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The Effect of L-Arginine and Creatine on Vascular Function and Homocysteine Metabolism

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Abstract

Background—Studies with L-arginine supplementation have shown inconsistent effects on endothelial function. The generation of guanidinoacetate (GAA) from L-arginine with subsequent formation of creatine and homocysteine and consumption of methionine may reduce the pool of L-arginine available for nitric oxide generation. Experimental studies suggest that creatine supplementation might block this pathway. We sought to determine the effects of L-arginine, creatine, or the combination on endothelium-dependent vasodilation and homocysteine metabolism in patients with coronary artery disease.

Methods—Patients with coronary artery disease were randomized to L-arginine (9gm/day), creatine (21gm/day), L-arginine plus creatine, or placebo for 4 days (n=26–29/group). Brachial artery flow-mediated dilation and plasma levels of L-arginine, creatine, homocysteine, methionine, and GAA were measured at baseline and follow up.

Results—L-arginine and creatine supplementation had no effects on vascular function. L-arginine alone increased GAA (P<0.01) and the ratio of homocysteine to methionine (P<0.01) suggesting increased methylation demand. The combination of creatine and L-arginine did not suppress GAA production or prevent the increase in homocysteine-to-methionine ratio. Unexpectedly, creatine supplementation (alone or in combination with L-arginine) was associated with an 11 to 20% increase in homocysteine concentration (P<0.05), which was not attributable to worsened renal function, providing evidence against an effect of creatine on decreasing methylation demand.

Conclusion—The present study provides no evidence that L-arginine supplementation improves endothelial function and suggests that L-arginine may increase methylation demand. Creatine supplementation failed to alter the actions of L-arginine on vascular function or suppress methylation demand. The unexpected increase in homocysteine levels following creatine supplementation could have adverse effects and merits further study, since creatine is a commonly used dietary supplement.

Keywords

L-arginine; creatine; homocysteine; nitric oxide; methylation; endothelial function

Introduction

Nitric oxide (NO) generated by the endothelium serves as a critical regulator of vascular homeostasis by modulating vascular tone, platelet reactivity, and inflammation. NO is generated from its precursor amino acid L-arginine by the enzyme nitric oxide synthase (NOS).

¹ Impairment of the normal biologic activity of endothelium-derived NO contributes to the development of atherosclerosis and its ischemic manifestations.^{2–4}

Supplying additional L-arginine in atherosclerotic disorders may augment NO production and improve endothelial function. Beneficial effects of L-arginine on endothelium-dependent vasodilation have been shown in animal models and human subjects.^{5–8} Clinical studies with L-arginine have also suggested beneficial actions in clinical ischemia.⁷ In contrast, other studies have failed to demonstrate beneficial effects of L-arginine on endothelial function.^{9–11} In fact, one group reported a paradoxical finding of L-arginine negating the protective effects of inducible NOS gene deficiency in apolipoprotein E knockout mice – raising the possibility that L-arginine contributed to the progression of atherosclerosis.¹² Furthermore, concern for potential detrimental effects of L-arginine on mortality following myocardial infarction has been raised.¹³

A possible explanation for the inconsistent results of L-arginine supplementation may reside in its numerous metabolic fates. NO synthesis has been shown to reflect only a minority (~1%) of L-arginine flux.¹⁴ In contrast, a much greater proportion of L-arginine flux is attributable to synthesis of creatine by the enzyme L-arginine:glycine amidinotransferase (AGAT).¹⁵ Generation of creatine is estimated to consume ~70% of labile methyl groups, with S-adenosyl methionine (SAM) serving as the methyl donor (Figure 1).¹⁶ This methylation demand may reduce the available methyl groups required in numerous biological reactions. In fact, hypomethylation and aberrant methylation have been shown to have adverse consequences for endothelial cell function and growth.^{17–19} In the process of donating methyl groups to guanidinoacetate (GAA), SAM is metabolized to S-adenosyl homocysteine (SAH) and eventually homocysteine. Given the known adverse effects of homocysteine on endothelial function, L-arginine has the potential to paradoxically impair endothelial function.^{20–22}

