Dose-dependent beneficial effects of citrulline supplementation in short bowel syndrome in rats

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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.

1. Introduction

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

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

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

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

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):

- The citrulline groups (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)
- The control group (n = 6) and the sham group (n = 6) consisted of rats receiving standard enteral nutrition.

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

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

2.5. Tissue removal:

Blood

Blood was sampled in heparinized tubes and then swiftly centrifuged.

Liver

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

Jejunum and ileum

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

Muscles

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

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

mitochondrial proteins, actin and myosin was calculated using the following equation: FSR = (Ei x 100) /(Eprec x t), where Ei is the enrichment as atom percent excess of 13C derived from combustion of valine from proteins at time t (minus basal enrichment), Eprec is the mean enrichment in the precursor pool (tissue fluid [13C] valine), and t is the incorporation time in hours [13]. Data are expressed as %/h.

2.6.3. Preparation of muscle lysates

Frozen muscles (*tibialis*) were ground in liquid nitrogen. Powdered muscles were weighed and homogenized in 10 volumes of buffer containing 150mM NaCl, 10mM Tris-HCl pH7.4, 5mM EDTA, 1% NP40, phosphatase inhibitors (20mM sodium fluoride, 1mM sodium pyrophosphate, 25mM sodium glycerophosphate, 1mM sodium vanadate), and protease inhibitors (complete protease inhibitor cocktail, Roche, Meylan, France). The samples were continuously homogenized (1 hour, 4°C) and then centrifuged at 1000 *g* for 30 min at 4°C. Supernatants were aliquoted and stored at -80°C until analysis. Protein concentrations were determined using the bicinchoninic acid method (BC Assay UP 40840A kit Uptima, Interchim, France)

2.6.4 Immunoblotting: determination of the phosphorylation state of the principal actors in mTORC1

Total protein (50 µg) in SDS sample buffer (Laemmli Sample Buffer, Biorad) was heated to 95°C, and the proteins were separated by SDS-PAGE using a denaturing gel. After electrophoresis, proteins were transferred to nitrocellulose (GE-Healthcare) and then blocked for 1 h with a blocking buffer (10mM Tris-HCl pH8.0, 150mM NaCl, 0.05% Tween20, 5% nonfat dry milk). Nitrocellulose blots were incubated overnight at 4°C with primary antibodies that recognize phosphorylated forms. After washing, membranes were

209 incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h in TBS/T
210 under gentle shaking. The blots were developed using the enhanced chemiluminescence
211 detection system (GE Healthcare) according to the manufacturer's protocol. Films were
212 scanned and quantified using the Bio Imaging System (Syngene). After quantification of the
213 relative intensity of the phosphorylated forms, membranes were stripped using a stripping
214 buffer (0.5% acetic acid) and then probed with primary antibodies that recognize total forms.
215
216 **2.7. Statistical analysis**
217 Data are expressed as means ± SEM. Comparisons between sets of data were performed
218 using one-way analysis of variance (ANOVA) followed by the Duncan test (all analysis
219 were performed on parameters measured at euthanasia and are statistically independent).
220 When statistical analysis did not show a dose-dependent effect of citrulline, we analyzed the
221 global effect of citrulline supplementation versus no citrulline, results of all supplemented
222 rats were pooled and compared to results of unsupplemented resected rats (controls).
223 Statistical analyses (ANOVA, Duncan test, correlation, quadratic analysis, linear regression
224 etc.) were performed using Statview software (Cary, NC). Threshold of significance was set
225 at $p<0.05$.
226

