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Maternal supplementation with citrulline or arginine during gestation impacts fetal amino acid availability in a model of intrauterine growth restriction

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ABSTRACT

Background. Supplementing maternal diet with citrulline or arginine during gestation was shown to enhance fetal growth in a model of IUGR induced by maternal dietary protein restriction in the rat.

Objective. The aims of this study were to determine in the same model whether maternal supplementation with citrulline or arginine would increase 1) citrulline and arginine concentration in fetal circulation; 2) the expression of placental amino acid transporters, and 3) the fetal availability of essential amino acids.

Methods. Pregnant rats (n=8) were fed either an isocaloric control (20% protein, NP) or a low protein (LP, 4% protein) diet, either alone or supplemented with 2g/kg/d of L-citrulline (LP+CIT) or isonitrogenous Arginine (LP+ARG) in drinking water throughout gestation. Fetuses were extracted by C-section on the 21st day of gestation. The gene expression of system A (*Slc38a1*, *Slc38a2*, and *Slc38a4*) and L (*Slc7a2*, *Slc7a5*, *Slc7a8*) amino acid transporters was measured in placenta and amino acid concentrations determined in maternal and fetal plasma.

Results. Maternal LP diet decreased fetal (4.01±0.03 vs. 5.45±0.07 g, p<0.0001) and placental weight (0.617±0.01 vs. 0.392±0.04g, p<0.001), by 26 and 36% respectively, compared with NP diet. Supplementation with either CIT or ARG increased fetal birth weight by ≈ 5 or 11%, respectively (4.21±0.05 and 4.48±0.05 g vs. 4.01±0.03 g, p<0.05). CIT supplementation produced a 5- and 2-fold increase in fetal plasma citrulline and arginine, whereas ARG supplementation only increased fetal arginine concentration. LP diet led to lower placental SNAT 4 mRNA, and higher LAT2 and SNAT1 expression, compared with NP. SNAT4, 4hFC, LAT2 mRNA were up-regulated in LP+CIT and LP+ARG group compared with the un-supplemented LP group. Higher level of LAT1 mRNA was also observed in the LP+CIT group than in the LP group (p<0.01). SNAT2 expression was

unchanged in response to CIT or ARG supplementation. Fetal amino acid concentrations were decreased by LP diet, , and were not restored by CIT or ARG supplementation.

Conclusions The current findings confirm supplementation with citrulline or arginine enhances fetal growth in a rat model of IUGR. They further suggest that: 1) citrulline and arginine administered orally to the pregnant mother may reach fetal circulation; 2) citrulline effectively raises fetal arginine availability; and 3) although it failed to increase the concentrations of essential amino acids in fetal plasma, citrulline or arginine supplementation upregulates the gene expression of several placental amino acid transporters,.

1. INTRODUCTION

Intrauterine growth restriction (IUGR), defined as a birth weight < 10th percentile for gestational age, is a common obstetrical complication [1] which exposes infants not only to an increased risk of stillbirth [2], neonatal mortality, and morbidity [3], requiring neonatal intensive care [4], but to a higher risk of developing metabolic or cardiovascular diseases in adulthood as well [5-7].

Whether IUGR is due to maternal undernutrition– or impaired utero-placental perfusion–the main causes of IUGR in developing and industrialized countries, respectively– insufficient nutrient availability is the main factor leading to fetal undernutrition [8,9].

Fetal amino acid supply is thought to be the driving force for fetal growth, as it determines protein accretion.

In normal pregnancy, the concentration of most amino acids in fetal blood largely exceeds those in maternal blood, as amino acids cross the placenta by active transport through several amino acid transporters systems [10]: system A, [11] which transports small, amino acids such as alanine or glycine[12], and system L which exchanges essential amino acids such as leucine against non-essential amino acids. [13]

In both humans [14] and animals [15,16], IUGR is associated with a decrease in the fetal plasma concentration of essential amino acids, (e.g., branched-chain amino acids). and alterations in placental nutrient transport and metabolism [17]. In human IUGR, a down regulation of placental system A and system L has been described both *in vitro* and *in vivo* [18-22]. Down regulation of placental amino acid transporters was observed in models of IUGR in rats [23, 24] and baboons [25, 26]. Such alterations occur before the onset of fetal growth failure, which suggests that impaired placental amino acid transport may be a cause rather than a consequence of growth restriction [23, 27]..

