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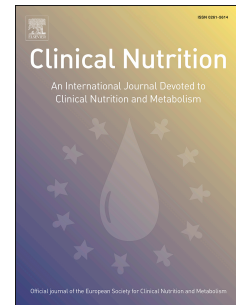
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Citrulline in health and disease. Review on human studies

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Citrulline in health and disease

1 **Abstract**

2 The amino acid L-citrulline (CIT) is safely used from the neonatal period onwards in
3 those with urea cycle defects and carbamyl phosphate synthetase or ornithine
4 transcarbamylase deficiencies, but several lines of enquiry indicate that it might have a
5 much wider therapeutic role.

6 When protein intake is low and there is a catabolic state, endogenous arginine (ARG)
7 synthesis cannot fully be met and its supplementation can prove challenging, particularly
8 in patients with critical and multisystem illness. Supplementary CIT could constitute a
9 safer but still focused means of delivering ARG to endothelial and immune cells as CIT is
10 efficiently recycled into these cells and as kidneys can convert CIT into ARG. Unlike
11 ARG, CIT is efficiently transported into enterocytes and bypasses liver uptake. It also
12 appears to prevent excessive and uncontrolled nitric oxide (NO) production. Animal
13 studies and early human data indicate positive effects of CIT on protein synthesis, in
14 which its contribution is thought mediated through the mTOR pathway.

15 It appears that CIT is an anabolic pharmacconutrient that can be safely administered even
16 in critically ill patients. Promising results in cardiovascular diseases and in disease-
17 related malnutrition can now be considered sufficient to justify formal clinical exploration
18 in these areas and in sarcopenia in general.

19 **Introduction and background**

20 Citrulline (CIT) is an amino acid which is an end product of glutamine metabolism and a
21 metabolite of arginine (ARG).

22 Its name is derived from *citrullus* the Latin name for watermelon. It was extracted in 1914
23 by Koga&Odake from watermelon⁽¹⁾ and identified by Wada in 1930. Of note, this
24 aminoacid is not incorporated into proteins⁽²⁾.

25 Glutamine is a precursor of ornithine, which can be converted to CIT by the intestine.
26 Arginine is also metabolized in CIT into enterocytes⁽²⁾. Because enterocytes do not
27 possess argininosuccinate synthase, CIT is released in the portal vein⁽³⁾. Since there only
28 small net CIT uptake by the liver⁽³⁾, in the presence of normal liver function, it enters the
29 systemic circulation and is then transformed in the kidney to ARG (fig.1). CIT net
30 production at the whole body level is therefore almost exclusively from the epithelium of
31 small intestine (Fig 1)⁽²⁾.

32 CIT can act as ARG regulator as it can control delivery of ARG to the liver. Intestinal
33 arginase and ornithine carbamoyl transferase yield citrulline in proporsion to delivery of
34 dietary protein. Fasting leads to less CIT and proportionally more ARG reaching liver.
35 ARG has a major impact on hepatic enzymes and up to 5 fold increase in ureagenesis.
36 This effect needs to be regulated, hence the important role of CIT in this physiological
37 context^(2,3)

38 These metabolic considerations explain why administration of CIT has been proposed to
39 increase systemic ARG concentrations. Of note, CIT is almost absent in food. Only

40 watermelon contains significant amounts of CIT; all parts of watermelon, rind, flesh and
41 seeds contain CIT in greatest amount, on a dry weight basis in the rind, which might offer
42 a convenient source of natural citrulline⁽⁴⁾

43 In healthy individuals the plasma concentration of citrulline is about 40 $\mu\text{mol/L}$ with
44 some racial variation (less in Chinese Asians)⁽⁵⁾.

45 CIT plasma concentration has been proposed as clinical tool for identification of small
46 bowel absorptive mass. Statistical significant correlations between plasma CIT
47 concentration and small bowel length as well as villous atrophy have been demonstrated.
48 CIT has been considered a reliable marker of intestinal malabsorption and its role in
49 clinical practice is currently under investigation⁽⁶⁾.

50 CIT as an organic supplement appears to be a powerful pharmacological nutrient, and early
51 experimental studies have suggested its therapeutic potential to restore ARG metabolism
52 in critically ill patients with sepsis^(7, 8).

53 CIT exhibits good bioavailability⁽⁹⁾, thanks to its ability to be handled by a wide number
54 of amino acid transporters⁽¹⁰⁾. In the liver CIT is a metabolic intermediate involved in the
55 elimination of a toxic component (ammonia) through another which is non-toxic (urea)
56 (Fig.1). Of note, CIT recycling in the urea cycle is mainly separated meaning that there is
57 neutral /balanced flux in the liver⁽³⁾ (Fig 1). The brain and some leukocytes can also
58 produce ARG from CIT⁽¹¹⁾ to a limited extent.

59 Oral CIT supplementation raises plasma ARG concentrations and augments nitric oxide
60 (NO)-dependent signalling proportionally⁽¹²⁾. Since CIT is not subject to pre-systemic

61 elimination in the liver whereas ARG is largely extracted there⁽¹³⁾ (Fig. 1) CIT serves as
62 an ARG precursor more productively than ARG itself^(2, 3, 12). In most human studies,
63 CIT has been used as a supplement, intending this as a substrate from which ARG can be
64 synthesized or as a NO precursor.

