Nutrition21
INNOVATIVE INGREDIENTS. REAL RESULTS.

Nitrosigine® Claims Substantiation Evaluation CARSE Report Version 4.0

(Competent and Reliable Scientific Evidence)

CLINICAL DOSE 1,500 MG NITROSIGINE

NUTRITION21 | Harrison, NY 10528

A Systematic Evaluation Conducted by Substantiation Sciences, Inc.

This document is a reference for potential claims to be used in products that contain Nitrosigine®. These claims are not meant to serve as an exhaustive list, an exact recitation of language required, or guaranteed to pass regulatory evaluation. As Nutrition 21 is committed to scientific excellence and continuously studies its product portfolio, this document may change. Please do not hesitate to contact us to ensure you have the most up-to-date version.

Disclaimer Notice

Statements in this document have not been evaluated by the Food and Drug Administration. Products using these claims should not be intended to diagnose, treat, cure, or prevent any disease.

The claims listed in this report are examples of potential structure/function claims. As with all product claims, use of these claims should be reviewed by your legal/regulatory advisors to make sure that they comply with current regulations.

Date: October 25, 2021

The purpose of this compendium is to detail marketing and advertising claims and substantiation for Nitrosigine® (ASI, inositol stabilized arginine silicate dietary supplement).

1. This compendium is of the substantiation of marketing and advertising claims as shared by Nutrition 21 with Substantiation Sciences. These claims are related to the dietary supplement product Nitrosigine. This report is considered a thorough evaluation of the published scientific literature, unpublished scientific data and in-house data as related to Nitrosigine.

Substantiation Sciences, Inc., led by Douglas S. Kalman PhD, RD and Susan Hewlings PhD, RD, has organized and compiled this Substantiation document utilizing the following methods:

1. Review direct claims as shared by Nutrition21.
2. Collect and collate all published, un-published and in-house data regarding Nitrosigine.
3. Coordinate, compare and contrast the stated claims with the data on hand. Compile added data, if needed.
4. Write the assessment packet whereby noting the claim, the general reference that supported that claim and further detail the exact aspect of the data that supports the claim.
5. Organize compendium to include the claim, paragraph description, general reference and claim specific reference.
6. Globally, any and all direct or indirect claims regarding any dietary supplement are not intended to mean that the supplement may prevent, cure or treat any disease or condition. All claims should be read within the purview of the Dietary Supplement Health Education Act of 1994.

It is the belief of Substantiation Sciences, Inc. and of Douglas S. Kalman PhD, RD and Susan Hewlings PhD, RD that the assessment of Nitrosigine and the set of claims shared within the document (as of October 25, 2021) have Competent and Reliable Scientific Evidence as generally accepted within the industry and discipline.

Respectfully Submitted by:

Douglas S. Kalman *(signed electronically 10-25-2021)*

Douglas S. Kalman PhD, RD, FISSN, CCRC, FACN

Susan Hewlings *(signed electronically 10-25-2021)*

Susan Hewlings PhD, RD

Nitrosigine Claim Substantiation Evaluation and Report

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Disclaimer		
†Results compared to baseline. ^In a post-hoc analysis.		

Nitrosigine General Claims

Claim: <u>Nitrosigine enhances performance and supports an active lifestyle by promoting blood flow factors† and by increasing focus and perceived energy.</u>
<u>†Results compared to baseline.</u>

Nitrosigine has been clinically shown to significantly enhance levels of arginine and silicon, as well as Kallistatin and nitric oxide levels, which are important factors in increasing blood flow to working muscles (Kalman, 2015; Komorowski, 2015; Kalman, 2016; Alvares, 2011). Nitrosigine increases plasma arginine by >70% compared to arginine hydrochloride (ArgHCl) and does so for a significantly longer duration (up to 6 hrs.), indicating that Nitrosigine is a more bioavailable form of arginine (Komorowski, 2016). In a double-blind, placebo-controlled trial, Nitrosigine significantly enhanced cognitive function and perceived energy levels without negatively impacting heart rate or blood pressure compared to placebo, supporting the use of Nitrosigine as a non-stimulant, energy enhancing ingredient (Harvey, 2015; Kalman, 2016; Rood-Ojalvo, 2015; Sylla, 2018). Nitrosigine is a bioavailable source of arginine and silicon that has been shown to significantly increase nitric oxide levels, cognitive function and perceived energy levels in humans, endpoints which support performance and active lifestyles.

Substantiation:

1. Kalman 2015 Page 108 Conclusion: The arginine silicate dietary supplement increases blood levels of arginine after a single dose within 30 minutes for up to 5 hours and increases blood levels of silicon for up to 1.5 hours ($p<0.05$). Page 107 second paragraph: After 14 days of use, baseline salivary nitrite levels increased in six subjects and stayed the same in four subjects; this shift was significant ($p=0.031$).
2. Komorowski 2015 Discussion: ASI supplementation significantly increased the protein Kallistatin ($p<0.01$), a potent vasodilator, which has been shown to protect against oxidative stress and suppress cellular apoptosis. Vasodilation increases blood flow and vessel flexibility, a key factor in sports nutrition and bodybuilding.
3. Kalman 2016 JISSN Abstract: This *in vitro* study was designed to compare the cellular production of NO (nitric oxide) of several sports nutrition ingredients including ASI, L-Arginine, L-Arginine AKG, L-Citrulline, L-Citrulline Malate and Agmatine Sulfate. At the doses used in this study, ASI significantly increased NO production over each of the five other compounds tested ($p<0.01$; Figure 1). There was a greater than 5X increase in NO production with ASI compared to the other tested sports nutrition ingredients. In addition, of the sports nutrition ingredients tested, only ASI significantly increased NO production versus control ($p<0.01$). In this *in vitro* study to evaluate NO production of a number of sports nutrition ingredients using the established Greiss assay to detect cellular NO production, ASI significantly enhanced NO levels compared to the other compounds and also versus control. Increasing NO can lead to substantial benefits for training athletes and fitness enthusiasts as it can result in enhanced delivery of oxygen and nutrients to working muscles, positively affecting workouts and recovery.
4. Alvares 2011 Page 242: Preliminary observations from our laboratory observed significant increases in blood volume – measured by near infrared spectroscopy – during the recovery

period of sets of resistance exercise performed 90 minutes after oral L-arginine supplementation (as free form), without simultaneous increases in strength performance.

5. Komorowski 2016 Abstract conclusion: This study showed that ASI supplementation significantly increased plasma arginine levels at multiple time-points up to six hours post-dose, while ArgHCl supplementation did so for only one hour. This study also showed that ASI supplementation resulted in a >70% increase in plasma arginine AUC compared to ArgHCl, and a lower standard deviation than that seen with ArgHCl, suggesting that ASI is a more bioavailable, and less variable, source of arginine.
6. Harvey 2015 Abstract: Faster times in TMT B are associated with enhanced visual search, speed of processing, mental flexibility, and executive functions under pressure. Approximately 10 minutes after taking the first dose, TMT B time decreased by 17.6 seconds in the ASI group (p=0.001) from a baseline time of 52.7 seconds (a 33% improvement), compared to a decrease of 4.9 seconds in the placebo group (p=0.384). The changes in TMT B times after 10 minutes were statistically significant between groups (p=0.024). After 3 days of dosing, TMT B time decreased 18.5 seconds compared to baseline (p<0.0005) a 35% improvement, whereas the placebo group decreased 5.1 seconds (p=0.517). These findings show that daily doses of ASI significantly improved TMT B times, with effects seen in as little as 10 minutes after dosing and continued improvement over 14 days. Improvement in TMT B test times preliminarily suggests improved complex processing speed in subjects treated with ASI.
7. Kalman 2016 Nutrients Abstract and Conclusion: Inositol-stabilized arginine silicate (ASI; Nitrosigine®) has been validated to increase levels of arginine, silicon and nitric oxide production. To evaluate potential enhancement of mental focus and clarity, ASI (1500 mg/day) was tested in two double-blind placebo-controlled crossover (DBPC-X) studies using the Trail Making Test (TMT, Parts A and B). In the two studies, healthy males took ASI for 14 and 3 days, respectively. In the first study, after 14 days of dosing, TMT B time decreased significantly from baseline (28% improvement, $p = 0.045$). In the second study evaluating shorter-term effects, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) in a 10-min period. After 3 days of dosing, TMT B time significantly decreased from baseline scores (35% improvement, $p < 0.001$). These findings show that ASI significantly improved the ability to perform complex cognitive tests requiring mental flexibility, processing speed and executive functioning. Results from two separate placebo-controlled clinical studies showed that daily doses of ASI significantly improved TMT B times, with effects seen in as little as 10 min after dosing and continued improvement with ongoing use. Expected practice effects were seen in placebo-treated subjects. Reductions in completion times seen with ASI supplementation were greater than would be expected from simple practice effects alone. Nitrosigine (ASI) led to a significant decrease in TMT B time compared to placebo, with an effect size of d = 0.80, which shows the strength of the effect to be considerable. Improvement in TMT B test times suggests improved complex processing speed in subjects treated with ASI.
8. Rood-Ojalvo 2015 Abstract Results: Hyperemia, measured using leg circumference, increased significantly in the ASI group by 1.8cm (p = 0.001) at 72 hours from pre-dose, compared to a non-significant increase in the placebo group by 0.8cm (p = 0.091); p = 0.070 between groups. Blood flow, measured by blood velocity through the femoral artery using a Doppler Ultrasound, increased 59.9 cm/s in the ASI group (p < 0.005) and

49.9cm/s in the placebo group ($p < 0.005$) after exercise on Day 3; $p = 0.2$ between groups. Perceived energy, measured using the POMS vigor-activity sub-scores, significantly increased after 72 hours compared to placebo ($p = 0.039$). At 72 hours, perceived fatigue, measured using the POMS fatigue-inertia sub-scores, significantly decreased in the ASI group ($p = 0.041$) from pre-dose, compared to a non-significant change in the placebo group ($p = 0.580$); $p = 0.055$ between groups.

9. Sylla 2018 Abstract Conclusion: In conclusion, currently available data show that ASI intake at daily doses of 1500 mg does not induce elevations in heart rate or blood pressure. While intake of other known energy-stimulating ingredients can increase heart rate and blood pressure even after a single dose, ASI intake did not change heart rate or blood pressure after both short term (after 25 minutes) and longer term (after 14 days) use. Overall, clinical and preclinical data suggest that ASI (Nitrosigine®) can be safely used as a non-stimulant ingredient to increase physical energy and cognitive function without adverse cardiovascular effects.

Substantiation 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. *Clinical Pharm: Adv and Applica.* 2015;7:103-109.
2. Komorowski J, Rood-Ojalvo S, El-Sohemy A. Arginine silicate supplementation decreases markers of cardiovascular, renal and metabolic dysfunction and increases markers of vasodilation and cardiovascular health in healthy adult males. *The FASEB Journal*. 2015;29:748.2.
3. Kalman D, Perez Ojalvo S, Komorowski J. Comparison of cellular nitric oxide production from various sports nutrition ingredients. *JISSN*. 2016;13(Suppl 1):P33.
4. 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(Suppl 1):690.17.
5. Alvares TS, Meirelles CM, Bhambhani YN, Paschoalin VM, Gomes PS. L-arginine as a potential ergogenic aid in healthy subjects. *Sports Med*. 2011;41(3):233-248.
6. Harvey P, Rood-Ojalvo S, Komorowski J. The effects of inositol-stabilized arginine silicate on cognitive function. *JACN.* 2015;34(6):544.
7. 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):736.
8. Rood-Ojalvo S, Sandler D, Veledar E, Komorowski J. The benefits of inositol-stabilized arginine silicate as a workout ingredient. *JISSN*. 2015;12(Suppl1):P14.
9. Sylla S, Perez Ojalvo S, Komorowski J. An evaluation of the effect of inositol-stabilized arginine silicate on heart rate and blood pressure. *The FASEB Journal*. 2018;32(Suppl 1):742.12.

Nitrosigine General Claims

Claim: **The advanced ingredient that works within 15 minutes – increasing focus and cognitive function.**

In a double-blind, placebo-controlled, crossover study, 16 male subjects consumed 1,500 mg of Nitrosigine daily for 4 days, and cognitive function was measured at baseline and after 15 minutes. The Trail Making Test (TMT) parts A and B were used to assess cognitive function. The TMT is a validated and commonly used neuropsychological test that measures cognitive processing speed, visual attention, task switching, and executive functioning. The test consists of two parts, A and B. Each test is measured by the time to completion, with lower scores indicating greater performance. TMT A involves connecting an ascending sequence of 25 numbers, while TMT B involves connecting an alternating sequence of 25 numbers and letters. Part B is considered to be a test of higher-level cognitive skills, such as mental flexibility (Drane, 2002). After 3 days of supplementation, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) within a 15-minute period post-dose (Harvey, 2015; Kalman, 2016). These clinical findings show that Nitrosigine significantly increases focus and cognitive function within 15 minutes of dosing.

Substantiation:

1. Drane 2002 Introduction: The Trail Making Test (TMT) is a visuomotor speeded task that is used routinely in clinical evaluations and consists of two parts: TMT-A and TMT-B. Trail Making Test-A is a simple visual-scanning task that requires one to draw a line connecting consecutive numbers from 1 to 25. Trail Making Test-B adds a dimension of "cognitive flexibility" by requiring one to draw a line connecting numbers and letters in alternating sequence. The first TMT index score, subtracting TMT-A time from TMT-B time (B -A), is thought to provide a "pure measure of cognitive efficiency." The rationale behind this suggestion assumes that both measures are equivalent in their demands for simple sequencing, visual scanning, and psychomotor functioning, making the difference score between them a more direct measure of the added cognitive flexibility required by TMT-B.
2. Harvey 2015 Abstract: Faster times in TMT B are associated with enhanced visual search, speed of processing, mental flexibility, and executive functions under pressure. Approximately 10 minutes after taking the first dose, TMT B time decreased by 17.6 seconds in the ASI group (p=0.001) from a baseline time of 52.7 seconds (a 33% improvement), compared to a decrease of 4.9 seconds in the placebo group (p=0.384). The changes in TMT B times after 10 minutes were statistically significant between groups (p=0.024). After 3 days of dosing, TMT B time decreased 18.5 seconds compared to baseline (p<0.0005) a 35% improvement, whereas the placebo group decreased 5.1 seconds (p=0.517). These findings show that daily doses of ASI significantly improved TMT B times, with effects seen in as little as 10 minutes after dosing and continued improvement over 14 days. Improvement in TMT B test times preliminarily suggests improved complex processing speed in subjects treated with ASI.

