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L-Arginine as a Potential Ergogenic Aid in Healthy Subjects

Thiago S. Alvares,[1,2] *Cláudia M. Meirelles*,[1,3] *Yagesh N. Bhambhani*,[4] *Vânia M.F. Paschoalin*[2] and *Paulo S.C. Gomes*[1]

1 Laboratory Crossbridges, Center for Interdisciplinary Research in Health, Department of Physical Education, Universidade Gama Filho, Rio de Janeiro, Brazil
2 Chemistry Institute, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
3 Escola de Educação Física do Exército, Rio de Janeiro, Brazil
4 Department of Occupational Therapy, University of Alberta, Edmonton, Alberta, Canada

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Abstract

Dietary supplements containing L-arginine, a semi-essential amino acid, are one of the latest ergogenic aids intended to enhance strength, power and muscle recovery associated with both aerobic and resistance exercise. L-arginine is claimed to promote vasodilation by increasing nitric oxide (NO) production in the active muscle during exercise, improving strength, power and muscular recovery through increased substrate utilization and metabolite removal, such as lactate and ammonia. Research on L-arginine has recently tested this hypothesis, under the assumption that it may be the active compound associated with the vasodilator effects of NO. There were only five acute studies retrieved from the literature that evaluated exercise performance after L-arginine supplementation, three of which reported significant improvements. Regarding studies on chronic effects, eight studies were encountered: four reported enhancements in exercise performance, whilst four reports showed no changes. Whether these improvements in exercise performance – regardless of the aerobic or anaerobic nature of the exercise – can be associated with increases in NO production, has yet to be demonstrated in futures studies. Low oral doses (≤20 g) are well tolerated and clinical side

effects are rare in healthy subjects. In summary, it is still premature to recommend dietary supplements containing L-arginine as an ergogenic aid for healthy physically active subjects.

In recent years, many supplements with ergogenic properties have been developed to optimize gains in muscular strength and hypertrophy during resistance training. Supplements containing L-arginine are the latest ergogenic supplements to become commercially available. The semi-essential amino acid, L-arginine, is the only substrate for endogenous synthesis of nitric oxide (NO). The acute effects of L-arginine supplementation supposedly promote vasodilation due to enhanced NO synthesis in the active muscle during exercise.

In animals, L-arginine supplementation has demonstrated positive effects on aerobic exercise performance and skeletal muscle adaptations. Maxwell et al.[1] observed that mice supplemented with L-arginine showed increases in post-exercise urinary nitrate excretion (an indicator of NO production) and aerobic capacity (measured by maximal oxygen consumption [$\dot{V}O_{2max}$]). Long et al.[2] reported increases in myotube density, total nuclei number and nuclear fusion index after L-arginine supplementation. The authors of both studies conclude that enhanced exercise performance and skeletal muscle adaptations might be explained in part by the augmented NO production from L-arginine supplementation.

In humans, L-arginine administration has been claimed to promote an increase in blood perfusion in the active muscle,[3] increasing substrates necessary for improving muscular recovery and protein synthesis during and/or after exercise. It also promotes greater removal of metabolites, such as lactate and ammonia,[4] which are related to muscle fatigue during intense physical exercise.

In a recent survey, Malinauskas et al.[5] observed that 17% (out of 89 males) and 7% (out of 56 females) of the athletes of the National Collegiate Athletic Association (NCAA) at a southeastern state university in the US were interested in taking supplements for increasing circulation. Among these athletes, 8% and 5% of males and females, respectively, were taking L-arginine.

It appears that there is an increasing interest in L-arginine-based supplements, and, therefore, more knowledge about its effects in healthy physically active subjects is needed.

In 2007, McConell[6] published a review analysing the effect of both oral and intravenous L-arginine administration on metabolism at rest and during exercise. The author concluded that L-arginine supplementation appears to improve exercise capacity in individuals with cardiovascular disease, but had little impact on aerobic exercise capacity in healthy individuals. Although the author had cited other types of exercise such as resistance and anaerobic power exercise, the conclusion of the review was limited to the effects of L-arginine on aerobic exercise capacity and further suggested that studies were required to elucidate the potential ergogenic effects of L-arginine.

Therefore, the aim of this review was to evaluate both the acute and chronic effects of L-arginine supplementation on physical performance during different types of exercise, including aerobic, resistance and anaerobic power exercise in healthy subjects. Furthermore, the proposed underlying mechanism by which L-arginine may function is also addressed.

Scientific articles were retrieved based on an extensive search of several databases, including MEDLINE (1966–2010), EMBASE (1974–2010), Cochrane Database of Systematic Reviews (1993–2010), Lilacs (1982–2010), Scielo (1997–2010) and Google Scholar (1980–2010). Computer search engines used the following keywords combined: 'L-arginine', 'supplementation', 'exercise'. After using these initial keywords, the search engines were limited to clinical trials, human studies and randomized controlled trials. As a result, 20 articles related to the effects of L-arginine supplementation on metabolism and performance in response to exercise in humans were considered, all of which were full-text articles and published in the English language. However, in order to reach

Table I. Acute effects of L-arginine supplementation on exercise performance in healthy subjects

