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

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17 Telephone number: none that I want to be public – thank you.

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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.

38 **Abbreviations:** NO, nitric oxide

40

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.

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

83 **Insulin sensitivity and the diabetes risk**

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

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

103 **Other cardiometabolic factors**

104 The effect of supplemental arginine on blood pressure were further documented by a well-
105 conducted, large, randomized single-blind trials in Iran where 90 individuals with obesity or visceral
106 obesity were supplemented with arginine at either 1g 3 times/d or 2g 3 times/d, or given a placebo
107 [22*]. Systolic blood pressure decreased by 4 mmHg and 8 mmHg with the 3g and 6g
108 supplementation doses, respectively. Arginine supplementation also resulted in body weight loss (3%
109 and 9%), which could explain part of the important reduction in blood pressure [22*].

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

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

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

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

128 The first studies of arginine supplementation were conducted at relatively high doses (mostly ~9-
129 12g/d). However, some studies have investigated the potential of lower doses, commensurable with
130 spontaneous dietary intake and its possible variation resulting from changes to the level and type of
131 dietary protein consumed. Studies at low doses are important because the favorable effects of some
132 foods are often ascribed, at least in part, to the amount of arginine they contain – e.g. nuts [30, 31].
133 As discussed above, the effects of low-dose arginine on endothelial function and insulin sensitivity

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

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

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

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

163 **Reduced arginine bioavailability in a context of cardiometabolic risk**

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

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

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

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

194 Supplemental arginine may have a physiological and pathophysiological impact that might originate
195 from its effects on the many pathways other than NO synthesis. In recent years, numerous studies
196 have been performed on homoarginine, a new player in the area of arginine and the cardiometabolic
197 risk. Homoarginine has been proposed as a protective risk factor for cardiovascular disease and
198 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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223 **Conflicts of interest: None.**

224

225

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