3. Kalman 2016 Nutrients Abstract and Conclusion: Inositol-stabilized arginine silicate (ASI; Nitrosigine®) has been validated to increase levels of arginine, silicon and nitric oxide production. To evaluate potential enhancement of mental focus and clarity, ASI (1500 mg/day) was tested in two double-blind placebo-controlled crossover (DBPC-X) studies using the Trail Making Test (TMT, Parts A and B). In the two studies, healthy males took ASI for 14 and 3 days, respectively. In the first study, after 14 days of dosing, TMT B time decreased significantly from baseline (28% improvement, $p = 0.045$). In the second study evaluating shorter-term effects, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) in a 10-min period. After 3 days of dosing, TMT B time significantly decreased from baseline scores (35% improvement, $p < 0.001$). These findings show that ASI significantly improved the ability to perform complex cognitive tests requiring mental flexibility, processing speed and executive functioning. Results from two separate placebo-controlled clinical studies showed that daily doses of ASI significantly improved TMT B times, with effects seen in as little as 10 min after dosing and continued improvement with ongoing use. Expected practice effects were seen in placebo-treated subjects. Reductions in completion times seen with ASI supplementation were greater than would be expected from simple practice effects alone. Nitrosigine (ASI) led to a significant decrease in TMT B time compared to placebo, with an effect size of d = 0.80, which shows the strength of the effect to be considerable. Improvement in TMT B test times suggests improved complex processing speed in subjects treated with ASI.

Substantiation References:

1. Drane DL, Yuspeh RL, Huthwaite JS, Klingler LK. Demographic characteristics and normative observations for derived trial making test indices. *Neuropsychiatry Neuropsychol Behav Neurol*. 2002;15(1):39-43.
2. Harvey P, Rood-Ojalvo S, Komorowski J. The effects of inositol-stabilized arginine silicate on cognitive function. *JACN.* 2015;34(6):544.
3. 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):736.

Nitrosigine General Claims

Claim: <u>Extensive preclinical (animal) and clinical (human) studies show that Nitrosigine has unique benefits important to the training athlete.</u>

Multiple preclinical studies have shown increased absorption of arginine from Nitrosigine compared to ArgHCl and Arginine base (L-arginine) (Proctor, 2005; Proctor, 2007; Bartos, 2016). In one of the same preclinical studies, Nitrosigine was also shown to significantly increase blood vessel relaxation greater than Arginine HCl, showing blood flow benefits as blood vessel relaxation, or vasodilation, causes an increase in blood flow (Proctor, 2005). In clinical studies Nitrosigine has been shown to significantly enhance blood levels of arginine and silicon, as well as nitric oxide levels, which is an important factor in increasing blood flow to working muscles (Kalman, 2015; Komorowski, 2016; Alvares, 2011). Nitrosigine increases plasma arginine by >70% compared to arginine hydrochloride (ArgHCl) and does so for a significantly longer duration (up to 6 hrs.), indicating that Nitrosigine is a more bioavailable form of arginine (Komorowski, 2016). In a double-blind, placebo-controlled trial, Nitrosigine significantly reduces markers of muscle damage and enhances cognitive function and perceived energy levels without negatively impacting heart rate or blood pressure compared to placebo, supporting the use of Nitrosigine as a non-stimulant, energy enhancing ingredient (Harvey, 2015; Kalman, 2016; Rood-Ojalvo, 2015; Sylla, 2018). In a separate clinical study, Nitrosigine also prevented the 51% decrease in TMT performance seen in the placebo group following strenuous exercise (Evans, 2018). Nitrosigine is a bioavailable source of arginine and silicon that has been shown to significantly increase nitric oxide levels, cognitive function and energy levels in humans, while also supporting recovery, endpoints supportive of and important to the training athlete.

Substantiation:

1. Proctor 2005 Pages 1928, 1929 and Table 2.
2. Proctor 2007 Figure 4. Plasma NOx concentrations.
3. Bartos 2016 Patent Application 0069: The results of the single dose oral pharmacokinetic study from testing in fasted male Sprague Dawley rates (equivalent dose of 500 mg/kg L-Arginine) for L-Arginine (free base), L Arginine HCl, ResArgin, and Arginine silicate inositol (ASI), measured in plasma cone of arginine (µg/ml) over time, are shown in Figure 1. Comparative plasma concentrations of arginine (in µg/mL) based on administration of the test compounds demonstrated that both AUCinf and Cmax were increased substantially for ASI compared with Arginine base, 3-fold and 2.35-fold, respectively. Stated in another way, AUCinf for ASI increased by 209% over Arginine base in plasma, while Cmax for ASI increased by 135% over Arginine base in plasma.
4. Kalman 2015 Page 108 Conclusion: The arginine silicate dietary supplement increases blood levels of arginine after a single dose within 30 minutes for up to 5 hours and increases blood levels of silicon for up to 1.5 hours ($p<0.05$). Page 107 second paragraph: After 14 days of use, baseline salivary nitrite levels increased in six subjects and stayed the same in four subjects; this shift was significant ($p=0.031$).

5. Komorowski 2016 Abstract Conclusion: This study showed that ASI supplementation significantly increased plasma arginine levels at multiple time-points up to six hours post-dose, while ArgHCl supplementation did so for only one hour. This study also showed that ASI supplementation resulted in a >70% increase in plasma arginine AUC compared to ArgHCl, and a lower standard deviation than that seen with ArgHCl, suggesting that ASI is a more bioavailable, and less variable, source of arginine.
6. Alvares 2011 Page 242: Preliminary observations from our laboratory observed significant increases in blood volume – measured by near infrared spectroscopy – during the recovery period of sets of resistance exercise performed 90 minutes after oral L-arginine supplementation (as free form), without simultaneous increases in strength performance.
7. Harvey 2015 Abstract: Faster times in TMT B are associated with enhanced visual search, speed of processing, mental flexibility, and executive functions under pressure. Approximately 10 minutes after taking the first dose, TMT B time decreased by 17.6 seconds in the ASI group ($p=0.001$) from a baseline time of 52.7 seconds (a 33% improvement), compared to a decrease of 4.9 seconds in the placebo group ($p=0.384$). The changes in TMT B times after 10 minutes were statistically significant between groups ($p=0.024$). After 3 days of dosing, TMT B time decreased 18.5 seconds compared to baseline ($p<0.0005$) a 35% improvement, whereas the placebo group decreased 5.1 seconds ($p=0.517$). These findings show that daily doses of ASI significantly improved TMT B times, with effects seen in as little as 10 minutes after dosing and continued improvement over 14 days. Improvement in TMT B test times preliminarily suggests improved complex processing speed in subjects treated with ASI.
8. Kalman 2016 Nutrients Abstract and Conclusion: Inositol-stabilized arginine silicate (ASI; Nitrosigine) has been validated to increase levels of arginine, silicon and nitric oxide production. To evaluate potential enhancement of mental focus and clarity, ASI (1500 mg/day) was tested in two double-blind placebo-controlled crossover (DBPC-X) studies using the Trail Making Test (TMT, Parts A and B). In the two studies, healthy males took ASI for 14 and 3 days, respectively. In the first study, after 14 days of dosing, TMT B time decreased significantly from baseline (28% improvement, $p = 0.045$). In the second study evaluating shorter-term effects, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) in a 10-min period. After 3 days of dosing, TMT B time significantly decreased from baseline scores (35% improvement, $p < 0.001$). These findings show that ASI significantly improved the ability to perform complex cognitive tests requiring mental flexibility, processing speed and executive functioning. Results from two separate placebo-controlled clinical studies showed that daily doses of ASI significantly improved TMT B times, with effects seen in as little as 10 min after dosing and continued improvement with ongoing use. Expected practice effects were seen in placebo-treated subjects. Reductions in completion times seen with ASI supplementation were greater than would be expected from simple practice effects alone. Nitrosigine (ASI) led to a significant decrease in TMT B time compared to placebo, with an effect size of $d = 0.80$, which shows the strength of the effect to be considerable. Improvement in TMT B test times suggests improved complex processing speed in subjects treated with ASI.
9. Rood-Ojalvo 2015 Abstract: Perceived energy, measured using the POMS vigor-activity sub-scores, significantly increased after 72 hours compared to placebo ($p = 0.039$). At 72 hours, perceived fatigue, measured using the POMS fatigue-inertia sub-scores, significantly

decreased in the ASI group ($p = 0.041$) from pre-dose, compared to a non-significant change in the placebo group ($p = 0.580$); $p = 0.055$ between groups. Hyperemia, measured using leg circumference, increased significantly in the ASI group by 1.8cm ($p = 0.001$) at 72 hours from pre-dose, compared to a non-significant increase in the placebo group by 0.8cm ($p = 0.091$); $p = 0.070$ between groups. Blood flow, measured by blood velocity through the femoral artery using a Doppler Ultrasound, increased 59.9 cm/s in the ASI group ($p < 0.005$) and 49.9cm/s in the placebo group ($p < 0.005$) after exercise on Day 3; $p = 0.2$ between groups. CK levels significantly decreased in the ASI group at 24 ($p = 0.040$), 48 ($p = 0.017$) and 72 ($p = 0.034$) hours post- exercise compared to the placebo group. Immediately post-exercise at the hour 0 visit, ASI supplementation led to 44% less muscle damage, measured by CK levels, than placebo ($p = 0.057$). LDH levels significantly increased from baseline immediately after exercise in the placebo group ($p = 0.015$), but not in the ASI group ($p = 0.366$); $p = 0.133$ between groups. Both primary and secondary endpoints show that daily doses of ASI prior to workout significantly increased pre-workout energy levels, increased muscle pump immediately following a workout, and decreased biomarkers of muscle damage immediately after a workout and during recovery. These results demonstrate multiple benefits of ASI as a functional workout ingredient.

10. Sylla 2018 Abstract Conclusion: In conclusion, currently available data show that ASI intake at daily doses of 1500 mg does not induce elevations in heart rate or blood pressure. While intake of other known energy-stimulating ingredients can increase heart rate and blood pressure even after a single dose, ASI intake did not change heart rate or blood pressure after both short term (after 25 minutes) and longer term (after 14 days) use. Overall, clinical and preclinical data suggest that ASI (Nitrosigine®) can be safely used as a non-stimulant ingredient to increase physical energy and cognitive function without adverse cardiovascular effects.
11. Evans 2018 Abstract: A single dose of ASI significantly improved cognitive function parameters of mental acuity, focus and processing speed after intense exercise, compared to placebo ($p \leq 0.05$). Following strenuous exercise, time to complete TMT-A and TMT-B increased by a significant 51% and 11% respectively in the placebo group, while it decreased by 5% for TMT- A and 7% for TMT-B when participants consumed an acute dose of ASI ($p \leq 0.05$; Figure 2,3). The results of this study showed that ASI prevents the decline in cognitive function seen following strenuous exercise. Acute consumption of ASI prevented the intense exercise induced cognitive function decline of 51% seen in the placebo. These results could be of interest to individuals looking to maintain a strong cognitive state after expending energy during intense athletics, as well as everyday life.

Substantiation References:

1. Proctor SD, Vine DF, 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;48:1925-1932.
2. Proctor SD, 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):1318-1325.
3. Bartos, JD. (2016). US Patent Application Publication No. US 2016/0081959 A1.

4. 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 Pharm: Adv and Applica.* 2015; 7:103-109.
5. 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(Suppl 1):690.17.
6. Alvares TS, Meirelles CM, Bhambhani YN, Paschoalin VM, Gomes PS. L-arginine as a potential ergogenic aid in healthy subjects. *Sports Med*. 2011;41(3):233-248.
7. Harvey P, Rood-Ojalvo S, Komorowski J. The effects of inositol-stabilized arginine silicate on cognitive function. *JACN.* 2015;34(6):544.
8. 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):736.
9. Rood-Ojalvo S, Sandler D, Veledar E, Komorowski J. The benefits of inositol-stabilized arginine silicate as a workout ingredient. *JISSN*. 2015;12(Suppl1):P14.
10. Sylla S, Perez Ojalvo S, Komorowski J. An evaluation of the effect of inositol-stabilized arginine silicate on heart rate and blood pressure. *The FASEB Journal*. 2018;32(Suppl 1):742.12.
11. 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. *JISSN*. 2018;15(Suppl 1):A50.

Nitrosigine Blood Arginine and Silicon Level (Bioavailability) Claims

Claim: <u>After a single dose, Nitrosigine increases blood arginine levels in as quickly as 30 minutes and lasts up to 6 hours.†</u>
<u>†Results compared to baseline.</u>

In an open-label, pharmacokinetics/pharmacodynamics/safety clinical trial, ten men took 1,500 mg of Nitrosigine for 14 days. On Day 1, plasma arginine levels increased at 0.5 hours, 1 hour, 1.5 hours, 2 hours, 3 hours, and 5 hours ($p<0.01$) (Kalman, 2015). Moreover, a randomized, double-blind, active-controlled, crossover study was carried out to evaluate the extent and duration of elevated plasma arginine levels in ten healthy adult male subjects taking Nitrosigine and arginine hydrochloride (ArgHCl). Men were randomly assigned to take a single oral dose of Nitrosigine or ArgHCl (each containing a total of 500 mg of arginine) qd for 14 days, and attended study visits on Days 1 and 15, with a 7-day washout period between test product administration. On day 1, plasma arginine levels in the Nitrosigine group significantly increased from baseline at 1, 1.5, 2, 3 and 6 hours ($p<0.05$). Plasma arginine levels in the ArgHCl group only increased at 1 hour ($p<0.05$) (Komorowski, 2016). These clinical studies show that Nitrosigine increases blood arginine levels in as quickly as 30 minutes and lasts up to 6 hours.

Substantiation:

1. Kalman 2015 Page 108 Conclusion: The arginine silicate dietary supplement increases blood levels of arginine after a single dose within 30 minutes for up to 5 hours ($p<0.05$).
2. Komorowski 2016 Abstract Conclusion: This study showed that ASI supplementation significantly increased plasma arginine levels at multiple time-points up to six hours post-dose, while ArgHCl supplementation did so for only one hour. This study also showed that ASI supplementation resulted in a >70% increase in plasma arginine AUC compared to ArgHCl, and a lower standard deviation than that seen with ArgHCl, suggesting that ASI is a more bioavailable, and less variable, source of arginine.

Substantiation 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. *Clinical Pharm: Adv and Applica.* 2015;7:103-109.
2. 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(Suppl 1):690.17.

Nitrosigine Blood Arginine and Silicon Level (Bioavailability) Claims

Claim: **Nitrosigine provides the benefits of arginine and silicon, plus additional synergistic benefits from its unique combination.†**
†Results compared to baseline.

Nitrosigine is a bioavailable source of arginine and silicon providing the benefits of each (Kalman, 2015; Komorowski, 2016). Arginine is the biological precursor of nitric oxide, which is critical for healthy blood flow and silicon is known to enhance the flexibility of arterial walls thereby making it an important nutrient for healthy blood flow. In a preclinical study, arginine and silicon absorption was compared in rats treated with Nitrosigine versus equivalent doses of the separate components of the complex (arginine hydrochloride, silicon, and inositol (A+S+I)) for 29 days. After 29 days, serum and joint tissue levels of arginine and silicon were significantly higher in the Nitrosigine group compared to the A+S+I group ($p<0.05$). Nitrosigine also significantly improved markers of inflammation and overall arthritis and inflammation score over the arthritic control group and the A+S+I group. These results demonstrate that the Nitrosigine complex is more effective than a combination of the individual ingredients (Sandler, 2016; Perez Ojalvo, 2017; Sahin, 2019). Nitrosigine is therefore more effective than a combination of the individual ingredients in increasing arginine and silicon absorption and efficacy. These data show that the Nitrosigine complex is a highly bioavailable source of arginine and silicon that can provide the benefits of these nutrients to a greater extent than the combination of the individual ingredients due to its unique formulation.