Since creatine reduces the activity of AGAT by feedback inhibition, augmenting creatine concentrations has the potential to decrease conversion of L-arginine to creatine.^{23, 24} This effect would be expected to increase the availability of L-arginine for NO synthesis, reduce methylation demand, and decrease homocysteine levels.^{25–27} Thus, we sought to determine the effects of short-term L-arginine and creatine supplementation on endothelial function and homocysteine metabolism. Our hypothesis was that the combination of L-arginine and creatine would enhance endothelium-dependent vasodilation and reduce homocysteine levels.

Methods

Study Population

Subjects with proven coronary artery disease were recruited from the patient population at Boston Medical Center. Coronary artery disease was confirmed by coronary angiography or clinical history of myocardial infarct. Subjects were excluded for the following reasons: creatine or L-arginine use within 1 month, myocardial infarction or intervention within 2 weeks, or change in medication within 1 week. Since the placebo supplement contained lactose, potential subjects with milk allergy or lactose intolerance were also excluded. The study was approved by the Institutional Review Board of Boston University Medical Center. All participants gave written informed consent.

Baseline demographic variables, biochemical assessment, and vascular function testing were assessed during the first visit. Repeat biochemical assessment and vascular function testing was performed on the 4th day of taking the study supplementation.

Vascular Function Assessment

All cardiovascular medications were continued during the study, but subjects were required to be on stable doses of these agents for at least four weeks prior to the study. Prior to each visit, all vasoactive medications, including nitrates, calcium channel blockers, angiotensin converting enzyme inhibitors, beta-adrenergic blockers, and other vasodilators, were withheld for 24 hours. Patients were asked to fast and refrain from smoking (if applicable) overnight.

Endothelium-dependent, flow-mediated and endothelium-independent, nitroglycerin-induced dilation were assessed using high-resolution ultrasonography of the brachial artery.^{28,29} Briefly, the brachial artery was imaged at baseline for assessment of resting arterial diameter and flow. After a 5-minute cuff occlusion, brachial artery flow was measured during the hyperemic phase. Images of the brachial artery were obtained 1 minute after cuff release to measure diameter and calculate flow-mediated dilation (FMD). After a 10 minute rest period, images were recorded before and 4 minutes after administration of a sublingual nitroglycerin tablet (0.4 mg). Nitroglycerin was omitted if the subject declined, had a previous adverse reaction to nitroglycerin, had a history of migraine headaches, had a systolic blood pressure less than 100 mmHg, or used a type 5 phosphodiesterase inhibitor within 1 week of the study. All images were digitized online and analyzed offline using customized software (Medical ImagingApplications, Inc) in a blinded manner.

L-Arginine and Creatine Supplementation

Subjects were randomized in a double-blind fashion to one of four treatment groups: L-arginine (9 gm/day), creatine (21 gm/day), L-arginine (9 gm/day) plus creatine (21 gm/day), or placebo. The materials used in the study included General Nutrition Centers (GNC) A-Z Arginine 1000 mg tablets, Twinlab Creatine Fuel Powder (Micronized Formula), and gelatin capsules and/or lactose powder for placebo controls. The supplementation protocol was given for 3 days as follows: 7 grams of creatine three times per day (with the powder mixed in juice), 3 grams of L-arginine three times a day, and placebo tablets and/or powder three times a day, based upon the treatment assignment. The 4th day of supplementation consisted of 7 gm of creatine once, 9 gm of arginine once, both creatine and L-arginine, or placebo in the morning 2–3 hours prior to undergoing repeat biochemical and vascular function evaluations.