Study	No. of subjects; sex (sample characteristic); study design	Supplementation	Exercise protocol	Result
Liu et al.[7]	10; M (judo athletes); r, db, co	L-arg (6 g) or PLA 3 d	Cycle ergometer (13 sets at 0.05 kp/kg, 1 min rest at 60 rpm after set 9)	[La], ammonia, nitrate, nitrite, L-citr, MP and P_{max}: L-arg = PLA
Stevens et al.[8]	13; M (NR); r, db, co	GAKic or PLA 45, 20 and 0 min before exercise	Isokinetic dynamometer (concentric/eccentric knee extension 35 reps at 90°/s (0 min, 5 min, 15 min and 24 h after GAKic or PLA)	PT, TW: GAKic > PLA FI: GAKic < PLA (except after 24 h)
Buford and Koch[9]	10; M (resistance trained); r, db	GAKic or PLA 45, 30 and 10 min before exercise	Cycle ergometer (5 sets of 10-s sprints, 50-s rest intervals	MP: GAKic > PLA P_{max} and FI: GAKic = PLA
McConell et al.[10]	9; M (endurance-trained); r, db, co	L-arg HCl (30 g IV) or PLA after 75 min of exercise	Cycle ergometer (120 min at 72 ± 1% $\dot{V}O_{2\ peak}$)	[La], insulin, $\dot{V}O_{2peak}$, RPE, FET: L-arg = PLA GCR: L-arg > PLA
Bailey et al.[11]	9; M (trained); r, db, co	L-arg (6 g) or PLA 3 d 1 h before exercise	Cycle ergometer (70–90 rpm)	[La]: L-arg = PLA FET, nitrite: L-arg > PLA $\dot{V}O_{2cost}$, $\dot{V}O_{2sc}$: L-arg < PLA

co = crossover; **db** = double-blind; **FET** = fatigue exercise time; **FI** = fatigue index; **GAKic** = 2 g glycine + 6 g L-arg HCl + 3.2 g α-ketoisocaproic acid; **GCR** = glucose clearance rate; **IV** = intravenous; **[La]** = lactate concentration; **L-arg** = L-arginine free form; **L-arg HCl** = L-arg hydrochloride; **L-citr** = L-citrulline; **M** = males; **MP** = mean power; **NR** = not reported; **PLA** = placebo; **P_{max}** = peak power; **PT** = peak torque; **r** = randomized; **reps** = repetitions; **RPE** = rating of perceived exertion; **rpm** = revolutions per minute; **TW** = total work; **$\dot{V}O_{2cost}$** = cost of oxygen consumption; **$\dot{V}O_{2peak}$** = peak oxygen consumption; **$\dot{V}O_{2sc}$** = slow component of oxygen consumption; > or < indicates significant difference between groups ($p < 0.05$); = indicates no significant differences between groups.

a more objective recommendation on the potential ergogenic effects of L-arginine supplementation, only the studies evaluating exercise performance were considered, which are duly represented in tables I and II of this review. All the studies considered were randomized, double-blind and placebo controlled. References cited on the retrieved articles were also considered in this review.

1. L-Arginine and Nitric Oxide (NO) Metabolism

L-arginine is a semi-essential amino acid, which becomes an essential amino acid in special conditions, such as catabolic stress, infant growth, intestinal and kidney dysfunction.[20]

L-arginine plays a role in some metabolic pathways. L-arginine is needed to synthesize creatine (Cr) and agmatine.[21] Its conversion into L-ornithine and urea, mediated by arginase, is essential in order to eliminate toxic nitrogen compounds (figure 1). Furthermore, L-arginine is important for the production of NO, [22] a potent vasodilator that acts by elevating the concentration of cyclic guanosine monophosphate (cGMP), resulting in the relaxation of smooth muscle and vasodilation (figure 2).

NO is a highly reactive molecule produced endogenously in gas form. The synthesis of NO is dependent upon a family of related enzyme encoded by separate genes called NO synthase (NOS). These enzymes convert L-arginine into NO and L-citrulline in the presence of some cofactors: calmodulin, tetrahydrobiopterine, nicotinamide adenosine dinucleotide phosphate, flavin adenine dinucleotide, nicotinamide adenine dinucleotide and molecular oxygen. There are three isoforms of NOS: two of them expressed constitutively, neuronal NOS (nNOS, or type I) and endothelial NOS (eNOS, or type III) and one, expressed in an inducible way, NOS (iNOS, or type II).

Although NO is primarily known for its vasodilatory effects, it is also an important regulatory molecule in many different tissues, including skeletal muscle. Studies have shown that both NOS type I (nNOS) and type III (eNOS) are expressed in skeletal muscle.[23,24]

Table II. Chronic effects of L-arginine supplementation on exercise performance in healthy subjects

Study	No. of subjects; sex (sample characteristic); study design	Supplementation	Exercise protocol	Results
Campbell et al.[12]	35; M (trained); r, db	AAKG (12 g) or PLA daily; 8 wk	RT (4 ×/wk 3 sets 8–10 reps, 70–85% 1RM), 1RM bench-press test, Wingate test. Aerobic activity (3 ×/wk 30 min at 70% of HR_{max})	1RM strength, AP: AAKG > PLA FET: AAKG > PLA
Abel et al.[13]	30; M (trained); r, db	Asp-Arg (14.4 g or 5.0 g) or PLA daily; 4 wk	Cycle ergometer (100 W increased every 3 min by 30 W until exhaustion)	FET, $\dot{V}O_2$, $\dot{V}CO_2$, [La]: Asp-Arg = PLA
Colombani et al.[14]	14; M (trained); r, db	Asp-Arg (15 g) or PLA daily; 4 wk (1 wk washout)	31 km run	[La], ammonia, TDC: Asp-Arg = PLA Urea: Asp-Arg > PLA
Little et al.[15]	35; M (trained); r, db	Cr + AAKG (0.1 g/kg/day Cr + 0.075 g/kg/day AAKG) or Cr or PLA; 10 d	1RM bench-press test; 3 sets 30-s Wingate cycle tests (2 min rest)	1RM strength: Cr + AAKG = Cr > PLA P_{max}: Cr + AAKG > Cr = PLA MP: Cr + AAKG = Cr = PLA
Santos et al.[16]	12; M (untrained); r, db, co	Asp-Arg (3 g) or PLA daily; 15 d	Isokinetic dynamometer (15 reps concentric knee flexion/extension 180°/s)	FI: Asp-Arg < PLA FRF (%): Asp-Arg = PLA
Fricke et al.[17]	23; F (PM); r, db	L-arg HCl (18 g) or PLA; 6 mo	Dynamometric grip force and counter-movement jumping on force plate	MIGF (N), PJP (W) and PJF (N): L-arg = PLA PJF/kg: L-arg > PLA
Chen et al.[18]	16; M (cyclists); r, db	L-arg (5.2 g powder form) or PLA; 3 wk	Cycle ergometer (until exhaustion at 60% MWR)	AT: L-arg > PLA [La], $\dot{V}O_{2max}$, MP: L-arg = PLA
Camic et al.[19]	50; M (untrained); r, db	L-arg (1.5 g or 3.0 g) or PLA; 4 wk	Cycle ergometer (80 W increasing 30 W each 2 min until exhaustion)	PWC_{FT}: L-arg > PLA

