2023, Volume 6 (Issue 1): 5 OPEN ACCESS

Inositol-Stabilized Arginine Silicate Reduces Exercise Induced Muscle Damage and Increases Perceived Energy

Original Research

Danielle Greenberg[1], Katie Emerson[1], Sara Perez Ojalvo[1], Sarah Sylla[2], and James Komorowski[2]

[1]*Nutrition21 LLC, Saddle Brook, New Jersey, USA*
[2]*JDS Therapeutics, LLC Harrison, New York, USA*

Open Access

Published: July 31, 2023

Journal of Exercise and Nutrition: *2023, Volume 6 (Issue 1): 5*

ISSN: 2640-2572

Abstract

Introduction: Inositol-stabilized arginine silicate (ASI; Nitrosigine®) significantly increases circulating arginine and nitric oxide (NO). We examined ASI effects on objective and subjective indicators of muscle recovery, blood flow and energy.

Methods: In a double-blind, placebo-controlled crossover-design, subjects (n=16) were given ASI (1,500 mg/day) or placebo for 4 days, with a 7-day washout period. Measurements occurred at baseline, 24, 48, and 72 h. On test days, subjects performed stress inducing leg extension exercises associated with muscle soreness. Following exercise, recovery markers creatine kinase (CK), myoglobin and lactate dehydrogenase (LDH), doppler ultrasound blood flow, leg circumference, salivary nitrite tests were measured. The Profile Mood States (POMS), VAS scales, vigor-activity cognitive tests were administered.

Results: Serum CK but not LDH was significantly reduced in the ASI group on day 1 and 24, 48, and 72 h post-exercise ($p<0.05$); myoglobin was reduced on d1 and at 24 h post-exercise. No negative heart rate or blood pressure effects were observed. Reactive hyperemia indicated by leg circumference showed greater increases in the ASI group at 72 h ($p<0.05$). No differences were found in salivary nitrite levels ($p=0.265$). Perceived energy POMS responses increased in the ASI group compared to placebo ($p=0.039$) but no differences were found in subjective muscle recovery as determined by VASs.

Conclusions: ASI may be beneficial for fitness goals by increasing blood flow, and reducing muscle damage and perceived energy.

.

Key Words: Nitric Oxide, hyperemia, myoglobin, salivary nitrite

Corresponding author: Danielle Greenberg, Ph.D, dg403@caa.columbia.edu

Introduction

Inositol-stabilized arginine silicate (ASI; Nitrosigine®) provides a highly bioavailable form of arginine[1,2]. Arginine is well known to increase nitric oxide (NO) production, which leads to vasodilation and increased blood flow[3-5]. While not universally agreed upon, silicon especially as silicic acid, may protect vascular integrity in vascular diseases[6,7] and is thought to be actively involved, and perhaps integral to bone mineralization and prevention of osteoporosis, as well as in collagen synthesis[8]. Previous research demonstrated that a single administration of ASI (1.5 g) increased blood levels of arginine for up to 6 hours and of silicon for up to 1.5 hours[9] such administration also led to rapidly increasing circulating arginine levels within 30 minutes[9,10]. ASI significantly increased salivary levels of nitrite, which is known to be converted into NO and is therefore a marker of NO production[9-11]. An *in vitro* study using the Greiss Method

compared ASI to other compounds known to raise NO levels and increase blood flow, ASI increased NO production significantly more than L-Arginine, Arginine *arginine-alpha-ketoglutarate (*AKG), L-Citrulline, L-Citrulline Malate, or Agmatine sulfate, ($p<0.01$)[12].

A single administration of ASI also increased plasma arginine by >70% compared to arginine hydrochloride (ArgHCl) and maintained this elevation for a significantly longer duration, indicating that ASI is likely to be a more bioavailable form of arginine as compared to ArgHCl[9]. This study also demonstrated that compared to ArgHCl, ASI supplementation inhibited levels of arginase, an enzyme that catabolizes arginine[9,13,14]. That ASI could inhibit arginase was confirmed in a study where 15 days of ASI supplementation led to significant reductions in arginase at multiple time points compared to when ArgHCl was given ($p< 0.05$). Thus arginase levels were reduced by ASI, while no changes in arginase was detected after ArgHCl supplementation[15]. The effects of ASI on arginase suggest a possible mechanism of action by which ASI enhances arginine bioavailability, and in turn, increases NO production[9,15].

Preclinical studies have also shown that ASI increased blood vessel relaxation, significantly more than does ArgHCl thereby showing more benefits for increased blood flow[16]. This preferential increase in blood flow has also been demonstrated in humans. Results of a double-blind, placebo-controlled trial demonstrated that 1.5g of ASI increased endothelial-dependent vasodilation as measured by a change in flow-mediated dilation compared to placebo and to the same extent as 8g of citrulline malate[17]. Arginine is a sometimes called a semi-essential or conditionally essential amino acid as endogenous arginine synthesis is inhibited under catabolic stress or with dysfunction of the small intestine or kidney[18,19]. Arginine strongly influences numerous metabolic pathways. It plays a pivotal role in the synthesis of biological compounds including creatine, citrulline, agmatine, and nitric oxide (NO). Although the arginine metabolic pathway has a variety of biological roles, one of its most important roles is in the cardiovascular system[5]. Because NO facilitates the dilation of blood vessels, decreases vascular resistance, and modulates smooth muscle cell proliferation, NO production is crucial for vasodilation and cardiovascular health[20,21]. Impaired endothelial NO production has been implicated in vascular dysfunction and diseases. For example, a reduction in NO bioavailability is a marker of the vascular dysfunction associated with atherosclerosis and coronary heart disease[21]. A meta-analysis of 11 double-blind, placebo-controlled trials showed that compared to placebo, arginine intervention ranging from 4 to 24 g/d significantly lowered systolic blood pressure by 5.39 mm Hg ($p=0.001$) and diastolic blood pressure by 2.66 mm Hg ($p<0.001$)[22]. These findings are supported by numerous animal and human studies which have found that acute and long-term administration of arginine improves blood flow, blood pressure, and cardiovascular health[14,23,24].

Considering arginine's vital role in facilitating vasodilation and blood flow, arginine supplementation could benefit athletes undergoing intense training by enhancing blood flow and resulting delivery of oxygen and other nutrients to muscle tissues. Moreover, increased blood flow could result in greater removal of metabolic waste products from muscles, thereby resulting in enhanced muscle recovery and reduced muscle soreness. While there have been numerous studies examining the effects of arginine on athletic performance, there are mixed results regarding arginine's role in muscle recovery. Yavuz et al.[25] found that after male wrestlers took a single administration of arginine, time to exhaustion increased significantly during anaerobic exercise compared to placebo ($p<0.05$). Other studies focusing on acute arginine supplementation and performance have shown that arginine positively affects power output and exercise tolerance[26,27]. On the other hand, Liu et al.[28] found that 6 g of arginine supplementation over 3 days did not significantly enhance power output.

