Effects of L-arginine supplementation on exercise metabolism

Glenn K. McConell

Purpose of review

To describe the influence of acute and chronic administration of L-arginine on metabolism at rest and during exercise.

Recent findings

There has been substantial examination of the effect of infusion and ingestion of L-arginine at rest. It has been clearly demonstrated that L-arginine administration improves endothelial function in various disease states. In addition, L-arginine infusion at rest increases plasma insulin, growth hormone, glucagon, catecholamines and prolactin. Such hormonal changes affect metabolism. There has, however, been very little examination of the effect of increases in L-arginine availability during exercise. This is important to study as there is preliminary evidence that L-arginine infusion, probably via increases in nitric oxide (NO), alters skeletal-muscle metabolism during exercise. There is a need for further research, especially to understand the mechanisms of how L-arginine affects exercise metabolism and also to determine whether the hormonal responses that occur in response to L-arginine at rest are also present to some extent during exercise.

Summary

This line of research may have important therapeutic implications as there are indications that L-arginine augments the effects of exercise training on insulin sensitivity and capillary growth in muscles.

Keywords

contraction, endothelial function, glucose uptake, nitric oxide

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Abbreviations

eNOS	endothelial NO synthase
nNOS	neuronal NOS
NO	nitric oxide
NOS	NO synthase

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Introduction

L-Arginine (2-amino-5-guanidinovaleric acid) is a conditionally essential amino acid that has several metabolic functions including involvement in the transport, processing and excretion of nitrogen, urea synthesis, and as a substrate in the synthesis of creatine and NO. This review will focus on the effect of L-arginine supplementation (ingestion or infusion) on metabolism, especially during exercise. There will also be discussion of the effect of L-arginine supplementation at rest, particularly in disease states.

L-Arginine and nitric oxide

Although L-arginine is involved in many processes it appears that its major influence on metabolism is via NO. L-Arginine is converted to NO and L-citrulline by NO synthases (NOSs). NO binds to guanylate cyclase to increase the production of the second messenger cGMP. The primary isoform of NOS in endothelial cells is endothelial NOS (eNOS), which plays a role in vasodilation. In skeletal muscle eNOS and neuronal NOS (nNOS) are expressed within the muscle fibres themselves [1•,2,3]. It appears that in humans nNOS is more highly expressed than eNOS in skeletal muscle [1•,3]. In rodents lacking nNOS there is no increase in skeletal-muscle cGMP with contraction, whereas increases occur in eNOS-knockout mice [4]. This suggests that nNOS is the major isoform of NOS involved in exercise metabolism. The inducible isoform of NOS (iNOS) is increased in skeletal muscle by inflammatory processes, including diabetes [5].

Effects of acute L-arginine supplementation at rest

In several disease conditions endothelial NO production is reduced, resulting in endothelial dysfunction. For instance, it appears that a defective endothelial NO pathway precedes the onset of essential hypertension [6]. Potential reasons for lower NO production include reduced NOS expression, reduced L-arginine availability, reduced L-arginine transport and lower levels of cofactors such as tetrahydrobiopterin that are required for NO generation by NOS [7,8].

The K_m value for nNOS is 1.4–2.2 μmol/l and for eNOS it is ~2.9 μmol/l [9]. Since the normal plasma concentration of L-arginine is ~100 μmol/l, which is much higher than the K_m values for nNOS and eNOS, one would not expect L-arginine infusion to alter NOS activity. L-Arginine infusion (usually 30 g given intravenously) causes the plasma

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concentration of L-arginine in humans to increase to 5000–7000 μmol/l. However, acute L-arginine infusions increase peripheral vasodilation *in vivo* in a dose-dependent manner. This phenonomen is referred to as the L-arginine paradox [9,10]. L-Arginine administration also inhibits platelet aggregation [11], acutely improves endothelium-dependent vasodilator responses, reduces macrophage adhesion to the endothelium and prevents atherosclerosis [11]. L-Arginine infusion decreases blood pressure with a compensatory increase in heart rate. In addition, L-arginine infusion improves endothelium-dependent vasodilation in people with hypercholesterolemia [12]. These effects appear to involve the NOS pathway because levels of plasma nitrate and urine cGMP increase during the L-arginine administration. It has been demonstrated that there is an endogenous inhibitor of NOS present in humans and this compound (asymmetric dimethylarginine) is increased in people with cardiovascular disease. It has been hypothesized that asymmetric dimethylarginine may explain the L-arginine paradox [10]. Infusion of L-arginine improves endothelium-dependent vascular function in people with high asymmetric dimethylarginine levels [10].