Substantiation:

1. Kalman 2015 Page 108 Conclusion: The arginine silicate dietary supplement increases blood levels of arginine after a single dose within 30 minutes for up to 5 hours and increases blood levels of silicon for up to 1.5 hours. ($p<0.05$).
2. Komorowski 2016 Abstract Conclusion: This study showed that ASI supplementation significantly increased plasma arginine levels at multiple time-points up to six hours post-dose, while ArgHCl supplementation did so for only one hour. This study also showed that ASI supplementation resulted in a >70% increase in plasma arginine AUC compared to ArgHCl, and a lower standard deviation than that seen with ArgHCl, suggesting that ASI is a more bioavailable, and less variable, source of arginine.
3. Sandler 2016 Abstract: Serum arginine, serum silicon, joint tissue arginine and joint tissue silicon were all significantly greater in the ASI group (IV) compared to the control groups (I and II) and the A+S+I group (III) ($p<0.05$; Table 1). ASI supplementation significantly increased arginine and silicon bioavailability not only in the blood, but also in joint tissue, to a greater degree than the supplementation of equivalent doses of arginine, silicon and inositol as individual components. This preclinical study showed that ASI is a more bioavailable source of arginine and silicon than the individual components, supporting its position as an active sports nutrition ingredient enhancing energy, focus and markers of muscle recovery. These results showing increased absorption of arginine and silicon from ASI, help explain and support the enhanced efficacy of ASI in sports and athletic applications.

4. Perez Ojalvo 2017 Abstract: Day 29 arthritis scores and inflammation scores were both significantly lower in the ASI group (IV) compared to the arthritic control group (II) and the A+S+I group (III). Seven inflammatory markers tested in the blood — TNF-α, IL-17, IL-6, ghrelin, obestatin, sclerostin, and DKK-1 — were significantly lower in the ASI group (IV) compared to the arthritic control group (II). Four of five inflammatory markers tested in the tissue — Cox-2, TNF-α, IL-6, NF-κB, and B-catenin — were significantly lower in the ASI group (IV) compared to the arthritic control group (II). ASI supplementation significantly improved markers of inflammation and overall arthritis and inflammation score over the arthritic control group and the A+S+I group. These results demonstrate that ASI may be effective in reducing inflammation and that the ASI complex is more effective than a combination of the individual ingredients. ASI may also be of physiological benefit to athletes and fitness enthusiasts concerned with joint health and inflammation and to those experiencing joint pain due to inflammation.
5. Sahin 2019 Abstract: The purpose of this study was to test the effects of arginine-silicate-inositol complex (ASI), compared to a combination of the individual ingredients (A+S+I) of the ASI, on inflammatory markers and joint health in a collagen-induced arthritis (CIA) rat model. A total of 28 Wistar rats were divided into four groups: (i) Control; (ii) Arthritic group, rats subjected to CIA induction by injection of bovine collagen type II (A); (iii) Arthritic group treated with equivalent doses of the separate components of the ASI complex (arginine hydrochloride, silicon, and inositol) (A+S+I); (iv) Arthritic group treated with the ASI complex. The ASI complex treatment showed improved inflammation scores and markers over the arthritic control and the A+S+I group. ASI group had also greater levels of serum and joint-tissue arginine and silicon than the A+S+I group. Joint tissue IL-6, NF-κB, COX-2, TNF-α, p38 MAPK, WISP-1, and β-Catenin levels were lower in the ASI group compared to the other groups ($P < 0.05$ for all). In conclusion, these results demonstrate that the ASI complex may be effective in reducing markers of inflammation associated with joint health and that the ASI complex is more effective than a combination of the individual ingredients.

Substantiation 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. *Clinical Pharm: Adv and Applica.* 2015;7:103-109.
2. 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(Suppl 1):690.17.
3. Sandler D, Perez Ojalvo S, Komorowski J. Absorption of bonded arginine silicate compared to individual arginine and silicon components. *JISSN*. 2016;13(Suppl 1):P19.
4. Perez Ojalvo S, Sahin K, Komorowski J. Effect of bonded arginine silicate on inflammatory markers and arthritis. *The FASEB Journal.* 2017;31(Suppl 1):166.5.
5. Sahin K, Perez Ojalvo S, Akdemir F, Orhan C, Tuzcu M, Sahin N, Ozercan IH, Sylla S, Koca SS, Yilmaz I, Komorowski JR. Effect of inositol -stabilized arginine silicate on arthritis in a rat model. *Food Chem Toxicol*. 2019;125:242-251.

Nitrosigine Blood Arginine and Silicon Level (Bioavailability) Claims

Claim: **Silicon is difficult to absorb through dietary intake, but Nitrosigine was shown in preclinical studies to dramatically increase silicon absorption and in a clinical study Nitrosigine supplementation significantly increased silicon levels for up to 1.5 hours.†**
†Results compared to baseline.

A preclinical study compared the absorption of Nitrosigine and arginine HCl (as a reference agent) in obese insulin-resistant male and female JCR:LA-cp rats. Male and female rats were treated with the preparations at 1.0 mg/(kg d) (expressed as arginine HCl) from 8 to 12 and 12 to 18 weeks of age, respectively. Plasma silicon concentrations were measured at the end of the treatment period. Both male and female rats given Nitrosigine had markedly higher plasma silicon concentrations than the untreated controls or the arginine HCl–treated rats ($p<0.001$ vs. control) (Proctor, 2007). In a separate preclinical study, arginine and silicon absorption was compared in rats treated with Nitrosigine versus equivalent doses of the separate components of the complex (arginine hydrochloride, silicon, and inositol (A+S+I)) for 29 days. After 29 days, serum and joint tissue levels of arginine and silicon were significantly higher in the Nitrosigine group compared to the A+S+I group ($p<0.05$) (Sandler, 2016; Sahin, 2019). These results were further supported by an open-label, pharmacokinetics/pharmacodynamics/safety clinical trial where ten men took 1,500 mg of Nitrosigine for 14 days. Serum silicon levels were measured pre-dose and 0.5 hours, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, and 6 hours post-dose on Day 1 and Day 14. Day 1 silicon levels increased at 1 hour and 1.5 hours ($p<0.05$). Day 14 silicon levels increased at 1 hour, 1.5 hours, 2 hours, and 3 hours ($p<0.01$) (Kalman, 2015).

Substantiation:

1. Proctor 2007 Page 1321: Plasma silicon concentrations (Fig. 3) were measured in the rats at the end of the treatment periods as an index of the efficacy of absorption of the arginine silicate inositol complex. The arginine silicate–treated rats of both sexes had markedly higher plasma silicon concentrations than the untreated controls or the arginine HCl–treated rats. The +/- rats (both male and female) had lower silicon concentrations than the *cp*/*cp* control animals ($P < .001$). The apparently lower silicon concentrations of the *cp*/*cp* female rats compared with the male rats were not significant ($P = .057$).
2. Sandler 2016 Abstract: Serum arginine, serum silicon, joint tissue arginine and joint tissue silicon were all significantly greater in the ASI group (IV) compared to the control groups (I and II) and the A+S+I group (III) ($p<0.05$; Table 1). ASI supplementation significantly increased arginine and silicon bioavailability not only in the blood, but also in joint tissue, to a greater degree than the supplementation of equivalent doses of arginine, silicon and inositol as individual components. This preclinical study showed that ASI is a more bioavailable source of arginine and silicon than the individual components, supporting its position as an active sports nutrition ingredient enhancing energy, focus and markers of muscle recovery. These results showing increased absorption of arginine and silicon from

ASI, help explain and support the enhanced efficacy of ASI in sports and athletic applications.

3. Sahin 2019 Page 245: As Table 4 clearly defines, in collagen-induced arthritic rats, the serum and joint tissue arginine and silicon levels were significantly reduced when compared to other treatment groups ($P < 0.001$). Treatment either with the ASI complex or A+S+I significantly in- creased the concentrations of arginine and silicon in serum and joint tissue ($P < 0.05$ for both). Interestingly, when animals were treated with the ASI complex, a statistically significant increase in serum concentrations of arginine and silicon was found relative to the effects of A+S+I on these parameters. These results show that the ASI complex significantly increased blood serum levels of arginine and silicon compared to equivalent doses of ASI's components individually.
4. Kalman 2015 Page 105 Figures 1 and 2. Results pages 106-107.

Substantiation References:

1. Proctor SD, 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):1318-1325.
2. Sandler D, Perez Ojalvo S, Komorowski J. Absorption of bonded arginine silicate compared to individual arginine and silicon components. *JISSN*. 2016;13(Suppl 1):P19.
3. Sahin K, Perez Ojalvo S, Akdemir F, Orhan C, Tuzcu M, Sahin N, Ozercan IH, Sylla S, Koca SS, Yilmaz I, Komorowski JR. Effect of inositol -stabilized arginine silicate on arthritis in a rat model. *Food Chem Toxicol*. 2019;125:242-251.
4. 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 Pharm: Adv and Applica.* 2015;7:103-109.

Nitrosigine Arginase Claims

Claims: **Decreases blood levels of arginase, an enzyme that breaks down arginine. Nitrosigine inhibits the metabolic pathway that breaks down arginine.**

Arginase is an enzyme that regulates nitric oxide production. By breaking down arginine into its metabolites, arginine cannot be converted into nitric oxide (Durante, 2007). Nitrosigine is hypothesized to be a highly bioavailable form of arginine due to its ability to inhibit arginase. In a double-blind, active-controlled, crossover pharmacokinetic study where subjects were supplemented with Nitrosigine and ArgHCl for 15 days, blood samples were collected pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours post-dose for plasma arginine and arginase measurements. After 15 days of supplementation, arginase levels were significantly different between the Nitrosigine and ArgHCl groups ($p<0.05$). At multiple time points after supplementation, arginase levels were reduced by Nitrosigine, whereas there were no changes detected after ArgHCl supplementation. Peaks of arginine absorption corresponded to timepoints of low arginase levels (Komorowski, 2018; Komorowski, 2016).

Substantiation:

1. Durante 2007 Summary: Arginase is the focal enzyme of the urea cycle hydrolyzing L-arginine to urea and L-ornithine. Emerging studies have identified arginase in the vasculature and have implicated this enzyme in the regulation of NO synthesis and the development of vascular disease. Arginase inhibits the production of nitric oxide (NO) via several potential mechanisms, including competition with NO synthase (NOS) for the substrate L-arginine.
2. Komorowski 2018 Abstract Results: Study results show that after 15 days of supplementation, arginase levels were significantly different between the ASI and ArgHCl groups ($p<0.05$). At multiple time points after supplementation, arginase levels were reduced by ASI, whereas there were no changes detected after ArgHCl supplementation.
3. Komorowski 2016 Abstract Conclusion: This study showed that ASI supplementation significantly increased plasma arginine levels at multiple time-points up to six hours post-dose, while ArgHCl supplementation did so for only one hour. This study showed that ASI is a more bioavailable, and less variable, source of arginine.

Substantiation References:

1. Durante W, Johnson FK, Johnson RA. Arginase: a critical regulator of nitric oxide synthesis and vascular function. *Clin Exp Pharmacol Physiol*. 2007;34(9):906–911.
2. 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. *Current Developments in Nutrition*. 2018;2(11):P08-054.
3. 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(Suppl 1):690.17.

Nitrosigine Arginase Claims

Claim: **Increases arginine bioavailability through arginase inhibition.**

Arginase is an enzyme that regulates nitric oxide production. By breaking down arginine into its metabolites, arginine cannot be converted into nitric oxide (Durante, 2007). Nitrosigine is hypothesized to be a highly bioavailable form of arginine due to its ability to inhibit arginase. In a double-blind, active-controlled, crossover pharmacokinetic study where subjects were supplemented with Nitrosigine and ArgHCl for 15 days, blood samples were collected pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours post-dose for plasma arginine and arginase measurements. After 15 days of supplementation, arginase levels were significantly different between the Nitrosigine and ArgHCl groups ($p<0.05$). At multiple time points after supplementation, arginase levels were reduced by Nitrosigine, whereas there were no changes detected after ArgHCl supplementation. Peaks of arginine absorption corresponded to timepoints of low arginase levels (Komorowski, 2018; Komorowski, 2016).

Substantiation:

1. Durante 2007 Summary: Arginase is the focal enzyme of the urea cycle hydrolyzing L-arginine to urea and L-ornithine. Emerging studies have identified arginase in the vasculature and have implicated this enzyme in the regulation of NO synthesis and the development of vascular disease. Arginase inhibits the production of nitric oxide (NO) via several potential mechanisms, including competition with NO synthase (NOS) for the substrate L-arginine.
2. Komorowski 2018 Abstract Results: Study results show that after 15 days of supplementation, arginase levels were significantly different between the ASI and ArgHCl groups ($p<0.05$). At multiple time points after supplementation, arginase levels were reduced by ASI, whereas there were no changes detected after ArgHCl supplementation.
3. Komorowski 2016 Abstract Conclusion: This study showed that ASI supplementation significantly increased plasma arginine levels at multiple time-points up to six hours post-dose, while ArgHCl supplementation did so for only one hour. This study showed that ASI is a more bioavailable, and less variable, source of arginine.

Substantiation References:

1. Durante W, Johnson FK, Johnson RA. Arginase: a critical regulator of nitric oxide synthesis and vascular function. *Clin Exp Pharmacol Physiol*. 2007;34(9):906–911.
2. 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. *Current Developments in Nutrition*. 2018;2(11):P08-054.
3. 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(Suppl 1):690.17.

Nitrosigine Blood Protein Claims

Claim: **Statistically significant increases in levels of Kallistatin, a blood protein known as a potent vasodilator.†**
†Results compared to baseline.

In an open-label, pharmacokinetics/pharmacodynamics/safety clinical trial, ten men took 1,500 mg of Nitrosigine for 14 days. In order to evaluate proteomic changes due to Nitrosigine, blood samples were taken pre-dose, 6-hours after single dose, and after 14 days of Nitrosigine administration. Plasma proteins were quantified by mass spectrometry-based multiple reaction monitoring. Results showed significant changes in plasma Kallistatin levels compared to baseline after 6 hours (+27%) and after 14 days (+32%) (Komorowski, 2015). Kallistatin is a blood protein known as a potent vasodilator which is the dilation of blood vessels which leads to increased blood flow and delivery of oxygen and nutrients to working muscles (Chao, 1997).

