



Arginine supplementation and cardiometabolic risk

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11 **Title of review article: Arginine supplementation and cardiometabolic risk**

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19

20 **Abstract**

21 *Purpose of review:* Because arginine is the substrate for nitric oxide synthesis, which is pivotal to
22 vascular homeostasis and linked to the insulin response, it has long been posited that supplemental
23 arginine could benefit cardiometabolic health.

24 *Recent findings:* Recent data have supported the view that supplemental arginine could alleviate the
25 initiation and development of endothelial dysfunction and also shown that it may reduce the risk of
26 type 2 diabetes. One important finding is that these effects may indeed vary as a function of the
27 amount of arginine, its form and notably the metabolic status of the population. Some studies have
28 shown that low doses of slow-release arginine are better used for NO synthesis and beneficial in
29 individuals with abnormal arginine metabolism/bioavailability. Pathophysiological data in rodents
30 have emphasized the importance of arginase activation during the development of cardiometabolic
31 risk, which lends credence to a potential benefit for arginine supplements. Likewise, epidemiological
32 evidence suggests that alterations to arginine bioavailability are important regarding the
33 cardiometabolic risk. However, other metabolic mechanisms linked to the multiple pathways of
34 arginine metabolism may also play a role.

35 *Summary:* Further studies are needed to confirm and analyze how and when supplemental arginine
36 is beneficial to cardiometabolic health.

37 **Keywords:** L-arginine; arginine metabolism; Endothelial function; insulin resistance; nitric oxide.

41 **Introduction:**

42 Since the discovery that arginine was the unique substrate for the synthesis of nitric oxide (NO)
43 which plays a critical and pivotal role in vascular homeostasis, it has been suggested that
44 supplemental arginine might be beneficial in alleviating or preventing atherosclerosis. Then, because
45 of growing recognition of the association between insulin sensitivity and endothelial function [1] and
46 the possible role that NO might play in systemic alterations to metabolic fluxes and hemodynamics
47 [2], it has also been proposed arginine could have potential benefits on a broad cluster of metabolic
48 abnormalities that constitute cardiometabolic risk factors which increase the risks of cardiovascular
49 disease and type 2 diabetes.

50 In this review, we will particularly focus on the most recent findings regarding the effects of
51 supplemental arginine on preventing or alleviating risk factors such as endothelial dysfunction and
52 insulin resistance. We will not present data on arginine supplementation on other endpoints, or in
53 specific populations, although these make the case for its metabolic impact, such as recent findings
54 regarding preeclampsia in animal models [3, 4] and clinical trials [5]. The review will focus on data in
55 humans but also report findings from animal studies useful to understand the possible mechanisms.
56 We will not directly consider data from cellular experiments that would not take account of the
57 fundamental compartmentation of the arginine nutritional system *e.g.* [6, 7]. Nor will we consider
58 studies where arginine supplementation has been combined with that of other nutrients [8, 9]
59 because it is not possible to disentangle the specific role of arginine.

60 **Endothelial function**

61 Recent reviews and meta-analyses have supported the view that supplemental arginine favors
62 endothelial function in humans [10, 11, 12*]. However, Rodrigues-Krause et al. reported that the
63 results were highly heterogeneous and that the positive effects of arginine supplementation on
64 endothelial function (evaluated as flow-mediated dilation) could only be identified by reducing
65 heterogeneity in order to discard extreme results [12*].

66 Those findings have also been supported by animal studies using diet-induced cardiometabolic risk.
67 For instance, in fructose-fed rats, supplemental arginine prevented the impairment of endothelium-
68 dependent vasodilation and hyper-reactivity to phenylephrine, to the same degree as aerobic
69 training [13]. Both treatments increased plasma nitrite, indicative of increased eNOS activity.

In a randomized double-blind trial in healthy humans with elevated triglycerides and enlarged waist, we found that supplemental arginine (1.5g 3 times/d) did not improve fasting endothelial function but lowered postprandial dysfunction in individuals with lower baseline plasma arginine concentrations [14*]. This finding was in line with an earlier report that a moderate amount of arginine (2.8g) added to a high-fat meal could specifically prevent postprandial endothelial dysfunction [15]. Meta-analyses and clinical trials have documented that arginine might have beneficial effects at levels of intake that could be manipulated by the diet (i.e. addition of 3-6g/d to an average baseline dietary intake of ~5g/d) [16, 17] in individuals displaying an impairment of baseline endothelial function [17]. In the elderly, arginine (3g) rescued the impairment in femoral blood flow and muscle microvascular blood volume in response to 15g amino acids [18]. In healthy young individuals, we would expect that the effect of moderate doses of arginine would mainly be seen in those with baseline metabolic/physiological alterations and/or during an allostatic challenge, such as that elicited by a high saturated fat meal.