ASI is formulated as a unique complex that binds arginine with silicon, a nutrient that appears to enhance the bioavailability of L-arginine and may support the strength and flexibility of arterial walls on its own [6,10]. In addition, it may be that the stabilization with inositol along with arginase inhibition underlies the greater bioavailability of ASI noted above. Considering ASI's unique structure and positive effects on NO production, blood flow and arginine and silicon absorption, we hypothesized that intake of this complex could improve muscle recovery. Therefore, the purpose of this study was to determine if ASI supplementation, in combination with exercise, can increase blood flow to working muscles and the body overall, and in turn, lead to greater muscle growth and/or recovery. Since we expected that there would also be increased blood flow to the brain in response to increased arginine,[29] we also hypothesized that perceived energy levels could increase with ASI administration.

Scientific Methods

Participants

The study was conducted in male subjects (N=16), aged 18 to 35, BMI between 19 to <30 kg/m2. Subjects were briefed as to the study protocol and the aims of the study and were required to read and sign an Institutional Review

Board-approved informed consent form prior to their participation in the study. The study was approved by the Aspire Institutional Review Board (Santee, CA). Subjects were non-smokers and exercised less than 150 minutes per week. The 150 minutes per week of exercise upper limit was chosen in order to be able to reveal any effects of ASI. We believed that if subjects were trained above this limit there could have been a ceiling effect. All subjects were required to be willing and able to comply with the protocol. Subjects agreed to not use any dietary/ herbal supplements or similar products until after study completion nor take any anti-inflammatory medications or any pain relievers for 24 hours prior to visits or use ice or compression socks between visits. Subjects were in overall good health and appropriate condition for exercise as determined by physical examination, medical history, and electrocardiogram (ECG). In addition, to assure study eligibility blood pressure was measured twice at the screening visit, with allowed rest in between. If blood pressure was ≥ 140/90 mmHg (that is, if the systolic BP was ≥ 140 or the diastolic BP was ≥ 90), the subject was excluded from participation in the study. At the screening visit prior to enrollment eligibility was also determined by standard blood tests including a comprehensive metabolic panel (glucose, BUN, Cr, AST, ALT, AP) and complete blood count (CBC) with differential (WBC, RBC, Hgb, Hct) under fasting conditions. Subjects with blood test results deemed unacceptable for participation by a medical investigator were not enrolled. Subjects were excluded if they had any of the following medical conditions: active heart disease, uncontrolled high blood pressure (≥140/90 mmHg), renal or hepatic impairment/disease, type I or II diabetes, bipolar disorder, active psychiatric disease (hospitalization within the past 12 months), Parkinson's disease, thyroid disease, immune disorder (such as HIV/AIDs), untreated/active periodontal disease or any active oral infection, or any other serious medical conditions.

Protocol

This study utilized a randomized, double-blind, placebo-controlled, crossover design to determine the effects of ASI (1,500 mg/day) or placebo. This amount of ASI has been shown to lead to improved blood flow and improved cognition in clinical trials[17,30,31]. We examined: markers of muscle damage for recovery, doppler ultrasound to assess blood flow, leg circumference for hyperemia, and salivary nitrite tests for NO assessment as objective markers. VAS scales were used to determine muscle recovery, and the Profile of Mood States (POMS) vigor-activity cognitive tests were used to assess perceived energy as subjective markers. The study consisted of a screening visit and 8 follow-up visits. Eligibility was determined at the screening visit. Screening procedures included determining height and weight, reviewing medical history, current medications, checking vital signs, and preforming an EEG and a comprehensive metabolic panel. At screening subjects also were tested to determine baseline strength. Subjects performed a one repetition maximum (1-RM) for leg extensions. Eligible subjects received either ASI or placebo in a randomized sequence and subsequently the other substance separated by a washout period of seven days. For each substance, the test period consisted of four visits (Day 1 = hour 0, Day 2 = hour 24, Day 3 = hour 48, and Day 4 = hour 72). Subjects were instructed not to exercise 24 hours prior to visits and to fast after midnight the night prior to visits. At the Day 1 visit, subjects were given either ASI or placebo and then performed an exercise protocol consisting of leg extensions that used a plate-loaded leg extension machine (Key Fitness Products, LP; Garland, TX) or comparable exercise equipment, to induce muscle damage. After the exercise subjects then rated their subjective discomfort by visual analog scales (VAS) and were administered the following: cognitive testing, measurements of blood flow with Doppler ultrasound, salivary nitrite testing, leg circumference measurements, POMS vigor-activity subscore questionnaire, vital-signs monitoring, and blood collections. For Day 2, 3, and 4 visits, subjects were provided with ASI (1,500 mg) or placebo, and performed the leg extension protocol as described. Subjects then rated discomfort by VAS, after which the following procedures were carried out: salivary nitrite testing, vital-signs monitoring, and blood collections. At the Day 4 visit, subjects were administered ASI (1,500 mg) or placebo and then repeated the leg extension protocol to induce muscle damage. All procedures given on Day 1 were then repeated.

Dietary and physical activity controls

Subjects were instructed to continue consuming their normal diets throughout the study. Subjects were required to fast (no food or beverage other than water, no caffeine and no alcohol) after midnight prior to all visits. A dichotomous questionnaire was administered at all study visits to determine if subjects met the agreed to criteria. Subjects were also instructed to refrain from intentional exercise 24 hours prior to all visits. At the screening visit, subjects were provided a nutrition bar (containing 150 to 250 calories) and water at least 15 minutes prior to the physical testing. During each study visit, subjects were provided a standardized breakfast at least 15 minutes prior to receiving either ASI (1,500 mg) or placebo. The standardized breakfast included the following: 1 bagel, 1 tablespoon cream cheese and 8 ounces of orange juice (foods not high in arginine). Subjects were allowed to drink water ad libitum at the visits (other than for the 30 minutes prior to the salivary nitrite tests). Other than pre-visit requirements, subjects were instructed to continue their usual food intake and exercise routines throughout the study.

Supplementation Procedures

After informed consent, study subjects were randomly assigned to receive either ASI (1,500 mg) or placebo in one of the following two orders; ASI followed by placebo or placebo followed by ASI. The ASI product contained 1,500 mg of an inositol-stabilized arginine silicate complex (arginine, silicon from potassium silicate, and inositol) along with the inactive ingredients: citric acid, maltodextrin, natural flavor, sucralose, acesulfame potassium and FD&C Red 40. The placebo product contained only the inactive ingredients. Both ASI and placebo were in powdered form and dissolved in 8 oz of water immediately prior to oral administration. Both study products were visually matched for appearance and color. Subjects were provided with a single administration of study product at each visit. The dissolved ASI or placebo was provided prior to exercise and subjects were required to drink the entire 8 oz of liquid.

