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DOSE-EFFECT OF CITRULLINE SUPPLEMENTATION IN SHOR BOWEL SYNDROME IN RATS : WHICH IMPACT ON MUSCLE ?

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1. Abstract

Background: Supplementing diet with citrulline has proved an efficient means of preserving nitrogen balance and improving nutritional status after massive intestinal resection. We aimed to model the action of citrulline in gut-resected rats using a dose-ranging study focused on skeletal muscle nitrogen homeostasis.

Methods: Forty-six rats were randomly assigned to one of the following groups: citrulline 0.5g/kg/day (n=9), citrulline 1g/kg/day (n=7), citrulline 2.5g/kg/day (n=8), citrulline 5g/kg/day (n=8), control (n=6), and sham (n=8). The sham group underwent transection and the other 6 groups underwent resection of 80% of the small intestine. All rats were then fed enteral nutrition (all diets were isocaloric and isonitrogenous). After 10 days, the rats were euthanized to measure and analyze animal weight, duodenum, jejunum and ileum weight, and muscle trophicity. Protein fractional synthesis rate (FSR) and mTORC1 activation were measured in the tibialis muscle.

Results: There was a significant dose-dependent association between rat weight and citrulline dose up to 2.5 g/kg/day (p=0.004). There was a significant improvement in tibialis weight correlated to plasma citrulline. Net protein FSR in the tibialis tended to be greater after resection and tended to return to baseline after citrulline supplementation. Citrulline supplementation significantly decreased the activated phosphorylated forms of S6K1 (p=0.003) and S6RP (p=0.003), with a significant positive association between myofibrillar FSR and activation of S6K1 (r=0.614, p=0.02) and S6RP (r=0.601, p=0.023). Jejunum weight was significantly positively correlated with plasma citrulline (r=0.319, p=0.0345).

Conclusion: Citrulline promotes body weight gain, preserves muscle trophicity, and enhances intestinal adaptation in a dose-dependent way in a model of resected rats.

49 **1. Introduction**

50 Short bowel syndrome (SBS) occurs when there is insufficient length of small intestine to
51 maintain adequate protein-energy, fluid, electrolyte, or micronutrient balance without
52 parenteral support [1]. SBS most frequently occurs following extensive surgical resection of
53 the intestine, congenital defect, or disease-associated loss of absorption. Current lines of
54 research involve the development of pharmacological treatments and/or the use of specific
55 nutrients with pharmacological properties to potentiate post-resectional gut adaptation and
56 reduce parenteral nutrition requirements [2].

57 Citrulline is a non-protein amino acid synthesized for systemic use almost
58 exclusively by the enterocytes of the proximal small bowel. It escapes splanchnic extraction.
59 Citrulline is mainly metabolized by the kidney and converted into arginine [3-7]. As there
60 are profound impairments of arginine metabolism in SBS [8,9], citrulline makes a good
61 candidate to generate arginine and improve nutritional status in SBS. However, to date, there
62 is only one clinical study of citrulline supplementation in SBS patients [10]. In 9 SBS
63 patients with good nutritional status in the late phase of intestinal adaptation—which is
64 likely too late—and with near-normal baseline citrulline homeostasis, a 7-day oral
65 supplementation with citrulline (0.18 $g \cdot kg^{-1} day^{-1}$) enhanced citrulline and arginine
66 bioavailability but had no anabolic effect on whole-body protein metabolism. However, the
67 experimental data and this clinical study are not necessarily contradictory. Indeed, work
68 carried out by Jourdan et al. [11] in healthy subjects found that citrulline intake significantly
69 increases muscle protein synthesis but does not affect protein synthesis at whole-body level.
70 Likewise, Kuçi et al [12] showed that in critically-ill rats, citrulline effect varies according to
71 type of injury and to the skeletal muscle under study, with a limited effect at whole-body
72 level. This result connects with the findings of Osowska et al. [13], who showed that old
73 malnourished rats re-fed with a citrulline-enriched diet had higher muscle protein synthesis

74 and lower hepatic protein synthesis than control animals. These data may explain the lack of
75 citrulline effect at whole-body level. Other hypotheses proposed by Jirka et al [10] to
76 explain their disappointing results included the patients' good nutritional status, the late
77 phase of intestinal adaptation, and the normal baseline citrulline level.

78 To date, there is no data available on the most appropriate therapeutic range and the
79 dose-response relationship of CIT in the acute-phase SBS population. Here, to address this
80 gap, we used a model of intestinal insufficiency mimicking SBS disease, i.e. gut-resected
81 rats, to characterize the action of citrulline in a dose-ranging study design, focusing on
82 muscle nitrogen homeostasis.

83

84

2. Materials and Methods

2.1. Animals:

Male Wistar rats (n=46) (230-250g, 2-month-old) were used. Before surgery, the rats were acclimatized for five days in metabolic cages with free access to standard laboratory chow (Safe, Epinay-sur-Orge, France) and water.

2.2. Surgical procedures:

As previously described [14], the rats were fasted for 12 hours before surgery, then anesthetized by isoflurane inhalation (3% isoflurane) using a regulated airflow apparatus (Minerve, Esternay, France). Enterectomy was performed on rats in the citrulline and control groups (see below for definition) by removing 80% of the small intestine, leaving 10% of the proximal jejunum and 10% of the distal ileum. Eight rats were sham-operated (transection). For both resected and transected animals, continuity was restored with an end-to-end anastomosis using a single running silk 6-0 suture. The gastrostomy tube was placed immediately following intestinal resection or transection. A silicone tube (Tygon, size 0.51 mm; Fisher Bioblock Scientific, Illkirch, France) was introduced into the lumen of the stomach and the remaining end of the catheter was tunneled subcutaneously to the back of the neck and attached to a spring coil-swivel mechanism (Harvard Apparatus, Les Ulis, France) allowing the rat to move freely in the cage. Before the surgical procedure, all the rats received a painkiller (Temgesic®; Schering-Plough) subcutaneously at a dose of 0.05 mg/kg of body weight.

One of the authors (CM) is authorized by the French government (No. 75522) to use this experimental model of surgery. Animal care complied with French regulations for the protection of animals used for experimental and other scientific purposes (D 2001-486) and

with European Community regulations (Official Journal of the European Community, L538 12:18:1986)..