Substantiation:
1. Komorowski 2015 Discussion: ASI supplementation significantly increased the protein Kallistatin ($p<0.01$).
2. Chao 1997: The tissue kallikrein-kinin system has been postulated to play an important role in blood pressure regulation. The best characterized function of tissue kallikrein is to generate kinin peptides from precursor kininogens by limited proteolysis. The actions of kinins, such as vasodilation, blood pressure reduction, smooth muscle contraction and relaxation, pain, and inflammation, are mediated by kinin receptors. Tissue kallikrein is synthesized as a proenzyme but is generally present in tissues and body fluids in an active form. The activity of tissue kallikrein is regulated by kallistatin, a newly identified tissue kallikrein-binding protein. Kallistatin is a potent vasodilator which may function directly through a vascular smooth muscle mechanism.

Substantiation Reference:

1. Komorowski J, Rood-Ojalvo S, El-Sohemy A. Arginine silicate supplementation decreases markers of cardiovascular, renal and metabolic dysfunction and increases markers of vasodilation and cardiovascular health in healthy adult males. *The FASEB Journal*. 2015;29:748.2.
2. Chao J, Stallone JN, Liang YM, Chen LM, Wang DZ, Chao L. Kallistatin is a potent new vasodilator. *J Clin Invest.* 1997;100(1):11-17.

Nitrosigine Blood Protein Claims

Claim: **Nitrosigine significantly enhances important blood proteins related to arterial strength and flexibility and overall cardiovascular health.†**
†Results compared to baseline.

In an open-label, pharmacokinetics/pharmacodynamics/safety clinical trial, ten men took 1,500 mg of Nitrosigine for 14 days. In order to evaluate proteomic changes due to Nitrosigine, blood samples were taken pre-dose, 6-hours after single dose, and after 14 days of Nitrosigine administration. Plasma proteins were quantified by mass spectrometry-based multiple reaction monitoring. Compared to baseline, the 6-hour post-dose protein analyses resulted in statistically significant changes in the levels of 73 out of 107 (68%) proteins. With 14 days of use, levels of 87 out of 107 (81%) proteins changed significantly from pre-dose baseline. Proteins found to have the greatest percent increase were those associated with vasodilation and cardiovascular health. Proteins found to have the greatest percent decrease were those associated with cardiovascular, renal and metabolic dysfunction. Post-dose changes in blood arginine and silicon levels were significantly correlated with changes in proteins (Komorowski, 2015). These data show that Nitrosigine significantly enhances blood protein levels related to arterial strength and flexibility and overall cardiovascular health.

Substantiation:

1. Komorowski 2015 Discussion: ASI supplementation significantly increased the protein Kallistatin, a potent vasodilator, which has been shown to protect against oxidative stress and suppress cellular apoptosis. Vasodilation increases blood flow and vessel flexibility, a key factor in sports nutrition and bodybuilding. ASI treatment increased the levels of several cardiovascular-protective proteins including Paraoxanase 1 (PON1) and Paraoxanase 3 (PON3) (7, 1). These results suggest that ASI may have cardiovascular-disease-preventative effects through the up-regulation of paraoxanase levels. The levels of a variety of proteins involved in vascular wound-healing were notably increased both 6-hours post initial ASI dosing and after 14 days of ASI supplementation. These proteins included Fibrinogen Gamma Chain, Fibrinopeptide-A, Alpha-2-Antiplasmin and Fibrinogen Beta Chain. In combination with ASI's ability to provide bioavailable silicon, these results suggest that ASI may be efficacious in improving the speed and efficiency of healing vascular injuries. The protein levels affected most by ASI treatment at both 6-hours post-dose and after 14 days of supplementation were those of Cystatin-C and Alpha-2-HS-Glycoprotein, two known markers of cardiovascular, renal and metabolic dysfunction.

Substantiation Reference:

1. Komorowski J, Rood-Ojalvo S, El-Sohemy A. Arginine silicate supplementation decreases markers of cardiovascular, renal and metabolic dysfunction and increases markers of vasodilation and cardiovascular health in healthy adult males. *The FASEB Journal*. 2015;29:748.2.

Nitrosigine Preclinical Study Claims

Claim: **<u>The Nitrosigine complex was shown in preclinical studies to be absorbed efficiently, raising plasma arginine and silicon levels.</u>**

In a preclinical study, insulin-resistance rats were treated for 5 weeks with either Nitrosigine or arginine-HCl at 1.0 g/kg/day, which provided equivalent arginine doses per body weight. Study results showed that plasma arginine concentrations were higher in Nitrosigine treated rats compared to controls ($p<0.05$), while rats treated with arginine-HCl had non-significantly higher concentrations compared to controls ($p>0.05$) (Proctor, 2005). Another preclinical study compared the absorption of Nitrosigine and arginine-HCl (as a reference agent) in obese insulin-resistant male and female JCR:LA-cp rats. Male and female rats were treated with the preparations at 1.0 mg/(kg d) (expressed as arginine HCl) from 8 to 12 and 12 to 18 weeks of age, respectively. Plasma silicon and arginine concentrations were measured at the end of the treatment period. Both male and female rats given Nitrosigine had markedly higher plasma silicon concentrations than the untreated controls or the arginine HCl–treated rats ($p<0.001$ vs. control). Concentrations of arginine were significantly higher in rats treated with Nitrosigine, in both male and female groups, compared to control ($p<0.05$). There were smaller increases in the plasma arginine levels in rats treated with arginine HCl, with significance only seen in the female animals (Proctor, 2007). Furthermore, in an oral, single dose pharmacokinetic study male rats were supplemented with either a 1:1 molar combination of L-arginine and resveratrol, free arginine or Nitrosigine at doses equivalent to 500 mg L-arginine/kg body weight. Results showed that mean plasma arginine levels were the highest in the Nitrosigine group at all timepoints compared to the other arginine treatment groups. Comparative plasma concentrations of arginine (in µg/mL) demonstrated that C_{max}(µg/mL), AUC_{last} (hr*µg/mL), AUC_{inf} (hr*µg/mL), and MRT_{last} (hr) demonstrated significance at $p<0.05$ in comparing Nitrosigine with free arginine. AUC_{inf} for Nitrosigine increased by 209% over free arginine in plasma, while C_{max} for Nitrosigine increased by 135% over free arginine in plasma (Bartos, 2016). Finally, in another preclinical study, arginine and silicon absorption was measured in rats treated with Nitrosigine versus equivalent doses of the separate components of the complex (arginine hydrochloride, silicon, and inositol (A+S+I)) for 29 days. After 29 days, serum and joint tissue levels of arginine and silicon were significantly higher in the Nitrosigine group compared to the A+S+I group ($p<0.05$) (Sandler, 2016; Sahin, 2019). Results of these preclinical studies show that Nitrosigine significantly raises both plasma arginine and silicon levels, and is therefore a highly absorbed complex.

Substantiation:

1. Proctor 2005 Page 1928 Results: The plasma concentrations of arginine in untreated +/*?* And *cp/cp* control rats were not significantly different. Rats treated with the arginine-silicate complex had significantly high plasma concentrations of arginine than the *cp/cp* controls (1.45± 0.09 vs. 1.18±0.05 nmol/l, $p<0.05$), In contrast, rats treated with arginine-HCl had non-significantly higher concentrations (1.33±0.06 nmol/l, $p>0.05$), despite the equivalent dose of arginine for each treatment.

2. Proctor 2007 Page 1320-1321: The concentrations of arginine were significantly higher in the rats treated with arginine silicate, in both male and female groups, compared with those in the *cp/cp* control rats ($P < .05$). There were smaller increases in the plasma arginine levels in rats treated with arginine HCl that were significantly only in the female animals ($P < .01$). Plasma silicon concentrations (Fig. 3) were measured in the rats at the end of the treatment periods as an index of the efficacy of absorption of the arginine silicate inositol complex. The arginine silicate–treated rats of both sexes had markedly higher plasma silicon concentrations than the untreated controls or the arginine HCl–treated rats. The +/- rats (both male and female) had lower silicon concentrations than the *cp*/*cp* control animals ($P < .001$). The apparently lower silicon concentrations of the *cp*/*cp* female rats compared with the male rats were not significant ($P = .057$).
3. Bartos 2016 Patent Application 0069: The results of the single dose oral pharmacokinetic study from testing in fasted male Sprague Dawley rates (equivalent dose of 500 mg/kg L-Arginine) for L-Arginine (free base), L Arginine HCl, ResArgin, and Arginine silicate inositol (ASI), measured in plasma cone of arginine (µg/ml) over time, are shown in Figure 1. Comparative plasma concentrations of arginine (in µg/mL) based on administration of the test compounds demonstrated that both AUCinf and Cmax were increased substantially for ASI compared with Arginine base, 3-fold and 2.35-fold, respectively. Stated in another way, AUCinf for ASI increased by 209% over Arginine base in plasma, while Cmax for ASI increased by 135% over Arginine base in plasma.
4. Sandler 2016 Abstract: Serum arginine, serum silicon, joint tissue arginine and joint tissue silicon were all significantly greater in the ASI group (IV) compared to the control groups (I and II) and the A+S+I group (III) ($p<0.05$; Table 1). ASI supplementation significantly increased arginine and silicon bioavailability not only in the blood, but also in joint tissue, to a greater degree than the supplementation of equivalent doses of arginine, silicon and inositol as individual components. This preclinical study showed that ASI is a more bioavailable source of arginine and silicon than the individual components, supporting its position as an active sports nutrition ingredient enhancing energy, focus and markers of muscle recovery. These results showing increased absorption of arginine and silicon from ASI, help explain and support the enhanced efficacy of ASI in sports and athletic applications.
5. Sahin 2019 Page 245: As Table 4 clearly defines, in collagen-induced arthritic rats, the serum and joint tissue arginine and silicon levels were significantly reduced when compared to other treatment groups ($P < 0.001$). Treatment either with the ASI complex or A+S+I significantly increased the concentrations of arginine and silicon in serum and joint tissue ($P < 0.05$ for both). Interestingly, when animals were treated with the ASI complex, a statistically significant increase in serum concentrations of arginine and silicon was found relative to the effects of A+S+I on these parameters. These results show that the ASI complex significantly increased blood serum levels of arginine and silicon compared to equivalent doses of ASI's components individually.

Substantiation References:

1. Proctor SD, Vine DF, 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;48:1925-1932.

2. Proctor SD, 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):1318-1325.
3. Bartos, JD. (2016). US Patent Application Publication No. US 2016/0081959 A1.
4. Sandler D, Perez Ojalvo S, Komorowski J. Absorption of bonded arginine silicate compared to individual arginine and silicon components. *JISSN*. 2016;13(Suppl 1):P19.
5. Sahin K, Perez Ojalvo S, Akdemir F, Orhan C, Tuzcu M, Sahin N, Ozercan IH, Sylla S, Koca SS, Yilmaz I, Komorowski JR. Effect of inositol -stabilized arginine silicate on arthritis in a rat model. *Food Chem Toxicol*. 2019;125:242-251.

Nitrosigine Preclinical Study Claims

Claim: **Nitrosigine was shown in preclinical studies to be better absorbed than arginine hydrochloride (HCl) and L-arginine.**

A preclinical study compared the absorption of Nitrosigine and arginine HCl in obese insulin-resistant male and female JCR:LA-cp rats. Male and female rats were treated with the preparations at 1.0 mg/(kg d) (expressed as arginine HCl) from 8 to 12 and 12 to 18 weeks of age, respectively. Concentrations of arginine were significantly higher in rats treated with Nitrosigine, in both male and female groups, compared to control ($p<0.05$). There were smaller increases in the plasma arginine levels in rats treated with arginine HCl, with significance only seen in the female animals (Proctor, 2007). In another preclinical study, insulin-resistance rats were treated for 5 weeks with either Nitrosigine or Arginine-HCl at 1.0 g/kg/day, which provided equivalent arginine doses per body weight. Study results showed that plasma arginine concentrations were higher in Nitrosigine treated rats compared to controls ($p<0.05$), while rats treated with arginine-HCl had non-significantly higher concentrations compared to controls ($p>0.05$) (Proctor, 2005). In a separate preclinical study, arginine and silicon absorption was compared in rats treated with Nitrosigine versus equivalent doses of the separate components of the complex (arginine hydrochloride, silicon, and inositol (A+S+I)) for 29 days. After 29 days, serum and joint tissue levels of arginine and silicon were significantly higher in the Nitrosigine group compared to the A+S+I group ($p<0.05$). These results demonstrate that the Nitrosigine complex is more bioavailable than a combination of the individual ingredients (Sandler, 2016; Sahin, 2019). Finally, in an oral, single dose pharmacokinetic study male rats were supplemented with either a 1:1 molar combination of L-arginine and resveratrol, free arginine or Nitrosigine in doses equivalent to 500 mg L-arginine/kg body weight. Results showed that mean plasma arginine levels were the highest in the Nitrosigine group at all timepoints compared to the other arginine treatment groups. Comparative plasma concentrations of arginine (in µg/mL) demonstrated that C_{max} (µg/mL), AUC_{last} (hr*µg/mL), AUC_{inf} (hr*µg/mL), and MRT_{last} (hr) demonstrated significance at $p<0.05$ in comparing Nitrosigine with free arginine. AUC_{inf} for Nitrosigine increased by 209% over free arginine in plasma, while C_{max} for Nitrosigine increased by 135% over free arginine in plasma (Bartos, 2016). The results of these three preclinical studies show that Nitrosigine is significantly better absorbed than arginine hydrochloride and free arginine (L-arginine).

Substantiation:

1. Proctor 2007 Page 1320: The concentrations of arginine were significantly higher in the rats treated with arginine silicate, in both male and female groups, compared with those in the *cp/cp* control rats ($P < .05$). There were smaller increases in the plasma arginine levels in rats treated with arginine HCl that were significantly only in the female animals ($P < .01$).
2. Proctor 2005 Page 1928 Results: The plasma concentrations of arginine in untreated +/*?* And *cp/cp* control rats were not significantly different. Rats treated with the arginine-silicate complex had significantly high plasma concentrations of arginine than the *cp/cp* controls (1.45± 0.09 vs. 1.18±0.05 nmol/l, $p<0.05$), In contrast, rats treated with arginine-

HCl had non-significantly higher concentrations (1.33±0.06 nmol/l, $p>0.05$), despite the equivalent dose of arginine for each treatment.