1RM = one-repetition maximum; **AAKG** = arginine alpha-ketoglutarate; **AP** = anaerobic power; **Asp-Arg** = arginine aspartate; **AT** = anaerobic threshold; **co** = crossover; **Cr** = creatine; **db** = double-blind; **F** = females; **FET** = fatigue exercise time; **FI** = fatigue index; **FRF** = fatigue resistance factor; **HR_{max}** = maximal heart rate; **[La]** = lactate concentration; **L-arg** = L-arginine free form; **L-arg HCl** = L-arg hydrochloride; **M** = males; **MIGF** = maximal isometric grip force; **MP** = mean power; **MWR** = maximal work rate; **PJF** = peak jump force; **PJP** = peak jump peak; **PLA** = placebo; **PM** = postmenopausal; **P_{max}** = peak power; **PWC_{FT}** = physical working capacity at the fatigue threshold; **r** = randomized; **reps** = repetitions; **RT** = resistance training; **TDC** = total distance covered; **$\dot{V}CO_2$** = carbon dioxide production; **$\dot{V}O_2$** = oxygen consumption; **$\dot{V}O_{2max}$** = maximal $\dot{V}O_2$; > or < indicates greater or lesser significant difference between groups ($p < 0.05$); = indicates no significant differences between groups; + indicates in association.

Skeletal muscle functions mediated by NO include force and power production,[25,26] vasodilation,[27] protein synthesis,[28,29] activation of satellite cells,[30] mitochondrial biogenesis[31,32] and glucose homeostasis.[33,34]

Due to a large amount of information on this topic, the reader should refer to other review articles that specifically address the underlying mechanism of NO on skeletal muscle.[35-37]

The most notable function of NO is its effect on regulating vascular tone.[38] However, this function may be compromised by situations that provoke endothelial dysfunction,[39] a condition in which inadequate production of NO has been observed.[40]

Many studies in humans have demonstrated the positive effects of L-arginine in modulating vascular tone via increased NO production,[41-46] which may benefit individuals with endothelial dysfunction; however, the positive effects of supplementation on modulating vascular tone in healthy and unhealthy humans are controversial.[47-49]

2. Markers of NO Production

Detection of NO in biological samples represents a challenge, since its biological half-life is only a few seconds.[50] The synthesis of NO is not the only way in which the endothelium alters

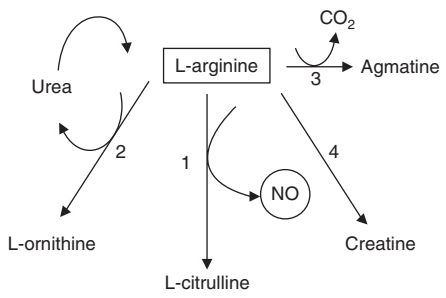

Fig. 1. Overview of the metabolism of L-arginine. (**1**) Synthesis of nitric oxide (NO) and L-citrulline from L-arginine by NO synthase (NOS); (**2**) synthesis of L-arginine to L-ornithine and urea by arginase; (**3**) decarboxylation of L-arginine to agmatine by arginine decarboxylase; (**4**) synthesis of L-arginine to creatine by L-arginine: glycine amidinotransferase.

vascular tone. The endothelium also triggers vasodilation via prostaglandins and/or endothelium-derived hyperpolarizing factor.[51] Acetylcholine, an endothelium-dependent vasodilator, may alter vascular tone via prostaglandins, NO and/or endothelium-derived hyperpolarizing factor synthesis.[51] Measuring NO is essential to understanding its role in many biological processes, including the L-arginine/NO pathway.

Several papers have described techniques to detect NO production, both directly and indirectly. Techniques such as electron paramagnetic resonance[52] and chemiluminescence,[53] as well as electrochemical detection using intravascular probes,[54] have been used to directly quantify NO synthesis in biological models, even though they are expensive and not commonly used. Thus, this review only describes studies utilizing indirect markers of NO production.

Quantifying cGMP and nitrate and nitrite in biological fluids are methods commonly used to determine the effects of NO on guanylate cyclase enzyme and on nitrate and nitrite oxidation.[55-59]

2.1 Cyclic Guanosine Monophosphate

Once released from the endothelial cells, NO quickly spreads to the smooth muscle cells, where it activates the soluble guanylate cyclase to form a second messenger molecule, cGMP, from the breakdown of guanosine triphosphate. The formation of cGMP activates the calcium pump inside smooth muscle cells, reducing intracellular calcium concentrations, and thus reducing vascular tone. This pathway is the mechanism by which NO regulates smooth muscle tone, and thus local blood flow. Böger et al.[55] and Bode-Böger et al.[56,57] observed significant increases in urinary cGMP concentrations after intravenous L-arginine infusion. Lucotti et al.,[58] also observed significant plasma concentrations of cGMP after oral L-arginine supplementation. These data indicate that endogenous NO synthesis increased after L-arginine supplementation via intravenous or oral administration. Levels of cGMP may, however, increase for other reasons besides NO synthesis. Agonists, such as atriopeptin II, released due to the increased plasma volume, may stimulate guanylate cyclase enzyme and, therefore, increase cGMP, triggering increased coronary blood flow, irrespective of NO.