Biochemical Measurements

Plasma homocysteine, L-arginine, and creatine concentrations were measured using chemiluminescence, radioimmunoassay extraction, and enzymatic assay, respectively, via standard techniques adapted for automated clinical chemistry laboratory analyzers. Guanidinoacetate (GAA) and methionine were measured using tandem mass spectroscopy.³⁰ Cystatin C was measured by a particle enhanced immunonephelometric assay.³¹ Glomerular filtration rate was calculated at baseline using the Cockcroft-Gault formula and at both visits from the cystatin C values (using the formula $y=77.24x^{-1.2623}$).³²

Statistical Analysis

All data are expressed as mean $\pm$ standard deviation, except as otherwise noted. Baseline clinical, biochemical, and vascular imaging variables were compared either by chi squared testing for categorical variables or one-way analysis of variance (ANOVA) for continuous variables. Group differences in response to all supplementation regimens were compared using two-way repeated measures ANOVA with post hoc testing (using the Student Newman Keuls

method). Repeated analyses were also performed for primary outcomes including baseline variables with a trend towards a difference, as well as calculated glomerular filtration rate (by the Cockcroft-Gault formula), as covariates in separate repeat measures ANOVA. Analysis was completed using SPSS for Windows version 12.0.1 (SPSS Inc, Chicago, Illinois). Statistical significance was defined as a *P* level <0.05.

The study was designed to test the hypothesis that FMD would be increased to a greater extent following treatment with L-arginine and creatine compared to L-arginine alone, creatine alone, or placebo. The study had 80% power ($\alpha = 0.05$) to detect an improvement in FMD of 2.1 percent with a sample size of 25 subjects per group.

Results

Subject Characteristics

A total of 119 subjects were recruited and randomized in the study. Seven subjects withdrew from the study prior to their follow up assessment on the 4th day of supplementation, which left a total of 112 patients (26–29 per group) with repeat biochemical and vascular function assessment for analysis. Baseline characteristics, including demographics, medical conditions, medication usage, and laboratory data, in each of the four groups are shown in Table 1. There were no significant differences in these baseline characteristics among the groups. However, a possible trend towards a difference between groups was seen with tobacco use, previous CABG, previous stroke or carotid disease, and multivitamin or folate supplementation.

Supplementation

The current supplementation regimen with L-arginine and creatine was effective in increasing their respective levels (Table 2). There was a 2 to 2.5-fold increase in plasma L-arginine levels with L-arginine supplementation and 15 to 20-fold increase in plasma creatine levels with creatine supplementation, either used alone or in combination. Creatine supplementation had no significant effect on L-arginine levels and L-arginine supplementation had no significant effect on creatine levels. The increase in creatine and L-arginine remained significant after adjusting for each covariate (including tobacco use, previous CABG, previous stroke, and multivitamin or folate supplementation, as well as glomerular filtration rate).

Vascular Function Assessment

The effects of treatment on vascular function are displayed in Table 3. As shown, brachial artery diameter, flow, reactive hyperemia, flow-mediated dilation, and nitroglycerin-mediated dilation were similar among groups. There were no significant effects of treatment on any measure of vascular function, even after adjusting for covariates (including tobacco use, previous CABG, previous stroke, and multivitamin or folate supplementation, as well as glomerular filtration rate).

Biochemical Assessment

Table 2 displays the effects of the four treatments on intermediates related to the metabolism of arginine. L-arginine supplementation alone was associated with increased plasma GAA concentration ($P < 0.01$), due to L-arginine conversion to GAA by the enzyme AGAT. Homocysteine and methionine levels did not change following L-arginine supplementation; however, the ratio of homocysteine-to-methionine increased significantly ($P = 0.008$). Taken together, increased GAA levels and an increase in the ratio of homocysteine-to-methionine suggest increased methylation demand and flux through this pathway (Figure 1).

Creatine supplementation alone was associated with increased creatinine levels ($P < 0.001$), which was expected since creatinine is formed by spontaneous cyclization of creatine.

