Effects of 7 Days of Arginine-Alpha-Ketoglutarate Supplementation on Blood Flow, Plasma L-Arginine, Nitric Oxide Metabolites, and Asymmetric Dimethyl Arginine After Resistance Exercise

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Background: Arginine-alpha-ketoglutarate (AAKG) supplements are alleged to increase nitric oxide production, thereby resulting in vasodilation during resistance exercise. This study sought to determine the effects of AAKG supplementation on hemodynamics and brachial-artery blood flow and the circulating levels of L-arginine, nitric oxide metabolites (NOx; nitrate/nitrite), asymmetric dimethyl arginine (ADMA), and L-arginine:ADMA ratio after resistance exercise. Methods: Twenty-four physically active men underwent 7 days of AAKG supplementation with 12 g/day of either NO2 Platinum or placebo (PLC). Before and after supplementation, a resistance-exercise session involving the elbow flexors was performed involving 3 sets of 15 repetitions with 70–75% of 1-repetition maximum. Data were collected immediately before, immediately after (PST), and 30 min after (30PST) each exercise session. Data were analyzed with factorial ANOVA (p < .05).

Results: Heart rate, blood pressure, and blood flow were increased in both groups at PST (p = .001) but not different between groups. Plasma L-arginine was increased in the NO2 group (p = .001). NOx was shown to increase in both groups at PST (p = .001) and at 30PST (p = .001) but was not different between groups. ADMA was not affected between tests (p = .26) or time points (p = .31); however, the L-arginine:ADMA ratio was increased in the NO2 group (p = .03). Conclusion: NO2 Platinum increased plasma L-arginine levels; however, the effects observed in hemodynamics, brachial-artery blood flow, and NOx can only be attributed to the resistance exercise.

Keywords: vasodilation, hemodynamics, amino acid, skeletal muscle

Nitric oxide is a gaseous signaling molecule known to contribute to the control of vascular tone (Thomas, Shaul, Yuhanna, Froehner, & Adams, 2003) and is considered to play a role in the vasodilation of muscle resistance vessels during exercise (Tschakovsky & Joyner, 2008). Nitric oxide is biosynthesized endogenously in the endothelium from L-arginine by endothelial nitric oxide synthase (eNOS; Böger & Bode-Böger, 2001); however, asymmetric dimethyl arginine (ADMA) can interact with eNOS and inhibit its activity (Leiper & Vallance, 1999). L-arginine (2-amino-5-guanidino-pentanoic acid), a conditionally essential, proteinogenic amino acid that is a natural constituent of dietary proteins (McConnell, 2007), has been used clinically to improve vasodilatory capacity and blood flow (McConnell, 2007), based on the premise that L-arginine supplementation has been shown to affect the release of nitric oxide (Hishikawa et al., 1992).

In recent years, various nutritional supplements have been developed containing L-arginine and other compounds (mainly arginine-alpha-ketoglutarate; AAKG) and are being marketed as ergogenic aids because of their function as “vasodilators” as a result of up-regulation of the endothelial L-arginine-nitric-oxide pathway. The AAKG-enhanced vasodilation and blood flow to working muscles during resistance exercise is alleged to provide an even greater impetus for increasing muscle strength and hypertrophy than exercise alone. This is based on the premise that as nitric oxide is elevated in circulation, blood flow increases to active muscles. However, it has been shown that L-arginine supplementation does not increase muscle blood flow after resistance exercise (Tang, Lysecki, Manolakos, Tarnopolsky, & Phillips, 2011). Furthermore, 8 weeks of resistance training combined with AAKG supplementation at a daily dose of 12 g appeared to be safe and well tolerated but had only caused modest improvements in muscle strength and power and had no effects on body composition or aerobic capacity (Campbell et al., 2006).