Exercise Procedures to elicit Muscle fatigue and Delayed Onset Muscle Soreness (DOMS)

To determine baseline strength, the screening visit began with subjects performing a one repetition maximum (1-RM) leg extension. To determine the 1-RM, lifting weight was steadily increased in 10% to 20% increments, with 2 to 4-minute rest periods in between, until subjects could only perform one repetition. Using the individual subject's estimated 1-RM, the 30%, 45%, 60% and 70% 1-RM were determined. After product administration on Day 1, subjects completed a leg extension protocol to induce muscle damage and soreness. Subjects performed 10 sets of 10 reps at 30%, 45% and 60% estimated 1-RM. Subjects rested 3 minutes between the first two sets and 15 to 20 minutes after the third set to allow for recovery. Subjects were permitted to rehydrate as needed with water. The total number of repetitions that subjects could perform until failure at 70% 1-RM was recorded. Subjects then rested 3 minutes and this procedure was repeated two more times. The total number of repetitions from these three sets were combined for a single score. During the sets to failure, subjects were encouraged by a trained professional to maximize the number of repetitions completed to maximize muscle damage and soreness.

Leg Extension Protocol for Exercise Induced Muscle Damage

After administration of ASI or placebo on Day 4, subjects completed a leg extension protocol to assess muscle recovery. Subjects completed 3 sets of 10 repetitions at 30%, 45% and 60% 1-RM. Subjects rested 3 minutes between the first two sets and 20 minutes after the third set to allow for recovery. Water was permitted as needed. The total number of repetitions that subjects could perform until failure at 70% 1-RM was determined. Subjects then rested 3 minutes and this procedure was repeated two more times. On Day 4 during the sets to failure, subjects were not encouraged by the trained professional to maximize the number of repetitions completed. Subjects were however instructed prior to beginning the protocol to exert their maximal effort. The total number of repetitions from these three sets were combined for a single score. To measure total workload, the following calculation was used:

Total Workload = Total # of Reps (from Sets 1, 2 and 3) X by 70% of 1-RM Weight.

Subjective Recovery Assessments

VAS for discomfort, was used to assess the discomfort caused by the DOMS inducing exercise protocol[32]. VAS responses were recorded on Day 1 pre-supplementation/exercise and post-supplementation/exercise, and on Day 2, Day 3, and Day 4 immediately post exercise. Subjects rated their feelings of discomfort on a scale of 1 to 10 with anchors of 1 being "No discomfort at all" and 10 being "Extreme discomfort." The Profile of Mood States 2nd ed short version (POMS) scores were used to assess perceived energy and fatigue[33]. The VAS scales and POMS were administered pre-supplementation and approximately 10 minutes post-supplementation (pre-exercise) on the Day 1 and Day 4 visits. We used the vigor-activity and fatigue-inertia subsets of POMS as efficacy endpoints. Subjects were asked to use a 5-point Likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely), to review a list of subjective feelings and rank the degree to which they were experiencing each feeling. For the vigor-activity subset, the higher the score, the greater the vigor-activity. For the fatigue-inertia subset, the lower the score, the lesser the fatigue-inertia[33].

Blood Flow Measurements

A Doppler ultrasound was used to measure femoral artery velocity (cm/s) on Day 1 (pre-supplementation/exercise) and Day 4 (post-supplementation/exercise). Higher velocity measurements indicated greater blood flow[34]. The Doppler ultrasound was also used to measure femoral artery diameter axial size (mm) on Day 1 (pre-supplementation/exercise) and Day 4 (post-supplementation/exercise). Larger measurements indicated greater blood vessel dilatation. Ultrasound images of the common femoral artery were obtained in sagittal and axial planes. Through Pulsed Doppler and Color Flow Imaging, a sample volume of red blood cell direction and velocity was obtained. This information also included antero-posterior (AP) and lateral measurements for diameter on all axial images. Blood flow

velocities and vessel diameters were recorded. All measurements were made at the same anatomical level to improve accuracy and sensitivity.

Biomarker Laboratory Tests

Serum creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin were measured as biomarkers of muscle recovery[26,27]. Increasing levels of these biomarkers indicated greater muscle breakdown/damage. Duplicate samples of blood were collected into tubes. Blood samples were taken to measure CK (U/L), lactate dehydrogenase (U/L), and myoglobin (ng/mL) levels two times on Day 1 (pre-supplementation/exercise and post-supplementation/exercise), and one time on the Day 2, Day 3, and Day 4 (post-supplementation/exercise). In addition, various protein concentrations were measured using a proteomics assay on Day 1 (pre-supplementation/exercise) and Day 4 (post-supplementation/exercise). Samples were sent to a certified medical laboratory (Quest Diagnostics, Wallingford, CT) to perform the analyses. Proteomics results are not reported in this publication and can be found in the *FASEB, 2015 Komorowski, J.* publication titled "Arginine Silicate Supplementation Decreases Markers of Cardiovascular, Renal and Metabolic Dysfunction and Increases Markers of Vasodilation and Cardiovascular Health in Healthy Adult Males".

Safety Measurements

As markers for safety endpoints, blood pressure and heart rate measurements were taken during each test visit. Systolic blood pressure (mmHg), diastolic blood pressure (mmHg), and heart rate (beats/min) measurements were taken at screening, two times on Day 1 (pre-supplementation/exercise and post-supplementation/pre-exercise), and one time on Day 2, Day 3, and Day 4 (pre-supplementation /exercise).

Statistical Analysis

Allowing for 20% attrition, the enrollment of 16 subjects provided a 80% power to obtain nominal significance ($p \leq 0.05$) when testing for a change over time within ASI or placebo if the mean change is at least 85% as large as the within-product standard deviation of the paired changes. When testing for a difference in the changes between ASI or placebo if the mean difference is 85% as large as the standard deviation of the paired differences between the two substances. For continuous variables, mean changes from baseline within and between groups were tested for nominal significance using the paired Student t-test, or by the non-parametric Wilcoxon signed-rank test if non-normally distributed. For categorical variables, differences between product groups were tested for nominal significance by the Fisher Ex-act test if possible or Chi-Square test if necessary. Statistical significance was set at $p \leq 0.05$. Excel 2010 (Microsoft Corp, Redmond, WA), was used for data entry, validation and restructuring. All data was analyzed using SPSS v.19 (IBM, Armonk, NY) including the generation of the descriptive tables, charts and statistical output.