3. Results

3.1. Body weight

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

3.2. Liver weight

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

3.3. Muscle weight

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

3.4. Intestinal adaptation

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

3.5. Amino acid concentrations in plasma and muscles

3.5.1. Plasma amino acid concentrations:

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

3.5.2. Muscles amino acid concentrations:

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

3.6. Evaluation of muscle protein synthesis

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

3.7. Evaluation of mTORC1 pathway activation

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

4. Discussion

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

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

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

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

acid-induced translation initiation, thereby promoting protein anabolism to a much greater extent than continuous enteral feeding [25]. Likewise, mTORC1 is mainly activated in the postprandial (bolus-style) state. 2) In patients with early-phase SBS, feeding fails to decrease proteolysis, in contrast to what is physiologically observed in healthy subjects. Note that the improvement in intestinal function and nutritional status achieved by rhGH treatment in SBS is mediated by the decrease in proteolysis in response to feeding [26]. We hypothesize that the same could occur in work. The rise in muscle FSR after resection could reflect an increased protein turnover due to excess proteolysis, and the stimulatory effect of citrulline on muscle protein synthesis could, at least in part, be mediated by a decrease in proteolysis. This potential effect of CIT on proteolysis was also hinted at in a proteomics study [27]. Note that the systemic relation between protein synthesis and muscle mass is inconstant, as it was previously shown that CIT could increase protein synthesis without increasing muscle mass [18].

In conclusion, our results confirm that citrulline has potential benefit in SBS as it promotes body weight gain, preserves muscle trophicity, and enhances intestinal adaptation in our model of resected rats. Here we demonstrated that this beneficial effect was dose-dependent, with a maximal beneficial effect for a citrulline dose of 2.5 g/kg/day. Citrulline reversed the rise in protein synthesis in skeletal muscle induced by intestinal resection through the mTOR pathway, prompting the hypothesis that citrulline could reverse the post-resection modification in protein turnover. This new approach to the pharmacological properties of citrulline in SBS warrants further research, but we anticipate it could help clinicians develop valuable clinical studies in the early-adaptation phase of SBS.

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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