3. Sandler 2016 Abstract: Serum arginine, serum silicon, joint tissue arginine and joint tissue silicon were all significantly greater in the ASI group (IV) compared to the control groups (I and II) and the A+S+I group (III) ($p<0.05$; Table 1). ASI supplementation significantly increased arginine and silicon bioavailability not only in the blood, but also in joint tissue, to a greater degree than the supplementation of equivalent doses of arginine, silicon and inositol as individual components. This preclinical study showed that ASI is a more bioavailable source of arginine and silicon than the individual components, supporting its position as an active sports nutrition ingredient enhancing energy, focus and markers of muscle recovery. These results showing increased absorption of arginine and silicon from ASI, help explain and support the enhanced efficacy of ASI in sports and athletic applications.
4. Sahin 2019 Page 245: As Table 4 clearly defines, in collagen-induced arthritic rats, the serum and joint tissue arginine and silicon levels were significantly reduced when compared to other treatment groups ($P < 0.001$). Treatment either with the ASI complex or A+S+I significantly increased the concentrations of arginine and silicon in serum and joint tissue ($P < 0.05$ for both). Interestingly, when animals were treated with the ASI complex, a statistically significant increase in serum concentrations of arginine and silicon was found relative to the effects of A+S+I on these parameters. These results show that the ASI complex significantly increased blood serum levels of arginine and silicon compared to equivalent doses of ASI's components individually.
5. Bartos 2016 Patent Application 0069: The results of the single dose oral pharmacokinetic study from testing in fasted male Sprague Dawley rates (equivalent dose of 500 mg/kg L-Arginine) for L-Arginine (free base), L Arginine HCl, ResArgin, and Arginine silicate inositol (ASI), measured in plasma cone of arginine (µg/ml) over time, are shown in Figure 1. Comparative plasma concentrations of arginine (in µg/mL) based on administration of the test compounds demonstrated that both AUCinf and Cmax were increased substantially for ASI compared with Arginine base, 3-fold and 2.35-fold, respectively. Stated in another way, AUCinf for ASI increased by 209% over Arginine base in plasma, while Cmax for ASI increased by 135% over Arginine base in plasma.

Substantiation References:

1. Proctor SD, 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):1318-1325.
2. Proctor SD, Vine DF, 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;48:1925-1932.
3. Sandler D, Perez Ojalvo S, Komorowski J. Absorption of bonded arginine silicate compared to individual arginine and silicon components. *JISSN*. 2016;13(Suppl 1):P19.
4. Sahin K, Perez Ojalvo S, Akdemir F, Orhan C, Tuzcu M, Sahin N, Ozercan IH, Sylla S, Koca SS, Yilmaz I, Komorowski JR. Effect of inositol -stabilized arginine silicate on arthritis in a rat model. *Food Chem Toxicol*. 2019;125:242-251.
5. Bartos, JD. (2016). US Patent Application Publication No. US 2016/0081959 A1.

Nitrosigine Preclinical Study Claims

Claim: <u>Blood vessel relaxation was almost 5x greater than Arginine HCl in a preclinical study, showing blood flow benefits.</u>

In a preclinical study, insulin-resistance rats were treated for 5 weeks with either Nitrosigine or Arginine-HCl at 1.0 g/kg/day, which provided equivalent arginine doses per body weight. Vascular function was assessed at the end of the treatment period by measuring aortic ring contractile and relaxant responses. The relaxation responses of endothelium-intact aortic rings were assessed through dose-response curves after treatment with the endothelial-NO releasing agent, acetylcholine (Proctor, 2005). Compared to control, relaxant response was 9% greater in the Nitrosigine group and 2% greater in the Arginine-HCl group. Therefore, relaxant response in rats treated with Nitrosigine was almost 5x greater compared to rats treated with Arginine-HCl ($p<0.05$). These results show that Nitrosigine increases blood vessel relaxation, thereby promoting vasodilation, which is a critical component for enhanced blood flow.

Substantiation:

1. Proctor 2005 Page 1929 Table 2.

Substantiation Reference:

1. Proctor SD, Vine DF, 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;48:1925-1932.

Nitrosigine Preclinical Study Claims

Claim: <u>In a preclinical study, Nitrosigine decreased inflammation and improved joint health.</u>

A preclinical study was conducted to compare the effects of Nitrosigine to a combination of the individual ingredients of Nitrosigine (A+S+I) on inflammation and joint health in a collagen-induced arthritis (CIA) rat model. Animals were randomized into one of the following treatments: i) Control (no treatment); (ii) Arthritic control group, rats subjected to CIA induction by intradermal injection of bovine collagen type II (A), (iii) Arthritic group treated with equivalent doses of the separate component parts (arginine HCl, silicon, and inositol) of Nitrosigine by oral gavage for 29 days (A+S+I), iv) Arthritic group treated with Nitrosigine by oral gavage for 29 days (ASI). Samples of joint tissue were evaluated for arthritis severity and perisynovial tissue inflammation severity using histopathological scoring. After 29 days, arthritis and inflammation severity scores were lower in the Nitrosigine group compared to the A+S+I and arthritic control groups ($p<0.05$). CIA rats treated with Nitrosigine and A+S+I did not exhibit the chronic inflammation of synovial tissue seen in the arthritic control group, however Nitrosigine was more effective than A+S+I. There were lower levels of inflammatory markers in the serum and joint tissue of the Nitrosigine group compared to the A+S+I and arthritic control groups ($p<0.05$) (Sandler, 2016; Perez Ojalvo, 2017; Sahin, 2019). These results demonstrate that Nitrosigine is effective in reducing inflammation and improving joint health, and does so to a greater extent, than a combination of the individual ingredients of the complex.

Substantiation:

1. Sandler 2016 Abstract: Serum arginine, serum silicon, joint tissue arginine and joint tissue silicon were all significantly greater in the ASI group (IV) compared to the control groups (I and II) and the A+S+I group (III) ($p<0.05$; Table 1). ASI supplementation significantly increased arginine and silicon bioavailability not only in the blood, but also in joint tissue, to a greater degree than the supplementation of equivalent doses of arginine, silicon and inositol as individual components. This preclinical study showed that ASI is a more bioavailable source of arginine and silicon than the individual components, supporting its position as an active sports nutrition ingredient enhancing energy, focus and markers of muscle recovery. These results showing increased absorption of arginine and silicon from ASI, help explain and support the enhanced efficacy of ASI in sports and athletic applications.
2. Perez Ojalvo 2017 Abstract: Day 29 arthritis scores and inflammation scores were both significantly lower in the ASI group (IV) compared to the arthritic control group (II) and the A+S+I group (III). Seven inflammatory markers tested in the blood — TNF-α, IL-17, IL-6, ghrelin, obestatin, sclerostin, and DKK-1 — were significantly lower in the ASI group (IV) compared to the arthritic control group (II). Four of five inflammatory markers tested in the tissue — Cox-2, TNF-α, IL-6, NF-κB, and B-catenin — were significantly lower in the ASI group (IV) compared to the arthritic control group (II). ASI supplementation significantly improved markers of inflammation and overall arthritis and

inflammation score over the arthritic control group and the A+S+I group. These results demonstrate that ASI may be effective in reducing inflammation and that the ASI complex is more effective than a combination of the individual ingredients. ASI may also be of physiological benefit to athletes and fitness enthusiasts concerned with joint health and inflammation and to those experiencing joint pain due to inflammation.

3. Sahin 2019 Abstract: The purpose of this study was to test the effects of arginine-silicate-inositol complex (ASI), compared to a combination of the individual ingredients (A+S+I) of the ASI, on inflammatory markers and joint health in a collagen-induced arthritis (CIA) rat model. A total of 28 Wistar rats were divided into four groups: (i) Control; (ii) Arthritic group, rats subjected to CIA induction by injection of bovine collagen type II (A); (iii) Arthritic group treated with equivalent doses of the separate components of the ASI complex (arginine hydrochloride, silicon, and inositol) (A+S+I); (iv) Arthritic group treated with the ASI complex. The ASI complex treatment showed improved inflammation scores and markers over the arthritic control and the A+S+I group. ASI group had also greater levels of serum and joint-tissue arginine and silicon than the A+S+I group. Joint tissue IL-6, NF-κB, COX-2, TNF-α, p38 MAPK, WISP-1, and β-Catenin levels were lower in the ASI group compared to the other groups ($P < 0.05$ for all). In conclusion, these results demonstrate that the ASI complex may be effective in reducing markers of inflammation associated with joint health and that the ASI complex is more effective than a combination of the individual ingredients.

Substantiation References:

1. Sandler D, Perez Ojalvo S, Komorowski J. Absorption of bonded arginine silicate compared to individual arginine and silicon components. *JISSN*. 2016;13(Suppl 1):P19.
2. Perez Ojalvo S, Sahin K, Komorowski J. Effect of bonded arginine silicate on inflammatory markers and arthritis. *The FASEB Journal.* 2017;31(Suppl 1):166.5.
3. Sahin K, Perez Ojalvo S, Akdemir F, Orhan C, Tuzcu M, Sahin N, Ozercan IH, Sylla S, Koca SS, Yilmaz I, Komorowski JR. Effect of inositol -stabilized arginine silicate on arthritis in a rat model. *Food Chem Toxicol*. 2019;125:242-251.

Nitrosigine Nitric Oxide and Blood Flow Claims

Claims: **Nitrosigine increases nitric oxide levels.**
Significantly boosts nitric oxide levels, a key factor for increasing blood flow to working muscles.

In an open-label, pharmacokinetics/pharmacodynamics/safety clinical trial, ten men took 1,500 mg of Nitrosigine for 14 days. Saliva samples were collected prior to subjects taking the test product (for baseline assessment) and 0.5 hours, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, and 6 hours post-product administration for the assessment of salivary nitrite (for both PK test visits). Although not a significant difference, nitric oxide, as measured as salivary nitrate, increased in four subjects and stayed the same in six subjects at 0.5 hours after the first dose (p=0.125). After 14 days of use, baseline nitric oxide levels increased in six subjects and stayed the same in four subjects; this shift was significant (p=0.031) (Kalman, 2015). In a double-blind randomized, placebo controlled, crossover study twenty-one healthy, normotensive and physically active participants (male=15, female=6) reported for three trials each preceded by a 7-day washout period. Baseline flow mediated dilation (FMD) measurement was obtained for each visit, followed by consumption of one clinical dose of 8 g citrulline malate (CM), 1.5 g Nitrosigine, or 8 g dextrose (placebo). Acute Nitrosigine supplementation effected the nitric oxide producing capacity of endothelium dependent vasodilation as measured by a Dual Mode Doppler ultrasound resulting in a 31% increase in brachial artery diameter compared to a decrease of 2% during placebo trial. Results from this study indicate that 1.5g of Nitrosigine significantly increase vasodilation, blood flow and nitric oxide levels as measured by FMD (Rogers, 2020). In an *in vitro* study designed to compare the cellular production of NO (nitric oxide) of several sports nutrition ingredients including Nitrosigine, L-Arginine, L-Arginine AKG, L-Citrulline, L-Citrulline Malate and Agmatine Sulfate, Nitrosigine significantly enhanced NO levels compared to the other compounds and also versus control (Kalman, 2016). By increasing nitric oxide levels, Nitrosigine is thought to increase blood flow and nutrient delivery to working muscles.

Substantiation:

1. Kalman 2015 Page 107 second paragraph: After 14 days of use, baseline salivary nitrite levels increased in six subjects and stayed the same in four subjects; this shift was significant (p=0.031).
2. Rood-Ojalvo 2015 Blood flow, measured by blood velocity through the femoral artery using a Doppler Ultrasound, increased 59.9 cm/s in the ASI group ($p < 0.005$) and 49.9cm/s in the placebo group ($p < 0.005$) after exercise on Day 3; $p = 0.2$ between groups.
3. Rogers 2020 Nitrosigine (1.5 g) increased endothelial-dependent vasodilation as measured by a change in brachial artery flow mediated dilation as measured by a Dual Mode Doppler ultrasound resulting in a 31% increase in brachial artery diameter compared to a decrease of 2% during placebo trial.
4. Kalman 2016 JISSN Abstract: This *in vitro* study was designed to compare the cellular production of NO (nitric oxide) of several sports nutrition ingredients including ASI, L-Arginine, L-Arginine AKG, L-Citrulline, L-Citrulline Malate and Agmatine Sulfate. At the

doses used in this study, ASI significantly increased NO production over each of the five other compounds tested ($p<0.01$; Figure 1). There was a greater than 5X increase in NO production with ASI compared to the other tested sports nutrition ingredients. In addition, of the sports nutrition ingredients tested, only ASI significantly increased NO production versus control ($p<0.01$). In this *in vitro* study to evaluate NO production of a number of sports nutrition ingredients using the established Greiss assay to detect cellular NO production, ASI significantly enhanced NO levels compared to the other compounds and also versus control. Increasing NO can lead to substantial benefits for training athletes and fitness enthusiasts as it can result in enhanced delivery of oxygen and nutrients to working muscles, positively affecting workouts and recovery.

Substantiation 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. *Clinical Pharm: Adv and Applica.* 2015;7:103-109.
2. Rood-Ojalvo S, Sandler D, Veledar E, Komorowski J. The benefits of inositol-stabilized arginine silicate as a workout ingredient. *JISSN*. 2015;12(Suppl 1):P14.
3. Rogers J, Gills J, Gray M. Acute effects of Nitrosigine® and citrulline malate on vasodilation in young adults. *JISSN*. 2020;17:12.
4. Kalman D, Perez Ojalvo S, Komorowski J. Comparison of cellular nitric oxide production from various sports nutrition ingredients. *JISSN*. 2016;13(Suppl 1):P33.

Nitrosigine Nitric Oxide and Blood Flow Claims

Claim: <u>With continued use, nitric oxide levels increase over time.†</u>
<u>†Results compared to baseline.</u>

In an open-label, pharmacokinetics/pharmacodynamics/safety clinical trial, ten men took 1,500 mg of Nitrosigine for 14 days. Saliva samples were collected prior to subjects taking the test product (for baseline assessment) and 0.5 hours, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, and 6 hours post-product administration for the assessment of salivary nitrite (for both PK test visits). Although not a significant difference, nitric oxide, as measured as salivary nitrate, increased in four subjects and stayed the same in six subjects at 0.5 hours after the first dose (p=0.125). After 14 days of use, baseline nitric oxide levels increased in six subjects and stayed the same in four subjects; this shift was significant (p=0.031) (Kalman, 2014; Kalman, 2015). By increasing nitric oxide levels over time, Nitrosigine is thought to increase blood flow and nutrient delivery to working muscles.

Substantiation:
1. Kalman 2014: Whole abstract.
2. Kalman 2015 Page 106 last paragraph: Baseline plasma arginine was higher at Day 14 than Day 1, and the difference trended toward statistical significance. Additionally, 4-hour plasma arginine was significantly higher at Day 14 than Day 1. Page 107 Results section "Salivary Nitrite": After 14 days of use, baseline salivary nitrite levels increased in six subjects and stayed the same in four subjects; this shift was significant (p=0.031).

Substantiation 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. *The FASEB Journal.* 2014;28:LB 418.
2. 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 Pharm: Adv and Applica.* 2015;7:103-109.