Results

Compliance and adverse events:

Sixteen subjects completed the study. One subject who was initially enrolled failed to complete the study and data from this subject was not included in the analyses. In the ASI group, two events occurred that were rated by the investigators as unlikely related to the study product based on the reported timing of the events. One subject reported dizziness and another reported tunnel vision; however, both events were reported as mild in severity. There was an additional mild in severity event that may have been related to the study product where one subject reported exacerbation of headaches in a time frame that could have been related to the product.

In the placebo group, one subject reported nasal congestion and another reported upper respiratory infection. However, it was determined that neither event was related to participation in the study. There were no serious adverse events (SAEs) observed in either group during this study.

Safety variables:

Heart rate and blood pressure were utilized as markers for safety and were measured at screening and pre-supplementation and approximately 25 minutes post-supplementation on Day 1, and pre-supplementation on Day 2, Day 3, and Day 4[35]. Blood pressure levels and heart rates were in the healthy range for this age group. There were no significant differences in heart rate (beats/min) or blood pressure (diastolic or systolic mmHg) between ASI and placebo groups at any measured time point (Table 1).

Table 1. Markers for safety: Heart Rate (beats/min) Systolic BP (mmHg) and Diastolic BP (mmHg).

Measure	Time	ASI	Placebo	*p* Value (ASI vs Placebo
Heart Rate	Hour 0: Baseline (BL) Pre-Dose	63.2 ± 8.2 63 (51 – 85)	64.9 ± 12.7 62 (50 – 87)	*p*=0.655
	Hour 0: 25 Minutes Post-Dose	62.2 ± 9.7 61 (50 – 78)	63.4 ± 10.6 62 (45 – 88)	*p*=0.724
	Hour 24: Pre-Dose	68.8 ± 9.9 69 (52 – 84)	67 ± 12.9 63.5 (49 – 89)	*p*=0.650
	Hour 48: Pre-Dose	67.3 ± 10.6 66 (48 – 87)	67.2 ± 9.8 67 (50 – 81)	*p*=0.976
	Hour 72: Pre-Dose	64.6 ± 10.6 62 (50 – 83)	65.7 ± 8.2 64 (51 – 83)	*p*=0.754
	Change from BL to 25 Minutes Post-Dose (Hour 0)	-1.1 ± 5.4 -1 (-9 – 9) *p* = 0.428	-1.5 ± 7.9 -2 (-12 – 10) *p* = 0.461	*p*=0.852
	Change from BL to Hour 24 Pre-Dose	5.6 ± 6.4 5 (-5 – 21) *p* = 0.002*	2.1 ± 9 1.5 (-12 – 22) *p* = 0.362	*p*=0.203
	Change from BL to Hour 48 Pre-Dose	4.1 ± 8.9 4 (-11 – 27) *p* = 0.075	2.3 ± 6.2 4 (-8 – 14) *p* = 0.158	*p*=0.508
	Change from BL to Hour 72 Pre-Dose	1.4 ± 8.6 -2 (-9 – 16) *p*= 0.516	0.8 ± 8.3 -0.5 (-14 – 17) *p* = 0.702	*p*=0.836
Systolic BP	Hour 0: Baseline (BL) Pre-Dose	118.9 ± 8.7 117 (109 – 138)	116.5 ± 8.4 117 (103 – 132)	*p*=0.419
	Hour 0: 25 Minutes Post-Dose	122.4 ± 7.1 123 (107 – 135)	121.9 ± 10.8 121 (102 – 145)	*p*=0.881
	Hour 24: Pre-Dose	117.4 ± 9.2 116 (100 – 136)	116.8 ± 9.4 115 (105 – 135)	*p*=0.868
	Hour 48: Pre-Dose	115.8 ± 7.8 114 (106 – 132)	114.9 ± 6.8 114.5 (104 – 125)	*p*=0.711
	Hour 72: Pre-Dose	115.9 ± 8.3 116.5 (98 – 130)	116.3 ± 6.5 118 (106 – 126)	*p*=0.869
	Change from BL to 25 Minutes Post-Dose	3.4 ± 7.9 2 (-9 – 19) *p* = 0.093	5.4 ± 13.4 4 (-14 – 35) *p* = 0.130	*p*=0.616
	Change from BL to Hour 24 Pre-Dose	-1.6 ± 8 0 (-17 – 11) *p* = 0.423	0.3 ± 12.2 -2 (-13 – 28) *p* = 0.920	*p*=0.597
	Change from BL to Hour 48 Pre-Dose	-3.1 ± 10.5 -1 (-31 – 8) *p* = 0.241	-1.6 ± 9.6 -3.5 (-15 – 19) *p* = 0.509	*p*=0.674
	Change from BL to Hour 72 Pre-Dose	-3.5 ± 11 -1.5 (-20 – 13) *p* = 0.222	-0.2 ± 10.3 -2.5 (-12 – 23) *p* = 0.943	*p*=0.385
Diastolic BP	Hour 0: Baseline (BL) Pre-Dose	77.2 ± 6.2 76 (69 – 93)	75.8 ± 6.1 *(16)* 75.5 (64 – 89)	*p*=0.492
	Hour 0: 25 Minutes Post-Dose	77.2 ± 6 78 (66 – 86)	76.3 ± 8.1 *(16)* 75.5 (65 – 93)	*p*=0.710
	Hour 24: Pre-Dose	74.9 ± 7.1 75 (62 – 86)	75.6 ± 5.9 76.5 (65 – 87)	*p*=0.768
	Hour 48: Pre-Dose	74.9 ± 5.2 75 (69 – 84)	74.9 ± 6.1 74 (65 – 86)	*p*=0.978
	Hour 72: Pre-Dose	74.8 ± 6.6 76 (62 – 85)	72.1 ± 5.5 71.5 (59 – 80)	*p*=0.221
	Change from BL to 25 Minutes Post-Dose (Hour 0)	0 ± 5.7 1 (-12 – 10) *p* = 1.000	0.6 ± 8.4 0.5 (-14 – 17) *p* = 0.792	*p*=0.822
	Change from BL to Hour 24 Pre-Dose	-2.3 ± 6.3 -2 (-15 – 8) *p* = 0.150	-0.1 ± 8.4 1 (-11 – 17) *p* = 0.953	*p*=0.404
	Change from BL to Hour 48 Pre-Dose	-2.4 ± 7 0 (-18 – 6) *p* = 0.186	-0.8 ± 8 -3.5 (-12 – 15) *p* = 0.691	*p*=0.561
	Change from BL to Hour 72 Pre-Dose	-2.6 ± 6.7 -3.5 (-11 – 9) *p* = 0.146	-3.6 ± 7.7 -5 (-17 – 9) *p* = 0.080	*p*=0.680