Insulin sensitivity and the diabetes risk

In 2018, Monti and collaborators reported their analysis of 90 months of follow-up of an 18-month, randomized, double-blind trial of arginine supplementation (6.4g/d) in 144 individuals with a cardiometabolic risk [19**]. The authors found a 34% lower risk of becoming diabetic in the arginine supplemented group, indicating the permanence of their findings at 12 months of follow-up. Another important study recently reported a beneficial effect of supplemental arginine on insulin sensitivity. In a randomized cross-over study, overweight men with prediabetes (i.e. elevated baseline plasma glucose or postprandial glucose intolerance) received 9g/d arginine or placebo [20**]. In men of European descent (but not those from southern Asian), arginine supplementation improved insulin sensitivity and glucose control (as assessed by an oral glucose tolerance test). This was concomitant with a marked increase in cold-induced glucose oxidation in men of European descent only. As with endothelial function, these findings have been supported by data in rats [13] and earlier findings in humans at moderate doses of arginine [16].

These important results in humans echo a recent analysis of data from the PREDIMED trial. Using a case-cohort design, the authors estimated the relationship between changes to plasma arginine during the trial in 892 individuals at high cardiovascular risk [21*]. They found a negative association between changes to plasma arginine and the risk of incident type 2 diabetes (-29% risk per 1 SD increment). However, it remains unclear whether the complex changes in arginine-related metabolites reflect changes to arginine intake or to arginine metabolism, particularly with regards to arginine availability for NO synthesis.

Other cardiometabolic factors

The effect of supplemental arginine on blood pressure were further documented by a well-conducted, large, randomized single-blind trials in Iran where 90 individuals with obesity or visceral obesity were supplemented with arginine at either 1g 3 times/d or 2g 3 times/d, or given a placebo [22*]. Systolic blood pressure decreased by 4 mmHg and 8 mmHg with the 3g and 6g supplementation doses, respectively. Arginine supplementation also resulted in body weight loss (3% and 9%), which could explain part of the important reduction in blood pressure [22*].

Although it could have been driven by changes to body weight, this reduction in blood pressure was in line with most evidence in this regard. Notably, in 2011, the meta-analysis by Dong et al. concluded that arginine supplementation reduced systolic blood pressure by ~5mmHg and diastolic blood pressure by ~3mmHg [10, 23]. Comparing the very high doses of 30g and 15g arginine supplements to a placebo in overweight/obese healthy individuals, McNeal also reported that 30g/d lowered systolic blood pressure (by ~5mmHg), in female participants only [24*].

The effects of arginine supplementation on body weight in the study by Dashtabi et al had already been reported by some previous trials and animal studies [10]. In my opinion, the data remain inconclusive in humans. Possible mechanisms include a reduced food intake (presumably by a direct central mechanism rather than mediated by the stimulation of gut hormone release, as recently proposed [25]), and changes to adipose tissue metabolism [26]. Arginine does not appear to affect the thermoregulatory response to stress in humans [27].

Few studies have reported effects of arginine supplementation on inflammatory pathways. Using moderate amounts of arginine to supplement the diet of rats, Hong et al. recently reported downregulation of nuclear factor kappaB p65 and a lowering of plasma CRP [28]. Kim et al. [29] also found that low amount of arginine supplementation reduced NF-KB expression in exercising rats fed a high-fat diet, and also reduced TNF α in rats fed a high-fat diet.

At which dose, in which form and for which people?

The first studies of arginine supplementation were conducted at relatively high doses (mostly ~9-12g/d). However, some studies have investigated the potential of lower doses, commensurable with spontaneous dietary intake and its possible variation resulting from changes to the level and type of dietary protein consumed. Studies at low doses are important because the favorable effects of some foods are often ascribed, at least in part, to the amount of arginine they contain – e.g. nuts [30, 31]. As discussed above, the effects of low-dose arginine on endothelial function and insulin sensitivity

have been reported at low doses in clinical trials [14*, 15, 16] and in the positive meta-analysis conducted by Bai et al., no relationship with the dose was evidenced [17].

Because dietary arginine is consumed naturally as dietary protein, it is made available much more slowly than the free arginine (i.e. arginine chlorhydrate or Arginine base) found in supplements. It has been proposed that the speed of absorption could impact the metabolic utilization of supplemental arginine, as is often the case for amino acids and nutrients in general. Using labeled arginine in a cross-over trial, we evidenced that arginine in sustained-release form was directed more to NO synthesis than arginine in the immediate release form [32*]. In this trial we also found that the utilization of supplemental arginine for NO synthesis was higher in individuals with cardiometabolic risk factors than in control subjects, thus offering the first observation at the metabolic level explaining that the benefits of arginine supplementation have mostly been demonstrated in individuals with cardiometabolic risk factors and altered baseline NO-related functions.

Against this background, low doses of sustained-release arginine have indeed been reported to exert favorable effects on endothelial function and insulin resistance in individuals with cardiometabolic risk factors [14*, 16, 33]. Recent studies in rats could be considered as contributing little to the topic because they were mostly conducted at doses (between 0.9 and 2.5g/kg bw) that are higher than what could be achieved in humans, even if the arginine was given by enriching the food or water, thus spreading delivery over the wakening period [13, 34, 35, 36*].