L-Arginine is also a potent hormone secretagogue. L-Arginine infusion at rest increases plasma insulin, glucagon, growth hormone, prolactin and adrenaline (epinephrine) and noradrenaline (norepinephrine) concentrations [13–15]. Intravenous infusion of several different amino acids stimulates insulin secretion, with L-arginine being the most potent. The increase in plasma insulin then generally causes a decrease in liver glucose output [16], although this will depend also on the extent of increase in plasma glucagon, which stimulates liver glucose output [17]. In aerobically trained individuals at rest, there appears to be diminished arginine-stimulated insulin secretion but not arginine-stimulated increases in plasma glucagon and growth hormone [18].

Although more physiologically relevant, oral L-arginine ingestion has much less effect than L-arginine infusion. This is probably due to the low bioavailability of ingested L-arginine, due in part to partial metabolizing in the intestinal mucosa. Indeed, ingestion of L-arginine at a level similar to a high-protein meal (1 mmol of arginine/kg of lean body mass) does not increase plasma insulin concentration in humans [19]. Higher oral L-arginine doses (3 g/h for 10 h) does increase plasma insulin concentrations and inhibit liver glucose output but only in individuals who have substantial conversion of L-arginine to L-citrulline (and presumably NO) [16].

Acute L-arginine supplementation and exercise

Although infusion of L-arginine, NO donors and NOS inhibitors has effects on blood pressure, heart rate and blood flow at rest [20,21], several studies have shown that these agents have little effect on haemodynamics during exercise in humans. For example, intravenous infusion of L-arginine during exercise in healthy humans [22] and patients with coronary heart disease [23] has no affect on heart rate or blood pressure compared with saline infusion. In addition, infusion of L-arginine has no effect on blood flow during exercise in humans [24,25].

L-Arginine administration and exercise both increase NO production by skeletal muscle. Skeletal-muscle NOS activity [26] and cGMP [2] are increased by contraction in rodents. In addition, electrical stimulation increases NO production in primary rat skeletal-muscle cell culture [27] and in isolated rat skeletal muscle [28]. Exercise also appears to increase skeletal-muscle NO production in humans based on increases in urinary nitrate/nitrite and cGMP levels [29]. Adding L-arginine to isolated rat muscles increases NO in the media [28].

The factors regulating glucose uptake into skeletal muscle during exercise are unclear, with calcium, calcium/calmodulin-dependent protein kinase, protein kinase C, AMP-activated protein kinase and NO all implicated [1•]. We recently found that infusion of L-arginine (0.5 g/min over 60 min) during prolonged exercise in young, healthy, endurance-trained men significantly increased glucose disposal during exercise compared with saline infusion (determined by infusion of a stable glucose tracer) [30••]. Given that we [24] and others [25] have previously shown that L-arginine infusion has no effect on blood flow during exercise in humans, and given that L-arginine infusion had no significant effect on plasma insulin concentration, we hypothesized that L-arginine infusion increased NO production by skeletal-muscle NOS, which then increased muscle glucose uptake.

Indeed, we have also found that femoral artery infusion of the NOS inhibitor, N^G-monomethyl L-arginine (L-NMMA), during cycle exercise attenuates the increase in leg glucose uptake during exercise in humans, with this inhibitory effect appearing to be reversed by infusion of L-arginine [24]. In a follow-up study we found that NOS inhibition attenuated the increase in glucose uptake during exercise more in people with type 2 diabetes than in matched controls [31]. In both studies there was no effect of NOS inhibition on leg blood flow, blood pressure or arterial plasma glucose or insulin concentration. In contrast, a recent study in humans found no effect of local (microdialysis) combined NOS and prostaglandin blockade on glucose uptake in skeletal muscle during exercise, despite decreases in local blood flow [32]. Studies in rodents examining the effect of NOS inhibition on contraction-stimulated glucose uptake have yielded conflicting results [33–37]; therefore, more studies are required

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to clarify the role of L-arginine/NOS in skeletal-muscle glucose uptake during exercise.