Citrulline is a non-essential amino acid that is not incorporated in protein and is produced endogenously in the small intestine; it escapes hepatic uptake, and is taken up by kidney where it is quantitatively converted to Arginine [29, 30]. Arginine, a conditionally essential amino acid for fetuses and growing mammals [31], is the sole endogenous source of nitric oxide (NO) which is involved in the regulation of placental growth and blood flow [32]. Arginine supplementation was therefore tested as a safe approach [33] to treat IUGR in humans [34] and in animal IUGR models induced by underfeeding [35], multiple pregnancy [36], or inhibitors of NO synthesis [37]. In earlier studies, we showed that maternal supplementation with citrulline or arginine during gestation was able to stimulate fetal growth and muscle protein synthesis in a rat model of IUGR induced by dietary protein restriction [38], and the expression of genes involved in placental growth such as insulin-like growth factor 2 (Igf2), and angiogenesis, such as Vegf and Flt-1 [39]. Yet several questions remained open. Do citrulline and arginine reach fetal bloodstream, as shown in an ovine model [30]? Does the effect of citrulline improve placental amino acid transport? Does it increase the bioavailability of essential amino acids to the fetus??

The aims of this study therefore were to determine whether maternal supplementation with CIT and ARG 1) increased the availability of CIT or ARG in fetal circulation; 2) enhanced placental amino acid transport, and 3) impacted the fetal availability of essential amino acids in a model of IUGR induced by protein restriction.

2. METHODS

2.1 Animals and experimental design

The study was carried out in accordance with current institutional guidelines in France and the EU Directive 2010/63/EU for animal experiments, and after approval from the animal ethics committee of Pays de La Loire [N°CEEA.2010.8].

Eight-week old, primiparous timed-pregnant Sprague-Dawley rats [n=7 to 8 per group], were purchased from Janvier [Le Genest Saint Isle, France], delivered to the animal facilities at gestation day 2 [GD2], and housed in individual cages in a room with constant air humidity, temperature ($22\pm 2^{\circ}\text{C}$), and a 12 h light/dark cycle with *ad libitum* access to chow [Arie Block, Woerden, The Netherlands] and water.

Upon arrival, pregnant rats were randomly assigned to one of four regimens (1) a control, semi-purified diet with an adequate protein content [NP, 20% casein]; (2) an isocaloric diet with a low protein content [LP; 4% casein] to induce IUGR; (3) a low protein diet, along with a 2 g/kg/d L-citrulline [0.48 g nitrogen/kg/d; obtained from Inresa, Bartenheim, France] supplementation in drinking water (LP+CIT diet) and (4) a LP diet, along with an isonitrogenous amount of L-arginine in drinking water (LP+ARG).

Composition of diets is shown in Supplemental **Table 1**. Amino acid solutions were prepared twice a week, stored at 4°C and added to drinking water. Arginine solution pH was adjusted to 7.0 with NaOH.

Maternal body weight, food and water consumption were recorded 3 times per week. On GD21, pregnant rats underwent C-section under general anesthesia as described [39]. The fetuses and placentas were rapidly extracted and dried, and litter size, placental weight and pup birth weight were recorded. Blood samples were drawn simultaneously from mother by cardiac puncture and from fetuses by decapitation, collected into heparinized tubes and pooled per litter. Placentas were washed in 0.9% NaCl, cut in four parts and quickly frozen in liquid

nitrogen. Blood samples were centrifuged at 4000g for 10 min at 4°C. Placental and plasma samples were stored at -80 °C until used for analysis. At the end of the procedure, female rats were euthanized by an overdose of isoflurane.

2.2 Amino acid analysis

Amino acid analysis was performed by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MSMS) as described for milk [40] and adapted for small volumes of plasma. Briefly, 20 µL of maternal or fetal plasma were mixed with 80µL of ultrapure water obtained from a Milli-Q® purifier [Millipore, Eschborn, Germany] and 50µL of labeled internal standard pool. Isotope-labeled amino acid internal standards were obtained from Cambridge Isotope Laboratories Inc. (Andover, USA), Tracer Technologies Inc. (Waterloo, Canada), or Eurisotop (Saint-Aubin, France). Samples were deproteinized with 10% sulfosalicylic acid, and centrifuged at 10,000 g for 15 min at 4°C. Free amino acids contained in the supernatant phase were collected and 10 µL were derivatized by adding 70 µL of AccQ•Tag™ Ultra Borate Buffer and 10 µL of supernatant and 20 µL of AccQ•Tag™ Ultra reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) [Waters Corporation, Milford, MA, USA] were incubated for 10 min at 55°C. 1µL of sample was injected in duplicate into an Acquity H-Class® UPLC system (Waters Corporation, Milford, USA) equipped with a quaternary solvent manager, an autosampler maintained at 4°C, a Waters AccQ•Tag™ Ultra® column (2.1 mm × 10 mm, 1.7 µm particles) with a pre-filter heated at 55°C, and coupled with a tandem quadrupole detector.