65 CIT is indeed one of the key organic compound leading to production of NO in most
66 cells, NO synthase (NOS) enzymes catalysing the conversion of ARG into CIT,
67 producing NO in an internally conservative cycle⁽¹³⁾ (fig 2). Although intracellular ARG
68 concentrations are sufficient to saturate NOS, therapy with excess ARG can enhance NO
69 production because the CAT-2 ARG transporter is closely associated to NOS within the
70 cell membrane and both are co-stimulated by signals such as pro-inflammatory cytokines
71⁽³⁾. In the situation of low protein intake, it is possible that the alternative pathway is
72 activated. To inhibit ARG derived ureagenesis and thus loss of proteins, intestinal
73 arginase and ornithine carbamoyl transferase are activated. This results in an increase in
74 prehepatic conversion of ARG to CIT, which (unlike ARG) passes more or less freely
75 through the liver and is released to the systemic circulation^(2,13,14).

76 CIT as dietary supplement appears to be a powerful pharmaconutrient, and early
77 experimental studies have suggested its therapeutic potential to restore ARG metabolism
78 in critically ill patients with sepsis^(7, 8).

79

80 **Safety of citrulline administration**

81 CIT is considered as safe for oral use⁽¹⁵⁾. It has no identified toxicity and is used as long-

82 term replacement therapy for children with urea cycle defects. In contrast to ARG and
83 ornithine, which induce gastrointestinal side effects at moderate dosage (e.g. 10g in one
84 bolus) ⁽¹⁶⁾, no side effects have been reported from CIT administration as an oral
85 supplement at doses up to 15g ⁽⁹⁾. Additional safety data come from interventional
86 studies.

87 In a randomized placebo-controlled double-blind trial of orally administered CIT in 40
88 children undergoing repair of congenital cardiac defects no adverse events were noted ⁽¹⁷⁾.

89 In a pharmacokinetic study of intravenously administered CIT no side effects or adverse
90 events were noted ⁽¹⁸⁾. This pharmacokinetic study formed the basis for an on-going
91 randomized, placebo controlled, double-blind trial of intravenous CIT in children at above
92 average postoperative risk after surgery for congenital heart disease ⁽¹⁹⁾.

93 CIT supplementation is now raising clinical interest for the treatment of paediatric
94 pulmonary hypertension however further controlled clinical trials are needed to draw an
95 impactful conclusion ⁽²⁰⁾.

96

97 **Effects on immunity, oxidative stress and related parameters**

98 CIT administration reduces the number of total leukocytes and of neutrophils in
99 circulation ⁽⁹⁾ and might induce ARG-derived NO-mediated vasoprotection, with
100 inhibition of cell adhesion and leukocyte activation, and suppression of endothelial
101 damage (Fig. 2) ⁽²¹⁾.

102 In red blood cells NO is oxidized to nitrate. Nitrite and nitrate are excreted in the urine.
103 PRMT (protein arginine methyl transferase) methylate L-arginine in proteins and
104 methylated proteins are hydrolysed to L-arginine derivate including ADMA (asymmetric
105 dimethylarginine) that is hydrolysed by DDAH (dimethylarginine
106 dimethylaminohydrolase) to L-citrulline. CIT acts as scavenger for oxidative lipoproteins
107 and ADMA⁽²²⁾ (Fig 2).

108 Citrulline mediated vasoprotection has now been demonstrated in a phase 2 study of
109 sickle cell disease⁽²³⁾ and further trials are on-going.

110 As further NO mediated effect with major changes in markers of oxidative stress was
111 demonstrated by supplementing a group of professional cyclists⁽²¹⁾ with a single pre-race
112 dose of 6g CIT malate. Higher concentrations of neutrophil nitrite suggested that these
113 effects were mediated by NO (Fig. 2), and there was no evidence of oxidative damage
114 (levels of malondialdehyde and creatine kinase, for example, remaining normal).

115 However, there is no definitive evidence that effects of CIT on immunity are mediated
116 through NO synthesis only. Polyamines derived from ARG and ornithine could also be
117 involved. Also, CIT has anti-oxidant properties, which could be involved in these effects.

118

119 **Effects on sports performance and recovery**

120 In addition to the study reported above⁽²¹⁾, several others are of interest in this field. Oral
121 CIT supplementation given for a week reduced the time needed to complete a cycle
122 ergometer exercise trial in healthy trained men in a double-blind randomized placebo-

123 controlled 2-way crossover study⁽²⁴⁾ CIT supplementation significantly increased plasma
124 ARG levels and reduced the exercise time by 1.5 % ($p < 0.05$). This was associated with
125 subjective improvements in muscle fatigue and ability to concentrate immediately after
126 exercise.

127 The effects of CIT on NO biomarkers, pulmonary O₂ uptake ($\dot{V}O_2$) kinetics, and exercise
128 performance were studied in a randomized, placebo-controlled, crossover study. Short-
129 term CIT, but not ARG supplementation can improve $\dot{V}O_2$ kinetics as expressed by $\dot{V}O_2$
130 mean response time (59 ± 8 and 53 ± 5 s with placebo and CIT respectively, $p < 0.05$)
131 during severe-intensity exercise, improving the tolerance (duration: $589s \pm 101$ vs $661s \pm$
132 107), and increasing the whole volume of work completed⁽²⁵⁾.

133 A further preliminary study suggested that consuming CIT malate before competition has
134 the potential to improve some elements of performance in masters level female tennis
135 players⁽²⁶⁾. In this lab-based study CIT yielded improved grip strength, peak and
136 explosive power compared to placebo. Direct application to “on court performance” is
137 requested to validate results.