2.3. Postoperative care:

Recovery period (D-5 to D0): Rats were housed individually in metabolic cages and allowed a five-day recovery period. On the first two days they had free access to a 5% glucose solution. On day 3, glucose was withdrawn, and the rats had access to standard laboratory chow (UAR A04, Protein 16%, Arginine content: 9.8g.kg^{-1} Dietex, France) and water. On day 4, enteral nutrition was introduced at a flowrate of 0.5 mL/h using a Harvard infusion pump (pump 11; Harvard Apparatus), and the rats also had free access to chow and water. Flowrate was increased gradually to a maximum on day 5 corresponding to an intake of 2 g N kg^{-1}day^{-1} [3]. From day 5 until the end of the experiment, rats received only enteral nutrition and had free access to water.

2.4. Experimental groups:

The rats were randomly assigned to one of the following groups (from D0 to D10):

- <u>The citrulline groups</u> (CITRULLINE) consisted of rats receiving standard enteral nutrition (Sondalis®; Nestlé Health Science, 1 kcal.mL^{-1} and 4.5 mg Arg-bound protein .mL^{-1}) supplemented with citrulline at different doses:
    - 0.5 g.kg^{-1}day^{-1} (n=9)
    - 1.0 g.kg^{-1}day^{-1} (n=7)
    - 2.5 g.kg^{-1}day^{-1} (n=8)
    - 5.0 g.kg^{-1}day^{-1} (n=8)
- <u>The control group</u> (n = 6) and <u>the sham group</u> (n = 6) consisted of rats receiving standard enteral nutrition.

134 To keep all groups isonitrogenous (2 g $N.kg^{-1}day^{-1}$) and isocaloric (200 kcal $kg^{-1}day^{-1}$) to
135 the CITRULLINE (5 g $kg^{-1}day^{-1}$) group, standard enteral nutrition was supplemented with a
136 mixture of amino acids consisting of alanine, asparagine, glycine, serine, histidine, and
137 proline in equimolar amounts (see Table 1, supplementary data).

138 Rats were weighed daily. Total enteral nutrition was administered for 10 days. Enteral
139 nutrition was stopped two hours before decapitation.

140

141 *2.5. Tissue removal*:

142 Blood

143 Blood was sampled in heparinized tubes and then swiftly centrifuged.

144

145 Liver

146 The abdominal cavity was opened. The liver was immediately removed and weighed, and a
147 sample was cut off, frozen in liquid nitrogen, and stored at -80°C until analysis.

148

149 Jejunum and ileum

150 The intestinal mucosa was washed with cold NaCl (0.9%), reverted and scraped, rapidly
151 frozen in liquid nitrogen, and stored at -80°C until analysis.

152

153 Muscles

154 Five muscles of the hindlimbs (*extensor digitorum longus* (EDL), *gastrocnemius*, *soleus*,
155 *plantaris*, and *tibialis anterior*) were rapidly removed, weighed, frozen in liquid nitrogen,
156 and stored at -80°C until analysis. These five muscles were selected because they differ
157 widely in their function and fiber type and show different metabolic responses to stress [15].

158

159 *2.6. Parameters studied and analytical methods*:

160 2.6.1. Amino acid concentrations in plasma and EDL muscle

161 As previously described [14], plasma was deproteinized with a 30% (w/v) sulfosalicylic acid
162 solution. Supernatants were stored at -80°C until analysis. Tissues were ground and
163 deproteinized with a 10% trichloroacetic acid solution containing 0.5 mM EDTA.
164 Supernatants were stored at -80°C until analysis. Amino acids were measured by ion
165 exchange chromatography using an amino acid autoanalyzer. The results of our participation
166 in the European Quality Control Scheme (ERNDIM) confirm the accuracy of our amino acid
167 determinations.

168

169 2.6.2. Fractional synthesis rate of proteins

170 Just before euthanasia, each rat received a subcutaneous injection of a large dose of L-[^{13}C]-
171 valine (99 atom%, 300 µmol/100 g, Cambridge Isotope Laboratories, Andover, MA) to
172 flood the protein synthesis precursor pool. The tracer incorporation time within the groups
173 was different for each rat. In each group, a rat was killed at 20, 25, 30, 35, 40, 45 or 50 min
174 after the tracer injection. Thus, the same kinetics of incorporation from 20 to 50 min was
175 performed for each group of animals. We used the kinetics of tracer incorporation in the
176 mitochondrial proteins, myosin and actin to calculate their synthesis rates (see the equation
177 below). A 200 mg piece of *tibialis anterior* was used for isolation of mitochondrial proteins,
178 myosin and actin, as previously described [16]. L-[^{13}C]-valine enrichment in hydrolyzed
179 proteins was measured using a GC-C-IRMS system (µGas System, Fisons Instruments, VG
180 Isotech, Middlewich, UK). Amino acids in the tissue fluid were derivatized, and valine
181 enrichments were used as precursor pool to calculate fractional synthesis rates (FSR) as
182 previously described [13] (Supplementary data, table 2). Another set of four rats per regimen
183 was used to determine basal isotopic abundance in mitochondrial proteins. The FSR of

184 mitochondrial proteins, actin and myosin was calculated using the following equation: FSR
185 = (Ei x 100) /(Eprec x t), where Ei is the enrichment as atom percent excess of 13C derived
186 from combustion of valine from proteins at time t (minus basal enrichment), Eprec is the
187 mean enrichment in the precursor pool (tissue fluid [13C] valine), and t is the incorporation
188 time in hours [13]. Data are expressed as %/h.
189
190 2.6.3. Preparation of muscle lysates
191 Frozen muscles (*tibialis*) were ground in liquid nitrogen. Powdered muscles were weighed
192 and homogenized in 10 volumes of buffer containing 150mM NaCl, 10mM Tris-HCl pH7.4,
193 5mM EDTA, 1% NP40, phosphatase inhibitors (20mM sodium fluoride, 1mM sodium
194 pyrophosphate, 25mM sodium glycerophosphate, 1mM sodium vanadate), and protease
195 inhibitors (complete protease inhibitor cocktail, Roche, Meylan, France). The samples were
196 continuously homogenized (1 hour, 4°C) and then centrifuged at 1000 *g* for 30 min at 4°C.
197 Supernatants were aliquoted and stored at -80°C until analysis. Protein concentrations were
198 determined using the bicinchoninic acid method (BC Assay UP 40840A kit Uptima,
199 Interchim, France)
200
201 2.6.4 Immunoblotting: determination of the phosphorylation state of the principal actors in
202 mTORC1
203 Total protein (50 µg) in SDS sample buffer (Laemmli Sample Buffer, Biorad) was heated to
204 95°C, and the proteins were separated by SDS-PAGE using a denaturing gel. After
205 electrophoresis, proteins were transferred to nitrocellulose (GE-Healthcare) and then
206 blocked for 1 h with a blocking buffer (10mM Tris-HCl pH8.0, 150mM NaCl, 0.05%
207 Tween20, 5% nonfat dry milk). Nitrocellulose blots were incubated overnight at 4°C with
208 primary antibodies that recognize phosphorylated forms. After washing, membranes were

incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h in TBS/T under gentle shaking. The blots were developed using the enhanced chemiluminescence detection system (GE Healthcare) according to the manufacturer's protocol. Films were scanned and quantified using the Bio Imaging System (Syngene). After quantification of the relative intensity of the phosphorylated forms, membranes were stripped using a stripping buffer (0.5% acetic acid) and then probed with primary antibodies that recognize total forms.

2.7. Statistical analysis

Data are expressed as means ± SEM. Comparisons between sets of data were performed using one-way analysis of variance (ANOVA) followed by the Duncan test (all analysis were performed on parameters measured at euthanasia and are statistically independent). When statistical analysis did not show a dose-dependent effect of citrulline, we analyzed the global effect of citrulline supplementation versus no citrulline, results of all supplemented rats were pooled and compared to results of unsupplemented resected rats (controls). Statistical analyses (ANOVA, Duncan test, correlation, quadratic analysis, linear regression etc.) were performed using Statview software (Cary, NC). Threshold of significance was set at $p<0.05$.

227 **3. Results**

228 ***3.1. Body weight***

229 There was no linear relationship between citrulline dose and body weight gain at day 10
230 (Wd10). However, a polynomial regression model evidenced a significant relationship
231 between citrulline supplementation dose and body weight (Figure 1): W(d10)
232 =78.2+11.9(dose)-2(dose^2) +0.6W(d0); p=0.02. Furthermore, a significant linear
233 relationship was found when the citrulline dose of 5 g/kg/d was excluded: W(d10)
234 =60.8+7.5(dose)+0.7W(d0), p=0.004.

235

236 ***3.2. Liver weight***

237 Liver weight was lower in the resected animals compared to the sham group (sham *vs* all
238 resected rats: 8.95 g *vs* 7.65 g, p=0.038). This difference was not corrected by citrulline
239 supplementation at any dose (Table 1).

240

241 ***3.3. Muscle weight***

242 Intestinal resection led to a significant lowering in soleus muscle mass and protein content
243 (Table 1) which was partly restored after citrulline supplementation: soleus weight in
244 unsupplemented resected rats (controls) *vs* all citrulline groups: p=0.029. However, we were
245 unable to find a clear relationship between citrulline dose and muscle mass for any muscle
246 (EDL, tibialis, soleus, plantaris anterior, gastrocnemius) or between citrulline
247 supplementation and protein content of EDL and soleus muscles (in absolute weight or in
248 percent of muscle mass). This was mainly due to major inter-individual variability in the
249 group of rats receiving the highest dose of citrulline. A linear regression model using the
250 plasma citrulline logarithm showed a 20% variation in tibialis anterior weight according to
251 plasma citrulline: W= 0.05log(plasma citrulline)+0.033; p=0.02 (Figure 2).

252

253 ***3.4. Intestinal adaptation***

254 The variation of jejunal and ileum weight according to citrulline load is presented in Table 1.
255 The variation of jejunal weight was significantly related to plasma citrulline:
256 W=0.05log(plasma citrulline)+97.12; p=0.035. However, there was no significant
257 association between ileum weight and plasma citrulline, and no significant association
258 between mucosal protein content of the intestines and citrulline dose or plasma
259 concentrations.

260

261 ***3.5. Amino acid concentrations in plasma and muscles***

262 3.5.1. Plasma amino acid concentrations:

263 Intestinal resection resulted in a lowering of plasma citrulline compared with the sham group
264 (control *vs* sham: 49 *vs* 75 µmol/L, p=0.002) and did not have any significant influence on
265 any of the other AA concentrations. Plasma concentrations of citrulline, arginine and
266 ornithine rose dose-dependently with citrulline supplementation (Table 2).

267 3.5.2. Muscles amino acid concentrations:

268 EDL levels of citrulline, arginine and ornithine tend to be lowering on average by 21–27%
269 following intestinal resection (control *vs* sham, p=0.07–0.08). Citrulline supplementation
270 significantly raised levels of citrulline, arginine and ornithine in the EDL muscle in a dose-
271 dependent manner (p<0.001, Table 2). Likewise, we found highly significant positive
272 associations between plasma citrulline and EDL levels of citrulline (r=0.96, p<0.001),
273 arginine (r=0.73, p<0.001) and ornithine (r=0.79, p<0.001) (Figure 3).

274

275

276

277 ***3.6. Evaluation of muscle protein synthesis***

278 Protein FSR tended to rise after intestinal resection, but the difference was only significant
279 for sarcoplasmic FSR (sham *versus* unsupplemented resected rats: 0.08%/h *vs* 0.12%/h,
280 p=0.014) and myofibrillar FSR (0.1%/h *vs* 0.15%/h, p=0.028). FSR levels tended to return to
281 baseline levels with citrulline supplementation (myofibrillar FSR: control *versus* all
282 citrulline-supplemented groups: p=0.018; p>0.05 for total FSR, mitochondrial FSR and
283 sarcoplasmic FSR) (Figure 4). No dose-dependent effect of citrulline were observed.

284

285 ***3.7. Evaluation of mTORC1 pathway activation***

286 Intestinal resection did not significantly modify the amounts of phosphorylated forms of
287 AKT, 4E-BP1 and eIF-4E, S6K1 or S6 ribosomal protein (S6RP) in the tibialis. However,
288 citrulline supplementation led to a significant lowering in the activated forms of S6K1
289 (p=0.003) and S6RP (p=0.003), regardless of citrulline dose (Figure 5). Furthermore, we
290 found a significant positive association between myofibrillar FSR and S6K1 (r=0.614,
291 p=0.02) and S6RP (r=0.601, p=0.023). No dose-dependent effect of citrulline were
292 observed.