Biomarkers of Muscle Recovery

Biomarkers of muscle recovery indicated that ASI reduced muscle damage and break-down (Table 2). Biomarkers of muscle damage, CK and LDH, were measured pre-supplementation/exercise and post-supplementation/exercise on Day 1 and post-exercise on Day 2, Day 3, and Day 4. Higher values indicated greater muscle damage and breakdown. CK levels were significantly lower in the ASI group on Day 2 (192.2 ± 106.4; p=0.040), Day 3 (170.3 ± 66.5; p=0.017) and Day 4 (193.5 ± 131; p=0.034) post-exercise compared to the placebo group Day 2 (389.8 ± 409.8); Day 3 (354.7 ± 311.9); Day 4 (448. 7 ± 505.5); indicated reduced damage and greater recovery. Immediately post-exercise on Day 1, ASI supplementation led to 44% less muscle damage, measured by CK levels, than placebo (p=0.057). There was no significant difference in the baseline values between the products (p=0.094), with a greater mean CK value for placebo when compared to ASI. Serum levels of myoglobin, a biomarker of muscle damage, were also measured pre-supplementation/exercise and post-supplementation/exercise on Day 1 and post-exercise on Day 2, Day 3, and Day 4. Higher values indicated greater muscle break-down and damage. From pre-supplementation/exercise to post-supplementation/exercise on Day 1, there was a significant decrease in serum myoglobin levels in the ASI group (p=0.015), while there was no change in the placebo group (p=0.348). The difference between groups at this timepoint approached significance (p=0.089). From pre-supplementation/exercise on Day 1 to post-supplementation/exercise on Day 2 there was a decrease in serum myoglobin levels in the ASI group approaching significance (p=0.083), while there was no change in the placebo group (p=0.302). There was a significant difference in serum myoglobin levels (ng/mL) between groups on Day 2 post-exercise, with levels being 59% higher in the placebo group compared to the ASI group (p=0.042). Serum myoglobin levels were 81% higher in the placebo group compared to the ASI group 48 hours post-exercise (p=0.077). There was a significant increase in LDH levels in the placebo group (+10.9 U/L; p=0.015) Day 1 (pre-supplementation /exercise to post-supplementation/exercise), an effect that was not seen in the ASI group. There were no significant differences in LDH levels for any of the other timepoints for either group.

Salivary Nitrite and Subjective Muscle Recovery

Salivary nitrite scores as an indicator of NO synthesis trended near significance for the ASI group on pre-supplementation /exercise and post-supplementation/exercise on Day 1 (p=0.058) compared to the placebo group. The placebo group showed no change from Day 1 to Day 4 (p=0.265). Subjective muscle recovery was measured by VAS-discomfort rating, with higher scores indicating more discomfort. There were no significant differences between groups at any timepoint. No differences were found in exercise capacity after the 4 day regime (data not shown).

Hyperemia and Blood Flow

Hyperemia, measured using leg circumference (cm), was measured pre-supplementation/exercise and post-supplementation/exercise on Day 1 and Day 4. Leg circumference increased in the ASI group by 1 cm (p=0.006) from pre-supplementation/exercise to post-supplementation/exercise on Day 1 and by 1.8 cm (p=0.001) from pre-supplementation/exercise on Day 1 to post-supplementation/exercise on Day 4. There was a non-significant increase in leg circumference in the placebo group of 0.8 cm (p=0.091) on Day 4. On Day 4 increases in leg circumference was even more pronounced in the ASI group compared to the placebo group, with a trend towards significance (p=0.070), suggesting that sustained or daily ASI supplementation continues to enhance hyperemia over time (Figure 1). Femoral artery velocity (a measurement of blood flow) and femoral artery diameter axial size (a measurement of blood vessel dilation) were measured on Day 1 (pre-supplementation/exercise) and Day 4 (post-supplementation/exercise). Femoral artery velocity tended towards an increase to a greater extent in the ASI group compared to placebo, 59.9 cm/s and 49.9 cm/s respectively, after 4 days of treatment. However, these differences were not statistically significant nor were differences observed between products at any measured timepoint. There were no differences in femoral artery diameter axial size observed between products. From baseline (pre-supplementation/exercise) to Day 4 (post-supplementation/exercise), there was a trend for a significant increase with ASI (p=0.090) while there was an antithetical significant decrease with placebo (p=0.042) (data not shown).

Table 2. Serum levels of markers of muscle damage: CK (U/L), LDH (U/L), and Myoglobin (ng/mL) by time point and by product. Higher levels suggest greater muscle damage.

	Creatine Kinase (U/L)		Lactate dehydrogenase (U/LA)		Myoglobin (ng/mL)	
	ASI	**Placebo**	**ASI**	**Placebo**	**ASI**	**Placebo**
Day 1: Pre-supplementation and exercise	157.06 ± 71.4	388.9 ± 668.2	159.1 ± 25.7	161.1 ± 29.2	28.4 ± 8.6	47.0 ± 63.3
Day 1: Post-supplementation and exercise	168.4 ± 76.6**	414.7 ± 695.6**	163.2 ± 25.7	171.9 ± 30.5**	35.4 ± 12.1**	51.3 ± 51.6
Day 2: Post-supplementation and exercise	192.2 ± 106.4*	389.8 ± 409.8	158.4 ± 30.7	162.4 ± 28.9	28.8 ± 6.9*	45.7 ± 37.2
Day 3: Post-supplementation and exercise	170.3 ± 66.5*	354.7 ± 311.9	154.4 ± 31	155.6 ± 25.1	40.1 ± 26.8	72.7 ± 83.2
Day 4: Post-supplementation and exercise	193.5 ± 131.0*	448.7 ± 505.5	151.9 ± 25	158.2 ± 37.2	46.9 ± 51.5	66.9 ± 74.6

Data are Means ± SD
*p<0.05 ASI compared to Placebo.
**p<0.05 ASI or Placebo compared to baseline