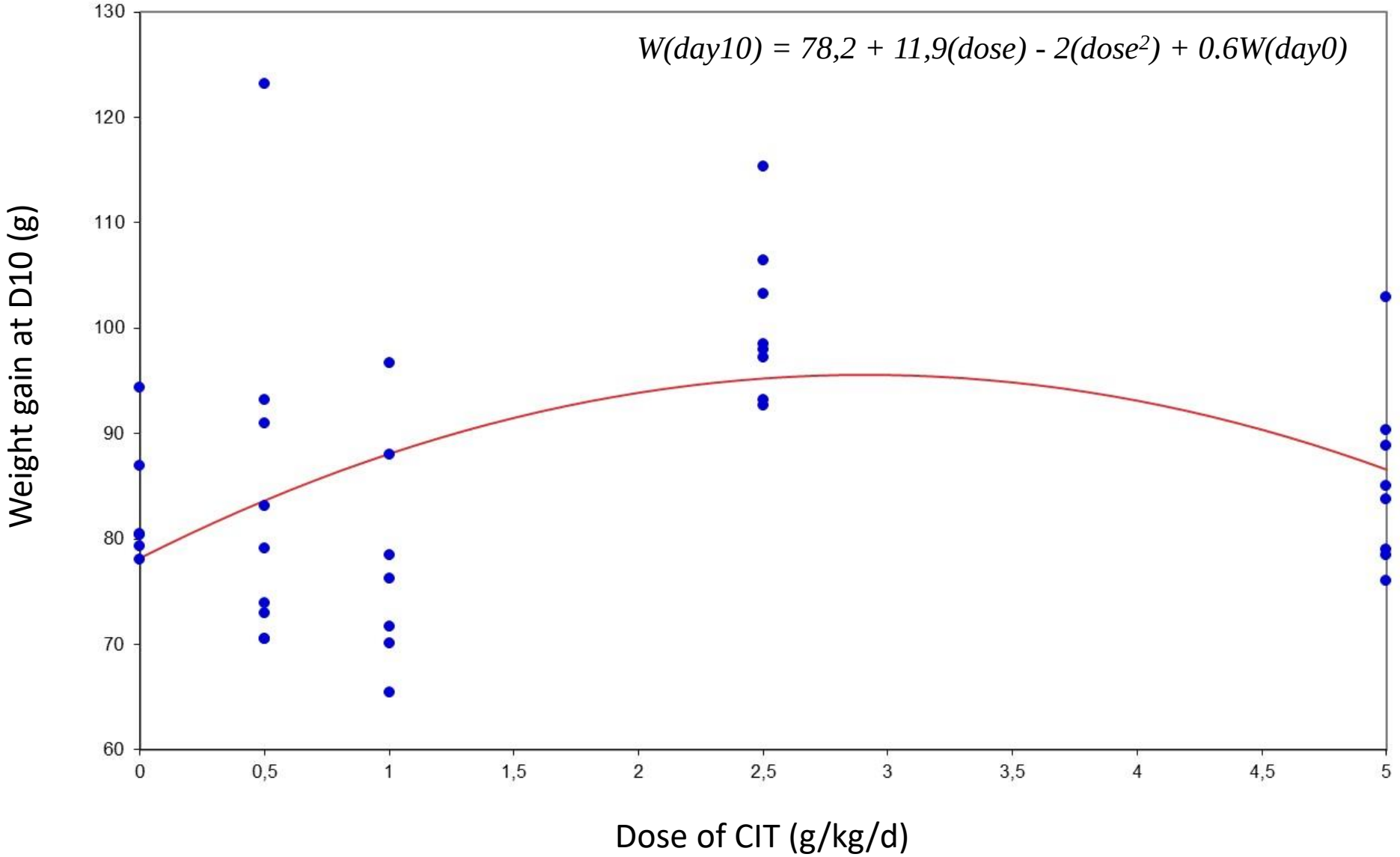

W(day10) = 78,2 + 11,9(dose) - 2(dose²) + 0.6W(day0)
Weight gain at D10 (g)
130
120
110
100
90
80
70
60
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
5
Dose of CIT (g/kg/d)