Nitrosigine Nitric Oxide and Blood Flow Claims

Claim: <u>Unique, bonded complex of arginine and silicon helps enhance nitric oxide levels.</u>

In an open-label, pharmacokinetics/pharmacodynamics/safety clinical trial, ten men took 1,500 mg of Nitrosigine for 14 days. Saliva samples were collected prior to subjects taking the test product (for baseline assessment) and 0.5 hours, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, and 6 hours post-product administration for the assessment of salivary nitrite (for both PK test visits). Although not a significant difference, nitric oxide, as measured as salivary nitrate, increased in four subjects and stayed the same in six subjects at 0.5 hours after the first dose (p=0.125). After 14 days of use, baseline nitric oxide levels increased in six subjects and stayed the same in four subjects; this shift was significant (p=0.031) (Kalman, 2015). In an *in vitro* study designed to compare the cellular production of NO (nitric oxide) of several sports nutrition ingredients including Nitrosigine, L-Arginine, L-Arginine AKG, L-Citrulline, L-Citrulline Malate and Agmatine Sulfate, Nitrosigine significantly enhanced NO levels compared to the other compounds and also versus control (Kalman, 2016). In a double-blind randomized, placebo controlled, crossover study twenty-one healthy, normotensive and physically active participants (male=15, female=6) reported for three trials each preceded by a 7-day washout period. Baseline flow mediated dilation (FMD) measurement was obtained for each visit, followed by consumption of one clinical dose of 8 g citrulline malate (CM), 1.5 g Nitrosigine, or 8 g dextrose (placebo). Acute Nitrosigine supplementation effected the nitric oxide producing capacity of endothelium dependent vasodilation as measured by a Dual Mode Doppler ultrasound resulting in a 31% increase in brachial artery diameter compared to a decrease of 2% during placebo trial. Results from this study indicate that 1.5g of Nitrosigine significantly increase vasodilation, blood flow and nitric oxide levels as measured by FMD (Rogers, 2020).

Substantiation:

1. Kalman 2015 Page 107 second paragraph: After 14 days of use, baseline salivary nitrite levels increased in six subjects and stayed the same in four subjects; this shift was significant (p=0.031).
2. Kalman 2016 JISSN Abstract: This *in vitro* study was designed to compare the cellular production of NO (nitric oxide) of several sports nutrition ingredients including ASI, L-Arginine, L-Arginine AKG, L-Citrulline, L-Citrulline Malate and Agmatine Sulfate. At the doses used in this study, ASI significantly increased NO production over each of the five other compounds tested (p<0.01; Figure 1). There was a greater than 5X increase in NO production with ASI compared to the other tested sports nutrition ingredients. In addition, of the sports nutrition ingredients tested, only ASI significantly increased NO production versus control (p<0.01). In this *in vitro* study to evaluate NO production of a number of sports nutrition ingredients using the established Greiss assay to detect cellular NO production, ASI significantly enhanced NO levels compared to the other compounds and also versus control. Increasing NO can lead to substantial benefits for training athletes and fitness enthusiasts as it can result in enhanced delivery of oxygen and nutrients to working muscles, positively affecting workouts and recovery.

3. Rogers 2020 Nitrosigine (1.5 g) increased endothelial-dependent vasodilation as measured by a change in brachial artery flow mediated dilation as measured by a Dual Mode Doppler ultrasound resulting in a 31% increase in brachial artery diameter compared to a decrease of 2% during placebo trial.

Substantiation 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. *Clinical Pharm: Adv and Applica.* 2015;7:103-109.
2. Kalman D, Perez Ojalvo S, Komorowski J. Comparison of cellular nitric oxide production from various sports nutrition ingredients. *JISSN*. 2016;13(Suppl 1):P33.
3. Rogers J, Gills J, Gray M. Acute effects of Nitrosigine® and citrulline malate on vasodilation in young adults. *JISSN*. 2020;17:12.

Nitrosigine Nitric Oxide and Blood Flow Claims

Claim: <u>Nitrosigine significantly increases vasodilation and blood flow.</u>

In a double-blind randomized, placebo controlled, crossover study twenty-one healthy, normotensive and physically active participants (male=15, female=6) reported for three trials each preceded by a 7-day washout period. Baseline flow mediated dilation (FMD) measurement was obtained for each visit, followed by consumption of one clinical dose of 8 g citrulline malate (CM), 1.5 g Nitrosigine, or 8 g dextrose (placebo). Acute Nitrosigine supplementation effected the nitric oxide producing capacity of endothelium dependent vasodilation as measured by a Dual Mode Doppler ultrasound resulting in a 31% increase in brachial artery diameter compared to a decrease of 2% during placebo trial. Results from this study indicate that 1.5g of Nitrosigine significantly increase vasodilation as measured by FMD (Rogers, 2020). Increased vasodilation leads to an increase in skeletal muscle blood flow.

Substantiation:

1. Rogers 2020 Nitrosigine (1.5 g) increased endothelial-dependent vasodilation as measured by a change in flow mediated dilation by 31% increase in brachial artery diameter compared to a decrease of 2% during placebo trial. (P=0.001). Increased vasodilation leads to an increase in skeletal muscle blood flow.

Substantiation Reference:

1. Rogers J, Gills J, Gray M. Acute effects of Nitrosigine® and citrulline malate on vasodilation in young adults. JISSN. 2020;17:12.

Nitrosigine Nitric Oxide and Blood Flow Claims

Claims: **Nitrosigine (1.5 g) significantly increases vasodilation and blood flow to the same extent as 8 g of citrulline malate.**
Nitrosigine only requires 1/5th the dose of citrulline malate to achieve the same degree of vasodilation and blood flow increase.

In a double-blind randomized, placebo controlled, crossover study twenty-one healthy, normotensive and physically active participants (male=15, female=6) reported for three trials each preceded by a 7-day washout period. Baseline flow mediated dilation (FMD) measurement was obtained for each visit, followed by consumption of one clinical dose of 8 g Citrulline Malate (CM), 1.5 g Nitrosigine, or 8 g dextrose (placebo). Analysis showed that Nitrosigine and CM supplementation resulted in significantly greater increases in FMD% (31% and 34%) when compared to placebo trial (2% decrease); however, Nitrosigine was given at 1/5th the dose of CM while achieving the same degree of vasodilation and blow flow increase. After allometric scaling of FDM in the brachial artery, Nitrosigine (23%) and CM (25%) generated significantly greater allometric scaling FMD values when compared to the placebo trial (0.60%) (Rogers, 2020).

Substantiation:

1. Rogers 2020 Nitrosigine (1.5 g) only requires 1/5th the dose of Citrulline Malate (8 g) to achieve the same degree of vasodilation and blood flow increase (31% and 34%) (P=0.001). This is a 5x difference in dose range between the supplement groups. Nitrosigine significantly increases both vasodilation and blood flow in the brachial artery post allometric scaling of 23% compared to the placebo trail (0.60%).

Substantiation Reference:

1. Rogers J, Gills J, Gray M. Acute effects of Nitrosigine® and citrulline malate on vasodilation in young adults. JISSN. 2020;17:12.

Nitrosigine Energy Claims

Claim: <u>Nitrosigine significantly increases perceived energy levels, quickly and safely.</u>

In a double-blind, placebo-controlled, crossover study, 16 male subjects consumed 1,500 mg of Nitrosigine daily for 4 days, and perceived energy levels were measured at baseline and after 24, 48, and 72 hours. The Profile of Mood States (POMS) scale was used to assess energy levels. The POMS is a self-report questionnaire used to assess six subscales of mood including tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment (Gibson, 1997). Perceived energy, measured using the POMS vigor-activity sub-scores, significantly increased after 72 hours compared to placebo ($p = 0.039$) (Rood-Ojalvo, 2015). Changes in heart rate and blood pressure were also measured from pre-dose to approximately 25 minutes post-dose on Day 1 and from pre-dose Day 1 to pre-dose Day 4. Results showed that while energy levels increased in the Nitrosigine group, there were no significant changes in heart rate or systolic and diastolic blood pressure versus placebo at any measured time point (Sylla, 2018). These data support the use of Nitrosigine as a safe energy source.

Substantiation:

1. Gibson 1997 Introduction: Of the more comprehensive inventories, the Profile of Mood States (POMS) questionnaire has become one of the most popular and widely used. A number of factor analytic studies during the development of the questionnaire have suggested six primary dimensions of mood: Depression-dejection, Tension-anxiety, Anger-hostility, Vigor-activity, Fatigue-inertia, and Confusion-bewilderment. This instrument is able to monitor several different dimensions of mood simultaneously, including feelings of depression, anxiety, and anger as well as more positive aspects of mood such as vigor and friendliness.
2. Rood-Ojalvo 2015 Profile of Mood States (POMS) Results: ASI increased energy levels compared to placebo ~15 minutes after the first dose on Day 1 (+0.6 vs. -0.8; p=0.08) and significantly compared to placebo ~15 minutes after the dose on Day 4 (+1.5 vs. -1.8; p=0.012). Summary section: ASI supplementation significantly increased POMS vigor-activity scores after 72 hours compared to placebo. These results show that ASI increases energy compared to placebo after 3 days of supplementation.
3. Sylla 2018 Abstract Conclusion: In conclusion, currently available data show that ASI intake at daily doses of 1500mg does not induce elevations in heart rate or blood pressure. While intake of other known energy-stimulating ingredients can increase heart rate and blood pressure even after a single dose, ASI intake did not change heart rate or blood pressure after both short term (after 25 minutes) and longer term (after 14 days) use. Overall, clinical and preclinical data suggest that ASI (Nitrosigine) can be safely used as a non-stimulant ingredient to increase physical energy and cognitive function without adverse cardiovascular effects.

Substantiation References:

1. Gibson J. The Measurement of Mood States in Older Adults. *J Gerontol B Psychol Sci Soc Sci.* 1997;52B(4):I67-I74.
2. Rood-Ojalvo S, Sandler D, Veledar E, Komorowski J. The benefits of inositol-stabilized arginine silicate as a workout ingredient. *JISSN*. 2015;12(Suppl 1):P14.
3. Sylla S, Perez Ojalvo S, Komorowski J. An evaluation of the effect of inositol-stabilized arginine silicate on heart rate and blood pressure. *The FASEB Journal*. 2018;32(Suppl 1):742.12.

Nitrosigine Cardiovascular Safety Claims

Claims: <u>Increases perceived energy without negatively affecting heart rate or blood pressure.</u>
<u>Nitrosigine is a non-stimulant source of perceived energy.</u>

In a double-blind, placebo-controlled, crossover study, 16 male subjects consumed 1,500 mg of Nitrosigine daily for 4 days, and perceived energy levels were measured at baseline and after 24, 48, and 72 hours. The Profile of Mood States (POMS) scale was used to assess energy levels. The POMS is a self-report questionnaire used to assess six subscales of mood including tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment (Gibson, 1997). Perceived energy, measured using the POMS vigor-activity sub-scores, significantly increased after 72 hours compared to placebo ($p = 0.039$) (Rood-Ojalvo, 2015). Changes in heart rate and blood pressure were also measured from pre-dose to approximately 25 minutes post-dose on Day 1 and from pre-dose Day 1 to pre-dose Day 4. Results showed that while energy levels increased in the Nitrosigine group, there were no significant changes in heart rate or systolic and diastolic blood pressure versus placebo at any measured time point (Sylla, 2018). These data support the use of Nitrosigine as a non-stimulant energy source.

Substantiation:

1. Gibson 1997 Introduction: Of the more comprehensive inventories, the Profile of Mood States (POMS) questionnaire has become one of the most popular and widely used. A number of factor analytic studies during the development of the questionnaire have suggested six primary dimensions of mood: Depression-dejection, Tension-anxiety, Anger-hostility, Vigor-activity, Fatigue-inertia, and Confusion-bewilderment. This instrument is able to monitor several different dimensions of mood simultaneously, including feelings of depression, anxiety, and anger as well as more positive aspects of mood such as vigor and friendliness.
2. Rood-Ojalvo 2015 Profile of Mood States (POMS) Results: ASI increased energy levels compared to placebo ~15 minutes after the first dose on Day 1 (+0.6 vs. -0.8; p=0.08) and significantly compared to placebo ~15 minutes after the dose on Day 4 (+1.5 vs. -1.8; p=0.012). Summary section: ASI supplementation significantly increased POMS vigor-activity scores after 72 hours compared to placebo. These results show that ASI increases energy compared to placebo after 3 days of supplementation.
3. Sylla 2018 Abstract Conclusion: In conclusion, currently available data show that ASI intake at daily doses of 1500mg does not induce elevations in heart rate or blood pressure. While intake of other known energy-stimulating ingredients can increase heart rate and blood pressure even after a single dose, ASI intake did not change heart rate or blood pressure after both short term (after 25 minutes) and longer term (after 14 days) use. Overall, clinical and preclinical data suggest that ASI (Nitrosigine) can be safely used as a non-stimulant ingredient to increase physical energy and cognitive function without adverse cardiovascular effects.

Substantiation References:

1. Gibson J. The Measurement of Mood States in Older Adults. *J Gerontol B Psychol Sci Soc Sci.* 1997;52B(4):I67-I74.
2. Rood-Ojalvo S, Sandler D, Veledar E, Komorowski J. The benefits of inositol-stabilized arginine silicate as a workout ingredient. *JISSN*. 2015;12(Suppl 1):P14.
3. Sylla S, Perez Ojalvo S, Komorowski J. An evaluation of the effect of inositol-stabilized arginine silicate on heart rate and blood pressure. *The FASEB Journal*. 2018;32(Suppl 1):742.12.

Nitrosigine Cognitive Function Claims

Claim: <u>Significantly increases cognitive function.</u>

Nitrosigine supplementation (1,500 mg/day) was tested in a double-blind, placebo-controlled, crossover study using the Trail Making Test (TMT, Parts A and B) to measure cognitive function. The TMT is a commonly used neuropsychological test that measures cognitive processing speed, visual attention, tasking switching, and executive functioning. The test consists of two parts, A and B. Each test is measured by the time to completion, with lower scores indicating greater performance. TMT A involves connecting an ascending sequence of 25 numbers, while TMT B involves connecting an alternating sequence of 25 numbers and letters. Part B is considered to be a test of higher-level cognitive skills, such as mental flexibility (Drane, 2002). After 4 days of supplementation, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) within a 15-minute period post-dose (Kalman, 2016; Harvey, 2015).

Substantiation:

1. Drane 2002 Introduction: The Trail Making Test (TMT) is a visuomotor speeded task that is used routinely in clinical evaluations and consists of two parts: TMT-A and TMT-B. Trail Making Test-A is a simple visual-scanning task that requires one to draw a line connecting consecutive numbers from 1 to 25. Trail Making Test-B adds a dimension of "cognitive flexibility" by requiring one to draw a line connecting numbers and letters in alternating sequence. The first TMT index score, subtracting TMT-A time from TMT-B time (B -A), is thought to provide a "pure measure of cognitive efficiency." The rationale behind this suggestion assumes that both measures are equivalent in their demands for simple sequencing, visual scanning, and psychomotor functioning, making the difference score between them a more direct measure of the added cognitive flexibility required by TMT-B.
2. Kalman 2016 Abstract: To evaluate potential enhancement of mental focus and clarity, ASI (1500 mg/day) was tested in two double-blind placebo-controlled crossover (DBPC-X) studies using the Trail Making Test (TMT, Parts A and B). In the two studies, healthy males took ASI for 14 and 3 days, respectively. In the first study, after 14 days of dosing, TMT B time decreased significantly from baseline (28% improvement, $p = 0.045$). In the second study evaluating shorter-term effects, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) in a 10-min period. After 3 days of dosing, TMT B time significantly decreased from baseline scores (35% improvement, $p < 0.001$). These findings show that ASI significantly improved the ability to perform complex cognitive tests requiring mental flexibility, processing speed and executive functioning. Results: In the second study (DBPC-X Study #2), approximately 10 min after taking the first dose, TMT B time (Figure 2) decreased by 17.6 s in the ASI group ($p = 0.001$) from a baseline time of 52.7 s (an effect size of $d = 0.80$), compared to a decrease of 4.9 s in the placebo group ($p = 0.384$). The change in TMT B time after 10 min was statistically significant between groups ($p = 0.024$).