Little research has been published to substantiate manufacturers’ claims of these allegedly vasodilating,
ergogenic nutritional supplements. In a study examining the effects of acute L-arginine supplementation and resistance exercise on arterial function in young men, there was no significant change in blood flow and hemodynamic and vascular responses when 7 g of L-arginine was given immediately before resistance exercise (Fahs, Heffernan, & Fernhall, 2009). It has been shown that single doses of alleged nitric-oxide-inducing supplements were ineffective at increasing circulating nitric oxide levels and blood flow in response to resistance exercise (Bloomer et al., 2010). Bode-Böger, Böger, Galland, Tsikas, and Frölich (1998) found that 6 g of L-arginine delivered either intravenously or orally did not result in any significant changes in blood pressure, heart rate, or cardiac output. Similarly, L-arginine provided orally at 6 g/day for 3 days was shown to have no effect on plasma nitric oxide levels and muscle power generated during an anaerobic cycle-ergometer test in well-trained male athletes (Liu et al., 2009).

In light of unsubstantiated product claims by supplement manufacturers supporting the notion that AAKG supplements increase blood flow, and because of the paucity of data involving AAKG supplementation and the conflicting results of available research, the purpose of this study was to determine the effects of 7 days of AAKG supplementation, using the nutritional supplement NO2 Platinum, on resting and resistance-exercise-induced hemodynamics (heart rate, blood pressure, and mean arterial blood pressure [MAP]), arterial blood flow, circulating levels of nitric oxide metabolites (NOx [nitrate/nitrite]) and ADMA, and the L-arginine:ADMA ratio.

Methods

Participants

Twenty-four apparently healthy, resistance-trained (regular, consistent resistance training—i.e., thrice weekly—for at least 1 year before the onset of the study) men age 18–25 with body-mass index of 18.5–30 kg/m² volunteered to participate in the double-blind study. Enrollment was open to men of all ethnicities. Only participants considered at low risk for cardiovascular disease, with no contraindications to exercise as outlined by the American College of Sports Medicine, who had not consumed any nutritional supplements (excluding multivitamins) 1 month before the study were allowed to participate. All participants provided written informed consent and were cleared for participation by passing a mandatory medical screening. With the exception of the exercise involved in the study, participants were instructed not to engage in any other resistance exercise during the course of the study. All eligible subjects signed university-approved informed-consent documents, and approval was granted by the Institutional Review Board for Human Subjects of Baylor University. All experimental procedures involved in the study conformed to the ethical considerations of the Helsinki Code. A diagram of the experimental design can be seen in Figure 1.

Baseline Muscle-Strength Testing

A familiarization/baseline muscle-strength testing session was scheduled approximately 72 hr before the first
resistance-exercise session. To determine maximum strength of the elbow flexors, participants performed a one-repetition maximum (1-RM) test on the same “preacher curl” machine to be used in the two resistance-exercise sessions. They warmed up by completing 5–10 repetitions at approximately 50% of their estimated 1-RM. Participants rested for 1 min and then completed 3–5 repetitions at approximately 70% of their estimated 1-RM. The weight was then increased conservatively, and the participants attempted to lift it for one repetition. If the lift was successful, the participant rested for 2 min before attempting the next weight increment. This procedure was continued until the participant failed to complete the lift. The 1-RM was recorded as the maximum weight that the participant was able to lift for one repetition.

Venous Blood Sampling
Venous blood samples were collected from the antecubital vein into 10-ml serum- and plasma-collection tubes using a standard Vacutainer apparatus. Blood samples were allowed to stand at room temperature for 10 min and then centrifuged. The serum and plasma were removed and frozen at –80 °C for later analysis. Three blood samples per participant were obtained at Testing Session 1 (T1) and 7 days later at Testing Session 2 (T2) for a total of six blood samples per participant. The first blood samples at T1 and T2 were obtained immediately before resistance exercise (PRE) and after an 8-hr fast, the second sample immediately after resistance exercise (PST), and the third sample 30 min after exercise (30PST).