Unexpectedly, creatine supplementation was associated with an increase in homocysteine concentration ($P=0.03$), arguing against our hypothesis that creatine supplementation would lead to a decrease in methylation demand (Figure 2). The increase in homocysteine concentration was unlikely to reflect a worsening of renal function following creatine supplementation, since cystatin C concentrations did not increase, and in fact, were significantly decreased following creatine supplementation ($P=0.03$). This was indicative of an increase in the calculated glomerular filtration rate or improvement in renal function. We anticipated that creatine supplementation would also reduce plasma GAA levels by decreasing the activity of AGAT, but, instead, observed an increase ($P=0.03$).

Treatment with the combination of L-arginine and creatine, was associated with the expected increases in GAA ($P<0.01$) and creatinine ($P<0.001$). As with L-arginine alone, combination treatment was associated with an increase in the ratio of homocysteine-to-methionine ($P<0.001$). In addition, the combination of L-arginine and creatine increased homocysteine levels ($P<0.05$) and decreased methionine levels ($P<0.05$), providing evidence against our hypothesis that supplemental creatine would decrease flux through this pathway. There was an unexpected increase in cystatin C levels with a reduction in calculated glomerular filtration rate with the combination of L-arginine and creatine ($P<0.05$).

The effect of time and treatment on homocysteine, homocysteine-to-methionine ratio, and GAA and cystatin C levels remained significant after adjusting for each covariate (including tobacco use, previous CABG, previous stroke, and multivitamin or folate supplementation, as well as glomerular filtration rate) in separate analyses.

Discussion

The present study demonstrated no significant effect of L-arginine supplementation on brachial artery flow-mediated dilation in patients with coronary artery disease. There also were no significant effects on baseline brachial artery diameter, flow, or extent of reactive hyperemia. The study had good statistical power to detect an effect on flow-mediated dilation comparable to that produced by other risk reducing interventions.³³ Creatine alone and creatine in combination with L-arginine also did not alter these measures of vascular function. Plasma analysis demonstrated the expected increases in arginine and creatine levels following supplementation. In addition, there was evidence of increased methylation demand following L-arginine supplementation with increased levels of GAA and an increase in the ratio of homocysteine-to-methionine that were not blunted by creatine supplementation. Unexpectedly, creatine supplementation was associated with an increase in plasma homocysteine levels that was not attributable to alterations in renal function.

The lack of an effect of L-arginine supplementation on endothelial function is consistent with the findings reported by some investigators,^{10, 11} but contrasts with others.^{5–9, 34} For example, hypercholesterolemic adults given L-arginine supplementation showed an improvement in endothelium dependent dilation.³⁴ By contrast, L-arginine supplementation for six months in patients with peripheral arterial disease reduced nitric oxide availability and endothelium-dependent vascular function compared to placebo.¹¹ Furthermore, a clinical trial found no improvement in vascular stiffness, and a possible association with higher post-infarction mortality.¹³

There are a number of potential explanations to account for the failure of L-arginine supplementation to improve NO-mediated vasodilation. The present study focused on the possibility that metabolism of L-arginine to GAA with subsequent formation of creatine and homocysteine might be playing a role in human subjects (Figure 1). Prior support for this possibility is provided by a study demonstrating that GAA supplementation increased

homocysteine concentration.²⁶ In the present study, we observed an increase in GAA levels with L-arginine supplementation, but did not find an increase in homocysteine levels. We did find, however, that homocysteine levels were elevated relative to its precursor substrate methionine, with an increase in the homocysteine to methionine ratio. This finding supports the concept that there is an increase in methylation demand (or a reduction in bioavailable methyl donors) with L-arginine supplementation.³⁵ Our current observation is in contrast with a decrease in homocysteine levels (and a decreased homocysteine to methionine ratio) seen following L-arginine supplementation in hypercholesterolemic subjects.³⁶

Consequences of increased methylation demand may have significant effects on vascular cell function.^{17–19} Several studies have proposed mechanisms through which hyperhomocysteinemia and a disturbance in methylation may contribute to cardiovascular disease.³⁷ Increased concentrations of homocysteine were shown to have inhibitory effects on cyclin A transcription and endothelial cell growth through hypomethylation related mechanisms.¹⁹ It was suggested that hyperhomocysteinemia induced endothelial NO system dysfunction may occur through aberrant methylation in the dimethylarginine dimethylaminohydrolase gene.¹⁸ Protein hypomethylation has also been shown to increase rates of endothelial cell apoptosis and premature senescence.¹⁷