293

294 **4. Discussion**

295 Citrulline is renowned for its homeostatic effect on nitrogen balance, and recent literature
296 has consistently shown that this effect was directed towards skeletal muscles [11, 13, 17-20],
297 by activation of the mTORC1/PI3K/MAPK pathway [21] and by modulation of muscle
298 energy metabolism [22]. We thus focused on muscle nitrogen homeostasis in our animal
299 model of SBS, and aimed to find a dose-ranging effect of citrulline supplementation.

300 In our study, citrulline supplementation generated dose-dependent rises in arginine and
301 ornithine contents in both plasma and muscles, modulated weight, and enhanced intestinal
302 adaptation. Furthermore, we demonstrated that this beneficial effect was dose-dependent,
303 mainly from 0.5 to 2.5g $kg^{-1}day^{-1}$ of citrulline supplementation.

304 Citrulline had a specific effect on muscles, i.e. on muscle weight, AA content, thus
305 corroborating the results of previous studies [11,13,17-19]. However, the action of citrulline
306 on muscle FSR in our resected animals was the opposite of what was expected. First,
307 intestinal resection resulted in a rise in skeletal-muscle protein FSR despite a decrease in
308 muscle mass. Among the possible explanations for this FSR variation, we posit that it could
309 reflect the onset of an intestinal adaptation process. Second, this FSR variation tended to be
310 normalized by citrulline supplementation, in particular for myofibrillar and sarcoplasmic
311 FSR. These results were not artifacts as they correlated with the mTOR pathway response to
312 citrulline, with a major reduction in the activated phosphorylated forms of S6k1 and S6R
313 concomitant with the lower values of FSR in citrulline-supplemented rats. We advance two
314 explanations to account for these unexpected results:

315 1) Our experimental model used a continuous enteral feeding mode. It has been shown that
316 continuous enteral feeding had a much less stimulatory effect on muscle and visceral protein
317 synthesis than intermittent bolus feeding [23,24]. Furthermore, Davis et al. (2015)
318 demonstrated that only intermittent bolus feeding could elicit a pulsatile pattern of amino

319 acid-induced translation initiation, thereby promoting protein anabolism to a much greater
320 extent than continuous enteral feeding [25]. Likewise, mTORC1 is mainly activated in the
321 postprandial (bolus-style) state. 2) In patients with early-phase SBS, feeding fails to decrease
322 proteolysis, in contrast to what is physiologically observed in healthy subjects. Note that the
323 improvement in intestinal function and nutritional status achieved by rhGH treatment in SBS
324 is mediated by the decrease in proteolysis in response to feeding [26]. We hypothesize that
325 the same could occur in work. The rise in muscle FSR after resection could reflect an
326 increased protein turnover due to excess proteolysis, and the stimulatory effect of citrulline
327 on muscle protein synthesis could, at least in part, be mediated by a decrease in proteolysis.
328 This potential effect of CIT on proteolysis was also hinted at in a proteomics study [27].
329 Note that the systemic relation between protein synthesis and muscle mass is inconstant, as
330 it was previously shown that CIT could increase protein synthesis without increasing muscle
331 mass [18].
332
333 In conclusion, our results confirm that citrulline has potential benefit in SBS as it promotes
334 body weight gain, preserves muscle trophicity, and enhances intestinal adaptation in our
335 model of resected rats. Here we demonstrated that this beneficial effect was dose-dependent,
336 with a maximal beneficial effect for a citrulline dose of 2.5 g/kg/day. Citrulline reversed the
337 rise in protein synthesis in skeletal muscle induced by intestinal resection through the mTOR
338 pathway, prompting the hypothesis that citrulline could reverse the post-resection
339 modification in protein turnover. This new approach to the pharmacological properties of
340 citrulline in SBS warrants further research, but we anticipate it could help clinicians develop
341 valuable clinical studies in the early-adaptation phase of SBS.
342

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Conflict of interest statement

JF, CM, SLP and LC are Citrage shareholders. Other authors have no conflict of interest.

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CRediT author statement

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Figure and table legends

Table 1: Organ weight and organ protein content in the sham group and in groups of resected and CIT-supplemented rats

Table 2: Plasma and EDL amino acid contents in the sham group and in groups of resected and CIT-supplemented rats

Figure 1: A polynomial regression model on rat weight at D10 (W10) and citrulline dose administered in sham and resected rats. X-Axis: Citrulline dose ($g.kg^{-1}.d^{-1}$), Y- Axis: Weight gain (g) (n=46)

$W(day10) = 78.2 + 11.9(dose) - 2(dose^2) + 0.6W(day0)$

Figure 2: Linear regression between log(citrullinemia) (X-Axis) and weight of *tibialis (mg)* (Y-Axis) in sham and resected rats under enteral nutrition (n=46)

Figure 3: Linear regression between plasma citrulline (Y-Axis) and levels of citrulline (X-Axis) (panel 1: $r=0.96$, $p<0.001$), arginine (panel 2: $r=0.73$, $p<0.001$) and ornithine (panel 3: $r=0.79$, $p<0.001$) in EDL muscle (n=46)

Figure 4: Protein fractional synthesis rate (FSR): total FSR, mitochondrial FSR, sarcoplasmic FSR, and myofibrillar FSR (expressed in %/h) in tibialis muscle in sham (transected rats fed standard enteral nutrition), control (resected rats fed standard enteral nutrition), and citrulline-supplemented rats (pooled results of resected rats fed enteral nutrition supplemented with either 0.5, 1, 2.5 or 5 mg/kg/day of citrulline) Results are presented as means ± SEM

Figure 5: Phosphorylated forms of S6K1 and S6RP in tibialis muscle (expressed as phosphorylated to total form ratio) in sham (transected rats fed standard enteral nutrition), control (resected rats fed standard enteral nutrition), and citrulline-supplemented rats

(pooled results of resected rats fed enteral nutrition supplemented with either 0.5, 1, 2.5 or 5 mg/kg/day of citrulline). Results are presented as means ± SEM.

.