2.3 RNA isolation and RT-qPCR.

To assess amino acid transporter relative mRNA abundance, total RNA was isolated from four placentas in 6 dams per group. Placental tissues were homogenized and RNA was extracted in 1 mL trizol reagent solution. RNA samples were treated with DNase I (Promega, Madison, WI, USA). Following extraction, total RNA was quantified and purity were

determined by UV spectrophotometry at 260 and 280 nm with NanoVue Spectrophotometer. The integrity of total RNA was confirmed by agarose gel electrophoresis. Quantitative real-time PCR analyses were performed with a 96-well plate carried out using SYBR Green detection on a CFX Connect™ Real Time PCR Detection System [Biorad, Hercules, CA, USA] First-strand cDNA was synthesized from 1 µg of total RNA with random hexamer primers and *M-MLV* reverse transcriptase [Invitrogen, Life Technologies, Carlsbad, USA]. Primers were designed using Perlprimer software (Sourceforge, <https://sourceforge.net/>). Sequences are shown in Supplemental Table 2.

In preliminary experiments, analysis using Bestkeeper, geNorm and NormFinder algorithm showed *Ywhaz* and GAPDH to be the most constant housekeeping genes under our experimental conditions, and to be expressed in placenta at levels of the same magnitude as our genes of interest. Data were normalized against the mean of *Ywhaz* and GAPDH expression in NP pups. Fold changes were calculated using the formula $2^{-\Delta Ct}$. The mean of normalized expression from 4 placentas of 6 dams per group is reported.

2.4 Biochemical parameters.

Glucose and lipids were analyzed in an automate analyzer.

2.5 Statistical analysis.

Values are means $\pm$ SEM. After normal distribution and equal variances were confirmed, differences between the four experimental groups were analyzed by one-way ANOVA using Prism 6.0® [GraphPad Software, San Diego, CA]. Fisher protected least significant difference (PLSD) method was applied for *post hoc* intergroup comparisons. In case of non-gaussian distribution, a non parametric, Kruskal-Wallis test was performed to determine significance and followed by Mann - Whitney U test. Alpha level for statistical significance was $p \leq 0.05$.

3. RESULTS

3.1 Gestation performance and food intake, fetal and placental growth

(Supplemental Table 3). On GD21, maternal body weight gain was lower in the LP group than in the NP group (163 ± 10 vs 61 ± 7 g, $P < 0.05$). Neither CIT nor ARG supplementation enhanced maternal gestational weight gain.

Average daily food intake was higher in the NP pregnant rats ($\text{g} \times \text{d}^{-1}$) than in the LP+CIT (22.9 ± 0.8 vs. 18.7 ± 0.8 $\text{g} \times \text{d}^{-1}$; $P < 0.05$) but did not significantly differ between the 3 groups fed a low protein diet (Supplemental Table 3).

Fetal and placental weights are detailed in Table 1. Feeding dams a low protein diet was associated with a 26% and 35% reduction in fetal weight (4.01 ± 0.03 vs. 5.45 ± 0.07 g, $p < 0.0001$), and placental weight, respectively, compared with the NP group. Though supplementation with amino acids did not restore fetal weight to those observed in NP group, CIT and ARG supplementation, increased fetal birth weight by ≈ 5 and 11%, respectively, compared to LP group (4.22 ± 0.05 and 4.48 ± 0.05 g vs 4.01 ± 0.03 g, $p < 0.05$). Neither CIT nor ARG supplementation enhanced placental weight.

The fetal/placental weight ratio was significantly ($P < 0.01$) increased in the 3 groups fed low protein diet, whether or not they were supplemented with amino acids.