Figure 2

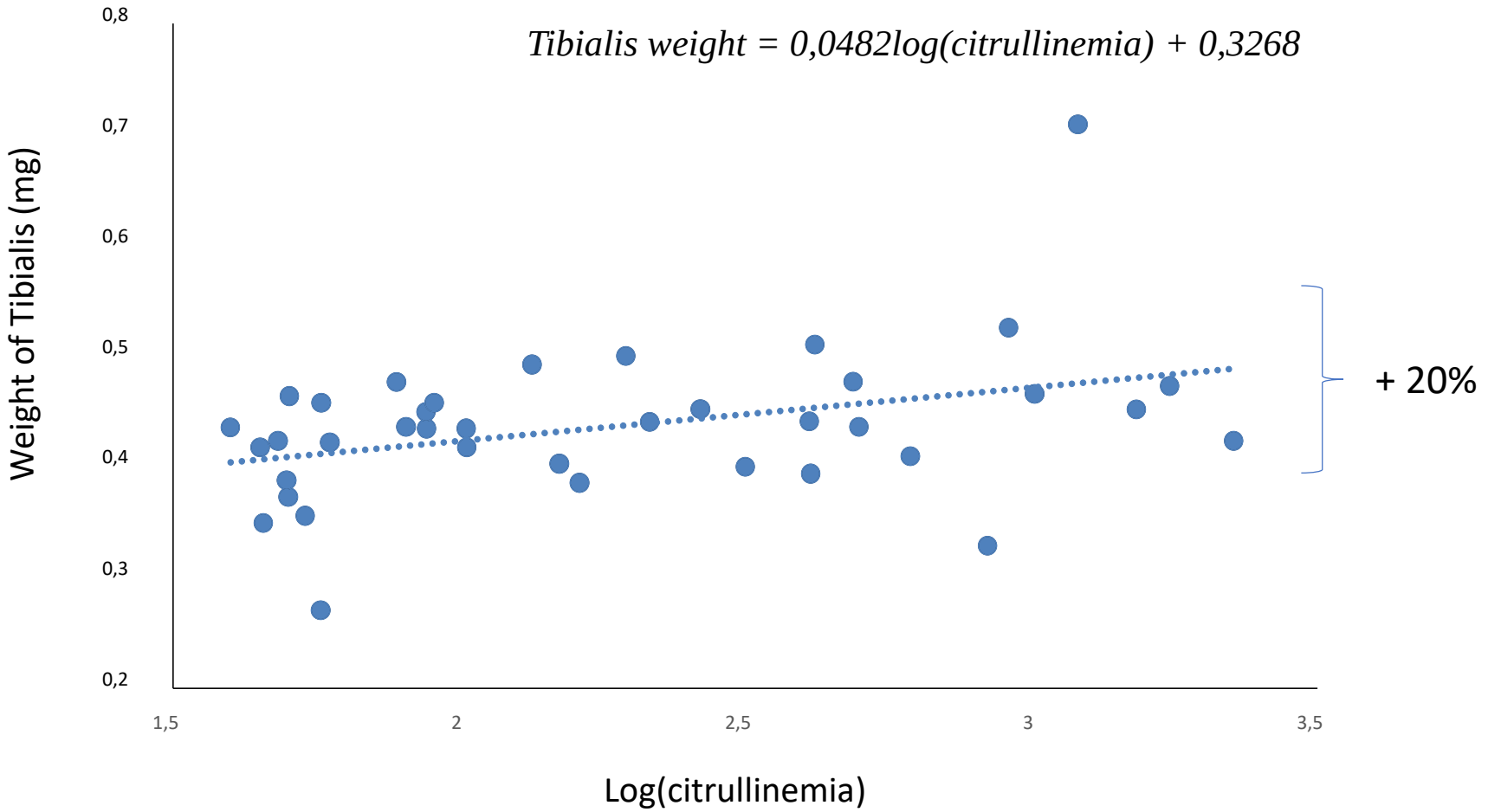
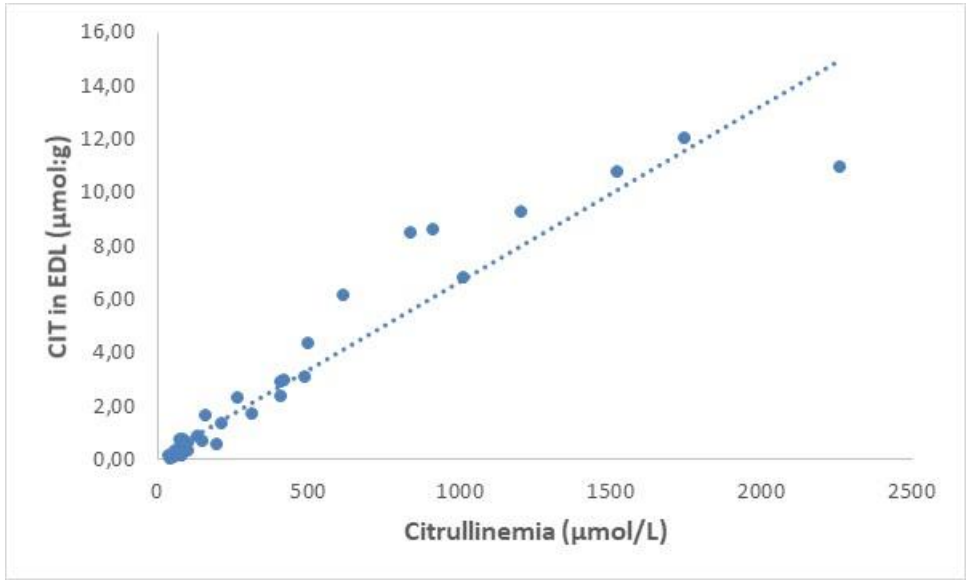
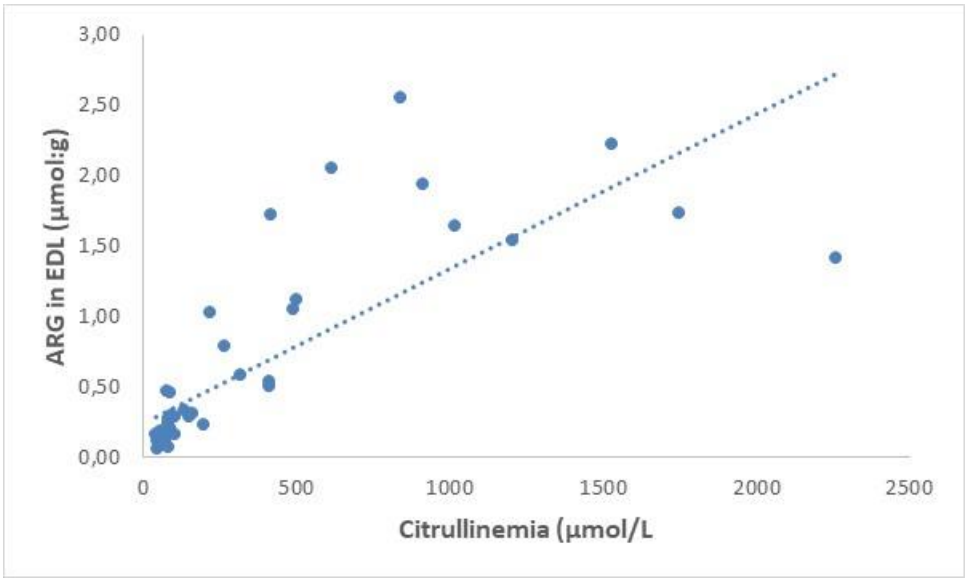