Since local muscle infusion of L-arginine [25] and femoral-artery infusion of L-arginine [24] have no effect on leg blood flow during exercise in humans, we feel that it is likely that L-arginine infusion increases glucose uptake during exercise by increasing glucose transporter 4 (GLUT-4) translocation to the plasma membrane rather than increasing skeletal-muscle blood flow. It should be kept in mind, however, that L-arginine/NOS inhibitors might effect the distribution of blood flow (the extent of capillary blood flow) during exercise without effecting total blood flow [25,38,39].

We also found that L-arginine infusion increased hepatic glucose output during exercise to a greater extent than exercise with saline infusion [30••]. The mechanism(s) behind this response are unclear but may relate, in part, to the relative hypoglycaemia caused by L-arginine infusion during exercise. Liver glucose output is exquisitely sensitive to small changes in plasma glucose levels during exercise in humans, so the decrease in plasma glucose would be expected to increase liver glucose output [40]. Therefore, it is possible that greater glucose uptake with L-arginine infusion causes a decrease in the plasma glucose concentration which then stimulated glucose output from the liver. It is also possible that L-arginine infusion increases plasma glucagon concentration during exercise, which then stimulates liver glucose output, since this has been shown to occur at rest in humans [17]. Finally, L-arginine infusion may augment the exercise-induced increases in hepatic glucose output by increasing NO, since NO donors have been shown to potentiate the effect of noradrenaline to increase liver glucose output in rats and cats [41]. Plasma noradrenaline increases during exercise in humans [42]. No study has examined the influence of L-arginine on hormonal responses to exercise, with the exception of insulin.

Lipolysis is reduced by L-arginine infusion during exercise, based on an attenuation of the increase in plasma glycerol and lower non-esterified fatty acid concentration [30••]. The lower lipolysis may have been due to greater NO production with L-arginine infusion since NO inhibits catecholamine-induced stimulation of lipolysis [43,44]. NO reduces glycerol release from isolated human adipocytes *in vitro* [45].

We found that L-arginine infusion has no effect on exercise performance which involved completion of a set amount of work as quickly as possible following 120 min of exercise [30••]. The set amount of work took around 15 min. Similarly, acute L-arginine infusion has been found to have no effect on $\dot{V}O_{2max}$-test exercise time in patients with chronic heart failure [46] and patients with hypercholesterolaemia [47].

It is necessary to now determine whether oral L-arginine supplementation, like L-arginine infusion, increases glucose disposal during exercise. Unfortunately several studies in humans involving oral supplementation of L-arginine have not used L-arginine on its own but rather have used L-arginine in combination with various other metabolites/salts, which makes interpretation of results difficult since the effects could be due to the L-arginine, the other metabolite or a combination. In addition, studies have sometimes involved very small numbers of participants. Ingestion of 20 g of L-arginine glutamate salt before exercising at 75–80% $\dot{V}O_{2max}$ caused a significant attenuation of the increase in plasma ammonia levels at the cessation of exercise (60 min) [48]. A concern about this study is that it only involved three participants. However, Schaefer *et al.* [49] also found lower plasma ammonia (and lactate) after maximal exercise when L-arginine was infused prior to exercise compared with a placebo infusion. These results suggest that L-arginine may decrease muscle energy imbalance during exercise; however, we found no effect of L-arginine infusion on plasma lactate during prolonged exercise in endurance-trained individuals [30••]. Studies with muscle biopsies are required to examine mechanisms in this regard.

Effects of chronic L-arginine supplementation at rest

It is obviously not possible to infuse L-arginine into humans for days or weeks. Therefore chronic effects of L-arginine supplementation are confined to studies utilizing oral L-arginine supplementation. Chronic oral L-arginine supplementation reduces cardiovascular disease risk factors. Four weeks of oral L-arginine supplementation (2 g, three times per day) improves angina class, lowers systolic blood pressure, increases maximum forearm blood flow and improves quality of life in hypertensive patients with microvascular angina [50]. It also raised the plasma L-arginine and cGMP concentration and increased the L-arginine/asymmetric dimethylarginine ratio [50].

In addition, long-term oral L-arginine supplementation improves insulin sensitivity and endothelial function in nonobese people with type 2 diabetes [51]. Recently, in a follow-up study, this group found that 21 days of oral L-arginine treatment augmented the beneficial effects of a hypocaloric diet and exercise training program on glucose metabolism, insulin sensitivity and markers of oxidative stress in obese type 2 diabetics [52•]. It is possible that L-arginine supplementation improves insulin sensitivity, at least in part, by increasing skeletal-muscle mitochondrial biogenesis. Mitochondrial volume is reduced in skeletal muscle of people with type 2

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diabetes [53]. NO donors increase mitochondrial biogenesis in L6 myocytes and eNOS-knockout mice have reduced muscle mitochondrial biogenesis markers [54]. We have recently found that 2 days of NOS inhibition reduces basal skeletal muscle mitochondrial biogenesis markers in rats [55].