Mean number of fetus was lower in dams fed the 20% protein diet than in dams fed the 4% protein diet. There was no effect of amino acid supplementation on litter size.

3.2 Effects of arginine and citrulline supplementation on fetal citrulline and arginine

Plasma citrulline concentrations was ≈ 5 -fold higher in the LP+CIT mothers and fetuses, compared with other groups ($p < 0.05$). Arginine concentrations rose 2.4 fold in maternal plasma and 2 fold in fetal plasma in response to CIT supplementation. Supplementation with citrulline was as effective as arginine supplementation in raising arginine concentrations in both maternal ($p=0.45$) and fetal plasma ($p=0.22$, LP+CIT vs. LP+ARG).

3.3 Effect of citrulline and arginine on mRNA expression of amino acid transporters in placenta (Fig 1)

The expression of SNAT 2, 4hFC, LAT1 mRNA relative to GAPDH and Ywhaz mRNA for each sample was unaltered in LP group compared with the NP group.

Maternal low protein diet led to a decrease in the SNAT 4 placental mRNA. In contrast, LAT2 and SNAT1 mRNA were higher in LP than in NP placentas.

SNAT4, 4hFC, LAT2 mRNA was significantly up-regulated in LP+CIT and LP+ARG group compared with the unsupplemented LP group and. Higher level of LAT1 mRNA was also observed in the LP+CIT group than in the LP group ($p < 0.01$).

SNAT2 expression was unchanged in response to CIT or ARG supplementation.

3.4 Effect of citrulline and arginine on maternal and fetal plasma amino acids concentrations (Table 2 and 3, Fig 2).

Concentrations of alanine and lysine in maternal plasma were not affected by maternal protein restriction. Concentrations of taurine, serine, glycine, phenylalanine, glutamate and glutamine were higher in LP than in NP mothers. All other maternal plasma amino acid concentrations including arginine, citrulline and branched amino acids (BCAA, leucine, isoleucine, valine) were decreased in the LP mothers compared with the NP group.

In fetal plasma, the concentration of most amino acids, including the majority of essential amino acids—except for lysine, phenylalanine, and tryptophan—was significantly lower in the LP than the NP group. Serine, glycine, glutamine, and phenylalanine were higher in LP fetuses. Tryptophan, arginine, citrulline, glutamate and alanine, were similar in the plasma from LP and NP fetuses.

In maternal plasma, ARG or CIT supplementation was associated with lower concentrations of glutamine, glycine and glutamic acid compared with the unsupplemented LP group.

Neither CIT nor ARG supplementation altered the concentration of most other amino acids

including BCAA or EAA, except for tryptophan which was higher, and lysine that was lower in LP+CIT mothers compared with the LP group (Table 2).

In fetal plasma, concentrations of glutamine, glycine and taurine were decreased in the LP+CIT group compared with the LP group [as observed in maternal plasma].(Table 3)

3.5 Biochemical parameters in maternal and fetal plasma (Supplemental Table 4)

Maternal plasma glucose was similar in the 4 groups. Regardless of diet, glucose concentrations were lower in fetal than in maternal plasma. LP diet was associated with a lower plasma glucose, compared with the NP group, and was not affected by either CIT or ARG supplementation.

DISCUSSION

The findings of the current study confirm supplementation with either citrulline or arginine enhances fetal growth in an animal model of intrauterine growth restriction. They further demonstrate that both, citrulline and arginine administered orally to the pregnant mother effectively raise fetal arginine availability. Finally, we provide evidence for an upregulation of placental amino acid transporters mRNA by citrulline and arginine.

4.1 Effect of citrulline and arginine on fetal growth.

As expected, maternal undernutrition impaired fetal growth, with a relative preservation of brain growth (Table 1), as observed in human IUGR [41]. The LP diet was associated with a lower concentration of glucose and of most essential amino acids in fetal plasma; as reported in human IUGR [14]. Consistent with our earlier studies [38,39], maternal supplementation with arginine or citrulline enhanced fetal growth. In contrast to our initial study, arginine was more effective than citrulline in the current study. Differences in study design may account for the discrepancy since: 1) supplementation was initiated from the 2nd, rather than the 8th day of gestation in our earlier study; and 2) food intake was 15% higher in LP+ARG group than in LP+CIT group, although the difference did not reach statistical significance. Alternatively, arginine may exert its effect in a specific time window in the earlier part of gestation; accordingly, in a recent clinical trial arginine prevented pre-eclampsia only when administered in the first trimester of pregnancy [42]. Finally, fetal plasma glucose, (Supplemental Table 4) tended to be higher in the arginine- than the citrulline-supplemented group, and a higher fetal plasma glucose may increase insulin secretion by fetal pancreas. Elevation of fetal insulin has long been known to drive fetal growth, as evidenced by the occurrence of macrosomia in infants from diabetic mothers [43]. We therefore speculate that arginine supplementation may enhance growth through enhanced fetal insulin secretion. As we did not measure fetal plasma insulin, such hypothesis remains to be tested.