Tibialis weight = 0,0482log(citrullinemia) + 0,3268
Weight of Tibialis (mg)
0,8
0,7
0,6
0,5
0,4
0,3
0,2
1,5
2
2,5
3
3,5
Log(citrullinemia)
+ 20%

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

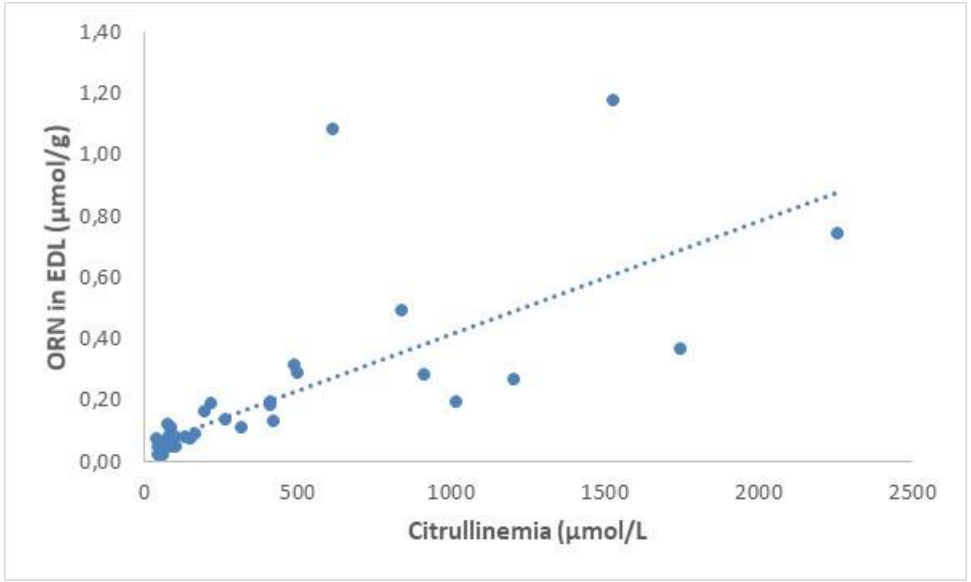

1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
ORN in EDL (µmol/g)
0
500
1000
1500
2000
2500
Citrullinemia (µmol/L