Figure 1

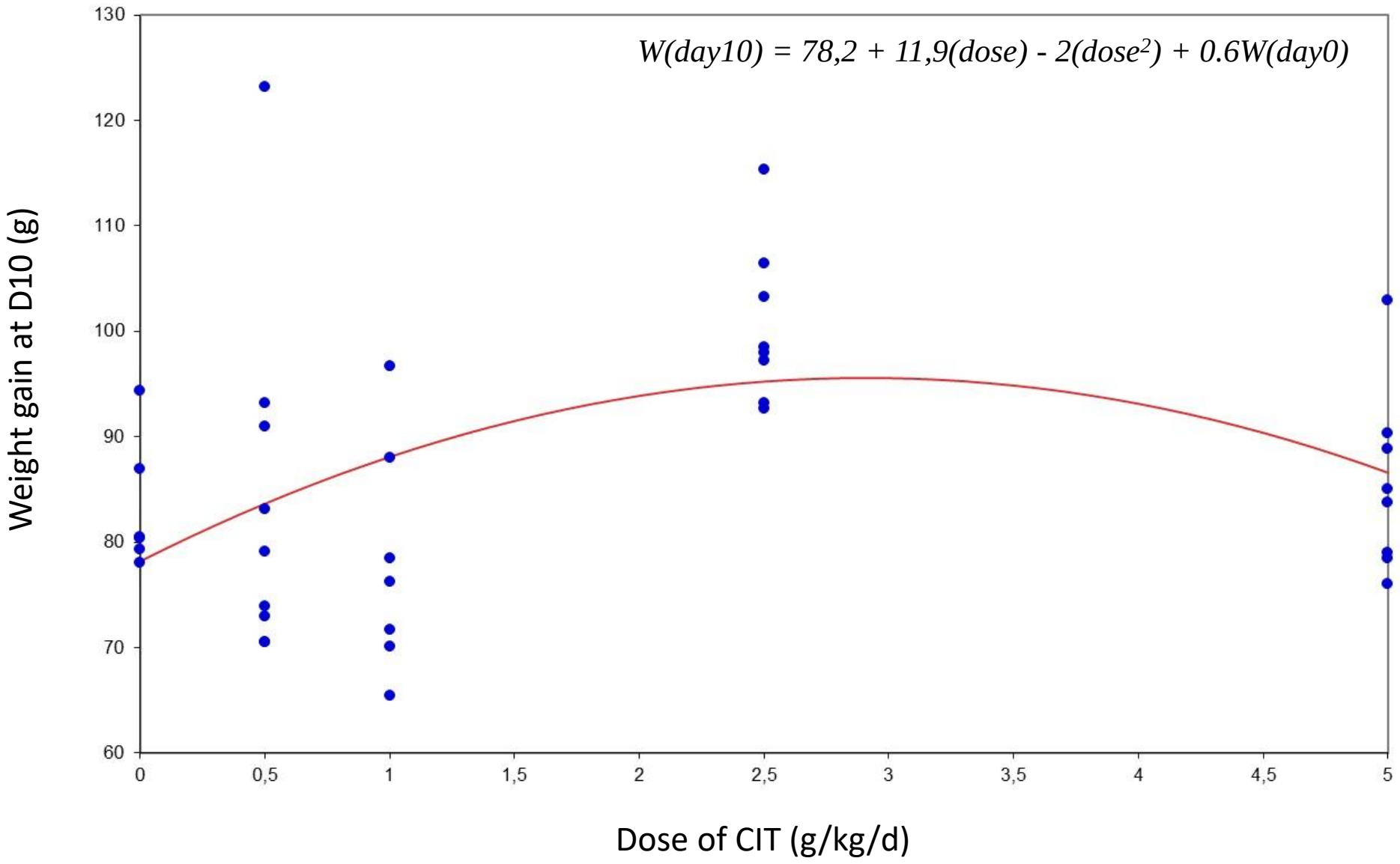

W(day10) = 78,2 + 11,9(dose) - 2(dose²) + 0.6W(day0)
Weight gain at D10 (g)
130
120
110
100
90
80
70
60
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
5
Dose of CIT (g/kg/d)