2.2 Nitrate and Nitrite

In cells and blood, oxidation of NO via several metabolic reactions results in the formation of nitrite and nitrate as the two major products.[60]

Nitrite is the principal oxidation product of NO synthesis in aqueous solutions (in the absence of biological constituents such as haemoproteins). The further oxidation to nitrate requires the presence of additional oxidizing species such as oxyhaemoproteins.[61] For example, NO is quickly oxidized to nitrite via autoxidation in aqueous solutions such as biological fluids, and may react with superoxide anions to produce peroxynitrites.

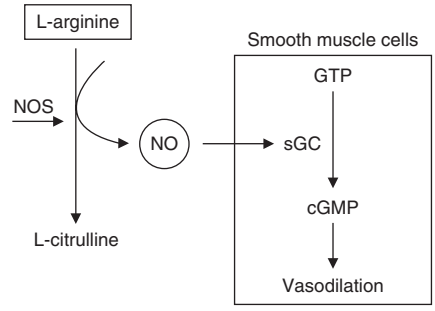

Fig. 2. Mechanism of vasodilation from L-arginine. After synthesis from L-arginine by nitric oxide synthase (NOS), NO diffuses to smooth muscle cells, in which it stimulates the soluble guanylate cyclase (sGC), resulting in enhanced synthesis of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). The increases of cGMP in the smooth muscle cells promote relaxation and, consequently, vasodilation.

In the presence of haeme groups in proteins such as haemoglobin and myoglobin, NO reacts with oxyhaemoglobin to produce metahaemoglobin and nitrate.

Most nitrite and nitrate comes from diet (vegetable products contain the highest levels of nitrate; meat and bean products contain the highest levels of nitrites),[62] mineral water and bacterial synthesis, which may alter the results of the analysis. Thus, endogenous synthesis of NO may not be adequately measured by nitrate and nitrite in plasma and urine when diet is not controlled. This problem may be mitigated by a diet low in nitrate and nitrite, as well as fasting. Dietary nitrate and nitrite excretion takes from 12 hours to 3 days, depending on the prior consumption and renal function.[63] In healthy subjects with a diet low in nitrate and nitrite (210 μmol/day), approximately 50% of urinary nitrate originates from systemic NO synthesis due to L-arginine.[64] After a 12-hour fast, plasma concentrations of nitrate and nitrite appear to reach a steady-state level in healthy subjects with a diet low in nitrate and nitrite.[65]

Current methods available for analysing nitrite and nitrate in plasma, serum and urine in experimental and clinical studies include colorimetric and ultraviolet spectrophotometric methods, fluorometric assays, chemiluminescence, high-performance liquid chromatography, capillary electrophoresis, gas chromatography, and gas chromatography/mass spectrometry.[63]

In general, nitrite and nitrate are stable metabolites of NO present both in blood and urine, and accessible to quantitative analysis. Therefore, measurement of nitrite and nitrate in various biological fluids, notably plasma or serum and urine appear to be the most suitable, practical and reliable non-invasive method to assess systemic NO synthesis *in vivo* under basal conditions, as well as upon pharmacological or physical training.[60,66,67]

3. The 'L-Arginine Paradox'

One of the factors that affect the velocity of a catalyzed reaction by an enzyme is the concentration of the substrate. L-arginine is the only substrate for the NOS, which converts L-arginine into NO and L-citrulline. Pollock et al.[68] reported that the *in vitro* Michaelis-Menten constant of endothelial NOS is ≈3 μmol/L, whereas the L-arginine concentrations in the plasma of both healthy and non-healthy individuals ranges from 40 to 100 μmol/L.[21] The data suggest that physiological concentrations of L-arginine are enough to saturate endothelial NOS, and that supplementary L-arginine does not promote increased enzyme activity – hence the condition known as the 'L-arginine paradox'.

Studies *in vivo* using L-arginine supplementation have demonstrated improved endothelial function, possibly due to increased NO production. It appears that L-arginine is a limiting factor for NO synthesis in patients at risk for atherosclerosis, but not for healthy individuals. Therefore, L-arginine supplementation may be necessary only for individuals with atherosclerosis risk factors.[41-45]

Among the possible explanations for this phenomenon is the presence of high levels of asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor. Higher concentrations of ADMA were encountered in individuals with atherosclerosis, as well as in individuals with atherosclerosis risk factors, such as hypercholesterolaemia, hypertension, diabetes mellitus, kidney failure, hyperhomocysteinaemia, smoking and aging.[69] Physiological levels of L-arginine and the presence of normal concentrations of ADMA saturate the endothelial NOS enzyme, promoting NO production. In these conditions, L-arginine supplementation does not affect enzyme activity. In contrast, in the presence of elevated plasma concentrations of ADMA the endothelial NOS activity diminishes, resulting in lower physiological levels of NO production. Under these conditions, L-arginine supplementation may re-establish the L-arginine/ADMA ratio in order to activate endothelial NOS.[70] Taken together, these results provide evidence that the endothelial NOS activity could be modulated by the extracellular ADMA and L-arginine levels. In general, the term 'L-arginine paradox' refers to specific situations in which L-arginine supplementation appears to stimulate NOS activity, even when endogenous levels are found in a physiological range.

Endothelial dysfunction also increases production of reactive oxygen species, mainly super-

oxide anion, which appears to react with NO, producing peroxynitrites that reduce the bioavailability of NO.[71,72] This reaction may also occur immediately following a resistance exercise session, due to the superoxide anion formation during resistance exercise post-ischaemic reperfusion, which results in an imbalance between superoxide anion production and removal.[73]

Hudson et al.[74] observed an increase in the plasma concentrations of protein carbonyl, an oxidative stress indicator, after two distinct resistance exercise protocols: one developed for strength and the other for hypertrophy, consisting of 11 sets of three repetitions at 90% of one-repetition maximum (1RM) strength, and four sets of ten repetitions at 75% of 1RM of a squat exercise, respectively. However, Bloomer et al.,[75] demonstrated that squatting at 70% of 1RM showed no increase in oxidative stress. Based on contrasting evidence, further studies are needed to evaluate the degree of oxidative stress produced by resistance exercise and its role on NO bioavailability.