Supplementation Protocol
In a randomized, double-blind, placebo-controlled design participants were assigned either an alleged nitric-oxide-inducing supplement containing AAKG (NO2 Platinum, Medical Research Institute, San Francisco, CA) or a placebo supplement (PLC: apple pectin, General Nutrition Corp., Pittsburgh, PA). The Medical Research Institute recommends that individuals weighing 160–200 lb (73–90 kg) take 8 tablets/day and those weighing over 200 lb take 10 tablets/day (http://www.mri-performance.com/no2.php). However, a previous study (Campbell et al., 2006) using NO2 Platinum provided 12 tablets/day; therefore, we chose to adopt this dosing protocol. In the current study, both supplements contained 1 g/tablet, and 12 tablets were ingested daily for 7 days. On Days 1–6, four tablets were ingested in the morning on an empty stomach 30 min before breakfast, four tablets 30 min before lunch, and four tablets on an empty stomach 30 min before dinner (Campbell et al., 2006). On Day 7, however, six tablets were ingested in the morning on an empty stomach 30 min before breakfast, and six tablets, 30 min before lunch. Supplementation compliance was monitored by participants’ returning empty containers of their supplement on Day 7 and also by documenting their daily supplement ingestion. With the exception of ingesting the supplement, participants were instructed not to change their normal dietary intake during the course of the study.

Resistance-Exercise Protocol
For T1, testing was performed between 12 and 2 p.m. To standardize the time of day for the two testing sessions, each participant performed T2 at the same time of day as T1. Each resistance-exercise session involved the “preacher curl” bicep-flexion exercise on a Selectorized weight machine (Body Master, Rayne, LA). Participants performed three sets of 15 repetitions with as much weight as they could lift per set (typically 70–75% of 1RM). Rest periods between sets were timed and lasted 10 s.

Assessment of Plasma L-Arginine Levels
Plasma L-arginine was assessed using high-performance liquid chromatography/mass spectrometry (Huang, Guo, Liang, Yang, & Cheng, 2004). Because we were primarily concerned about whether NO2 Platinum was effective at increasing circulating L-arginine, only the PRE blood samples obtained at T1 and T2 were evaluated. Whole blood was collected in heparinized tubes, and plasma was obtained by centrifugation for 10 min. Twenty milligrams of 5-SSA were added to 1 ml of plasma, and the mixture was incubated on ice for 10 min. The precipitated protein was removed by centrifugation for 10 min. Separations were performed on a Shimadzu model system that consisted of an LC-10Advp solvent delivery pump, a FCV-10ALvp low-pressure gradient unit, a DGU-14A degasser, a CTO-10Avp column oven, and a SPD-M10Avp photo-diode array detector. The column used for separation was a 2.0 × 150-mm Shimadzu VP-ODS column with a particle size of 5 μm. The analytical column was protected by a C18 Guard-Pak cartridge (2.0 × 10 mm, 5 μm, Waters, Milford, MA). The mobile phase consisted of water/acetonitrile (90/10, v/v) containing 0.5% TFA, which was degassed ultrasonically before use. Each component of the mobile phase was filtered through a 0.22-μm membrane. All separations were at ambient temperature and a flow rate of 0.2 ml/min. The wavelength of the photo-diode array detector was 200–300 nm. The amount of injection was 5 μl.

Mass-spectrometry experiments were performed using a LCMS-2010 quadrupole mass spectrometer (Shimadzu Kyoto, Japan) interfaced with the Shimadzu model system coupling with an atmospheric-pressure chemical-ionization interface. The mass spectrum of L-arginine was obtained by positively scanning between m/z 100 and 400/s. Selective ion-monitoring mode involved the use of the positively protonated molecular ion [M+H]⁺ at m/z 175 (IS ion). The L-arginine standard and IS were injected into the LCMS system, and sensitivity optimization was performed by injection of an L-arginine standard (20 μmol/L). Mass-spectrometric detection conditions for
both scan and selective ion monitoring were as follows: atmospheric-pressure chemical ionization temperature 380 °C, curved desolvation line temperature 240 °C, block temperature 200 °C, probe voltage 1.6 kV, curved desolvation line voltage –30 V, Q-array Bios voltage 22 V, and nebulizing gas flow 3.0 L/min.

Assessment of Serum NOx and ADMA

From the six blood samples obtained at T1 and T2, serum NOx (nitrate/nitrite) was determined using a commercially available colorimetric assay kit (Cayman Chemical, Ann Arbor, MI) according to the procedures provided by the manufacturer. This assay determines the measurement for total nitrate and nitrite concentrations involving the conversion of nitrate to nitrite using nitrate reductase. The absorbance was then detected photometrically at 540 nm. Quantification was performed with calibration curves using nitrate and nitrite standards of known concentrations. The sensitivity of the assay is 2.0 μM. The manufacturer has demonstrated the interassay coefficient of variation on five samples to be 3.4%. We have demonstrated the interassay coefficient of variation for this assay in our laboratory on approximately 500 samples to be 6.4%.