138 A randomized double-blind cross-over study⁽²⁷⁾ examined the effect of a single 8g dose
139 of CIT malate on the performance of flat barbell bench presses (pectoral training) as an
140 anaerobic exercise and to test muscle soreness after this exercise. The study showed a
141 significant increase in the number of repetitions achieved (52% more in the 4th set than in
142 the equivalent placebo session where 40mL lemon juice, 10 g powdered sugar, 60 mg
143 sodium saccharine, and tap water 200 mL were used) and there was a 40% decrease in

144 muscle soreness at 24 and 48 hours ⁽²⁷⁾. A further randomized double-blind study ⁽²⁸⁾
145 examined the effect of a pre-exercise dose of CIT (6g), watermelon juice (to provide CIT
146 1g), or placebo (7.5% sucrose placebo drink) on the total number of repetitions completed
147 over 5 sets, time to exhaustion, maximal oxygen consumption (VO₂max), anaerobic
148 threshold, and flow-mediated vasodilation. In this study pre-exercise supplementation
149 appeared to be ineffective in improving exercise performance ⁽²⁸⁾.
150 It thus appears that CIT may improve exercise performance in young healthy adults under
151 some conditions, but these acute effects still need further investigation ⁽²⁹⁾.
152 Of note, in several of these studies, CIT has been used as a malate salt, not as the native
153 amino acid. As malate is an intermediary of the Krebs cycle increasing cellular energy
154 production it is unclear whether the observed effects are due to malate, to CIT or to both.
155 There is no study comparing the effects of CIT malate and CIT. In Table 2 the doses of
156 citrulline from citrulline malate have been corrected to subtract the contribution of malate
157 in those cases where this salt was used.

158

159 **MELAS syndrome**

160 As CIT plays a key role in the production of NO in most cells, due to its great ability to
161 increase intracellular ARG availability, it has been used in children with the
162 Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS)
163 syndrome ⁽³⁰⁾. In a recent clinical study stable isotope infusion techniques were used to
164 assess NO production in children with MELAS syndrome and in healthy controls. In

165 children with MELAS syndrome, CIT supplementation resulted in important increases in
166 NO production, ARG flux, plasma ARG, and CIT flux, which were greater than obtained
167 with ARG supplementation⁽³¹⁾. In an earlier clinical trial⁽³²⁾, the effect of ARG or CIT on
168 lactic acidemia had been studied in adults with MELAS syndrome. Plasma lactate
169 decreased significantly after CIT supplementation, whereas the effect of ARG
170 supplementation did not reach statistical significance. These promising results justify
171 additional controlled trials to assess the therapeutic effects of CIT on clinical features and
172 complications of MELAS syndrome.

173 **Cardiovascular diseases**

174 In most of the work in the cardiovascular area, CIT has been given with the intention of
175 boosting levels of ARG and as a NO precursor. The conditions studied have therefore
176 included those where absolute or relative deficiencies of ARG and NO are known or
177 suspected, and include arteriosclerosis, pulmonary and systemic hypertension and cardiac
178 failure. In general, oral CIT is seen to improve cardiac performance with exercise. A
179 causal link is supported by the study on professional cyclists already referred to above⁽¹⁹⁾.
180 In a double-blind, randomized, placebo-controlled trial⁽³³⁾ 15 otherwise healthy middle-
181 aged male subjects with evidence of early arteriosclerosis were given 5.6 g/day of CIT
182 (n=8) or placebo (n=7) for 7 days. Their initial arterial stiffness was abnormal, as
183 indicated by a brachial-ankle pulse wave velocity (baPWV) of >1400 cm/s. CIT
184 supplementation increased plasma CIT (p<0.05), plasma ARG (p<0.01), and the ratio of

185 ARG to ADMA ($p < 0.05$). The circulating concentrations of nitrogen oxides (the sum of
186 nitrite and nitrate) and other metabolic products of NO also rose significantly ($p < 0.05$)
187 ⁽³³⁾. Associated with these biochemical changes there was a clinically significant fall in
188 the baPWV in the CIT group ($p < 0.01$) with no change in systemic blood pressure,
189 suggesting the potential for functional improvements in arterial stiffness from CIT
190 supplementation, independent of blood pressure.

191 Other studies in patients and in healthy volunteers do however demonstrate effects of CIT
192 on systemic blood pressure. In the cold pressor test (CPT), it is normal to see substantial
193 increases in systemic blood pressure, pulse pressure and a number of other
194 haemodynamic parameters. After 4 weeks of daily CIT supplementation (6 g orally)
195 these effects were all attenuated (each by 4 to 6 mmHg) ⁽³⁴⁾.

196 Beneficial effects of CIT and ARG on endothelial function are shown by their
197 normalization of the MAT/TT index in patients with early cardiac failure. This index is
198 the ratio of the maximum amplitude time (MAT) on finger plethysmography to the total
199 time (TT) of the curve. After 60 days during which 3 g oral CIT malate was given daily,
200 the basal MAT/TT had fallen from a mean of 41.1 (± 13.47) to 23.6 (± 6.74) ($p = 0.007$)
201 (where 30 is the upper limit of normal). Testing was repeated after brief, experimentally
202 induced, digital ischaemia, and when ARG was given rather than CIT, and in both cases
203 similar improvements were seen at 60 days ⁽³⁵⁾, which suggests a common mechanism of
204 action, likely through NO production.

205 In a second paper ⁽³⁶⁾, on the same patients, the authors recorded the effects of CIT on

206 pulmonary artery pressure, which fell by 16% (56.7 ± 7.96 mmHg to 47.7 ± 8.59 mmHg;
207 $p < 0.05$) over 60 days in association with an improvement in right ventricular ejection
208 fraction, blood pressure and treadmill tolerance. ARG was equally effective, but required
209 the higher dose of 8 g⁽³⁶⁾. This may be explained by the fact that ARG is metabolized in
210 splanchnic area whereas CIT is not (see above for details).