It is believed that L-arginine supplementation, in addition to restoring systemic NO production, may also reduce superoxide anions released by the endothelium, particularly in hypercholesterolaemia.[76]

4. Contribution of NO to Exercise-Induced Vasodilation

In response to acute exercise, numerous phenomena interact to increase blood flow to active muscles, including NO and prostaglandins.[51,77] The production of NO that occurs at the vascular level is directly related to the increase in shear stress. During an exercise session, cardiac output increases and the blood is redistributed to the active muscles. The increased blood flow induced by exercise provokes a rise in shear stress, thus creating a relationship between exercise, increased blood flow and endogenous production of NO.[78,79]

There is evidence demonstrating the role of NO in exercise-induced vasodilation by the increased levels of plasma and urinary markers of NO in humans: nitrate, nitrite[67,79-81] and cGMP.[66] Jungersten et al.[79] and Maeda et al.[80,81] observed significant increases in these markers after an acute and 2–3 month protocol of incremental cycle ergometer exercise, respectively. However, other authors did not observe any significant changes after an acute treadmill test[82] and cycle ergometer exercise.[83] Bode-Böger et al.[67] observed increases in urinary nitrate, nitrite and cGMP only during incremental cycle ergometer exercise, when compared 1 hour after exercise.

Despite the suitable, practical and reliable non-invasive method to assess changes in systemic NO synthesis *in vivo*, measuring nitrate and nitrite in plasma and urine require rigorous control. For example, consuming certain foods (vegetable, meat and bean products) may increase endogenous levels of these metabolites, which may bias the measurements.[62]

Other techniques have been applied to determine the contribution of NO to vasodilation induced by different exercise protocols. By applying the NOS-inhibiting substance, NG-monomethyl-L-arginine (L-NMMA), Gilligan et al.,[84] Dyke et al.[85] and Katz et al.[86] observed a significant 7–11%, 20–30% and 10–21% reduction in forearm blood flow during a rhythmic handgrip exercise, respectively. Schrage et al.[51] reported an ~80% reduction in blood flow during a rhythmic handgrip exercise after applying another NOS inhibiting substance, NG-nitro-L-arginine-methyl ester (L-NAME). The data suggest that NO contributes to the vasodilation observed during rhythmic handgrip exercise in healthy subjects. However, Radegran and Saltin[87] did not observe any significant changes in blood flow during dynamic knee extension exercises (30–50% of peak power output), but did demonstrate that NO is responsible for approximately 52% of arterial blood flow measured in the femoral region during rest and approximately 34% for the period of post-exercise recovery after L-NMMA infusion. Endo et al.[88] reported significant reductions in forearm blood flow immediately after static handgrip exercise in response to administration of L-NMMA.

The contribution of NO to vasodilation may vary depending on the type of exercise. For example, exercise involving large muscle groups greatly increase blood flow and pressure, and may cause greater shear stress on endothelial cells, which is

a stimulus for NO production. Green et al.[89] reported that after L-NMMA administration, blood flow during cycle ergometer exercise reduced significantly more than with rhythmic handgrip exercise. In another study, L-NMMA had no significant effect on blood flow measured during two intensities of wrist flexor exercises (0.2 and 0.4 W).[90] However, other studies showed significant reduction in blood flow during rhythmic handgrip exercise after L-NMMA administration.[84-86]

It is important to mention that muscle vasodilation occurring during exercise is the result of a combination of factors besides those attributed to NO, such as prostaglandins, endothelium-derived hyperpolarizing factor, adenosine and bradykinin, among others.

Boushel et al.[91] and Kalliokoski et al.[92] inhibited NO and prostaglandin production simultaneously by infusing L-NAME and indomethacin. By using near-infrared spectroscopy and the infusion of indocyanine green as a tracer, the authors observed that both L-NAME and indomethacin reduced blood flow during dynamic knee extension exercise. On the other hand, Schrage et al.[51] demonstrated that NO and prostaglandins act independently in the control of blood flow during exercise. The authors inhibited the NO production with L-NAME and observed a reduction in blood flow of approximately 17%, whereas inhibiting prostaglandins production with ketorolac, the indole moiety of indomethacin, resulted in a 32% reduction in blood flow during dynamic handgrip exercise at 10% of the maximum voluntary contraction.

In summary, NO is a potent endogenous vasodilator responsible for increasing blood perfusion via shear stress. It contributes to changes in blood flow during dynamic exercise and post-exercise recovery. However, NO is only one of many vasodilator substances produced by the endothelium.

5. L-Arginine Supplementation on Exercise Performance

5.1 Acute Effects

The claim that L-arginine supplementation supposedly modulates NO production and consequently increases blood perfusion to the tissues is of great interest to those who participate in aerobic- and resistance-type exercise. However, the majority of the research regarding L-arginine supplementation has utilized aerobic exercise in order to evaluate its supposed effects on performance. Table I summarizes the results of the studies that evaluated the acute effects of L-arginine supplementation on exercise performance in healthy subjects.