The serum level of ADMA (Alexis Biochemicals, San Diego, CA) was determined using a commercially available ELISA kit, based on manufacturer’s guidelines. The ADMA assay is a competitive ELISA involving polyclonal capture and secondary antibodies specific for human ADMA. Samples were assessed at a wavelength of 450 nm using a standard curve generated from a known concentration of ADMA with a Wallac 1420 Multilabel Counter (Wallac, Turku, Finland). Data analysis was performed using MicroWin microplate data-reduction software (Mikrotek Laborsysteme, Germany). The sensitivity of the assay is 0.05 μmol/L. The interassay coefficient of variation on six samples established by the manufacturer is 4.5%. In our laboratory, we have demonstrated the interassay coefficient of variation on approximately 200 samples to be 5.6%.

Assessment of Brachial-Artery Blood Flow

Blood flow (peak velocity) of the brachial artery was assessed before each blood draw and determined immediately before and after (within 2–3 min) and 30 min after exercise by way of high-resolution, real-time pulsed-wave Doppler ultrasound (SonoSite M-Turbo, SonoSite, Inc., Bothell, WA), employing an electronic 13-6-MHz multi-frequency and 25-mm linear array with a maximum depth of 6 cm. The brachial artery was located by palpation while the participants lay supine with the elbow in full extension. Using the Doppler probe, maximal arterial blood flow (cm/s) was determined by using the average value from a 30-s sampling period at each time point. Doppler ultrasonography has been well studied and is widely used in medical practice as the gold standard for vascular examination. Multiple studies have concluded that Doppler ultrasound is a reliable and valid objective measure of blood flow (Billinger & Kluding, 2009; Hoteleanu, Fodor, & Suciu, 2010; Matthiessen, Zeitz, Richard, & Klemm, 2004; Thomson, Thomson, Woods, Lannos, & Sage, 2001).

Assessment of Heart Rate, Blood Pressure, and MAP

At T1 and T2, heart rate and blood pressure were assessed and MAP calculated at PRE, PST, and 30PST. Heart rate and blood pressure were assessed in the supine position with an automated blood pressure monitor (Arial BP 2400, Medquip, Bluffton, SC) using standard procedures. MAP was determined using the equation MAP = DBP + [0.333(SBP – DBP)], where DBP = diastolic blood pressure and SBP = systolic blood pressure.

Reported Side Effects From Supplements

At T2, after 7 days of supplementation, participants reported by questionnaire whether they had tolerated the supplement and supplementation protocol and reported any medical problems or symptoms they may have encountered.

Statistical Analysis

For plasma L-arginine and L-arginine:ADMA, separate two-way (Group × Testing Session) factorial ANOVAs were used to determine differences between groups and testing sessions and interactions (p ≤ .05). For all other data, separate three-way (Group × Testing Session × Time Point) factorial ANOVAs were used to determine differences between groups, testing sessions, and time points and interactions (p ≤ .05). Tukey’s post hoc tests were used to locate significant differences among time points (p ≤ .05). Effect sizes are presented as partial eta-squared (η^2) values.

Results

Participant Demographics, Supplement Compliance, and Reported Side Effects

Of the 24 participants who began the study, all finished successfully. For the PLC group, participants had a mean height, body mass, and age of 179.62 ± 6.19 cm, 83.82 ± 12.51 kg, and 21.75 ± 2.17 years, respectively. The respective height, body mass, and age for the NO2 group were 175.66 ± 9.98 cm, 84.17 ± 12.51 kg, and 22.58 ±
Results showed no significant differences between groups for any of these variables, indicating homogeneity between groups.

Overall participant compliance with supplement ingestion was 95%; 1 participant in the NO₂ group accidentally only ingested 9 tablets/day rather than 12; however, a review of his data showed no difference from the rest of the group, so they were retained. In addition, none of the participants reported any negative side effects associated with ingesting either of the supplements.