Figure 4

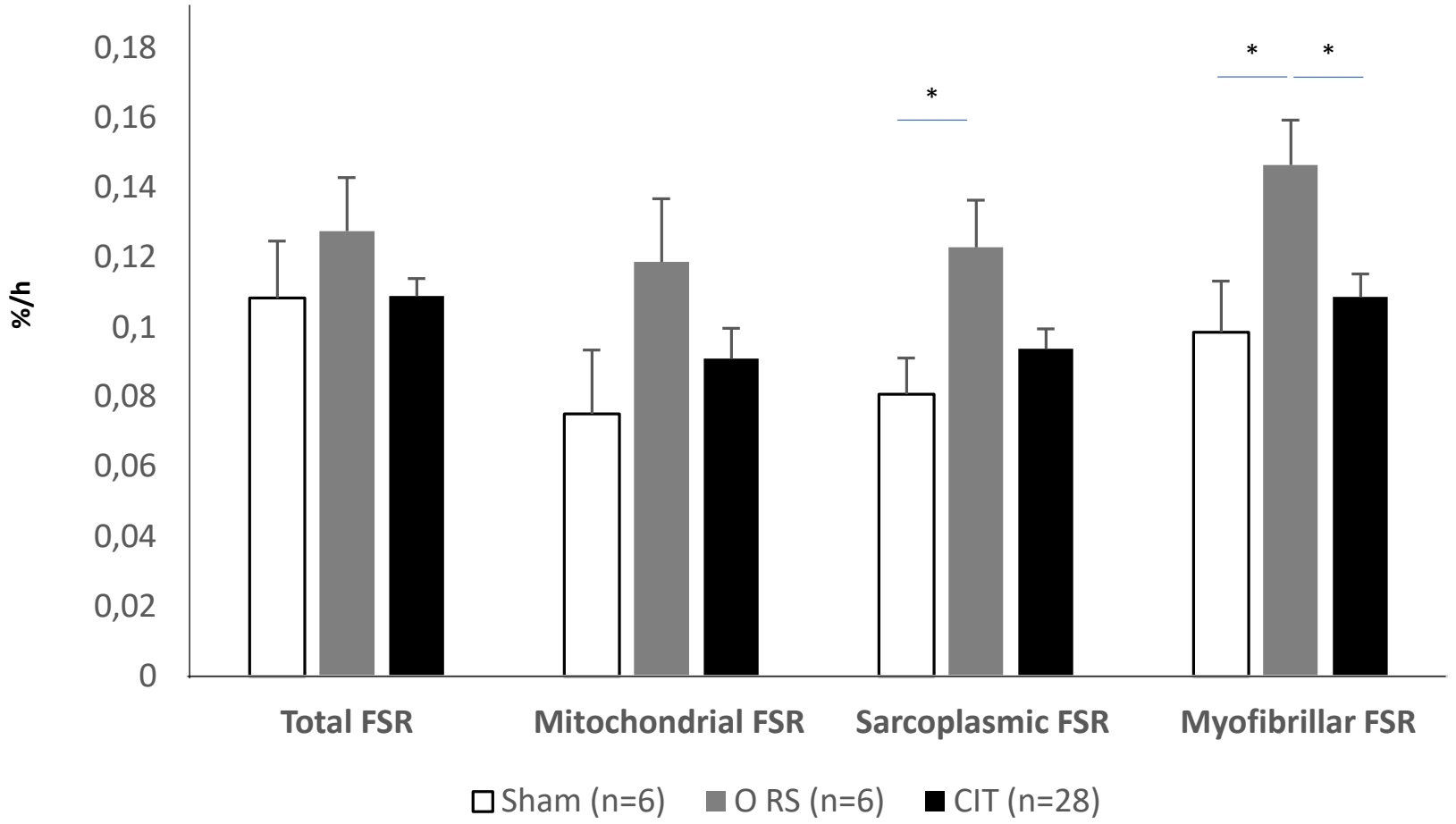

0,18
0,16
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
%/h
*
*
*
Total FSR
Mitochondrial FSR
Sarcoplasmic FSR
Myofibrillar FSR
Sham (n=6)
O RS (n=6)
CIT (n=28)