211 Another group of investigators recently reported that adults with heart failure had
212 improvements in left ventricular ejection fraction, functional class, and endothelial
213 function as assessed by photoplethysmography after treatment with oral CIT for 4 months
214⁽³⁷⁾.

215 Intensive CIT supplementation (oral or intravenous) was previously proposed as a
216 possible means of preventing post-operative pulmonary hypertension, with a subsequently
217 suggested target plasma CIT concentration in excess of $37 \mu\text{mol/L}$ ⁽³⁷⁾. This has been
218 partially tested in children undergoing surgical procedures for congenital heart lesions.
219 Oral CIT supplements safely increased plasma CIT and ARG concentrations compared
220 with placebo, and improved NO production⁽¹⁷⁾. The expected decreases in plasma CIT
221 and ARG concentrations after cardiopulmonary bypass seen in the placebo group were
222 prevented by CIT. This was associated with a decreased risk of postoperative pulmonary
223 hypertension (15% in those treated with CIT compared to 30% in the controls). It was
224 thought that this effect was causally derived from the production of L-ARG from CIT,
225 and to stimulation of NO pathway in the hepatic and pulmonary tissues. The cytosolic
226 portion of the urea cycle was thought to be enabling localized, intracellular production of

227 L-ARG from CIT within the pulmonary endothelium as well as in hepatocytes. Curiously
228 these papers, which report on work from more than a decade ago, do not seem to have
229 been followed-up by their authors or others in the field ^(38,39).

230 Electrophysiological mechanisms may also be important. A study in healthy individuals
231 given CIT (3.2g 60-90 minutes before testing) demonstrated a reduction in QT interval on
232 electrocardiography, indicative of a shortening of the time required to de/repolarize the
233 myocardium ⁽⁴⁰⁾.

234 Taken as a whole these results are impressive but most enrolled a limited number of
235 subjects and only surrogate markers were studied. The time appears ripe for study of
236 larger cohorts of patients and evaluating the effects of CIT on morbidity and mortality.

237

238 **Anabolic effects**

239 Through various underlying mechanisms, CIT has the potential to affect protein
240 metabolism ⁽⁴¹⁾. Osowska et al ⁽⁴²⁾ showed that when malnourished elderly rats were re-
241 fed with a CIT-enriched diet, muscle protein synthesis was greater, while hepatic protein
242 synthesis was less than in control rats fed an isonitrogenous supplement of non-essential
243 amino acids (NEAA). These data are now being supported by human studies of muscle
244 protein synthesis ⁽⁴³⁾. Eight healthy participants were investigated in a crossover study in
245 which, following 3 days of standardised low protein intake, CIT or a NEAA mixture was
246 given orally as small boluses over the course of 8 hours. Stable isotopes of phenylalanine
247 [¹³C] and tyrosine [¹⁵N] were administered as tracers to assess protein metabolism. The

248 fractional synthesis rate (FSR) of muscle protein was measured using phenylalanine
249 enrichment in muscle tissue fluid as the precursor pool. The FSR of mixed muscle
250 protein was found to be higher after the period on CIT than when on NEAA (NEAA:
251 0.049 ± 0.005 ; CIT: 0.060 ± 0.006 ; $p=0.03$). Muscle mitochondrial protein FSR and
252 whole-body protein turnover did not differ between the two phases of the study⁽⁴³⁾.

253 In a randomized controlled study of 10 healthy subjects, oral CIT supplementation was
254 associated with a 57% improvement in nitrogen balance at 12 hours (from 683 (± 246) to
255 970 (± 187) mmol nitrogen/12 h; $p=0.0053$ for the comparison with placebo)⁽¹⁵⁾.

256 In a more recent study⁽⁴⁴⁾ on sixty-six healthy volunteers, supplementation with CIT and
257 reduced glutathione were associated with an improvement in cGMP activity, suggesting
258 direct effects on muscle protein synthesis and muscle performance. As in Jourdan's study
259⁽⁴³⁾, in a further study⁽⁴⁵⁾ of healthy, well-nourished volunteers, oral CIT could not be
260 shown to affect whole-body protein kinetics in the post-absorptive state.

261 Muscle protein synthesis contributes only about 25% of whole body protein synthesis⁽³⁴⁾
262 and an increase of (for example) 20% in muscle protein synthesis would therefore
263 contribute less than a 7% increment in whole body protein synthesis. Together with
264 Osowska's data⁽⁴²⁾ of lower hepatic protein synthesis rates in rats fed with CIT, this may
265 explain the apparent lack of a CIT effect at the whole-body level despite a statistically
266 significant effect on muscle protein synthesis. Bouillanne et al⁽⁴⁶⁾ show, in a prospective
267 randomized multicentre study, that 3-week's CIT supplementation (10 g/day) in 29
268 moderately malnourished elderly subjects led to higher muscle mass and fat free mass,

269 and lower fat mass than controls supplemented with NEAA, whereas whole body protein
270 synthesis was similar in the two groups. In other words, the effects of CIT on nitrogen
271 handling are neither ubiquitous nor uniform, and its anabolic effects are likely to be
272 specific to muscle.