**Hemodynamics**

Heart rate \( (p = .001, \eta^2 = 0.17) \), systolic \( (p = .001, \eta^2 = 0.44) \) and diastolic blood pressure \( (p = .001, \eta^2 = 0.07) \), and MAP \( (p = .001, \eta^2 = 0.27) \) were shown to significantly increase at PST but were not different between groups \( (p > .05) \) or testing sessions \( (p > .05) \). Resistance exercise significantly increased brachial-artery blood flow in both groups \( (p = .001, \eta^2 = 0.42) \) at PST, but it was not different between groups \( (p = .14, \eta^2 = 0.02) \) or testing sessions \( (p = .47, \eta^2 = 0.04) \); Table 1).

**Plasma L-Arginine**

From the preexercise blood samples at T1 and T2, a significant Group \( \times \) Time interaction was observed \( (p = .003, \eta^2 = 0.18) \). Results demonstrated that L-arginine significantly increased in the NO₂ group compared with T1 and PLC values (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>PLC T1</th>
<th>PLC T2</th>
<th>NO₂ T1</th>
<th>NO₂ T2</th>
<th>( p &lt; .05 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>PRE</td>
<td>60.66 ± 8.74</td>
<td>62.91 ± 11.42</td>
<td>61.58 ± 10.47</td>
<td>63.91 ± 12.59</td>
</tr>
<tr>
<td></td>
<td>30PST</td>
<td>66.16 ± 11.99</td>
<td>66.75 ± 10.26</td>
<td>65.75 ± 12.22</td>
<td>65.75 ± 12.22</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>PRE</td>
<td>119.08 ± 11.30</td>
<td>119.50 ± 10.24</td>
<td>120.01 ± 13.91</td>
<td>122.17 ± 11.07</td>
</tr>
<tr>
<td></td>
<td>PST</td>
<td>142.58 ± 16.45</td>
<td>140.75 ± 9.10</td>
<td>150.50 ± 16.47</td>
<td>143.75 ± 12.57</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>30PST</td>
<td>124.33 ± 13.93</td>
<td>122.08 ± 10.65</td>
<td>124.33 ± 9.79</td>
<td>126.75 ± 9.56</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>PRE</td>
<td>71.83 ± 7.22</td>
<td>75.83 ± 10.32</td>
<td>73.83 ± 6.97</td>
<td>72.25 ± 6.51</td>
</tr>
<tr>
<td></td>
<td>PST</td>
<td>74.50 ± 8.82</td>
<td>74.33 ± 8.46</td>
<td>78.75 ± 6.92</td>
<td>76.75 ± 9.55</td>
</tr>
<tr>
<td>Brachial artery blood flow (cm/s)</td>
<td>30PST</td>
<td>69.33 ± 8.91</td>
<td>69.50 ± 13.63</td>
<td>72.25 ± 7.31</td>
<td>70.97 ± 6.14</td>
</tr>
<tr>
<td></td>
<td>PRE</td>
<td>87.56 ± 7.95</td>
<td>90.37 ± 9.15</td>
<td>89.26 ± 8.12</td>
<td>89.03 ± 6.56</td>
</tr>
<tr>
<td></td>
<td>PST</td>
<td>97.17 ± 9.39</td>
<td>96.45 ± 7.31</td>
<td>102.64 ± 9.04</td>
<td>99.06 ± 8.08</td>
</tr>
<tr>
<td></td>
<td>30PST</td>
<td>88.64 ± 7.95</td>
<td>87.01 ± 10.34</td>
<td>89.59 ± 7.06</td>
<td>89.51 ± 5.04</td>
</tr>
</tbody>
</table>

Note. PLC = placebo; T1 = Testing Session 1; T2 = Testing Session 2; NO₂ = NO₂ Platinum; PRE = preexercise; PST = immediately postexercise; 30PST = 30 min PST.
Table 2 Plasma and Serum Variables in Response to 7 Days of Arginine-Alpha-Ketoglutarate Supplementation and Resistance Exercise, M ± SD