The failure to detect an increase in plasma homocysteine levels, despite an apparent effect of L-arginine on its metabolism, may reflect its multiple metabolic pathways. Homocysteine may undergo remethylation to methionine in a folate-dependent process, catabolism to cysteine in the transsulfuration pathway, or export to the extracellular space (Figure 1).³⁸ Given these multiple fates of homocysteine, it may be difficult to detect a change in homocysteine levels with intact pathways and replete folate status to handle an increased load. Thus, it remains possible that L-arginine metabolism imposes a methylation demand that counterbalances the effects of NO generated despite the absence of a change in measured plasma homocysteine.

There are a number of other possible explanations to account for the lack of an effect of L-arginine on endothelial function. For example, it is conceivable that the dose and duration of L-arginine treatment were inadequate. These possibilities seem unlikely, since the dose was similar to prior studies, and we observed a 2-fold increase in plasma L-arginine levels. Although the duration of supplementation in this study was less than some other protocols,^{13, 34} beneficial effects have been reported after very short-term L-arginine administration.⁷ Another possibility is hydrolysis of L-arginine to L-ornithine and urea by the enzyme L-arginase. An increase in the activity of L-arginase may deplete intracellular L-arginine levels, and decrease NO production.³⁹ Similarly, an endogenous inhibitor of NOS, such as asymmetric dimethylarginine (ADMA), may prevent L-arginine augmentation in NO production.⁴⁰ It is recognized that medical conditions including coronary artery disease and renal impairment have been shown to have high levels of endogenous NOS inhibitors, including ADMA.^{41,42}

The subjects in our study were selected due to the presence of coronary artery disease; however, many of the subjects also had chronic kidney disease. This is evident by a mean glomerular filtration rate of 68.7 ± 27.7 mL/min (by the Cockcroft-Gault formula) -consistent with stage 2 disease by the National Kidney Foundation-Dialysis Outcomes Quality Initiative Clinical Practice Guidelines.⁴³ This provides two major risk factors for elevated levels of endogenous inhibitors of NOS that might have contributed to the negative results of our study. It has previously been shown that supplementation with L-arginine does not affect endothelial function, or renal perfusion, in chronic kidney disease.^{44–46} Unfortunately, measurement of ADMA levels, or L-arginase activity, were beyond the scope of the present study.

To gain further insight into the contribution of increased methylation demand as an explanation for the lack of benefit of L-arginine, we measured vascular function and plasma markers before

and after supplementation with creatine alone and in combination with L-arginine. We anticipated that creatine supplementation would reduce GAA and homocysteine production.^{24,27} We hypothesized that this effect of creatine would increase L-arginine levels and, thus, improve NO-dependent vasodilation. In contrast to prior observations that creatine supplementation reduce GAA levels,²⁶ we did not find a reduction in GAA levels or a change in endothelial function.

A potential explanation for this failure to inhibit GAA production may be inadequate dose or duration of creatine supplementation. We however selected our creatine regimen based upon prior studies revealing creatine supplementation within this general dose and duration has a significant effect of the biochemical pathways of interest. It has been shown that GAA levels may be reduced by 50% following a creatine load of 20 grams for 1 week.²⁴ Furthermore, much lower doses of creatine are required to provide twice the daily demand - about 2–5.5 gms daily.²⁷ This results in a modest reduction in homocysteine levels (change of $-0.9 \mu\text{mol/L}$ compared with $+0.2 \mu\text{mol/L}$) with 4 weeks of therapy. In addition, it is acknowledged that after ~ 2 days of a creatine load that maximal tissue accumulation occurs.⁴⁷ Despite these effects it remains possible that the use of a longer duration of creatine or usage of alternative approaches to inhibit conversion of L-arginine to GAA might have allowed for the testing of our hypothesis.