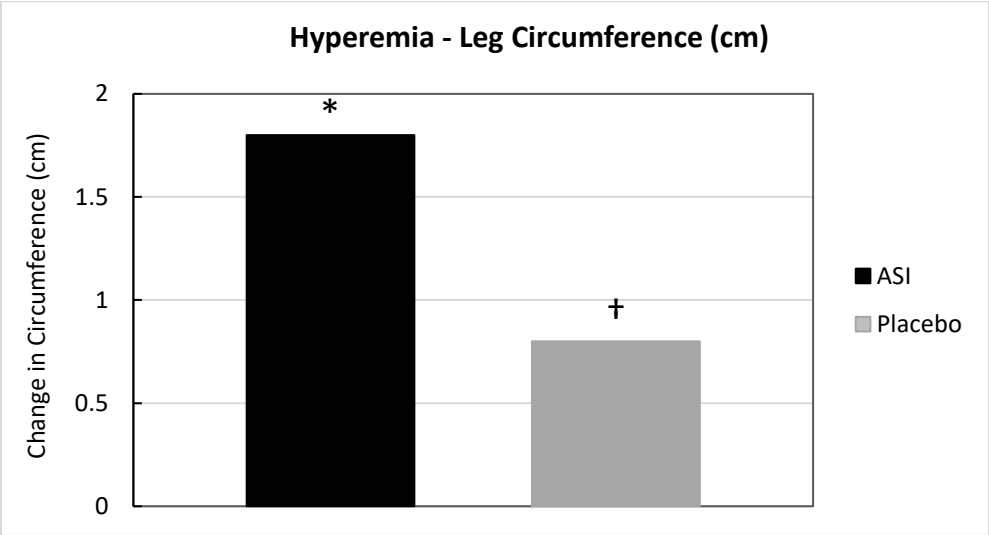

Figure 1. Change in hyperemia, measured by leg circumference (cm), from pre- to post-supplementation of ASI and placebo. ASI significantly increased leg circumference compared to baseline (*p=0.001) and compared to placebo was trending (p=0.070). Placebo had a non-significant change from baseline (†*p*=0.091).

Perceived Energy and Fatigue-Inertia

Perceived energy, measured by POMS vigor-activity subscores, was evaluated pre-supplementation and post-supplementation (prior to exercise) on Day 1 and Day 4. On Day 1, approximately 15 minutes after administration, perceived energy scores increased by 3% in the ASI group (+0.6), while scores decreased by 4% in the placebo group (-0.8; *p*=0.080). From pre-supplementation on Day 1 to pre-supplementation on Day 4, ASI perceived energy scores increased significantly more than placebo (*p*=0.012). Perceived energy scores increased by 8% in the ASI group (+1.5) and decreased by 8% (-1.8) in the placebo group (Figure 2). Fatigue-Inertia, measured by POMS fatigue-inertia sub-scores, was also evaluated pre-supplementation and post-supplementation (prior to exercise) on Day 1 and Day 4. On Day 1, scores in the ASI group stayed constant, while scores in the placebo group decreased (*p*=0.052). On Day 4, scores in the ASI group significantly decreased (*p*=0.041), while there was no change in scores in the placebo group (*p*=0.580; *p*=0.055 between groups; data not shown).

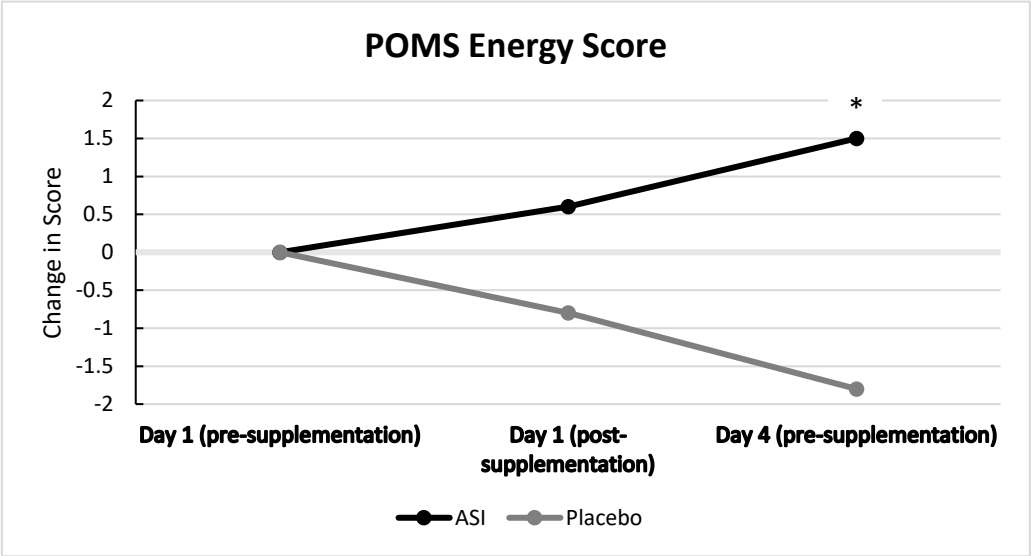

Figure 2. Perceived energy levels, measured by POMS vigor-activity subscores, pre-supplementation and post-supplementation (prior to exercise) on Day 1 and Day 4 (pre-supplementation). On Day 4, ASI perceived energy levels increased significantly more than placebo (*p=0.012).

Discussion

The major finding of the present study was that daily intake of ASI at 1,500 mg for 4 days prior to workout significantly decreased biomarkers of muscle damage immediately after a workout and during recovery. In addition, ASI increased hyperemia and pre-workout perceived energy levels as measured by the POMS vigor-activity subscore. Importantly, ASI elicited these outcomes without increasing heart rate or blood pressure compared to placebo, showing that ASI can be used as a non-stimulant promoter of muscle recovery that may also serve as a source of perceived energy that could improve exercise performance. While blood flow was not significantly greater in the ASI group compared to placebo, it is important to note that the effects of ASI may have been obscured due to the normal blood flow enhancing effects of the resistance exercise that was part of the study protocol alone. Past research has demonstrated that ASI is a highly bioavailable form of arginine that increases nitric oxide (NO) production and cognitive function[1,31]. The results from the salivary nitrite test in the current study, while small, also supports a role for NO.

Arginine is the only substrate for the endogenous synthesis of NO and therefore adequate levels are vital for healthy NO production and blood flow[36]. Consequently, arginine has become a popular ergogenic supplement to promote vasodilation by increasing NO production, thereby allowing for increased substrate utilization and metabolite removal in working muscles. Moreover, arginine has been shown to increase the production of growth hormone and creatine[20], two important factors for increasing muscle strength and size. Despite arginine's vital role in NO production and healthy blood flow, there is conflicting data regarding the efficacy of both acute and long-term arginine supplementation for exercise performance. While some studies have found that arginine improves muscular strength, fatigue, and recovery, other studies have found no effect on those performance variables[24,37].