The fetal to placenta weight ratio, was higher in groups fed an LP diet, and further increased by arginine and citrulline supplementation. Interpreting fetal/placental weight ratio, however, is complex. Fetal/placental weight ratio correlates with amino acid transport system A activity measured on placental vesicles from infants with adequate weight for gestational age [44]. This suggests such ratio may reflect placental transport efficiency. The increased birth/placental weight ratio commonly observed in human IUGR and in animal models of IUGR is thought to reflect an adaptative response of placental function to IUGR [45-48]. Yet impaired placental amino acid transport has been documented in animal and human IUGR [17-23, 25-27]. In the current report, both fetal /placental weight ratio and the gene expression of SNAT4, 4hFC, LAT2 mRNA were enhanced by CIT or ARG, but SNAT2 was not, and actual amino acid fluxes from mother to fetus were not measured. It therefore remains unclear whether the higher fetal/placental weight ratio reflects enhanced placental function.

4.2 Effect of citrulline and arginine on fetal plasma arginine and citrulline

Though our earlier studies documented an anabolic effect of citrulline on fetal growth, the specific site(s) of action of citrulline remained elusive. Citrulline may impact maternal metabolism, placenta, or fetal metabolism itself. The current report demonstrates that citrulline administered orally to the pregnant mother 1) raises the concentration of citrulline in fetal circulation, and 2) is as effective as arginine itself to increase arginine bioavailability in the fetus. This is consistent with data comparing intravenous citrulline vs. arginine infusion in pregnant ewes [30]. Citrulline may thus exert its effect—either *per se* or through its conversion to arginine—directly on fetal tissues. One potential advantage of citrulline over arginine is the fact that only arginine supplementation was associated with a rise in maternal plasma urea concentration (Table 7). Such rise is consistent with the fact that arginine may be

substantially extracted in maternal liver and degraded to urea by hepatic arginase. Extensive arginine catabolism was one of the hypotheses raised to explain the lack of efficacy of oral arginine supplementation in clinical trials [49]. In contrast, citrulline is known to escape hepatic uptake, and may be a better candidate for clinical trials in human IUGR.

4.3 Effect of citrulline and arginine on placental amino acid transport

In the current study, we observed a positive effect of citrulline and arginine on the expression of placental amino acid transporter LAT1 and LAT2 in charge of large neutral amino acids such as BCAA, as well as SNAT4. As a change in mRNA expression does not necessarily translate into a parallel change in protein expression or transport activity, these observations would obviously warrant confirmation using immunocytochemistry and Western blotting.

Previous work showed that cord blood amino acid concentrations are significantly reduced in human IUGR, compared to normal pregnancy [50]. Literature suggests impaired amino acid transport is a common feature of fetal growth restriction, regardless of its cause: down-regulation of placental A and L amino acid transporters has been reported both in human IUGR due to altered placental blood flow, as well as in animals models of IUGR induced by dietary protein restriction. *In vivo* and *in vitro* studies have shown lower transport of labelled amino acids or a reduced activity or expression of placental amino acid transporters in syncytiotrophoblast membrane from IUGR pregnancies [20, 22, 23, 51-57]. The fact that such alterations occurred before the onset of growth deceleration suggests that alterations in amino acid transport play a causative role in the growth failure associated with maternal underfeeding. Accordingly, treatment of rats fed a normal diet with an inhibitor of the A transport system was sufficient to produce growth restriction in rats [58]. In that context, the enhanced expression of placental amino acid transporters, supplementation may contribute to

the anabolic effect of citrulline and arginine on fetal growth through improved amino acid transport to the fetus.