3. Harvey 2015 Abstract: Faster times in TMT B are associated with enhanced visual search, speed of processing, mental flexibility, and executive functions under pressure. Approximately 10 minutes after taking the first dose, TMT B time decreased by 17.6 seconds in the ASI group (p=0.001) from a baseline time of 52.7 seconds (a 33% improvement), compared to a decrease of 4.9 seconds in the placebo group (p=0.384). The changes in TMT B times after 10 minutes were statistically significant between groups (p=0.024). After 3 days of dosing, TMT B time decreased 18.5 seconds compared to baseline (p<0.0005) a 35% improvement, whereas the placebo group decreased 5.1 seconds (p=0.517). These findings show that daily doses of ASI significantly improved TMT B times, with effects seen in as little as 10 minutes after dosing and continued improvement over 14 days. Improvement in TMT B test times preliminarily suggests improved complex processing speed in subjects treated with ASI.

Substantiation References:

1. Drane DL, Yuspeh RL, Huthwaite JS, Klingler LK. Demographic characteristics and normative observations for derived-trial making test indices. *Neuropsychiatry Neuropsychol Behav Neurol.* 2002;15(1):39-43.
2. 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):736.
3. Harvey P, Rood-Ojalvo S, Komorowski J. The effects of inositol-stabilized arginine silicate on cognitive function. *JACN.* 2015;34(6):544.

Nitrosigine Cognitive Function Claims

Claim: <u>Significantly increased mental acuity and focus within 15 minutes with a 33% improvement in a double-blind, placebo-controlled study.</u>

Nitrosigine supplementation (1,500 mg/day) was tested in a double-blind, placebo-controlled crossover study using the Trail Making Test (TMT, Parts A and B) to measure cognitive function. The TMT is a commonly used neuropsychological test that measures cognitive processing speed, visual attention, tasking switching, and executive functioning. The test consists of two parts, A and B. Each test is measured by the time to completion, with lower scores indicating greater performance. TMT A involves connecting an ascending sequence of 25 numbers, while TMT B involves connecting an alternating sequence of 25 numbers and letters. Part B is considered to be a test of higher-level cognitive skills, such as mental flexibility (Drane, 2002). After a single dose, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) within 15-minutes post-dose (Kalman, 2016; Harvey, 2015).

Substantiation:

1. Drane 2002 Introduction: The Trail Making Test (TMT) is a visuomotor speeded task that is used routinely in clinical evaluations and consists of two parts: TMT-A and TMT-B. Trail Making Test-A is a simple visual-scanning task that requires one to draw a line connecting consecutive numbers from 1 to 25. Trail Making Test-B adds a dimension of "cognitive flexibility" by requiring one to draw a line connecting numbers and letters in alternating sequence. The first TMT index score, subtracting TMT-A time from TMT-B time (B -A), is thought to provide a "pure measure of cognitive efficiency." The rationale behind this suggestion assumes that both measures are equivalent in their demands for simple sequencing, visual scanning, and psychomotor functioning, making the difference score between them a more direct measure of the added cognitive flexibility required by TMT-B.
2. Kalman 2016 Abstract: To evaluate potential enhancement of mental focus and clarity, ASI (1500 mg/day) was tested in two double-blind placebo-controlled crossover (DBPC-X) studies using the Trail Making Test (TMT, Parts A and B). In the two studies, healthy males took ASI for 14 and 3 days, respectively. In the first study, after 14 days of dosing, TMT B time decreased significantly from baseline (28% improvement, $p = 0.045$). In the second study evaluating shorter-term effects, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) in a 10-min period. After 3 days of dosing, TMT B time significantly decreased from baseline scores (35% improvement, $p < 0.001$). These findings show that ASI significantly improved the ability to perform complex cognitive tests requiring mental flexibility, processing speed and executive functioning. Results: In the second study (DBPC-X Study #2), approximately 10 min after taking the first dose, TMT B time (Figure 2) decreased by 17.6 s in the ASI group ($p = 0.001$) from a baseline time of 52.7 s (an effect size of $d = 0.80$), compared to a decrease of 4.9 s in the placebo group ($p = 0.384$). The change in TMT B time after 10 min was statistically significant between groups ($p = 0.024$).

3. Harvey 2015 Abstract: Faster times in TMT B are associated with enhanced visual search, speed of processing, mental flexibility, and executive functions under pressure. Approximately 10 minutes after taking the first dose, TMT B time decreased by 17.6 seconds in the ASI group (p=0.001) from a baseline time of 52.7 seconds (a 33% improvement), compared to a decrease of 4.9 seconds in the placebo group (p=0.384). The changes in TMT B times after 10 minutes were statistically significant between groups (p=0.024). After 3 days of dosing, TMT B time decreased 18.5 seconds compared to baseline (p<0.0005) a 35% improvement, whereas the placebo group decreased 5.1 seconds (p=0.517). These findings show that daily doses of ASI significantly improved TMT B times, with effects seen in as little as 10 minutes after dosing and continued improvement over 14 days. Improvement in TMT B test times preliminarily suggests improved complex processing speed in subjects treated with ASI.

Substantiation References:

1. Drane DL, Yuspeh RL, Huthwaite JS, Klingler LK. Demographic characteristics and normative observations for derived-trial making test indices. *Neuropsychiatry Neuropsychol Behav Neurol.* 2002;15(1):39-43.
2. 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):736.
3. Harvey P, Rood-Ojalvo S, Komorowski J. The effects of inositol-stabilized arginine silicate on cognitive function. *JACN.* 2015;34(6):544.

Nitrosigine Cognitive Function Claims

Claim: <u>Improved cognitive flexibility within 15 minutes by 41% vs. placebo after a single dose in a double-blind, placebo-controlled study.^</u>
<u>^In a post-hoc analysis.</u>

Nitrosigine supplementation (1,500 mg/day) was tested in a double-blind, placebo-controlled, crossover study using the Trail Making Test (TMT, Parts A and B) to measure cognitive function. The TMT is a commonly used neuropsychological test that measures cognitive processing speed, visual attention, tasking switching, and executive functioning. The test consists of two parts, A and B. Each test is measured by the time to completion, with lower scores indicating greater performance. TMT A involves connecting an ascending sequence of 25 numbers, while TMT B involves connecting an alternating sequence of 25 numbers and letters. While Part A of the TMT is a simpler test, Part B is a more complex test that requires strong executive functioning, including the ability to mentally switch between tasks. Cognitive flexibility, or the mental ability to switch between concepts, is important for task-switching. Therefore, the difference between TMT B and TMT A scores, the TMT B-A score, has been established to emphasize the complexity of TMT B and be a more direct measure of cognitive flexibility (Drane, 2002). After a single dose, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) within 15-minutes post-dose (Kalman, 2016). A reduction in the TMT B-A score demonstrates higher cognitive flexibility. Within 15 minutes after a single dose, the decrease in mean TMT B-A score from baseline was significantly greater in the Nitrosigine group (-14.4 sec; -45%) compared to placebo (-1.5 sec; -4%) ($p < 0.05$ between groups) (Kalman, 2018). When the placebo change in TMT B-A score of -4% is subtracted from the Nitrosigine change in TMT B-A score of -45%, there is a 41% difference between groups in TMT B-A, or cognitive flexibility, seen within 15 minutes in this clinical study.

Substantiation:

1. Drane 2002 Introduction: The Trail Making Test (TMT) is a visuomotor speeded task that is used routinely in clinical evaluations and consists of two parts: TMT-A and TMT-B. Trail Making Test-A is a simple visual-scanning task that requires one to draw a line connecting consecutive numbers from 1 to 25. Trail Making Test-B adds a dimension of "cognitive flexibility" by requiring one to draw a line connecting numbers and letters in alternating sequence. The first TMT index score, subtracting TMT-A time from TMT-B time (B -A), is thought to provide a "pure measure of cognitive efficiency." The rationale behind this suggestion assumes that both measures are equivalent in their demands for simple sequencing, visual scanning, and psychomotor functioning, making the difference score between them a more direct measure of the added cognitive flexibility required by TMT-B.
2. Kalman 2016 Abstract: To evaluate potential enhancement of mental focus and clarity, ASI (1500 mg/day) was tested in two double-blind placebo-controlled crossover (DBPC-X) studies using the Trail Making Test (TMT, Parts A and B). In the two studies, healthy males took ASI for 14 and 3 days, respectively. In the first study, after 14 days of dosing,

TMT B time decreased significantly from baseline (28% improvement, $p = 0.045$). In the second study evaluating shorter-term effects, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) in a 10-min period. After 3 days of dosing, TMT B time significantly decreased from baseline scores (35% improvement, $p < 0.001$). These findings show that ASI significantly improved the ability to perform complex cognitive tests requiring mental flexibility, processing speed and executive functioning. Results: In the second study (DBPC-X Study #2), approximately 10 min after taking the first dose, TMT B time (Figure 2) decreased by 17.6 s in the ASI group ($p = 0.001$) from a baseline time of 52.7 s (an effect size of $d = 0.80$), compared to a decrease of 4.9 s in the placebo group ($p = 0.384$). The change in TMT B time after 10 min was statistically significant between groups ($p = 0.024$).

3. Kalman 2018 Abstract Results and Conclusion: After a single dose, the decrease in the mean TMT B-A score from baseline was significantly greater in the ASI group (-14.4 sec; -45%) compared to placebo (-1.5 sec; -4%) (Figure 1) ($p < 0.05$ between groups). The results of this analysis show that ASI significantly improves TMT B-A scores, supporting the use of ASI to boost cognitive flexibility and improve athletic performance.

Substantiation References:

1. Drane DL, Yuspeh RL, Huthwaite JS, Klingler LK. Demographic characteristics and normative observations for derived-trial making test indices. *Neuropsychiatry Neuropsychol Behav Neurol.* 2002;15(1):39-43.
2. 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):736.
3. Kalman D, Hewlings S, Sylla S, Perez Ojalvo S, Komorowski J. An evaluation of the effects of inositol-stabilized arginine silicate (ASI; Nitrosigine®) on cognitive flexibility. *JISSN*. 2018;15(Suppl 1):A54.

Nitrosigine Cognitive Function Claims

Claim: <u>Improves mental flexibility^ and executive functioning 15 minutes after a single dose.</u>
<u>^In a post-hoc analysis.</u>

Nitrosigine supplementation (1,500 mg/day) was tested in a double-blind, placebo-controlled, crossover study using the Trail Making Test (TMT, Parts A and B) to measure cognitive function. The TMT is a commonly used neuropsychological test that measures cognitive processing speed, visual attention, tasking switching, and executive functioning. The test consists of two parts, A and B. Each test is measured by the time to completion, with lower scores indicating greater performance. TMT A involves connecting an ascending sequence of 25 numbers, while TMT B involves connecting an alternating sequence of 25 numbers and letters. While Part A of the TMT is a simpler test, Part B is a more complex test that requires strong executive functioning, including the ability to mentally switch between tasks. Cognitive flexibility, or the mental ability to switch between concepts, is important for task-switching. Therefore, the difference between TMT B and TMT A scores, the TMT B-A score, has been established to emphasize the complexity of TMT B and be a more direct measure of cognitive flexibility (Drane, 2002). After a single dose, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) within 15-minutes post-dose (Kalman, 2016). A reduction in the TMT B-A score demonstrates higher cognitive flexibility. Within 15 minutes after a single dose, the decrease in mean TMT B-A score from baseline, measuring cognitive flexibility, was significantly greater in the Nitrosigine group (-14.4 sec; -45%) compared to placebo (-1.5 sec; -4%) ($p < 0.05$ between groups) (Kalman, 2018).

Substantiation:

1. Drane 2002 Introduction: The Trail Making Test (TMT) is a visuomotor speeded task that is used routinely in clinical evaluations and consists of two parts: TMT-A and TMT-B. Trail Making Test-A is a simple visual-scanning task that requires one to draw a line connecting consecutive numbers from 1 to 25. Trail Making Test-B adds a dimension of "cognitive flexibility" by requiring one to draw a line connecting numbers and letters in alternating sequence. The first TMT index score, subtracting TMT-A time from TMT-B time (B -A), is thought to provide a "pure measure of cognitive efficiency." The rationale behind this suggestion assumes that both measures are equivalent in their demands for simple sequencing, visual scanning, and psychomotor functioning, making the difference score between them a more direct measure of the added cognitive flexibility required by TMT-B.
2. Kalman 2016 Abstract: To evaluate potential enhancement of mental focus and clarity, ASI (1500 mg/day) was tested in two double-blind placebo-controlled crossover (DBPC-X) studies using the Trail Making Test (TMT, Parts A and B). In the two studies, healthy males took ASI for 14 and 3 days, respectively. In the first study, after 14 days of dosing, TMT B time decreased significantly from baseline (28% improvement, $p = 0.045$). In the second study evaluating shorter-term effects, TMT B time decreased significantly compared to placebo (33% improvement, $p = 0.024$) in a 10-min period. After 3 days of

dosing, TMT B time significantly decreased from baseline scores (35% improvement, $p < 0.001$). These findings show that ASI significantly improved the ability to perform complex cognitive tests requiring mental flexibility, processing speed and executive functioning. Results: In the second study (DBPC-X Study #2), approximately 10 min after taking the first dose, TMT B time (Figure 2) decreased by 17.6 s in the ASI group ($p = 0.001$) from a baseline time of 52.7 s (an effect size of $d = 0.80$), compared to a decrease of 4.9 s in the placebo group ($p = 0.384$). The change in TMT B time after 10 min was statistically significant between groups ($p = 0.024$).

3. Kalman 2018 Abstract Results and Conclusion: After a single dose, the decrease in the mean TMT B-A score from baseline was significantly greater in the ASI group (-14.4 sec; -45%) compared to placebo (-1.5 sec; -4%) (Figure 1) ($p < 0.05$ between groups). The results of this analysis show that ASI significantly improves TMT B-A scores, supporting the use of ASI to boost cognitive flexibility and improve athletic performance.

Substantiation References:

1. Drane DL, Yuspeh RL, Huthwaite JS, Klingler LK. Demographic characteristics and normative observations for derived-trial making test indices. *Neuropsychiatry Neuropsychol Behav Neurol.* 2002;15(1):39-43.
2. 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):736.
3. Kalman D, Hewlings S, Sylla S, Perez Ojalvo S, Komorowski J. An evaluation of the effects of inositol-stabilized arginine silicate (ASI; Nitrosigine®) on cognitive flexibility. *JISSN*. 2018;15(Suppl 1):A54.