<table>
<thead>
<tr>
<th>Variable</th>
<th>PLC T1</th>
<th>PLC T2</th>
<th>NO2 T1</th>
<th>NO2 T2</th>
<th>p &lt; .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-arginine (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>92.83 ± 13.12</td>
<td>91.01 ± 16.27</td>
<td>104.17 ± 30.50</td>
<td>179.33 ± 75.79</td>
<td>NO2 &gt; PLC</td>
</tr>
<tr>
<td>NOx (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>9.20 ± 5.29</td>
<td>8.28 ± 7.64</td>
<td>11.22 ± 5.26</td>
<td>15.27 ± 6.38</td>
<td></td>
</tr>
<tr>
<td>PST</td>
<td>17.63 ± 1.96</td>
<td>15.77 ± 8.98</td>
<td>27.79 ± 9.03</td>
<td>22.79 ± 12.66</td>
<td>PST &gt; PRE</td>
</tr>
<tr>
<td>30PST</td>
<td>22.21 ± 13.96</td>
<td>18.28 ± 7.62</td>
<td>11.61 ± 10.50</td>
<td>16.45 ± 10.73</td>
<td></td>
</tr>
<tr>
<td>ADMA (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>1.53 ± 0.76</td>
<td>1.31 ± 0.72</td>
<td>1.38 ± 0.75</td>
<td>1.10 ± 0.59</td>
<td>NO2 &lt; PLC</td>
</tr>
<tr>
<td>PST</td>
<td>1.38 ± 0.75</td>
<td>1.31 ± 0.86</td>
<td>1.01 ± 0.65</td>
<td>1.19 ± 0.62</td>
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</tr>
<tr>
<td>30PST</td>
<td>1.34 ± 0.76</td>
<td>1.42 ± 0.84</td>
<td>0.97 ± 0.75</td>
<td>1.12 ± 0.65</td>
<td></td>
</tr>
<tr>
<td>L-arginine:ADMA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>83.84 ± 58.69</td>
<td>95.78 ± 54.18</td>
<td>165.37 ± 261.10</td>
<td>234.80 ± 211.24</td>
<td>NO2 &gt; PLC</td>
</tr>
</tbody>
</table>

Note. PLC = placebo; T1 = Testing Session 1; T2 = Testing Session 2; NO2 = NO2 Platinum; PRE = preexercise; NOx = nitric oxide metabolites; PST = immediately postexercise; 30PST = 30 min PST; ADMA = asymmetric dimethyl arginine.

**Serum NOx, eNOS, ADMA, and L-Arginine:ADMA Ratio**

Resistance exercise was shown to significantly increase NOx in both groups at PST (p = .001, η² = 0.10) but was not different between groups (p = .73, η² = 0.01) or testing sessions (p = .44, η² = 0.04). For ADMA, NO2 was significantly less than for PLC (p = .04, η² = 0.15) at PRE for T2; however, there were no significant differences between testing sessions (p = .26, η² = 0.01) or time points (p = .31, η² = 0.05). In regard to L-arginine:ADMA ratio, NO2 was significantly greater than PLC at T2 (p = .03, η² = 0.10), but there were no significant differences between testing sessions (p = .419, η² = 0.02; Table 2).

**Discussion**

This study examined the effects of 7 days of AAKG supplementation using the nutritional supplement NO2 Platinum on arterial blood flow after a single bout of resistance exercise. Despite a significant increase in plasma L-arginine, the primary finding of this study was that 7 days of AAKG supplementation at 12 g/day had no significant impact on hemodynamic function, brachial-artery blood flow, NOx, or ADMA in response to a single bout of resistance exercise.