Schaefer et al.[4] investigated metabolic changes with 3 g of intravenous L-arginine hydrochloride (HCl) during incremental cycle ergometer exercise. The authors observed a significantly lower increase in plasma lactate concentration and ammonia, besides substantially higher concentrations of L-citrulline (by-product of NO synthesis). This suggests that part of the L-arginine may have been diverted for L-citrulline and NO synthesis during exercise. Theoretically, higher lactate concentration and ammonia concentrations indicate an increase in hydrogen ions and, consequently, intramuscular acidity that reduce both strength and muscular work capacity. If so, L-arginine supplementation may be effective in reducing the aforementioned metabolite concentrations, thereby improving strength and muscle work capacity during exercise. However, Liu et al.[7] did not observe any significant differences in maximum and average anaerobic power during several sets of a cycle ergometer exercise test after orally supplementing ten elite male college judo athletes with 6 g of L-arginine (as free form) or placebo for 3 days. They also did not observe any significant difference in plasma lactate concentration, ammonia, nitrate and nitrite concentrations between groups.

Bailey et al.[11] trialled nine healthy recreationally active men with a supplement that contained 6 g of L-arginine (dissolved in 500 mL of water) or placebo 1 hour before a series of moderate- and severe-intensity exercise bouts performed on an electronically braked cycle ergometer for 3 days. On day 1 of supplementation, the subjects completed two 6-minute bouts of moderate-intensity cycling (at 70–90 revolutions per minute [rpm]); on day 2, they completed one 6-minute bout of moderate-intensity cycling followed by

one 6-minute bout of severe-intensity cycling, and on day 3, they completed one 6-minute bout of moderate-intensity cycling followed by one bout of severe-intensity cycling that was continued until task failure, as a measure of exercise tolerance. No significant difference was observed in plasma lactate concentration between L-arginine and placebo groups. There were, however, significant increases observed in plasma nitrite and time to task failure. There was also a significantly reduced oxygen consumption ($\dot{V}O_2$) cost of moderate-intensity cycle exercise and reduced $\dot{V}O_2$ slow component amplitude observed between groups.

It is important to note that this study associated other amino acids besides L-arginine, including L-citruline (quantities not expressed in the study), which have been shown to increase NO production, as measured by plasma concentrations of nitrite[93] and urinary excretion of nitrate and cGMP[94].

Interestingly, the authors did not measure plasma nitrite at baseline; they had just done so 1 hour after supplementation, which is a major methodological limitation, since it is not known whether there were any differences in the samples prior to supplementation. Furthermore, taking into consideration that diet can influence nitrite plasma concentrations, no dietary control to limit the consumption of foods rich in nitrite and nitrate was conducted. The authors' conclude that the precise mechanisms responsible for improving exercise efficiency and exercise tolerance remain to be elucidated.

Upon supplementing 13 subjects orally with a product comprised of L-arginine (6 g) plus glycine (2 g) plus α-ketoisocaproic acid (3.2 g) or 9.46 g sucrose isocaloric control in three equal aliquots at 45, 30 and 10 minutes before exercise, Stevens et al.,[8] observed significant increase in peak torque, total work and fatigue index using an isokinetic dynamometer. By using a similar supplement protocol, Buford and Koch[9] observed significant improvement of average power during repeated sets of supra-maximal exercise during cycle ergometry. However, no significant differences in plasma lactate concentration were observed between the groups. The authors did not evaluate any underlying mechanism that may explain the observed physical enhancement after supplementation.

It is well known that muscle glycogen is an essential fuel source for optimizing performance during moderate- to high-intensity aerobic and anaerobic exercise.[95] Therefore, replenishment of depleted muscle glycogen levels after strenuous exercise is paramount to complete recovery. Muscle glycogen synthesis may be optimized from increased skeletal muscle glucose uptake, which is enhanced by translocation of the GLUT-4 glucose transporter from intracellular vesicles to the plasma membrane in response to insulin.[96] There is evidence that NO may be playing an essential role in the regulation of skeletal muscle glucose uptake during exercise in humans.[97,98]

Some studies have examined the effects of increasing endogenous NO production from L-arginine – the only endogenous nitrogen-containing substrate of NOS – on insulin and NO release, which may increase muscle glucose uptake.[10,99-101] Yaspelkis and Ivy[99] supplemented 12 trained subjects with either oral L-arginine HCl (0.08 g/kg of bodyweight) plus carbohydrate (CHO; 1 g/kg of bodyweight) or only CHO at the following intervals: 0, 1, 2 and 3 hours after 2 hours of cycle ergometer exercise at 50–90% of $\dot{V}O_{2max}$. They observed that the group supplemented with L-arginine plus CHO had a significantly lower CHO oxidation rate compared with the CHO-only group. They suggested that the lower rate of post-exercise CHO oxidation could increase the availability of glucose for muscular glycogen synthesis during the recovery period. However, the authors' suggestion cannot be supported since no significant differences in plasma insulin and muscular glycogen concentrations between groups were observed during the recovery period.

In a recent study,[100] 12 healthy male judo athletes performed a single bout of treadmill exercise (at 75% $\dot{V}O_{2max}$) during 60 minutes, and then supplemented with oral L-arginine (0.1 g/kg bodyweight of instant powder) or placebo. The authors observed significantly higher concentrations of serum glucose 15 minutes after supplementation and insulin after 30 minutes, when

compared with the placebo group. No differences in the levels of plasma lactate concentration, ammonia, nitrite and nitrate were observed between the two groups.

Robinson et al.[101] observed that whole-blood glucose and plasma insulin concentrations after ingesting oral L-arginine (10 g) plus CHO (70 g) were not significantly different from placebo conditions when administered 30 minutes after different exercise protocols (non-exercised, resistance exercise or cycling exercise). McConell et al.[10] submitted nine endurance-trained males to a steady-state cycle ergometer exercise for 120 minutes at $72 \pm 1\%$ $\dot{V}O_{2peak}$. During the last 60 minutes of exercise, either a placebo or L-arginine HCl (30 g at 0.5 g/min) was administered intravenously. L-arginine had no significant effects on plasma insulin concentration and on cycling exercise performance as measured by mean power output in Watts and total performance time. However, L-arginine infusion significantly increased skeletal muscle glucose clearance compared with placebo. Given that plasma insulin concentration was unaffected by L-arginine infusion, the authors suggested that L-arginine increased NO production, which then increased muscle glucose uptake by skeletal muscle.