273 However, in one study⁽⁴⁷⁾ of 22 healthy, elderly subjects, an effect of CIT on myofibrillar
274 protein synthesis was not confirmed. CIT co-ingestion with a low quantity (15 g) of
275 protein was ineffective in augmenting anabolism compared with NEAA.
276 Hyperargininaemia was interestingly demonstrated after ingestion of CIT in this study.

277 The mechanisms of action of CIT begin to be understood. Data suggest an involvement
278 of the mTOR (mammalian/mechanistic target of rapamycin) pathway in the effect of CIT
279 on protein synthesis^(41, 48). In general mTOR coordinates protein synthesis and
280 mitochondrial functions by selectively modulating synthesis of a series of nuclear-
281 encoded mitochondrial proteins as well as by regulating mRNA translation⁽⁴⁸⁾.

282 In addition, it has been shown that NOS activity is necessary for calcium-induced
283 activation of the Akt pathway (involved in translation initiation and thus muscle protein
284 synthesis) through a cGMP/PI3K-dependent pathway⁽⁴⁹⁾. Nitrite has been shown to
285 enhance mTOR activity and cell proliferation of myoblasts⁽⁵⁰⁾. CIT has relevance in both
286 of these contexts.

287 **Conclusion**

Exogenous CIT is a potent precursor for ARG and it functions as a donor of NO in many
clinical contexts. Its administration appears safe but there are currently few clinical

studies from which to draw conclusions on its therapeutic efficacy. Preliminary data indicate that it could be of value in systemic and pulmonary hypertension, in cardiac failure, in the management of arteriosclerosis, and in sarcopenia in the elderly (Table 1 and 2). Several new clinical research studies have been designed to address these interesting possibilities and are on-going.

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References

- 1 Fearon WR. The carbamido diacetyl reaction: a test for citrulline. *Biochem J.* 1939; 6: 902-907
- 2 Cynober L, Le Boucher J, Vasson MP. Arginine metabolism in mammals. *J Nutr Biochem* 1995; 6:402-413.
- 3 Curis E, Nicolis I, Moinard C, Osowska S, Zerrouk N, Bénazeth S et al. Almost all about citrulline in mammals. Review. *Amino Acids* 2005; 29:177-205.
- 4 Rimando AM, Perkins-Veazie PM. Determination of citrulline in watermelon. *J Chromatogr* 2005; 1078:196-200.
- 5 Papadia C. International Seminars on nuclear war and planetary emergencies. 46 session. Edited by Raganini World Scientific 2014. 368-371
- 6 Papadia C, Sherwood R, Kalantzis C, Wallis K, Volta U, Fiorini E, Forbes A. Plasma citrulline concentration: a reliable marker of small bowel absorptive capacity independent of inflammation. *Am J Gastroenterol.* 2007; 102: 1474-1482.

- 7 Cynober L. Citrulline: just a biomarker or a conditionally essential amino acid and a pharmaconutrient in critically ill patients? *Crit Care* 2013; 17:122.
- 8 Luiking YC, Poeze M, Ramsay G, Deutz NEP. Reduced citrulline production in sepsis is related to diminished de novo arginine and nitric oxide production. *Am J Clin Nutr* 2009; 89: 142-152.
- 9 Moinard C, Nicolis I, Neveux N, Darquy S, Benazeth S, Cynober L. Dose ranging effects of citrulline administration on plasma amino acids and hormonal patterns in healthy subjects: the citrudose pharmacokinetic study. *Br J Nutr* 2008; 99: 855-862.
- 10 Bahri S, Curis E, El Wafi FZ, Aussel C, Chaumeil JC, Cynober L. et al. Mechanisms and kinetics of citrulline uptake in a model of human intestinal epithelial cells. *Clin Nutr* 2008; 27: 872-880.
- 11 Wijnands KAP, Castermans TMR, Hommen MPJ, Meesters DM, Poeze M. Arginine and citrulline and the immune response in sepsis. *Nutrients* 2015; 7: 1426-1463.
- 12 Schwedhelm E, Maas R, Freese R, Jung D, Lukacs Z, Jambrecina A et al. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. *Br J Clin Pharmacol* 2008; 65: 51–59.

- 13 Cynober L, Moinard C, De Bandt JP. The 2009 ESPEN Sir David Cuthbertson. Citrulline: A new major signaling molecule or just another player in the pharmaconutrition game? *Clin Nutr* 2010; 29: 545-551.
- 14 Erez A, Nagamani SC, Shchelochkov OA, Premkumar MH, Campeau PM, Chen Y et al. Requirement of argininosuccinate lyase for systemic nitric oxide production. *Nat Med* 2011;17: 1619–1626.
- 15 Rougé C, Des Robert C, Robins A, Le Bacquer O, Volteau C, De La Cochetière MF et al. Manipulation of citrulline availability in humans. *Am J Physiol Gastrointest Liver Physiol* 2007; 293:1061-1067.
- 16 Grimble G. Adverse Gastrointestinal Effects of Arginine and Related Amino Acids. *J Nutr* 2007; **137** : 1693S-1701S.
- 17 Smith HA, Canter JA, Christian KG, Drinkwater DC, Scholl FG, Christman, BW et al. Nitric oxide precursors and congenital heart surgery: a randomized controlled trial of oral citrulline. *J Thorac Cardiovasc Surg* 2006; 132: 58-65.
- 18 Barr EF, Tirona RG, Taylor MB, Rice G, Arnold J, Cunningham G et al. Pharmacokinetics and safety of intravenously administered citrulline in children undergoing congenital heart surgery: potential