Figure 2

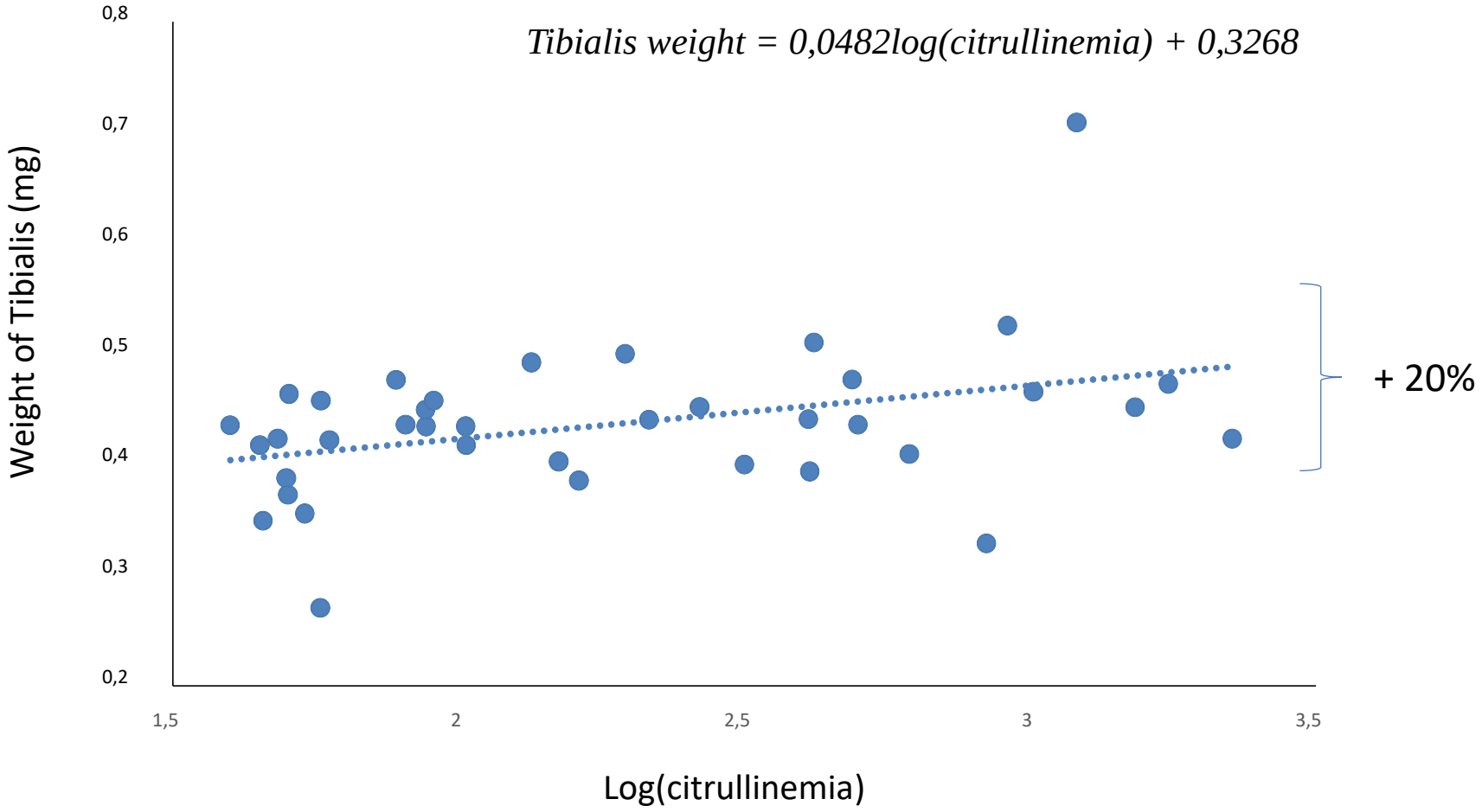
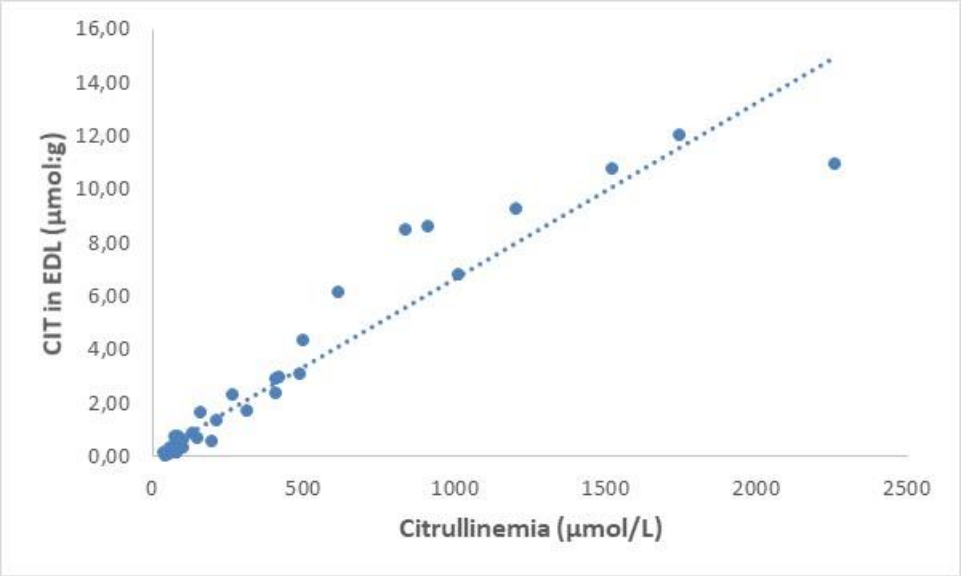
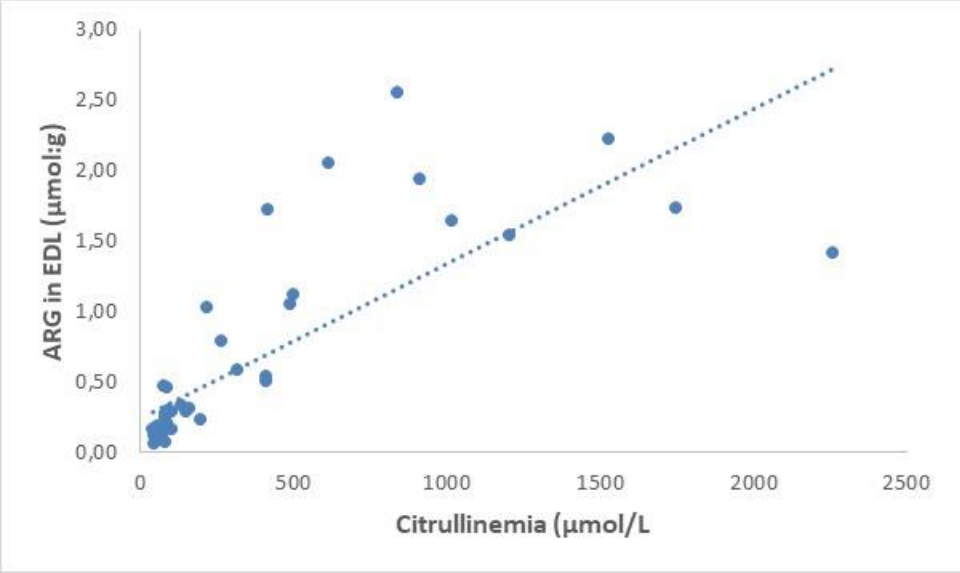

Tibialis weight = 0,0482log(citrullinemia) + 0,3268
Weight of Tibialis (mg)
0,8
0,7
0,6
0,5
0,4
0,3
0,2
1,5
2
2,5
3
3,5
Log(citrullinemia)
+ 20%

Figure 3


16,00
14,00
12,00
10,00
8,00
6,00
4,00
2,00
0,00
CIT in EDL (µmol:g)
0
500
1000
1500
2000
2500
Citrullinemia (µmol/L)


3,00
2,50
2,00
1,50
1,00
0,50
0,00
ARG in EDL (µmol:g)
0
500
1000
1500
2000
2500
Citrullinemia (µmol/L

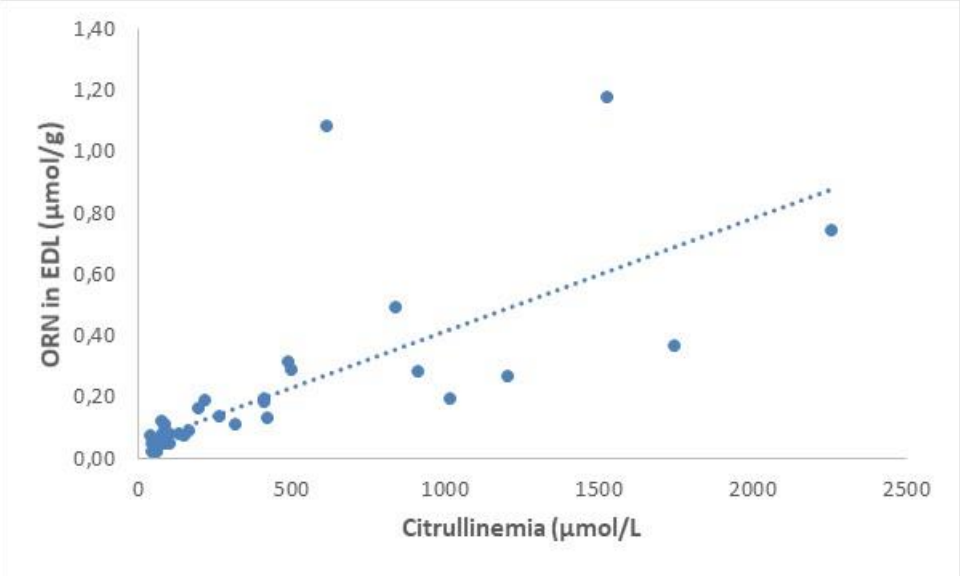

1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
ORN in EDL (µmol/g)
0
500
1000
1500
2000
2500
Citrullinemia (µmol/L