Exercise training and a combination of antioxidants and L-arginine reduce athererosclerotic lesions and spontaneous athererosclerotic plague rupture in hypercholesterolemic mice [56]. Importantly, the combined therapy of exercise training and supplementation improved these outcomes more than either treatment alone [56]. Six weeks of oral L-arginine supplementation in rats potentiates the exercise-training-induced increases in angiogenesis in skeletal muscle and the left ventricle by, it appears, increasing vacular endothelial growth factor expression [57]. Indeed, NOS inhibition has been shown to attenuate the increases in skeletal-muscle vacular endothelial growth factor mRNA with exercise in rats [58].

Chronic L-arginine supplementation and exercise

Chronic dietary L-arginine supplementation increases aerobic capacity during treadmill exercise (~8–9% increase in $\dot{V}O_{2max}$) in hypercholesterolemic and normal mice, which was linked to increases endothelial NO function [59]. In humans, results have been contradictory [60], but on balance it would appear that chronic oral L-arginine supplementation improves maximal ($\dot{V}O_{2max}$ test) exercise capacity in patients with cardiovascular disease, congestive heart failure, stable angina, and pulmonary hypertension [61–64]. In patients with stable angina pectoris, oral supplementation of L-arginine (6 g/day for 3 days) increased exercise capacity, as determined by a maximum exercise test [61]. In addition, Doutreleau *et al.* [65] found that 6 weeks of oral L-arginine supplementation improved a standard endurance-exercise tolerance test in patients with heart failure (compared with a placebo group). Heart rate and plasma lactate concentration were also lower during exercise after chronic L-arginine supplementation.

Several studies have examined the effect of chronic L-arginine ingestion on aspects of metabolism during exercise in humans. In one study 10 days of arginine aspartate supplementation resulted in a reduction in plasma ammonia levels at 15 min during 45 min of cycling at 80% $\dot{V}O_{2max}$ [66]. However, in another study 2 weeks of arginine aspartate supplementation had no effect on plasma ammonia concentration during or 2 h after a marathon run [67]. It also had no effect on plasma glucose, lactate, pyruvate, free fatty acids, glycerol, β-hydroxybutyrate, cortisol, insulin, lactate dehydrogenase or creatine kinase, although it increased growth hormone, glucagon, urea and L-arginine itself [67]. Campbell *et al.* [68] examined the effect of 8 weeks of oral L-arginine α-ketoglutarate supplementation on strength and other measures in resistance-trained men. Twenty men ingested L-arginine α-ketoglutarate three times per day (12 g/day) and 15 men ingested a placebo. The L-arginine α-ketoglutarate group had significantly greater gains in strength during the bench-press exercise and during a predominantly anaerobic 30-s sprint test on a bicycle ergometer (the Wingate peak power test). L-arginine α-Ketoglutarate did not influence body composition, muscular strength endurance or aerobic capacity [68]. The finding that L-arginine α-ketoglutarate supplementation did not improve aerobic capacity supports earlier findings that L-arginine improves $\dot{V}O_{2max}$ in various disease populations but not in healthy individuals [69].

Conclusion

It is clear that L-arginine supplementation improves aerobic exercise capacity in various cardiovascular disease states which are associated with endothelial dysfunction. It is likely that the improvement in exercise capacity is due to L-arginine increasing the production of NO in these individuals with reduced basal NO production. Accordingly, in healthy individuals with normal NO production it appears that L-arginine administration has little impact on aerobic exercise capacity.

Little research has been conducted to examine the effect of L-arginine supplementation on exercise metabolism. There is some evidence that L-arginine infusion increases glucose uptake during prolonged exercise and reduces lipolysis. It is possible that these effects are due to increases in NO production but more research is required to confirm this. There is also some evidence that oral L-arginine supplementation can interact with exercise training to increase the beneficial effects of exercise on capillary growth and insulin sensitivity. Further studies are required to elucidate the potential ergogenic and therapeutic potential of L-arginine.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 117).

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