Through inhibition of GAA production, we anticipated that creatine supplementation would have a favorable effect on homocysteine metabolism. This is supported by previous study findings that creatine supplementation may reduce homocysteine levels, although not supported by others studies finding no effect.^{26, 27, 48,49} In contrast, our study found an unanticipated increase in homocysteine of 11 to 20% following creatine supplementation. We sought to explain the mechanism to account for our observed increase in homocysteine levels with creatine supplementation. The kidney plays a significant role in homocysteine metabolism with homocysteine levels increasing as glomerular filtration rate (GFR) decreases.⁵⁰ There is the potential for subtle alterations in renal function that occur during the acute phase of creatine supplementation due to water retention as creatine loads into muscles. This concept was supported by a reduction in GFR in rats receiving creatine.⁵¹ Our finding of an increase in creatinine with creatine supplementation is not reflective of alteration in renal function as creatine undergoes spontaneous cyclization to form creatinine. To elucidate this potential mechanism further we measured cystatin C levels since this is a sensitive marker of GFR that is independent of creatine.^{50,52} We, however, found no consistent effect of creatine on renal function, as reflected by changes in cystatin C levels (levels increased with creatine alone and decreased with L-arginine plus creatine supplementation). Thus, it does not appear that worsened renal function, as assessed by a decline in glomerular filtration rate, accounts for the increase in homocysteine levels seen with short-term creatine.

As mentioned above with L-arginine supplementation, the inclusion of subjects with chronic kidney disease may be relevant to the interpretation of our study results. Creatine supplementation does not appear to effect homocysteine in chronic hemodialysis, already replete with folate and B vitamins.⁴⁹ We however found that the paradoxical effect of creatine on homocysteine remained significant after adjusting for differences in renal function as well as multivitamin (or folate) use. Thus, the mechanism to explain the increase in homocysteine levels with creatine supplementation in our study remains uncertain and may warrant further investigation. There are potential implications of this increase in homocysteine, including adverse vascular effects, such as activation of coagulation, stimulation of monocyte adhesion, oxidation of LDL, and impairment of NO-mediated vascular responses.²⁰

In conclusion, our study showed no benefit of short-term L-arginine supplementation on vascular function in patients with stable coronary artery disease, providing further evidence against its clinical utility in this patient population, although chronic kidney disease was a

common coexistent illness. Furthermore, our study provided evidence for increased methylation demand with an increase in GAA and homocysteine-to-methionine ratio with L-arginine supplementation. The clinical implications of this finding may merit further study. In addition, we administered creatine to inhibit L-arginine metabolism to GAA in an attempt to investigate further whether this mechanism played a role in the vascular responses to L-arginine supplementation. Despite a marked increase in plasma creatine concentrations, we observed no favorable effects on GAA levels or vascular function. There was an unexpected increase in homocysteine levels following creatine supplementation not attributed to alteration in renal function, which could have clinical implications since creatine is commonly used as dietary supplement in athletes and body builders. The consequences of long-term creatine supplementation in patients with atherosclerotic disease remain unknown.

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- 1) Arginine: Glycine Amidinotransferase
 - 2) Guanidinoacetate Methyltransferase
 - 3) Methionine Synthase
 - 4) Nonenzymatic Cyclization
 - 5) L-arginase
 - 6) Nitric Oxide Synthase
- SAM- S-Adenosyl Methionine
 SAH- S-Adenosyl Homocysteine
 THF- Tetrahydrofolate

Figure 1.

Relationship between L-arginine, creatine and homocysteine metabolism.

Figure 2.

Change in plasma concentration of homocysteine with supplementation of creatine (Cre), L-arginine (Arg), L-arginine plus creatine (Arg/Cre) and placebo. Figure is represented as change from baseline $\pm$ standard error.

Table 1

Baseline characteristics.