- therapy for postoperative pulmonary hypertension. *J Thorac Cardiovasc Surg* 2015; 134: 319-326.
- 19 Clinicaltrials.gov NCT00335244. Intravenous L-citrulline to treat children undergoing heart bypass surgery.
- 20 Yang S and So TY. The use of CIT for pediatric pulmonary hypertension. *Res J Clin Pediatr* 2017 1:1
- 21 Sureda A, Cordova A, Ferrer MD, Tauler P, Perez G, Tur JA et al. Effects of L-citrulline oral supplementation on polymorphonuclear neutrophils oxidative burst and nitric oxide production after exercise. *Free Radic Res* 2009; 43 :828-835.
- 22 Masahiko M., Masami S., Fumiko W. et al. Effects of oral L-citrulline supplementation on Lipoprotein Oxidation and Endothelial Dysfunction in Humans with Vasospastic Angina. *Immunol Endocrinol Metab Agents Med Chem.*2013; 3: 214-220.
- 23 Waugh WH, Daeschner CW, Files BA, McConnell ME, Strandjord SE. Oral citrulline as arginine precursor may be beneficial in sickle cell disease: early phase two results. *J Natl Med Assoc* 2001; 93: 363-371.
- 24 Suzuki T, Morita M, Kobayashi Y, Kamimura A. Oral L-citrulline supplementation enhances cycling time trial performance in healthy trained men: Double-blind randomized placebo-controlled 2-way

- crossover study. *J Int Soc Sports Nutr* 2016; 19: 13-16.
- 25 Bailey SJ, Blackwell JR, Lord T, Vanhatalo A, Winyard PG, Jones AM. Citrulline supplementation improves O₂ uptake kinetics and high-intensity exercise performance in humans. *J Appl Physiol* 2015; 119: 385-395.
- 26 Glenn JM, Gray M, Jensen A, Stone MS, Vincenzo JL. Acute citrulline-malate supplementation improves maximal strength and anaerobic power in female, masters athletes tennis players. *Eur J Sport Sci* 2016; 16: 1095-1103.
- 27 Pérez-Guisado J, Jakeman PM. Citrulline malate enhances athletic anaerobic performance and relieves muscle soreness. *J Strength Cond Res* 2010; 24: 1215-1222.
- 28 Cutrufello PT, Gadowski SJ, Zavorsky GS. The effect of l-citrulline and watermelon juice supplementation on anaerobic and aerobic exercise performance. *J Sports Sci* 2015; 33: 1459-1466.
- 29 Figueroa A, Wong A, Jaime SJ, Gonzales JU. Influence of L-citrulline and watermelon supplementation on vascular function and exercise performance. *Curr Opin Clin Nutr Metab Care* 2017; 20 :92-98.
- 30 Ginguay A, Cynober L, Curis E, Nicolis I. Ornithine aminotransferase, an important glutamate-metabolizing enzyme at the crossroads of multiple metabolic pathways. *Biology* 2017; 6: 18.

- 31 El-Hattab AW, Emrick LT, Hsu JW, Chanprasert S, Almannai M, Craigen WJ et al. Impaired nitric oxide production in children with MELAS syndrome and the effect of arginine and citrulline supplementation. *Mol Genet Metab* 2016; 117 : 407-412.
- 32 El-Hattab AW, Emrick LT. The effect of citrulline and arginine supplementation on lactic acidemia in MELAS syndrome. *Meta Gene* 2013; 1: 8-14.
- 33 Ochiai M, Hayashi T, Morita M, Ina K, Maeda M, Watanabe F et al. Short-term effects of L-citrulline supplementation on arterial stiffness in middle-aged men. *Int J Cardiol* 2012;155: 257-261.
- 34 Figueroa A, Trivino JA, Sanchez-Gonzalez MA, Vicil F. Oral L-citrulline supplementation attenuates blood pressure response to cold pressor test in young men. *Am J Hypertension* 2010; 23: 12-16
- 35 Orea-Tejeda A, Orozco-Gutiérrez JJ, Castillo-Martínez L, Keirns-Davies C, Montano-Hernández P, Vázquez-Díaz O et al. The effect of L-arginine and citrulline on endothelial function in patients in heart failure with preserved ejection fraction. *Cardiol J* 2010; 17: 464-470.
- 36 Orozco-Gutiérrez JJ, Castillo-Martínez L, Orea-Tejeda A, Vázquez-Díaz O, Valdespino-Trejo A, Narvaez-David R et al. Effect of L-arginine or L-citrulline oral supplementation on blood pressure and

- right ventricular function in heart failure patients with preserved ejection fraction. *Cardiol J* 2010;17: 612-618.
- 37 Balderas-Munoz K, Castillo-Martinez L, Orea-Tejeda A, Infante-Vazquez, Utrera-Lagunas M, Martinez-Memije R et al. Improvement of ventricular function in systolic heart failure patients with oral L-citrulline supplementation. *Cardiol J* 2012;19: 612-617.
- 38 Badesch DB, Abman SH, Ahearn GS, Barst RJ, McCrory DC, Simonneau G et al. Medical therapy for pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. *Chest* 2004; 126: 35S-62S.
- 39 Fike CD, Summar M, Aschner JL. L-citrulline provides a novel strategy for treating chronic pulmonary hypertension in newborn infants. *Acta Paediatr* 2014; 103: 1019-1026.
- 40 Kameda N, Okigawa T, Kimura T, Fujibayashi M, Asada T, Kinoshita R et al. The effect of L-citrulline ingestion on ECG QT interval and autonomic nervous system activity. *J Physiol Anthropol* 2011; 30: 41–45.
- 41 Breuillard C, Cynober L, Moinard C. Citrulline and nitrogen homeostasis: an overview. *Amino Acids* 2015; 47: 685-91.
- 42 Osowska S, Duchemann T, Walrand S, Paillard A, Boirie Y, Cynober L et al. Citrulline modulates muscle protein metabolism in old

