	0.929	0.060	0.149
Proline	Placebo	124.07±218.04	51.00±53.43	171.47±59.29
	APS	221.87±338.23	65.63±59.15	174.29±32.64
	p-value	0.421	0.708	0.200
Valine	Placebo	56.96±153.77	62.61±79.00	257.08±49.13
	APS	142.99±468.64	38.75±66.92	251.42±43.63
	p-value	0.413	0.297	0.501
Isoleucine	Placebo	8.71±24.60	44.38±74.21	65.22±15.15
	APS	18.64±51.62	32.50±60.92	67.71±14.99
	p-value	0.427	0.597	0.449
Leucine	Placebo	101.00±145.16	80.00±85.27	144.00±28.80
	APS	66.60±107.78	63.13±80.96	140.51±25.75
	p-value	0.305	0.546	0.508
Methionine	Placebo	44.13±146.08	34.38±66.12	29.83±4.53
	APS	45.08±103.12	52.50±69.80	29.81±4.32

	p-value	0.981	0.408	0.987
Tyrosine	Placebo	17.96±73.01	8.75±25.38	68.78±14.44
	APS	9.37±35.57	3.13±9.87	72.69±10.27
	p-value	0.619	0.340	0.200
Phenylalanine	Placebo	38.83±87.69	41.25±65.4	73.84±11.07
	APS	15.60±47.14	15.63±37.40	68.06±12.07
	p-value	0.240	0.137	0.074
Asparticacid	Placebo	185.75±213.65	106.25±62.85	10.01±4.68
	APS	182.59±227.37	116.88±56.12	11.35±4.84
	p-value	0.959	0.520	0.223
Glutamate	Placebo	1603.65±1579.64	86.25±51.82	100.01±31.27
	APS	1822.22±1574.46	79.38±48.95	113.03±33.66
	p-value	0.567	0.675	0.093
Histidine	Placebo	28.15±102.12	46.88±74.32	94.70±15.37
	APS	29.26±71.12	49.38±75.65	91.33±14.80
	p-value	0.968	0.917	0.293
Lysine	Placebo	730.08±788.70	86.88±67.52	247.38±51.79
	APS	759.17±1306.08	70.63±71.39	240.92±38.17
	p-value	0.935	0.413	0.625
Tyrptophan	Placebo	147.14±289.64	29.38±43.67	86.74±25.52
	APS	98.11±162.86	26.25±42.48	93.37±17.03
	p-value	0.478	0.833	0.177

* Data were expressed as mean ±standard deviation and a *p*-value less than 0.05 was considered statistically different