Nitrosigine Cognitive Function Claims

Claims: <u>Nitrosigine helps maintain cognitive function after intense exercise.</u>
<u>In a double-blind, placebo-controlled clinical study, Nitrosigine prevented the 51% decline in cognitive function caused by intense exercise.</u>

In a double-blind, placebo-controlled, crossover study, 24 male subjects took a single dose of Nitrosigine (1,500 mg) or placebo 30 minutes prior to a treadmill maximally Graded Exercise Test (mGXT). For the mGXT, participants wore a telemetric heart rate monitor and were fitted with a mouthpiece that directed all expired breath to a metabolic cart (CardioCoach^{TM} CO_2, KORR Medical Technologies Inc.) throughout the test and for 5 minutes following test cessation. Participants underwent a 5-min warm-up beginning at 2.5mph and increased speed to a self-paced walk/jog, a speed (X) they could maintain for 20 minutes. There were 6 stages of the test:

i. Stage 1 (minutes 0-2): Ran at speed X at a 1.5% elevation.
ii. Stage 2 (minutes 2-4): Ran at speed X at a 4.5% elevation.
iii. Stage 3 (minutes 4-6): Ran at speed X at a 7.5% elevation
iv. Stage 4 (minutes 6-8): Ran at speed X at a 10% elevation
v. Stage 5 (minuets 8-10): Ran at speed X + 0.5 at a 10% elevation
vi. Stage 6 (minutes 10-12): Ran at speed X + 1.0 at a 10% elevation

A Trail Making Test (TMT), composed of two parts (TMT A and TMT B) was used to measure cognitive function prior to dosing and immediately after exercise. The TMT is a commonly used neuropsychological test that measures cognitive processing speed, visual attention, tasking switching, and executive functioning. The test consists of two parts, A and B. Each test is measured by the time to completion, with lower scores indicating greater performance. TMT A involves connecting an ascending sequence of 25 numbers, while TMT B involves connecting an alternating sequence of 25 numbers and letters (Drane, 2002). Following strenuous exercise, time to complete TMT A and TMT B increased by a significant 51% and 11% respectively in the placebo group, while it decreased by 5% for TMT A and 7% for TMT B when participants consumed an acute dose of Nitrosigine. A single dose of Nitrosigine significantly improved cognitive function parameters of mental acuity, focus and processing speed after intense exercise, compared to placebo ($p \leq 0.05$). Nitrosigine therefore prevented the 51% decrease in TMT A performance seen in the placebo group following intense exercise as well as helping to maintain cognitive function after intense exercise (Evans, 2020).

Substantiation:

1. Drane 2002 Introduction: The Trail Making Test (TMT) is a visuomotor speeded task that is used routinely in clinical evaluations and consists of two parts: TMT-A and TMT-B. Trail Making Test-A is a simple visual-scanning task that requires one to draw a line connecting consecutive numbers from 1 to 25. Trail Making Test-B adds a dimension of "cognitive flexibility" by requiring one to draw a line connecting numbers and letters in alternating sequence. The first TMT index score, subtracting TMT-A time from TMT-

B time (B -A), is thought to provide a "pure measure of cognitive efficiency." The rationale behind this suggestion assumes that both measures are equivalent in their demands for simple sequencing, visual scanning, and psychomotor functioning, making the difference score between them a more direct measure of the added cognitive flexibility required by TMT-B.

2. Evans 2020: A single dose of ASI significantly improved cognitive function parameters of mental acuity, focus and processing speed after intense exercise, compared to placebo ($p \leq 0.05$). Following strenuous exercise, time to complete TMT-A and TMT-B increased by a significant 51% and 11% respectively in the placebo group, while it decreased by 5% for TMT-A and 7% for TMT-B when participants consumed an acute dose of ASI ($p \leq 0.05$; Figure 2,3). The results of this study showed that ASI prevents the decline in cognitive function seen following strenuous exercise. Acute consumption of ASI prevented the intense exercise induced cognitive function decline of 51% seen in the placebo. These results could be of interest to individuals looking to maintain a strong cognitive state after expending energy during intense athletics, as well as everyday life.

Substantiation References:

1. Drane DL, Yuspeh RL, Huthwaite JS, Klingler LK. Demographic characteristics and normative observations for derived-trial making test indices. *Neuropsychiatry Neuropsychol Behav Neurol.* 2002;15(1):39-43.
2. Evans M, McDonald A, Crowley D, Zakaria N, Guthrie N. Inositol-stabilized arginine silicate improves post-exercise cognitive function in recreationally active, healthy males: a randomized, double-blind, placebo-controlled crossover study. *JEN*. 2020;3(3):9.

Nitrosigine Muscle Damage and Recovery Claims

Claims: **Reduces markers of muscle damage from exercise, improving muscle recovery. Nitrosigine helps with muscle recovery after exercise.**

In a double-blind, placebo-controlled, crossover study, 16 male subjects consumed 1,500 mg of Nitrosigine daily for 4 days. Biomarkers of muscle recovery such as creatine kinase (CK) and lactate dehydrogenase (LDH) were measured at baseline (hour 0), hour 24, hour 48, and hour 72 following a leg-extension exercise protocol. CK levels significantly decreased in the Nitrosigine group at 24 ($p = 0.040$), 48 ($p = 0.017$) and 72 ($p = 0.034$) hours post exercise compared to the placebo group. Immediately post-exercise at the hour 0 visit, Nitrosigine supplementation led to 44% less muscle damage, measured by CK levels, than placebo ($p = 0.057$). LDH levels significantly increased from baseline immediately after exercise in the placebo group ($p = 0.015$), but not in the Nitrosigine group ($p = 0.366$); $p = 0.133$ between groups (Rood-Ojalvo, 2015). These results show that Nitrosigine reduces markers of muscle damage from exercise including CK and LDH, which in turn improves muscle recovery.

Substantiation:

1. Rood-Ojalvo 2015 Abstract: CK levels significantly decreased in the ASI group at 24 ($p = 0.040$), 48 ($p = 0.017$) and 72 ($p = 0.034$) hours post- exercise compared to the placebo group. Immediately post-exercise at the hour 0 visit, ASI supplementation led to 44% less muscle damage, measured by CK levels, than placebo ($p = 0.057$). LDH levels significantly increased from baseline immediately after exercise in the placebo group ($p = 0.015$), but not in the ASI group ($p = 0.366$); $p = 0.133$ between groups. Both primary and secondary endpoints show that daily doses of ASI prior to workout significantly decreased biomarkers of muscle damage immediately after a workout and during recovery. Results Section: Significant reduction in Creatine Kinase and the Lactate Dehydrogenase (CK and LDH, respectively) levels favoring the Nitrosigine group.

Substantiation Reference:

1. Rood-Ojalvo S, Sandler D, Veledar E, Komorowski J. The benefits of inositol-stabilized arginine silicate as a workout ingredient. *JISSN*. 2015;12(Suppl 1):P14.

Nitrosigine Muscle Pump and Volume Claims

Claim: **Boosts muscle pump, significant increases in muscle volume.†**
†Results compared to baseline.

In a double-blind, placebo-controlled, crossover study, 16 male subjects consumed 1,500 mg of Nitrosigine daily for 4 days. To assess hyperemia, or muscle pump (volume), the right upper thigh leg circumference was measured at baseline (hour 0), hour 24, hour 48, and hour 72 following a leg-extension exercise protocol. Hyperemia, measured using leg circumference, increased significantly in the Nitrosigine group by 1.8cm ($p = 0.001$) at 72 hours from pre-dose, compared to a non-significant increase in the placebo group by 0.8cm ($p = 0.091$); $p = 0.070$ between groups (Rood-Ojalvo, 2015). This data shows that Nitrosigine significantly increases muscle pump, or muscle volume, with exercise, while the placebo group did not.

Substantiation:

1. Rood-Ojalvo 2015 Results: Hyperemia, measured using leg circumference, increased significantly in the ASI group by 1.8cm ($p = 0.001$) at 72 hours from pre-dose, compared to a non-significant increase in the placebo group by 0.8cm ($p = 0.091$); $p = 0.070$ between groups.

Substantiation References:

1. Rood-Ojalvo S, Sandler D, Veledar E, Komorowski J. The benefits of inositol-stabilized arginine silicate as a workout ingredient. *JISSN*. 2015;12(Suppl 1):P14.

Nitrosigine Muscle Pump and Volume Claims

Claim: **Significant increases in blood flow to working muscles, increasing muscle pump.†**
†Results compared to baseline.

In a double-blind, placebo-controlled, crossover study, 16 male subjects consumed 1,500 mg of Nitrosigine daily for 4 days. To assess hyperemia, or muscle pump (volume), the right upper thigh leg circumference was measured at baseline (hour 0), hour 24, hour 48, and hour 72 following a leg-extension exercise protocol. Hyperemia, measured using leg circumference, increased significantly in the Nitrosigine group by 1.8cm ($p = 0.001$) at 72 hours from pre-dose, compared to a non-significant increase in the placebo group by 0.8cm ($p = 0.091$); $p = 0.070$ between groups (Rood-Ojalvo, 2015). This data shows that Nitrosigine significantly increases muscle pump, or muscle volume, with exercise, while the placebo group did not.

In a second and separate double-blind randomized, placebo controlled, crossover study twenty-one healthy, normotensive and physically active participants (male=15, female=6) reported for three trials each preceded by a 7-day washout period. Baseline brachial artery flow mediated dilation (FMD) measurement was obtained for each visit, followed by consumption of one clinical dose of 8 g citrulline malate (CM), 1.5 g Nitrosigine, or 8 g dextrose (placebo). Following a 60-minute digestion period, FMD was repeated. Repeated measures ANOVA yielded a significant supplement (3) x time (2) effect ($p>0.001$), such that Nitrosigine yielded a greater improvement in FMD response than placebo (31%) and after allometric scaling of the FMD values Nitrosigine increased by 23% (Rogers, 2020). Increased vasodilation and blood flow can lead to an increase in skeletal muscle blood flow resulting in increased muscle pump.

Substantiation:
1. Rood-Ojalvo 2015 Results: Hyperemia, measured using leg circumference, increased significantly in the ASI group by 1.8cm ($p = 0.001$) at 72 hours from pre-dose, compared to a non-significant increase in the placebo group by 0.8cm ($p = 0.091$); $p = 0.070$ between groups.
2. Rogers 2020 Nitrosigine (1.5 g) increased endothelial-dependent vasodilation as measured by a change in flow mediated dilation by 31% increase in brachial artery diameter compared to a decrease of 2% during placebo trial. ($P=0.001$). Increased vasodilation leads to an increase in skeletal muscle blood flow resulting in increased muscle pump.

Substantiation References:

1. Rood-Ojalvo S, Sandler D, Veledar E, Komorowski J. The benefits of inositol-stabilized arginine silicate as a workout ingredient. *JISSN*. 2015;12(Suppl 1):P14.
2. Rogers J, Gills J, Gray M. Acute effects of Nitrosigine® and citrulline malate on vasodilation in young adults. *JISSN*. 2020;17:12.

Nitrosigine CARSE Report References

Alvares TS, Meirelles CM, Bhambhani YN, Paschoalin VM, Gomes PS. L-arginine as a potential ergogenic aid in healthy subjects. *Sports Med*. 2011; 41(3):233-248.

Bartos, JD. (2016). US Patent Application Publication No. US 2016/0081959 A1.

Chao J, Stallone JN, Liang YM, Chen LM, Wang DZ, Chao L. Kallistatin is a potent new vasodilator. *J Clin Invest.* 1997;100(1):11-17.

Drane DL, Yuspeh RL, Huthwaite JS, Klingler LK. Demographic characteristics and normative observations for derived-trial making test indices. *Neuropsychiatry Neuropsychol Behav Neurol.* 2002;15(1):39-43.

Durante W, Johnson FK, Johnson RA. Arginase: a critical regulator of nitric oxide synthesis and vascular function. *Clin Exp Pharmacol Physiol*. 2007;34(9):906–911.

Evans M, McDonald A, Crowley D, Zakaria N, Guthrie N. Inositol-stabilized arginine silicate improves post-exercise cognitive function in recreationally active, healthy males: a randomized, double-blind, placebo-controlled crossover study. *JEN*. 2020;3(3):9.

Gibson J. The Measurement of Mood States in Older Adults. *J Gerontol B Psychol Sci Soc Sci.* 1997;52B(4):I67-I74.

Harvey P, Rood-Ojalvo S, Komorowski J. The effects of inositol-stabilized arginine silicate on cognitive function. *JACN.* 2015;34(6):544.

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 Pharm: Adv and Applica.* 2015;7:103-109.

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. *The FASEB Journal.* 2014;28:LB 418.

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):736.

Kalman D, Hewlings S, Sylla S, Perez Ojalvo S, Komorowski J. An evaluation of the effects of inositol-stabilized arginine silicate (ASI; Nitrosigine®) on cognitive flexibility. *JISSN*. 2018;15(Suppl 1):A54.

Kalman D, Perez Ojalvo S, Komorowski J. Comparison of cellular nitric oxide production from various sports nutrition ingredients. *JISSN*. 2016;13(Suppl 1):P33.

Komorowski J, Rood-Ojalvo S, El-Sohemy A. Arginine silicate supplementation decreases markers of cardiovascular, renal and metabolic dysfunction and increases markers of vasodilation and cardiovascular health in healthy adult males. *The FASEB Journal*. 2015;29:748.2.

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(Suppl 1):690.17.

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. *Current Developments in Nutrition*. 2018;2(11):P08-054.

Perez Ojalvo S, Sahin K, Komorowski J. Effect of bonded arginine silicate on inflammatory markers and arthritis. *The FASEB Journal.* 2017;31(Suppl 1):166.5.

Proctor SD, Vine DF, 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;48:1925-1932.

Proctor SD, 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):1318-1325.

Rogers J, Gills J, Gray M. Acute effects of Nitrosigine® and citrulline malate on vasodilation in young adults. *JISSN*. 2020;17:12.

Rood-Ojalvo S, Sandler D, Veledar E, Komorowski J. The benefits of inositol-stabilized arginine silicate as a workout ingredient. *JISSN*. 2015;12(Suppl1):P14.

Sahin K, Perez Ojalvo S, Akdemir F, Orhan C, Tuzcu M, Sahin N, Ozercan IH, Sylla S, Koca SS, Yilmaz I, Komorowski JR. Effect of inositol -stabilized arginine silicate on arthritis in a rat model. *Food Chem Toxicol*. 2019;125:242-251.

Sandler D, Perez Ojalvo S, Komorowski J. Absorption of bonded arginine silicate compared to individual arginine and silicon components. *JISSN*. 2016;13(Suppl 1):P19.

Sylla S, Perez Ojalvo S, Komorowski J. An evaluation of the effect of inositol-stabilized arginine silicate on heart rate and blood pressure. *The FASEB Journal*. 2018;32(Suppl 1):742.12.