Figure 5

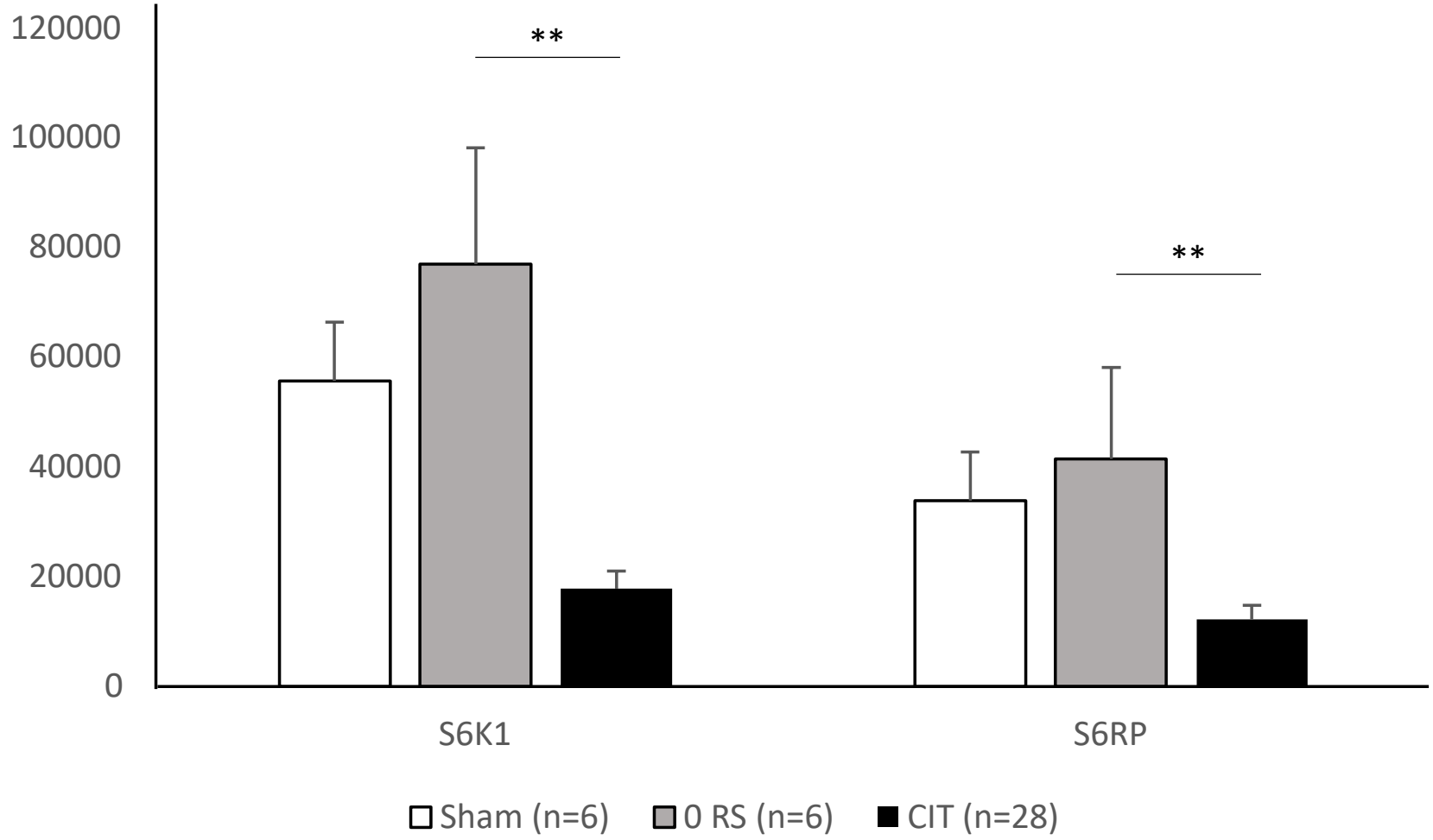

120000
100000
80000
60000
40000
20000
0
**
**
S6K1
S6RP
Sham (n=6)
0 RS (n=6)
CIT (n=28)

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 $p<0.05$.