To enhance L-arginine's ergogenic properties, arginine has been combined with alpha ketoglutarate, which is an intermediate to the Krebs cycle. While there have been some reports of ergogenic benefits[20,38], several studies have concluded that arginine alpha-ketoglutarate (AAKG) does not increase muscle protein synthesis, strength or endurance[39,40]. The inconstancy regarding the ergogenic benefits of arginine could be attributed to the fact that arginine is normally susceptible to being broken down in the liver by the enzyme arginase. Arginase catalyzes the conversion of L-arginine to L-ornithine which competes with NO synthase, an enzyme that activates the production of NO from arginine. There-fore, arginase acts to regulate and control the production of NO in the body[41,42]. While high arginase activity is associated with cardiovascular dysfunction and disease[41], and arginine supplementation has been shown to improve blood flow and NO production, healthy individuals may not respond to arginine supplementation, as arginase will naturally break down the nutrient to regulate NO production. The contribution of exercise to the within-product increases in blood flow possibly obscured any measurable differences be-tween groups. However, previous clinical research has demonstrated that ASI reduces arginase levels after 14 days of supplementation compared to arginine HCl. It should be noted that at the timepoints where arginase levels are the lowest, arginine levels are the highest when supplemented with ASI[43]. Therefore, it can be hypothesized that unlike most forms of arginine, ASI is a highly bioavailable, long-lasting form of arginine, due to its ability to lower arginase levels and therefore diminish the catabolizing effects of arginase on arginine.

Due to its unique delivery system, strong absorption properties, and direct impact on the NO production pathway, it is speculated that ASI allows for increased delivery of nutrients, such as oxygen, to muscle tissues and removal of metabolic waste away from muscle tissues. It should be noted these effects were seen acutely and in a more pronounced manner after 4 days of supplementation. This suggests that ASI acts quickly and also in a sustained manner to support vascular function/response. It is also impressive that these effects are seen at a fraction of the dose typically used for L-arginine alone[44]. As demonstrated by these effects, ASI may allow for greater exercise performance, recovery, and subsequent muscle growth. Moreover, the current study demonstrates that ASI increases perceived energy which may result in improvements in performance. It is also possible that ASI's positive effects on perceived energy come from the inositol component of the complex, as inositol is known to be a substrate of several neurotransmitters and neurotransmitter receptor signaling[45].

Limitations of this study include the short study duration and relatively small sample size. Considering that noticeable muscle growth can take weeks to achieve, a longer study duration might have resulted in measurable differences in

muscle growth, but that was not addressed in this study. Future studies should be of longer duration to allow observation of muscle growth and incorporate less vigorous exercise routines between study visits to create realistic circumstances for an individual who is using an exercise and supplement regime to improve muscle growth and function.

Conclusions

The results of this double-blind, placebo-controlled, crossover clinical trial show that daily intake of 1,500 mg of inositol-stabilized arginine silicate (ASI; Nitrosigine®) after an anerobic exercise bout: decreases biomarkers of muscle damage, increases perceived energy levels prior to working out, and results in greater hyperemia.. Combined with previous research demonstrating that ASI increases NO levels, arginine and silicon absorption, and cognitive functioning, these data further support the use of ASI as a functional ingredient for improving exercise outcomes.

Acknowledgements

The authors thank Dr. Catherine Kwik-Uribe for critical review of the manuscript.

References

1. Kalman DS, Feldman S, Samson A, Krieger DR. A clinical evaluation to determine the safety, pharmacokinetics, and pharmacodynamics of an inositol-stabilized arginine silicate dietary supplement in healthy adult males. *Clin Pharmacol.* 2015;7:103-109.
2. Komorowski JR, Hewlings S. Chapter 59 - Inositol-Stabilized Arginine Silicate: A Novel Nitric Oxide-Boosting Ingredient for Sports Nutrition and Improved Cognition. In: Bagchi D, Nair S, Sen CK, eds. *Nutrition and Enhanced Sports Performance (Second Edition).* Academic Press; 2019:683-689.
3. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *New England Journal of Medicine.* 1993;329(27):2002-2012.
4. Proctor SD, Kelly SE, Vine DF, Russell JC. Metabolic effects of a novel silicate inositol complex of the nitric oxide precursor arginine in the obese insulin-resistant JCR:LA-cp rat. *Metabolism: clinical and experimental.* 2007;56(10):1318-1325.
5. Levine AB, Punihaole D, Levine TB. Characterization of the Role of Nitric Oxide and Its Clinical Applications. *Cardiology.* 2012;122(1):55-68.
6. Buffoli B, Foglio E, Borsani E, Exley C, Rezzani R, Rodella LF. Silicic acid in drinking water prevents age-related alterations in the endothelium-dependent vascular relaxation modulating eNOS and AQP1 expression in experimental mice: An immunohistochemical study. *Acta Histochemica.* 2013;115(5):418-424.
7. Jurkić LM, Cepanec I, Pavelić SK, Pavelić K. Biological and therapeutic effects of ortho-silicic acid and some ortho-silicic acid-releasing compounds: New perspectives for therapy. *Nutrition & metabolism.* 2013;10(1):2.
8. Martin KR. Silicon: The Health Benefits of a Metalloid. In: Sigel A, Sigel H, Sigel RKO, eds. *Interrelations between Essential Metal Ions and Human Diseases.* Dordrecht: Springer Netherlands; 2013:451-473.
9. Komorowski J, Perez Ojalvo S. A Pharmacokinetic Evaluation of the Duration of Effect of Inositol-Stabilized Arginine Silicate and Arginine Hydrochloride in Healthy Adult Males. *The FASEB journal.* 2016;30:690.617.
10. Kalman DS, Feldman S, Samson A, Krieger DR. A clinical evaluation to determine the safety, pharmacokinetics, and pharmacodynamics of an inositol-stabilized arginine silicate dietary supplement in healthy adult males. *Clinical pharmacology: advances and applications.* 2015;7:103.
11. Babateen AM, Shannon OM, Mathers JC, Siervo M. Validity and reliability of test strips for the measurement of salivary nitrite concentration with and without the use of mouthwash in healthy adults. *Nitric Oxide.* 2019;91:15-22.
12. Kalman D. Comparison of cellular nitric oxide production from various sports nutrition ingredients: PG.16 p33. *Journal of the International Society of Sports Nutrition.* 2016;13(1):33.
13. Boucher JL, Moali C, Tenu JP. Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization. *Cellular and Molecular Life Sciences.* 1999;55(9):1015.
14. Böger RH, Bode-Böger SM. The clinical pharmacology of L-arginine. *Annual review of pharmacology and toxicology.* 2001;41(1):79-99.
15. Komorowski J, Perez Ojalvo S, Sylla S, Veledar E. Arginase inhibition by inositol-stabilized arginine silicate (ASI.; Nitrosigine®); a novel mechanism by which ASI enhances arginine bioavailability. *Dietary Bioactive Components Curr Dev Nutr.* 2018;2:49.