	Placebo	Arginine	Creatine	Arginine/Creatine	P value
	N=29	n=28	n=26	n=29	
Age	58 ± 12	60 ± 9	60 ± 11	57 ± 8	0.44
Men, No. (%)	22 (76)	23 (89)	18 (69)	23 (82)	0.36
Caucasian, No. (%)	24 (83)	22 (85)	17 (65)	20 (71)	0.46
BMI	30.8 ± 5.7	27.2 ± 9.6	29.9 ± 6.6	29.6 ± 7.7	0.35
Systolic BP (mm Hg)	139 ± 22	137 ± 19	134 ± 23	129 ± 19	0.25
Diastolic BP (mm Hg)	76 ± 10	74 ± 10	71 ± 11	72 ± 9	0.22
Past Medical History, No. (%)					
Diabetes	9 (31)	6 (23)	6 (23)	10 (36)	0.67
Hypertension	18 (62)	17 (65)	19 (73)	17 (61)	0.78
Hypercholesterolemia	24 (83)	23 (89)	22 (85)	22 (78.6)	0.8
Tobacco use within last year	14 (48)	11 (42)	6 (23)	14 (50)	0.17
Family history of CAD	17 (59)	13 (50)	14 (54)	14 (50)	0.9
Myocardial infarction	19 (66)	20 (77)	17 (65)	17 (61)	0.63
Previous CABG	6 (21)	4 (15)	11 (42)	9 (32)	0.13
Previous PCI	23 (79)	19 (73)	16 (62)	18 (64)	0.46
Stroke or carotid disease	4 (14)	1 (4)	1 (4)	0 (0)	0.13
Medication, No. (%)					
ASA/Clopidogrel	24 (82.8)	22 (84.6)	25 (96.2)	25 (92.6)	0.34
ACE inhibitor	12 (41.4)	11 (42.3)	10 (38.5)	13 (48.1)	0.91
Beta blocker	21 (72.4)	22 (84.6)	21 (80.8)	20 (74.1)	0.67
Statin	22 (75.9)	21 (80.8)	22 (84.6)	23 (85.2)	0.79
MVI/Folate	5 (17.2)	7 (26.9)	9 (34.6)	2 (7.4)	0.08
Baseline Laboratory Values					
Total cholesterol (mg/dl)	163.9 ± 29.8	169.8 ± 39.4	165.8 ± 56.1	160 ± 35.4	0.86
HDL-cholesterol (mg/dl)	41.9 ± 7.8	44.5 ± 10.1	45.5 ± 16.1	42 ± 9.7	0.6
LDL-cholesterol (mg/dl)	93 ± 24.7	93.2 ± 30.7	82.7 ± 23.5	87.6 ± 29.4	0.48

	Placebo	Arginine	Creatine	Arginine/Creatine	P value
	N=29	n=28	n=26	n=29	
Triglyceride (mg/dl)	152.2 ± 86.5	156.2 ± 87.1	168.3 ± 174.4	149.3 ± 82.7	0.93
Glucose (mg/dl)	131.6 ± 62.3	120 ± 38.1	116.3 ± 43	111.9 ± 34.9	0.42

Table 2

Biochemical measurements before and after supplementation.