Figure 4

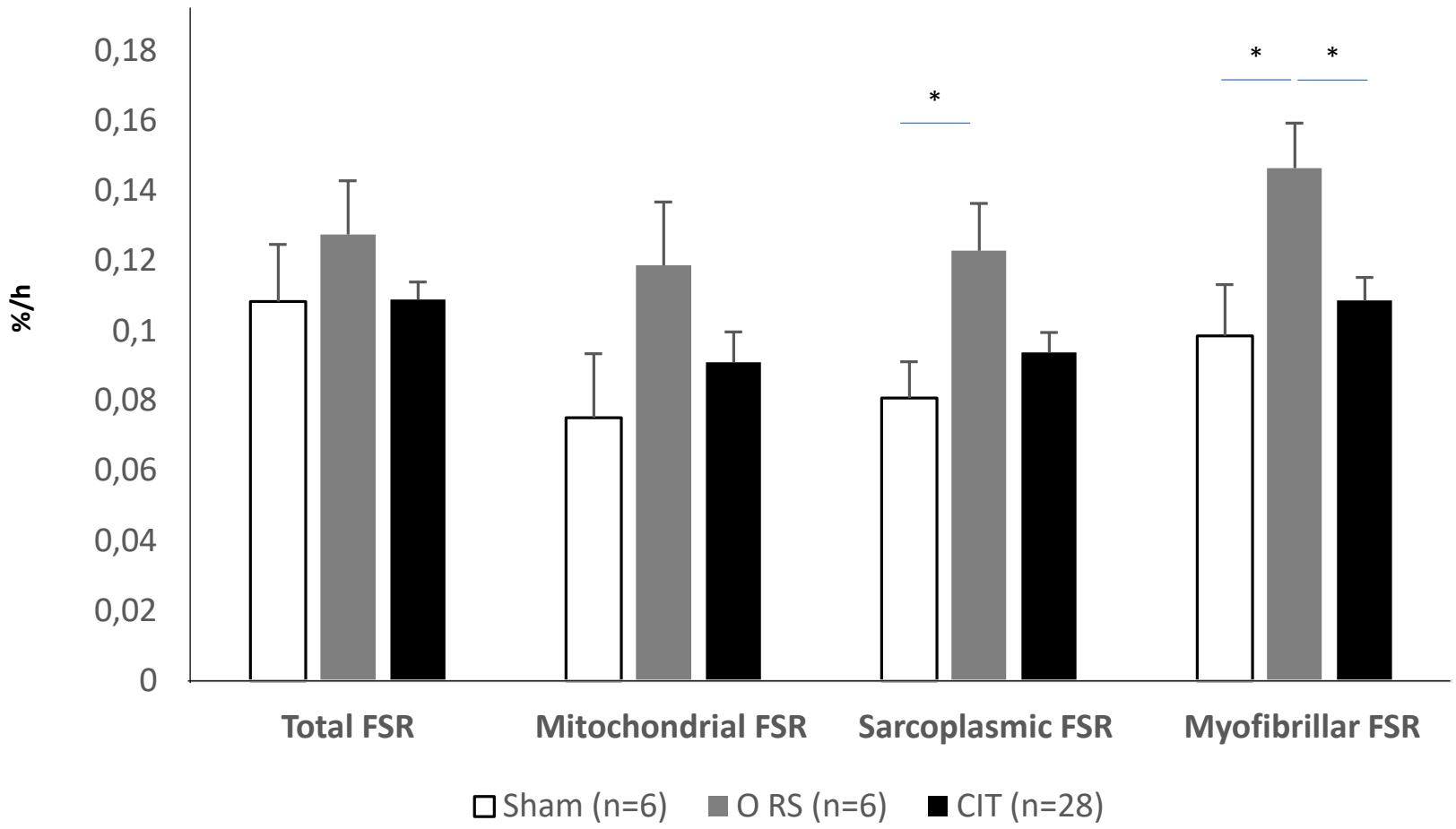

0,18
0,16
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
%/h
*
*
*
Total FSR
Mitochondrial FSR
Sarcoplasmic FSR
Myofibrillar FSR
Sham (n=6)
O RS (n=6)
CIT (n=28)