- malnourished rats. *Am J Physiol Endocrinol Metab* 2006; 291: E582–E586.
- 43 Jourdan M, Nair KS, Carter RE, Schimke J, Ford GC, Marc J et al. Citrulline stimulates muscle protein synthesis in the post-absorptive state in healthy people fed a low-protein diet. A pilot. *Clin Nutr* 2015; 34: 449-456.
- 44 McKinley-Barnard S, Andre T, Morita M, Willoughby DS. Combined L-citrulline and glutathione supplementation increases the concentration of markers indicative of nitric oxide synthesis. *J Int Soc Sports Nutr* 2015; 10: 12:27.
- 45 Thibault R, Flet L, Vavasseur F, Lemerle M, Ferchaud-Roucher V, Picot D, Darmaun D. Oral citrulline does not affect whole body protein metabolism in healthy human volunteers: results of a prospective, randomized, double-blind, cross-over study. *Clin Nutr* 2011; 30: 807-811.
- 46 Bouillanne O, Melchior JC, Faure C, Canouï-Poitrine F, Paul M, Boirie Y et al. Effects of Citrulline (CIT) Oral Supplementation During 21 Days on Body Composition in Malnourished Elderly Patients. *Clin Nutr* 2015; 34: S27-S28.
- 47 Churchward-Venne TA, Cotie LM, MacDonald MJ, Mitchell CJ, Prior T, Baker SK et al. Citrulline does not enhance blood flow,

- microvascular circulation, or microvascular circulation, or myofibrillar protein synthesis in elderly men at rest or following exercise. *Am J Physiol Endocrinol Metab* 2014; 307: E71-83.
- 48 Morita M, Gravel SP, Hulea L, Larsson O, Pollak M, St-Pierre J, et al. mTOR coordinates protein synthesis, mitochondrial activity and proliferation. *Cell Cycle* 2015;14: 473-480.
- 49 Drenning JA, Lira VA, Soltow QA, Canon CN, Valera LM, Brown DL et al. Endothelial nitric oxide synthase is involved in calcium-induced Akt signalling in mouse skeletal muscle. *Nitric Oxide* 2009; 21: 192-200.
- 50 Totzeck M, Schicho A, Stock P, Kelm M, Rassaf T, Hendgen-Cotta UB. Nitrite circumvents canonical cGMP signalling to enhance proliferation of myocyte precursor cells. *Mol Cell Biochem.* 2015; 401:175-183.

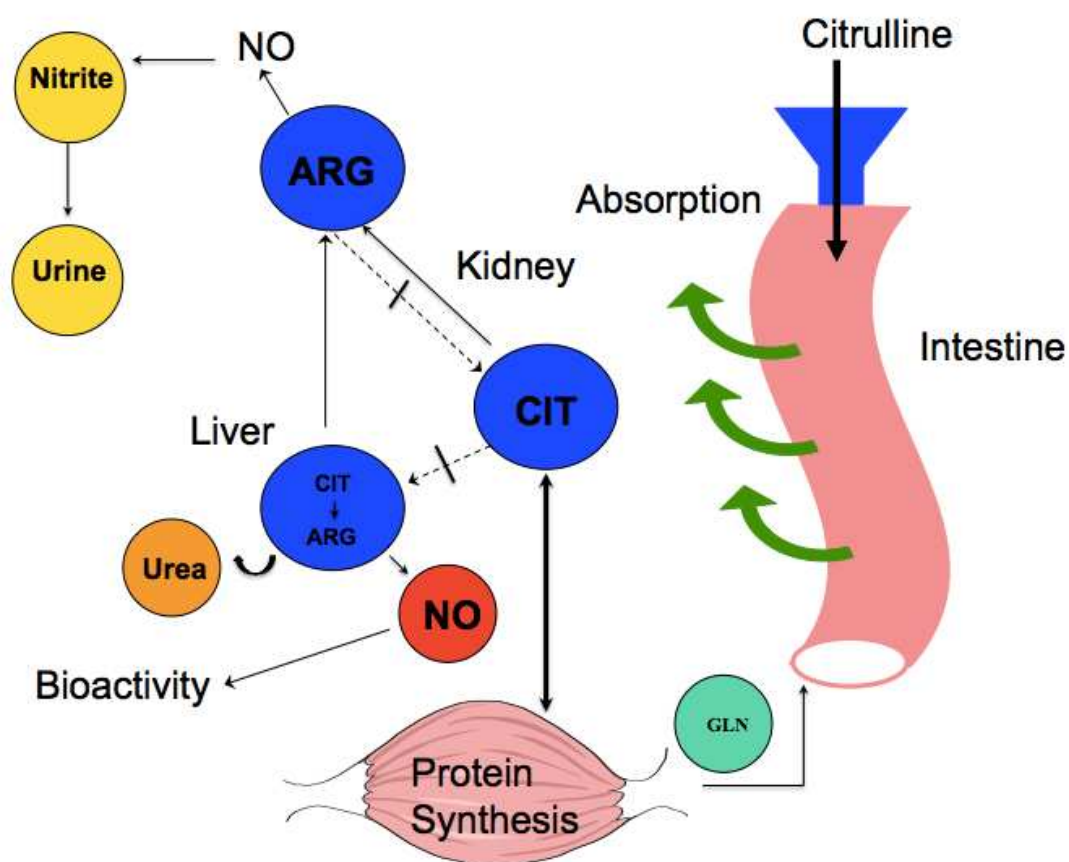
Table1. L- Citrulline: Interventional studies in humans