	Placebo	Arginine	Creatine	Arginine/Creatine	Overall P Value
Arginine Plasma Concentration (umol/L)					
Pre-treatment	71.7 ± 25.2	80.7 ± 30.4	60.9 ± 21.7	69 ± 21.7	<0.001
After intervention	81.1 ± 21.9	173.9 ± 61.3 *	74.6 ± 30.9	175.5 ± 93 *	
Creatine Plasma Concentration (mg/dl)					
Pre-treatment	0.6 ± 0.3	0.6 ± 0.3	0.8 ± 0.5	0.5 ± 0.3	<0.001
After intervention	0.8 ± 0.7	0.8 ± 1.0	15.1 ± 7.5 *	7.9 ± 6.8 *	
Homocysteine (umol/L)					
Pre-treatment	10.7 ± 3.2	11.1 ± 3.3	11.1 ± 3.6	10.7 ± 3.1	0.006
After intervention	10.7 ± 3.5	11.2 ± 3.1	12.3 ± 3.5 *	12.9 ± 4.2 *	
Methionine (umol/L)					
Pre-treatment	18 ± 4	17.4 ± 3.4	20.1 ± 3.8	19.5 ± 5.4	0.05
After intervention	18 ± 3.3	15.4 ± 7.9	22.1 ± 4	16.1 ± 4.4 *	
Homocysteine/Methionine Ratio					
Pre-treatment	0.66 ± 0.31	0.7 ± 0.34	0.61 ± 0.18	0.67 ± 0.42	0.002
After intervention	0.61 ± 0.24	0.9 ± 0.44 *	0.64 ± 0.18	0.96 ± 0.52 †	
Guanidinoacetate (umol/L)					
Pre-treatment	1.6 ± 0.9	1.6 ± 0.6	1.4 ± 0.7	1.5 ± 0.6	0.007
After intervention	1.6 ± 0.6	2.5 ± 1.2 †	2.1 ± 1 *	2.6 ± 1 †	
Creatinine (mg/dl)					
Pre-treatment	0.9 ± 0.3	0.9 ± 0.2	1.0 ± 0.3	0.9 ± 0.2	<0.001
After intervention	0.9 ± 0.3	0.9 ± 0.2	1.2 ± 0.3 †	1.1 ± 0.3 †	
Cystatin C (mg/L)					
Pre-treatment	1.074 ± 0.5	1.023 ± 0.2	1.137 ± 0.4	1.033 ± 0.2	0.003
After intervention	1.075 ± 0.4	1.024 ± 0.2	1.081 ± 0.3 *	1.101 ± 0.3 *	
Calculated GFR (ml/min)					
Pre-treatment	81.9 ± 29.6	78.0 ± 15.2	71.8 ± 17.4	77.5 ± 15.2	0.03

	Placebo	Arginine	Creatine	Arginine/Creatine	Overall P Value
After intervention	80.6 ± 27.7	78.0 ± 15.0	75.6 ± 17.6 *	73.2 ± 20.1 *	

Values are represented as mean ± standard deviation.

* P<0.05.

† P<0.001.

Table 3

Vascular function parameters before and after supplementation.

	Placebo	Arginine	Creatine	Arginine/Creatine	Overall P Value
Baseline arterial diameter (mm)					
Pre-treatment	4.75 ± 0.56	4.68 ± 0.53	4.52 ± 0.69	4.78 ± 0.81	
After intervention	4.76 ± 0.46	4.68 ± 0.54	4.55 ± 0.7	4.72 ± 0.74	0.77
Baseline arterial flow (cm/sec)					
Pre-treatment	287 ± 123	264 ± 89	210 ± 93	217 ± 88	
After intervention	569 ± 1558	242 ± 78	370 ± 587	219 ± 87	0.56
Hyperemic flow (cm/sec)					
Pre-treatment	1357 ± 519	1432 ± 439	1227 ± 547	1288 ± 348	
After intervention	1379 ± 482	1366 ± 496	1321 ± 595	1355 ± 5343	0.63
Flow-Mediated Dilation (%)					
Pre-treatment	7.8 (3.8)	6.9 (3.6)	9.1 (5.8)	7.4 (3.1)	
After treatment	8.1 (4.3)	8 (3.6)	7.6 (4.3)	7.9 (4.8)	0.18
Nitroglycerin-Mediated Dilation (%)					
Pre-treatment	11.2 (4.7)	11.2 (4.5)	13.2 (6.3)	12.2 (5.2)	
After treatment	9.2 (4.4)	9.3 (4.2)	11.0 (5.0)	9.2 (3.8)	0.88

Values are represented as mean ± standard deviation. The number of subjects undergoing testing with NMD were 18 in placebo, 17 in L-arginine, 9 in creatine, and 13 in L-arginine plus creatine.