The endothelium produces numerous paracrine substances, including nitric oxide, that help regulate vasomotor function. Nitric oxide is a labile, lipid-soluble gas synthesized in endothelial cells from the amino acid L-arginine through the action of eNOS (Palmer, Rees, Ashton, & Moncada, 1988). It is released both basally and in response to pharmacological stimulation (Vallance, Collier, & Moncada, 1989) and shear stress (Cabral, Hong, & Garvin, 2010). Because shear stress is a consequence of blood flow and viscosity, the likely physiological stimulus to endothelial nitric oxide production has been identified as increased flow through the vessel lumen (Pohl, Holtz, Busse, & Bassenge, 1986), with acute nitric-oxide-mediated vasodilation tending to normalize shear stress (Dimmeler & Zeiher, 2003). This raises the possibility that nitric oxide may contribute to exercise hyperemia, because exercise is associated with increased pulse pressure and pulsatility that results in concomitant increases in shear stress. However, nitric oxide may also affect exercise hyperemia by mechanisms associated with flow mediation (Maiorana, O’Driscoll, Taylor, & Green, 2003), mechanical vessel distortion (Clifford, Kluess, Hamann, Buckwalter, & Jasperse, 2006), muscle activation (Van Teeffelen & Segal, 2006), and red blood cell oxyhemoglobin desaturation (Ellsworth, 2004). In addition, indirect evidence for a role of nitric oxide in skeletal-muscle exercise hyperemia is provided by the increased levels of plasma and urinary nitrite in response to prolonged aerobic exercise but does not directly reflect endothelium-derived nitric oxide (Bode-Böger, Böger, Schröder, & Frölich, 1994). Furthermore, it has been shown that there is a significant correlation between the changes in forearm blood flow and serum nitrite concentration, suggesting that serum nitrite reflects changes in endothelial nitric oxide formation in human forearm circulation (Kelm, Preik-Steinhoff, Preik, & Strauer, 1999).
The infusion of 30 g of L-arginine has been shown to affect heart rate, blood pressure, and blood flow at rest, but they were not affected by 6 g of either intravenous or oral L-arginine (Bode-Böger et al., 1998). In the current study, we observed 7 days of AAKG supplementation at 12 g/day to have no effect on the resting levels of heart rate, blood pressure, MAP, blood flow, and NOx (Tables 1 and 2). This is also in line with a study that involved providing alleged nitric-oxide-inducing supplements before resistance exercise and found no change in the baseline heart rate and NOx values after the supplementation period (Bloomer et al., 2010). With respect to acute exercise, 6 mg/kg L-arginine has been shown to have little effect on hemodynamics in healthy humans in response to a 12-min exercise test (Haram, Kemi, & Wisloff, 2008). Similarly, no changes in heart rate or NOx levels were noted after single bouts of anaerobic or resistance exercise after 7 days of supplementation with alleged nitric-oxide-inducing supplements (Bloomer et al., 2010). However, in the current study, we observed increases in heart rate, blood pressure, MAP, blood flow, and NOx immediately after single bouts of resistance exercise (Tables 1 and 2). In comparison with Bloomer et al.’s study, the results of the current study may differ because we used a smaller muscle mass with the arm-curl exercise compared with the bench press. In addition, Bloomer et al. estimated arterial blood flow by way of muscle-tissue oxygen saturation using near-infrared spectroscopy, whereas the current study measured arterial blood flow using Doppler ultrasound.

ADMA is derived from the proteolysis of methylated arginine residues on various proteins. The methylation is carried out by a group of enzymes referred to as protein-arginine-methyltransferases (Leiper & Vallance, 1999). On proteolysis of methylated proteins, free methylarginines are released and can function as competitive inhibitors of nitric oxide activity. Many factors such as inflammatory cytokines (which have been shown to increase in response to resistance exercise; Izquierdo et al., 2009) have been shown to up-regulate ADMA accumulation, thereby inhibiting nitric oxide synthesis (Stuhlinger et al., 2001). Increased circulating ADMA levels and reduced L-arginine:ADMA ratio are correlated with a decreased endothelial-dependent flow-mediated vasodilation (Sydow et al., 2003). As such, an inhibition of eNOS activity can potentially be overcome with increases in the extracellular L-arginine:ADMA ratio through excess L-arginine substrate (i.e., AAKG supplementation).