16. Proctor SD, Kelly SE, Russell JC. A novel complex of arginine–silicate improves micro- and macrovascular function and inhibits glomerular sclerosis in insulin-resistant JCR:LA-cp rats. *Diabetologia.* 2005;48(9):1925-1932.
17. Rogers JM, Gills J, Gray M. Acute effects of Nitrosigine® and citrulline malate on vasodilation in young adults. *Journal of the International Society of Sports Nutrition.* 2020;17(1).
18. Morris Jr SM. Arginine: beyond protein. *The American journal of clinical nutrition.* 2006;83(2):508S-512S.
19. Flynn NE, Meininger CJ, Haynes TE, Wu G. The metabolic basis of arginine nutrition and pharmacotherapy. *Biomedicine & Pharmacotherapy.* 2002;56(9):427-438.
20. Campbell BI, La Bounty PM, Roberts M. The ergogenic potential of arginine. *Journal of the International Society of Sports Nutrition.* 2004;1(2):1-4.
21. Napoli C, de Nigris F, Williams-Ignarro S, Pignalosa O, Sica V, Ignarro LJ. Nitric oxide and atherosclerosis: an update. *Nitric oxide.* 2006;15(4):265-279.
22. Dong J-Y, Qin L-Q, Zhang Z, et al. Effect of oral l-arginine supplementation on blood pressure: A meta-analysis of randomized, double-blind, placebo-controlled trials. *American Heart Journal.* 2011;162(6):959-965.
23. Wu G, Meininger CJ, Knabe DA, Baze FW, Rhoads MJ. Arginine nutrition in development, health and disease. *Current Opinion in Clinical Nutrition & Metabolic Care.* 2000;3(1):59-66.
24. Alvares TS, Meirelles CM, Bhambhani YN, Paschoalin VM, Gomes PS. L-Arginine as a potential ergogenic aid in healthy subjects. *Sports Medicine.* 2011;41(3):233-248.
25. Yavuz HU, Turnagol H, Demirel AH. Pre-exercise arginine supplementation increases time to exhaustion in elite male wrestlers. *Biology of sport.* 2014;31(3):187.
26. Bailey SJ, Winyard PG, Vanhatalo A, et al. Acute L-arginine supplementation reduces the O2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. *Journal of applied physiology.* 2010;109(5):1394-1403.
27. Buford. Glycine-arginine-ketoisocaproic acid improves performance of repeated cycling sprints. *Medicine and science in sports and exercise.* 2004;36.
28. Liu T-H, Wu C-L, Chiang C-W, Lo Y-W, Tseng H-F, Chang C-K. No effect of short-term arginine supplementation on nitric oxide production, metabolism and performance in intermittent exercise in athletes. *The Journal of nutritional biochemistry.* 2009;20(6):462-468.
29. Yamada M, Huang Z, Dalkara T, et al. Endothelial Nitric Oxide Synthase-Dependent Cerebral Blood Flow Augmentation by <scp>L</scp>-Arginine After Chronic Statin Treatment. *Journal of Cerebral Blood Flow & Metabolism.* 2000;20(4):709-717.
30. Evans M, Zakaria N, Marzuk M, Perez Ojalvo S, Sylla S, Komorowski J. An evaluation of the effects of inositol-stabilized arginine silicate (ASI; Nitrosigine®) in preventing the decline in cognitive function caused by strenuous exercise. *Journal of the International Society of Sports Nutrition.* 2018;15:3.
31. Kalman D, Harvey PD, Perez Ojalvo S, Komorowski J. Randomized Prospective Double-Blind Studies to Evaluate the Cognitive Effects of Inositol-Stabilized Arginine Silicate in Healthy Physically Active Adults. *Nutrients.* 2016;8(11).
32. Lau WY, Muthalib M, Nosaka K. Visual Analog Scale and Pressure Pain Threshold for Delayed Onset Muscle Soreness Assessment. *Journal of Musculoskeletal Pain.* 2013;21(4):320-326.
33. Curran SL, Andrykowski MA, Studts JL. Short Form of the Profile of Mood States (POMS-SF): Psychometric information. *Psychological Assessment.* 1995;7(1):80-83.
34. Hoskins PR. Measurement of arterial blood flow by Doppler ultrasound. *Clinical Physics and Physiological Measurement.* 1990;11(1):1.
35. Hoeper MM, Oudiz RJ, Peacock A, et al. End points and clinical trial designs in pulmonary arterial hypertension: clinical and regulatory perspectives. *Journal of the American College of Cardiology.* 2004;43(12S):S48-S55.
36. Jobgen WS, Fried SK, Fu WJ, Meininger CJ, Wu G. Regulatory role for the arginine–nitric oxide pathway in metabolism of energy substrates. *The Journal of nutritional biochemistry.* 2006;17(9):571-588.
37. Centeno A, Fernández-Sanjurjo M, Revuelta C, Royuela L, Jimenez-Saiz S. L-Arginine as a Nutritional Supplement in Physical Exercise. *Nutrition & Food Science International Journal.* 2017;2(4).
38. Campbell B, Roberts M, Kerksick C, et al. Pharmacokinetics, safety, and effects on exercise performance of l-arginine α-ketoglutarate in trained adult men. *Nutrition.* 2006;22(9):872-881.
39. Wax B, Kavazis AN, Webb HE, Brown SP. Acute L-arginine alpha ketoglutarate supplementation fails to improve muscular performance in resistance trained and untrained men. *Journal of the International Society of Sports Nutrition.* 2012;9(1):1-6.

40. Greer BK, Jones BT. Acute arginine supplementation fails to improve muscle endurance or affect blood pressure responses to resistance training. *The Journal of Strength & Conditioning Research.* 2011;25(7):1789-1794.
41. Durante W, Johnson FK, Johnson RA. Arginase: a critical regulator of nitric oxide synthesis and vascular function. *Clinical and experimental pharmacology & physiology.* 2007;34(9):906.
42. Caldwell RB, Toque HA, Narayanan SP, Caldwell RW. Arginase: an old enzyme with new tricks. *Trends in pharmacological sciences.* 2015;36(6):395-405.
43. Sahin K, Orhan C, Tuzcu M, Hayirli A, Komorowski JR, Sahin N. Effects of dietary supplementation of arginine-silicate-inositol complex on absorption and metabolism of calcium of laying hens. *PloS one.* 2018;13(1):e0189329.
44. Evans RW, Fernstrom JD, Thompson J, Morris SM, Kuller LH. Biochemical responses of healthy subjects during dietary supplementation with L-arginine. *The Journal of nutritional biochemistry.* 2004;15(9):534-539.
45. Downes CP. Inositol phospholipids and neurotransmitter-receptor signalling mechanisms. *Trends in Neurosciences.* 1983;6:313-316.