Matsumoto et al.[102] submitted eight subjects (4 males) to a single oral supplement of either a drink containing 2 g of branched chain amino acids (BCAA) and 0.5 g of L-arginine or an isoenergetic placebo at 10 minutes into the first exercise bout. The exercise consisted of three bouts of 20-minute cycling exercise at approximately 126 W, which corresponded to 50% of the maximal work intensity. The authors found that ingestion of BCAA plus L-arginine resulted in a significant suppression of skeletal muscle proteolysis induced by endurance exercise at a moderate intensity compared with the placebo group. The addition of L-arginine to the BCAA supplement in this study was utilized to induce an additional anabolic effect by an increase in insulin level and blood flow, although no difference in either was observed. The L-arginine dosage (500 mg) in the present study was much smaller when compared with other studies that have shown positive results (6 g).[12] This supplementation was probably insufficient to induce an additional metabolic effect via increases in the blood flow and insulin level.

Only two studies have analysed the acute effect of L-arginine supplementation on blood flow during resistance exercise, neither of which demonstrated significant changes in blood flow when compared with the control group.[101,103] However, 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.

The lack of evidence demonstrates the need to develop acute studies to evaluate the underlying mechanism that may be triggered by L-arginine supplementation in association with exercise – in particular, resistance exercise – such as changes in blood volume and/or flow, muscular oxygenation, NO production and strength performance.

5.2 Chronic Effects

The results of the studies pertaining to the chronic effects of L-arginine supplementation on exercise performance in healthy subjects are summarized in table II. Burtscher et al.[104] submitted 16 trained males to 3 weeks of oral supplementation with either arginine aspartate (3 g/day) or placebo in order to evaluate the effects of prolonged supplementation with L-arginine on metabolic and cardiorespiratory responses to submaximal exercise in healthy subjects. Incremental submaximal cycle ergometer exercise (up to 150 W) was performed before and after the supplementation period. Three weeks of arginine aspartate supplementation resulted in significantly lower plasma lactate concentration, diminished glucose oxidation and reduced ventilation and CO_2 production during exercise when compared with the placebo group. Despite having observed some submaximal metabolic and cardiorespiratory improvements, the authors did not evaluate maximum exercise capacity or other physical performance indicators. Another study evaluating only cardiorespiratory response associated to L-arginine supplementation found

no significant difference in $\dot{V}O_{2max}$ and ventilatory threshold in 18 trained male cyclists after 28 days of oral L-arginine supplementation (12 g [6 g twice daily]).[105]

Nevertheless, Abel et al.[13] observed no significant difference in lactate concentration, carbon dioxide output and $\dot{V}O_2$ during incremental cycle ergometer exercise after 4 weeks of either high (5.7 g of arginine and 8.7 g of aspartate) or low (2.8 g of arginine and 2.2 g of aspartate) concentrations of oral arginine aspartate supplementation in 30 male endurance-trained athletes. Furthermore, the authors found no improvement in physical performance as measured by time to exhaustion. Colombani et al.[14] also observed no improvement in the time required to run 31 km after 14 days of supplementation with 15 g of oral arginine aspartate in 20 endurance-trained male athletes. They also found no change in lactate concentration and ammonia after the supplementation period. Koppo et al.[106] observed no significant difference in plasma lactate concentration in response to a cycle ergometer test at a frequency of ~70 rpm after 14 days of supplementing seven physically active males with 7.2 g of L-arginine HCl (3 × 3 capsules of 805 mg). No significant difference was observed in urinary nitrite/nitrate (utilized as a nitric oxide production indicator). Chen et al.[18] reported a significant increase in anaerobic threshold in sixteen elderly men cyclists after 3 weeks of ingesting 5.2 g of L-arginine (in powder form). However, no significant differences were observed in plasma lactate concentration, $\dot{V}O_{2max}$ and power output between L-arginine and placebo groups.

Many of the current commercial nutritional supplements that claim to enhance NO levels utilize arginine α-ketoglutarate (AAKG) as the main 'active ingredient'. α-Ketoglutarate is an important intermediate in the Kreb's cycle, following isocitrate and prior to succinyl coenzyme A. Campbell et al.[12] reported significant increases in 1RM strength and anaerobic power (Wingate test) after 8 weeks of oral AAKG (6 g of L-arginine and 6 g of α-ketoglutarate) supplementation. Little et al.,[15] reported that both Cr (0.1 g/kg/day) and Cr + AAKG (0.075 g/kg/day) supplementation increased the total number of repetitions that could be performed over three sets of bench-press exercise compared with placebo. Only Cr + AAKG supplementation induced significant performance improvements in peak power during three repeated Wingate cycling tests. No effect was observed from Cr supplementation alone on repeated Wingate cycle performance.

Cr supplementation increases the intramuscular stores of total Cr (i.e. Cr and phosphocreatine [PCr]), leading to an increased capacity to replenish adenosine triphosphate through PCr hydrolysis. PCr is an important energy substrate for repeated resistance exercise bouts.[107] Therefore, increased PCr availability after both Cr and Cr + AAKG supplementation could have enhanced total work capacity.

The significant increase in peak power during the Wingate test after Cr + AAKG supplementation might suggest that AAKG improves the ability to generate power on repeated bouts. These results support the work of Campbell et al.[12] who found a significant increase in peak power after supplementing 35 resistance-trained healthy males with 12 g of oral AAKG. Also, Camic et al.[19] observed a significant increase on physical working capacity at the fatigue threshold (the highest power output that can be maintained without neuromuscular evidence of fatigue) in fifty untrained men performing an incremental cycle ergometer test to exhaustion after 4 weeks of 1.5 g or 3.0 g of L-arginine supplementation. Santos et al.[16] observed increased resistance capacity to muscular fatigue evaluated by isokinetic dynamometer (15 repetitions of concentric knee flexion/extension at 180°/s) after 15 days of oral supplementation with arginine aspartate (3 g/day). Fricke et al.[17] observed no significant difference in maximal isometric grip force (N), utilizing a hand dynamometer as well as jump-height (cm), peak jump power (W) and peak jump force (N) performed on a force plate, after 6 months of L-arginine HCl supplementation (18 g) in postmenopausal women. Peak jump force relative to bodyweight (N/kg) was the only variable that showed a significant increase in the L-arginine group, although this variable is not as important as the other jump variables to assess changes in performance.