Because ADMA competes with L-arginine for binding to eNOS, it is considered an endogenous inhibitor of nitric oxide synthesis. Because L-arginine is a substrate for eNOS activity, we were curious to see if any changes in the levels of NOx because of 7 days of AAKG supplementation might provide an exacerbated increase in blood flow in response to resistance exercise because of eNOS inhibition. However, based on our inability to directly assess endothelial eNOS activity in our experimental model, we chose to indirectly assess it by evaluating the levels of ADMA and the L-arginine:ADMA ratio. After 7 days of supplementation, we observed decreases in ADMA of ~14% and ~25% at PRE in the PLC and NO2 groups, respectively, with the decrease in the NO2 group being significantly different (p = .035; Table 2). In addition, at the same time points, we also observed increases in the L-arginine:ADMA ratio of ~15% and ~42%, respectively, with NO2 being greater than PLC (p = .032; Table 2). Based on our data, we observed increases in circulating L-arginine with concomitant decreases in ADMA with AAKG supplementation. Therefore, because we observed decreases in ADMA and the increase in the L-arginine:ADMA ratio in NO2 as a result of the increase in L-arginine substrate, it is unlikely that ADMA had any inhibitory effect on eNOS-induced nitric oxide synthesis.

In the current study, the fact that the AAKG supplement had no effect on blood level could be a result of the amount of L-arginine ingested. Even so, the lack of effect on blood flow from the AAKG supplement may be a result of the bioavailability of L-arginine from the daily ingested dose. A single dose as high as 30 g of L-arginine administered intravenously during a 30-min period has been shown to induce vasodilation in humans (Bode-Böger et al., 1998). L-arginine-induced vasodilation was associated with increased release of nitric oxide metabolites—nitrate and nitrite—into urine, suggesting that nitric oxide release induced by such high doses of L-arginine contributed to the vasodilation effect. In a study examining the effects of 7 g of acute L-arginine supplementation and resistance exercise on arterial function in young men, there was no significant change in blood flow and hemodynamic and vascular responses when L-arginine was given immediately before resistance exercise (Fahs et al., 2009). Similarly, in the current study, we observed that 12 g/day of AAKG supplementation for 7 days had no preferential effects on the resting and exercise values for blood flow, hemodynamics, NOx, and ADMA. However, we must caution that a limitation of our results is that we measured nitric oxide metabolites and not actual levels of circulating nitric oxide.

There is no empirical evidence to date demonstrating any acute vasodilating effects of oral L-arginine with doses below 15 g/day. Apparently, an acute vasodilator effect has been shown only in studies in which L-arginine was administered parenterally, particularly at a dose greater than 15 g (Böger, 2007). Any acute hemodynamic effects of L-arginine at higher parenteral doses are most likely related to endocrine secretagogue vasodilator actions, which are absent with lower doses. Although intracellular L-arginine levels have been demonstrated to be considerably higher than L-arginine levels in the extracellular fluid or in plasma (Böger et al., 2000), extracellular L-arginine can be taken up rapidly by endothelial cells, thereby contributing to nitric oxide production (Schmidt et al., 1988). Moreover, the
issue of L-arginine’s bioavailability must be taken into consideration. It is absorbed in the small intestine and transported to the liver, where the majority is taken up and used in the hepatic urea cycle; however, a small part of dietary L-arginine passes through the liver and is used as a substrate for nitric oxide production (Böger, 2004). This can be further illustrated from the standpoint that the bioavailability of 6 g of orally ingested L-arginine has been shown to be only ~68% (Bode-Böger et al., 1998). As a result, it is conceivable that lower doses of L-arginine (either alone or within AAKG supplements) may lack the bioavailability needed to stimulate eNOS. Therefore, it is plausible that the lack of effect on blood flow we observed in the current study from AAKG supplementation resulted from an inadequate amount of bioavailable L-arginine, and it is possible that with a dosage greater than 12 g/day we may have observed a more positive response to the AAKG supplement.

At the dosage used, we have presented data herein that appear to refute the alleged supposition and manufacturers’ claims that “vasodilating supplements” containing L-arginine are effective at causing vasodilation, thereby resulting in increased blood flow to active skeletal muscle during resistance exercise. We have specifically demonstrated that a single bout of resistance exercise increases vasomotor function, arterial blood flow, and circulating NOx levels but that the AAKG supplement provided no additive, preferential response compared with placebo. Therefore, based on our collective data, we conclude that 7 days of AAKG supplementation at a dose of 12 g/day with NO2 Platinum effectively increased plasma L-arginine levels; however, the effects observed in brachial-artery blood flow and serum NOx were attributed to resistance exercise rather than the AAKG supplement.

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References