References	Conditions	Effects
8, 36, 39, 42, 43	Protein malnutrition	Increases fractional synthesis rate of muscle proteins, increases muscle mass and fat free mass, increases nitrogen balance
18-20	Healthy controls	Increases muscle performance
15, 17	Professional athletes	Reduces post-exercise oxidative stress
15, 17, 22, 26	Arteriosclerosis	Improves arterial stiffness, increases NO metabolites, decreases lipid oxidation
27	Hypertension	Reduces blood pressure on the cold pressor test
16	Sickle cell disease	Reduces hypertension in crises
28-30	Cardiac failure	Normalizes MAT/TT index, improves right ventricular ejection fraction and reduces hypertension
13, 29, 31	Pulmonary hypertension	Reduces pulmonary artery pressure

Table 2. L-Citrulline: Design of human studies

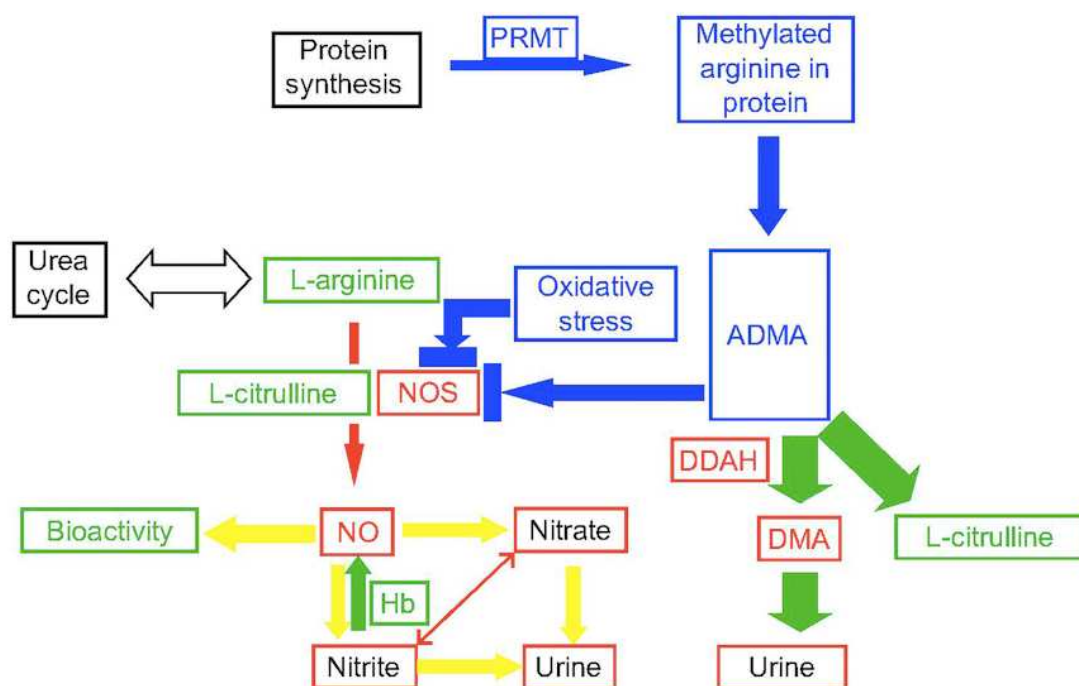
Author	References	Dose/day	Days	Subjects	Design
Schwedhelm E	8	0.75, 1.5, 3 g BD	7	20	Double-blind randomized placebo-controlled cross over
Rouge C	11	0.18 g/kg	3	10	Pilot randomized-controlled
Smith HA	13	9.5g / m ²	1	40	Randomized placebo controlled
Sureda A	15	3.4 g	1	16	Prospective, randomized single blinded study
Waugh WH	16	0.1 g/kg	28	5	Early phase II
Suzuki T	17	2.4 g	7	22	Double-blind randomized placebo-controlled 2-way crossover
Bailey SJ	18	6 g	7	10	Randomized placebo-controlled, crossover
Glenn JM	19	5.3 g	1	17	Double-blind, randomized
Pérez-Guisado J	20	4.5 g	1	41	Randomized, double-blind, 2-period crossover
Morita M		80 mg/day	60	22	Prospective pilot
Figueroa A	27	6 g/day	28	17	Randomized controlled crossover
Orea-Tejeda A	28	3g	60	30	Prospective controlled
Orozco-Gutiérrez JJ	29	3g	60	15	Prospective controlled
Balderas-Munoz K	30	3g	120	20	Prospective randomized placebo controlled
Jourdan M	36	11 – 24 g	1	8	Randomized cross-over pilot
Bouillanne O	39	10 g	21	29	Randomized multicentre

Abbreviations: BD, bis die (twice daily). In this table the citrulline doses have been corrected to subtract the contribution of malate in those cases where citrulline malate was used (15,19,20).



Abbreviations : ARG L-Arginine; CIT L-Citrulline, GLN Glutamine, NO Nitric Oxide

Figure 1: Effects of L-Citrulline supplementation



Abbreviations: ADMA: asymmetric dimethylarginine; DDAH: dimethylarginine dimethylaminohydrolase; DMA: dimethylamine; NOS nitric oxide synthase

Figure 2: Citrulline and Oxidative Stress