Conversely, it has long been suggested that supplemental arginine might be detrimental in certain situations of overt disease because NO synthesis may mostly be operating by the inducible form of NO synthase (iNOS) which generates oxidative and nitrosative stress. In this situation, supplemental arginine might indeed fuel this detrimental process. This was first proposed many years ago [37] and recently further illustrated by the finding that an increase in iNOS activity and the resulting nitrosative stress was pivotal in a model of heart failure [38]. The benefits of arginine supplement may therefore vary depending on the degree of activation of iNOS over constitutive NOS isoform (eNOS), and the reorganization of NO synthesis regulation during the development of obesity and/or associated cardiometabolic risks [37, 39, 40]. Nonetheless, the detrimental effects of arginine supplements may be limited to these specific situations of acute disease, and arginine supplementation has been documented as being safe in a wide variety of situations [10, 41, 42].

Reduced arginine bioavailability in a context of cardiometabolic risk

There is still widespread agreement that the development of cardiometabolic risk is associated with a reduction in NO synthesis [43], resulting from alterations to the metabolism of arginine and its bioavailability for NO synthesis [21]. However, no good markers for arginine bioavailability yet exist

in humans, so it is mostly studied from the ratio of arginine to other metabolites (ornithine and citrulline or methylated arginine) in fasting plasma, whereas arginine metabolism is highly compartmented and plasma arginine is not the metabolic precursor of NO synthesis [44].

Animal models have been of value to understanding the alterations to arginine metabolism that operate during the onset and development of obesity and cardiometabolic risk, and have shown that increased arginase activity may be the key to reducing NO synthesis-. In a well-conducted study using high-fat high-sucrose diets in rodents and endothelial cells culture, Bhatta et al. showed that the deletion or inhibition of arginase activity prevents endothelial dysfunction, arterial rigidity, the impairment of endothelial cell NO synthesis and increases in oxidative and nitrosative stress, as well as preserving a normal ratio of arginine over ornithine and citrulline [45**]. Other studies have recently highlighted the role of arginase in the development of endothelial function, and notably the importance of the up-regulation of arginase 1 in red blood cells, secondary to an increase in oxidative stress [46]. It has also been shown that perivascular adipose tissue could also contribute to diet-related endothelial dysfunction by the induction of arginase [47]. An increase in asymmetric dimethyl arginine is still thought to be implicated in endothelial dysfunction and insulin resistance, which may operate by the competitive inhibition of NO synthesis [48]. Taken together, these findings therefore indicate that an increase in L-arginine availability (e.g. by arginine administration or the inhibition of arginase) results in the prevention of cardiometabolic abnormalities [46, 47] which remains strongly compatible with the potential of supplemental arginine to provide a supplemental substrate to overcome or alleviate the reduction in NO synthesis.

In a more novel manner, recent studies have suggested that arginine supplementation could affect the microbiota and intestinal epithelial functions, which might ultimately modulate systemic pro-inflammatory conditions [49]. Recently, the attenuation of fructose-induced non-alcoholic steatohepatitis using supplemental arginine was shown to be accompanied by an alleviation of decreased tight junction protein levels in the upper portions of the small intestine, of increased levels of bacterial endotoxins in portal plasma and a rise in hepatic toll-like receptor-4 mRNA [36]. The precise mechanism still needs to be elucidated.

Supplemental arginine may have a physiological and pathophysiological impact that might originate from its effects on the many pathways other than NO synthesis. In recent years, numerous studies have been performed on homoarginine, a new player in the area of arginine and the cardiometabolic risk. Homoarginine has been proposed as a protective risk factor for cardiovascular disease and diabetes [50], although the underlying mechanism remains highly unclear [51*]. Importantly,

199 arginine administration has been reported to increase homoarginine synthesis in various models
200 including rats, pigs and humans in the acute and chronic settings [52, 53].

201 **Conclusion**

202 There is now further evidence regarding the benefits of supplemental arginine during the initiation
203 and development of cardiometabolic risk, namely with respect to endothelial dysfunction and the
204 risk of type 2 diabetes. These benefits may indeed vary as a function of the amount of arginine, its
205 type and notably the metabolic status of the population. Pathophysiological data and
206 epidemiological findings have highlighted abnormalities in arginine bioavailability that lend credence
207 to a potential benefit for arginine supplements. Further studies are now needed to confirm and
208 analyze how and when supplemental arginine could be beneficial to cardiometabolic health.

209

210 **Key points:**

211 (3-5 key points/sentences that summarize your article)

- 212 • There is now more evidence that supplemental arginine may benefit endothelial dysfunction
213 and insulin sensitivity, and possibly other cardiometabolic risk factors.
- 214 • The benefits may vary depending on its dose and type, and notably the metabolic status of
215 the population.
- 216 • Supplemental arginine may alleviate the development of the arginine metabolism
217 abnormalities that constitute important background mechanisms for increases in the
218 cardiometabolic risk.
- 219 • Further studies could interestingly address variations in the effects of supplemental arginine
220 as a function of the background arginine metabolism of individuals.

221

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224

225

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