Figure 5

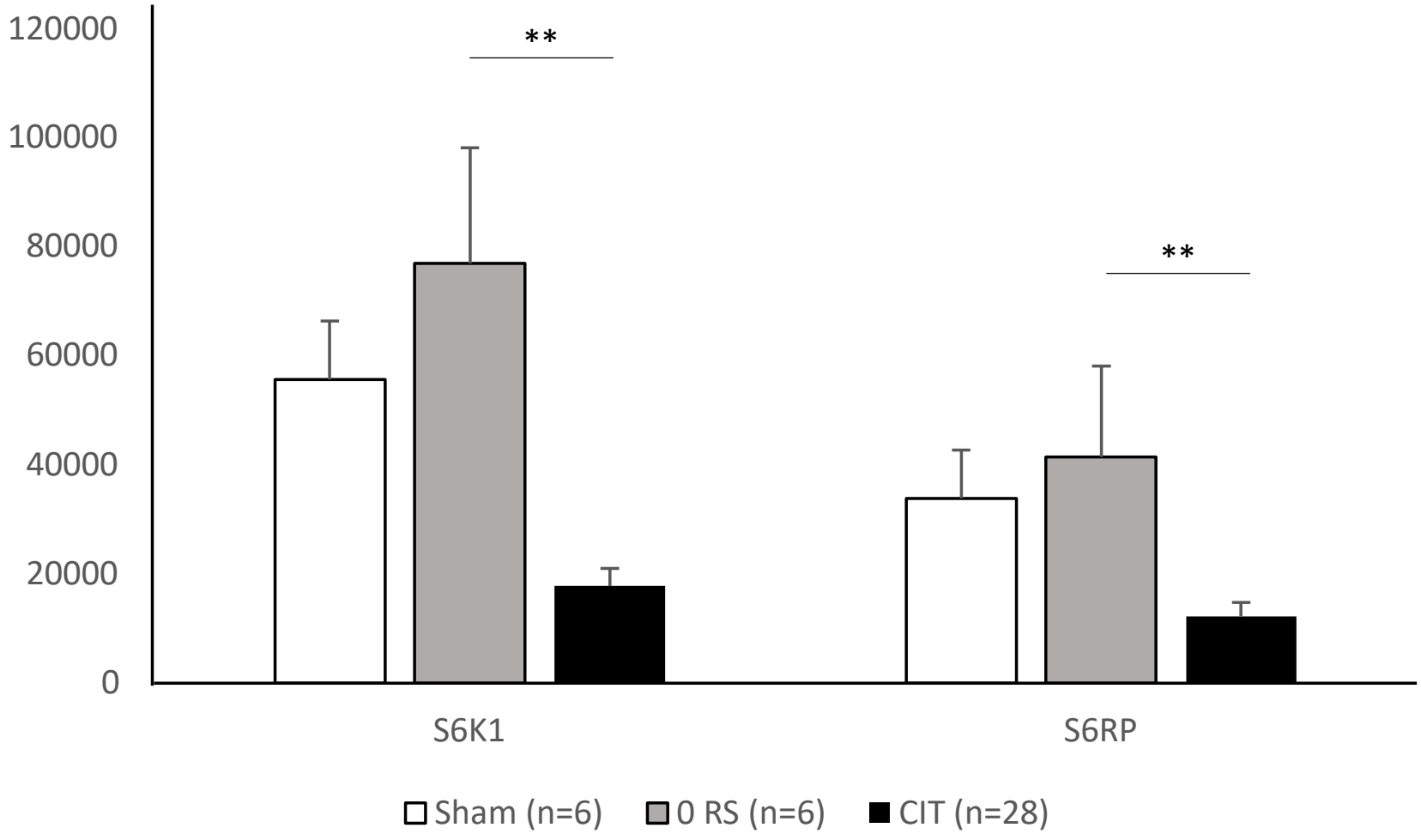

120000
100000
80000
60000
40000
20000
0
**
**
S6K1
S6RP
Sham (n=6)
0 RS (n=6)
CIT (n=28)

Table 1: *Organ weight and organ protein content in the sham group and in groups of resected citrulline-supplemented rats (from 0 to 5g.kg^{-1}.d^{-1}) by enteral route during 10 days*

		Sham (n=6)	*Control (n=6)*	*0.5 CIT (n=9)*	*1 CIT (n=7)*	*2.5 CIT (n=8)*	*5 CIT (n=8)*
Soleus	*Weight (mg)*	106±5 [a]	90±3 [b]	101±3 [a,c]	96±7 [c]	103±13 [a,c]	104±4 [a,c]
	Protein (mg)	11.7±0.8 [a]	9.0±1.0 [b]	11.7±1.2 [a]	11.8±1.5 [b]	11.7±1.0 [b]	10.6±0.8 [b]
EDL	*Weight (mg)*	110±4	106±4	104±3	104±6	103±3	109±6
	Protein (mg)	13.2±1.1	11.2±0.5	12.0±0.9	12.1±0.7	12.9±0.9	13.0±1.3
Gastrocnemius	*Weight (mg)*	1175±54	1087±67	1113±36	1090±84	1119±37	1133±78
Tibialis	*Weight (mg)*	450±20	417±19	426±13	422±28	437±15	473±39
Plantaris	*Weight (mg)*	194±9	181±9	175±9	194±13	180±14	192±13
Liver	*Weight (g)*	9.0±0.7 [a]	8.0±0.7 [b]	7.6±0.5 [b]	7.3±0.5 [b]	7.5±0.3 [b]	8.0±0.3 [b]
	Protein (mg)	NA	914±75	987±112	1095±212	955±86	1142±188
Jejunum	*Weight (mg/10 cm)*	118±16	108±21	94±14	93±11	107±17	161±44
	Protein (mg/10 cm))	4.3±0.3	4.6±1.1	4.0±0.4	5.8±0.5	4.5±0.9	6.0±1.1
Ileum	*Weight (mg)*	105±9	123±21	88±14	122±20	128±29	123±30
	Protein (mg)	63±10	53±10	54±9	74±9	58±8	54±14

Values are means ± SEM. ANOVA + Tukey–Kramer test.
Values with different superscripts are statistically different at $p<0.05$.

Table 2: *Plasma and EDL amino acid contents (respectively in µmol/L and µmol/g) in the sham group and in groups of resected citrulline-supplemented rats (from 0 to 5g.kg^{-1}.d^{-1}) by enteral route during 10 days*

Plasma µmol/L	*Sham (n=6)*	*Control (n=6)*	*0.5 CIT (n=9)*	*1 CIT (n=7)*	*2.5 CIT (n=8)*	*5 CIT (n=8)*
Citrulline	75±3^{a}	49±2^{b}	91±15^{a}	110±22^{c}	372±40^{d}	1265±192^{e}
Ornithine	55±4^{a}	47±4^{a}	54±4^{a}	71±10^{b}	157±15^{c}	330±46^{d}
Arginine	94±8^{a}	79±8^{a}	114±10^{b}	147±21^{c}	299±24^{d}	528±35^{e}
EDL µmol/g						
Citrulline	0.2±0.02^{a}	0.16±0.04^{a}	0.4±0.08^{b}	0.66±0.16^{c}	2.67±0.31^{d}	9.14±0.73^{e}
Ornithine	0.15±0.03^{a}	0.11±0.02^{a}	0.21±0.04^{b}	0.39±0.11^{c}	0.83±0.17^{d}	1.89±0.13^{e}
arginine	0.06±0^{a}	0.05±0.01^{a}	0.07±0.02^{a}	0.09±0.02^{a}	0.18±0.03^{b}	0.58±0.14^{c}

Values are means ± SEM. ANOVA + Tukey–Kramer test.
Values with different superscripts are statistically different at $p<0.05$.