Although having observed positive results in 1RM strength,[12] anaerobic power[12,15] and muscular endurance[16] after L-arginine supplementation, the authors of these studies have not evaluated the underlying mechanism that would lead to such effects. Further studies are necessary to identify the physiological mechanism behind strength, power and muscle endurance gains reported. There is still too little scientific evidence to recommend chronic L-arginine supplementation for both aerobic and resistance exercise.

6. Potential Side Effects

Studies using high doses of intravenous L-arginine (30 g) have shown side effects in normotensive healthy subjects, such as hypotension with tachycardia,[108] reduced peripheral arterial resistance[65] and an increase in cardiac output.[108] Allergic reactions, including anaphylaxis, may also result from L-arginine supplementation in some individuals,[109] suggesting that such supplementation should be avoided for individuals with allergic tendencies. Hyperkalaemia and hyperphosphataemia have been observed in patients with kidney and liver failure[110,111] and diabetes[112,113] after intravenous L-arginine administration.

Evans et al.[114] supplemented healthy subjects with different levels of oral free-form L-arginine (3, 9, 21 or 30 g/day), during 1 week and observed that four of the 12 subjects supplemented with 21 g had diarrhoea, one had nausea and another had nose bleeding. At 30 g/day, nine of ten subjects experienced diarrhoea. Campbell et al.,[12] reported no significant clinical side effects by orally supplementing healthy subjects with 12 g of AAKG for 8 weeks. Besides the dosage, it may be that the form of L-arginine supplementation (free-form vs AAKG) caused the observed side effects in the Evans study, compared with the Campbell study.

Schulman et al.[115] observed higher mortality in patients supplemented with 9 g of L-arginine for 6 months after myocardial infarction. Therefore, the authors concluded that L-arginine supplementation is not recommended for patients post-infarction. However, Bednarz et al.,[116] did not report any serious adverse effects after supplementing 792 patients with myocardial infarction with 9 g of L-arginine for 30 days. According to the authors, the supplementation was well tolerated, although it showed no benefits. Furthermore, no other study has shown high mortality rates or any other adverse effect as a result of L-arginine supplementation in the dosage as administered by Schulman et al.[115]

Sun et al.[117] recently published a meta-analysis with the purpose of analysing the effect of oral L-arginine supplementation on clinical outcomes of patients with acute myocardial infarction. Only two trials (927 participants) were included (Schulman and Bednarz studies, both described above). None of the studies showed a significant difference in event rate between the L-arginine and placebo groups. In an overall pooled estimate, there was a 7% reduction in mortality in the L-arginine treatment group compared with the control group. The authors concluded that oral L-arginine supplementation had no effect on the clinical outcomes of patients with acute myocardial infarction.

Shao and Hathcock[118] implemented a methodology for risk assessment – the observed safe level (OSL) – of L-arginine supplementation and concluded that, based on the available published human clinical trial data, there is a strong evidence indicating the absence of adverse effects up to 20 g/day, and these levels are identified as OSL for normal healthy adults.

Whereas high doses of both oral and intravenous L-arginine showed adverse effects in specific groups, low oral doses (≤20 g) are well tolerated and adverse effects are rare in healthy subjects.[20] However, one should be conservative in recommending L-arginine supplementation until further studies can establish its safety and effectiveness in patients with myocardial infarction, and particularly in non-symptomatic individuals with silent myocardial infarction.

7. Conclusions

NO is a potent endogenous vasodilator responsible for increasing blood perfusion via shear stress, and which contributes to changes in blood flow during dynamic exercise and post-exercise recovery. L-arginine is a semi-essential

amino acid that is the precursor of NO, which has led many to believe that oral supplementation with this amino acid may serve as a NO stimulator.

Of the five acute studies retrieved from the literature regarding L-arginine supplementation and exercise performance (table I), three studies reported significant increases in exercise performance: one reported increases in muscular peak torque, total work and reduced muscular fatigue, another study reported increases in anaerobic power and the remaining one reported increases in exercise time to fatigue.

Of the eight chronic studies retrieved from the literature that evaluated exercise performance (table II), four showed significant improvements in exercise performance: three studies reported increases in anaerobic power – one of which also demonstrated significant increases in 1RM strength, and one reported a significant reduction in muscular fatigue after L-arginine supplementation.

L-arginine supplementation seemed to be safe and well tolerated in the reported studies with healthy subjects, although the dosage used in the studies ranged only from 3 g to 18 g orally. No further dosages have been used in similar groups with the purpose of improving performance. Further studies are required to determine the potential ergogenic aid as well as its side effects.

Based on the current information available, it cannot be assumed that the positive results on exercise performance, whether acute and/or chronic, and regardless of the different types of exercise (aerobic or anaerobic) performed, were due to increased NO production via L-arginine supplementation, since none of the reports investigated the underlying mechanisms. There is clearly a need for more studies to verify if L-arginine enhances strength, power performance and muscular recovery associated with increases in NO production in healthy subjects.

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Correspondence: Professor *Paulo Sergio Chagas Gomes*, Laboratório Crossbridges, Centro de Pesquisas Interdisciplinares em Saúde, Universidade Gama Filho, Rua Manoel Vitorino 553, Piedade, Rio de Janeiro, RJ, 20740-900, Brazil.
E-mail: crossbridges@ugf.br