4.4 Effect of citrulline and arginine on fetal plasma amino acids, and fetal energy substrates.

In the current study, the enhanced expression of placental amino acid transporters observed with CIT or ARG supplementation did not translate into higher concentrations of essential amino acids in fetal plasma. It should be borne in mind, however, that the concentration of an essential amino acid in fetal plasma, only reflects the balance between the appearance of such amino acid from placental transfer, or from fetal protein breakdown on one hand, and its utilization for fetal protein synthesis and oxidation, on the other hand. We therefore speculate that fetal amino acid concentrations remained unaltered because increased utilization of essential amino acids for protein synthesis may offset the increased placental transfer of essential amino acids. In an earlier study [38], we indeed showed that citrulline had a powerful effect on protein synthesis rate in fetal skeletal muscle. The enhanced growth fetal observed in the current study supports the hypothesis. Such effect is unlikely to be due to nonspecific nitrogen supply, since a mixture of non-essential amino acids failed to stimulate protein synthesis in our earlier study [38].

In summary, the current study demonstrates that, in an animal model of IUGR, arginine and citrulline supplementation efficiently raise fetal plasma citrulline and arginine concentrations in fetal plasma, and enhance the expression of placental amino acid transporters. Such effect likely contributes to the effect of citrulline and arginine on fetal growth and anabolism, and suggests that, as recently proposed [59], trials of citrulline or arginine supplementation may be warranted in human IUGR.

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Statement of authors' contributions to manuscript.

330 Aurélie Bourdon: conceptualization, performance of experiments, data analysis, manuscript
331 writing

332 Jacob Hannigsberg: conceptualization, performance of experiments, data analysis, manuscript
333 writing

334 Emilie Misbert : conceptualization, performance of experiments, data analysis, manuscript
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336 Thang Nhat Tran conceptualization, performance of experiments, data analysis, manuscript
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338 Valérie Amarger : conceptualization, data analysis, manuscript writing

339 Véronique Ferchaud-Roucher : data analysis, manuscript writing

340 Norbert Winer : funding acquisition, conceptualization, performance of experiments, data
341 analysis, manuscript writing

342 Dominique Darmaun : funding acquisition, conceptualization, performance of experiments,
343 data analysis, manuscript writing

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TABLE 1. Litter size, fetal and placental weights on day 21 of pregnancy of dams fed either a control (NP), or a protein-restricted (LP) diet as such or supplemented with L-ARG (LP+ARG) or L-CIT (LP+CIT) in drinking water throughout gestation

	<i>Group</i>				<i>P</i>
	NP	LP	LP+CIT	LP+ARG	
mean litter size	10±0.9 ^a	13.8±0.7 ^b	11.5±0.9 ^b	13.1±0.9 ^b	0.03
fetal weight, g	5.48±0.07 ^a	4.01±0.03 ^b	4.22±0.05 ^c	4.48±0.04 ^d	<0.001
placental					
weight, g	0.617±0.01 ^a	0.392±0.04 ^b	0.391±0.008 ^b	0.413±0.01 ^b	<0.001
Placenta					
efficiency ²	9.1±0.2 ^a	10.3±0.1 ^b	10.9±0.2 ^b	11.1±0.2 ^c	<0.002
Brain/body					
weight ratio	0.038 ± 0.002	0.047 ± 0.002 ^c	0.044 ± 0.003 ^c	0.046 ± 0.003 ^c	<0.05

¹Data are expressed as means ± SEM, n=7-8 dams/group. Means in a row with letter superscripts without a common letter differ, *P*<0.05.

²Placenta efficiency was estimated by the fetal/placental weight ratio

TABLE 2. Amino acid concentrations in maternal plasma at GD21 in pregnant dams fed a control diet (NP), a low protein diet alone(LP), or a low protein diet plus citrulline (LP+CIT) or plus Arginine (LP+ARG).

Amino acid	Group				<i>P</i>
	NP	LP	LP+CIT	LP+ARG	
	<i>μmol/L</i>				
Histidine	58±3 ^a	26±2 ^b	29±4 ^b	25±2 ^b	<0.001
Taurine	91±18 ^b	259±29 ^a	102±23 ^a	150±26 ^a	<0.001
Serine	278±16 ^b	511±29 ^a	457±49 ^a	428±23 ^a	<0.001
Glutamine	728±37 ^{b,c}	1091±66 ^a	645±47 ^c	833±64 ^b	<0.001
Arginine	125±8 ^a	96±4 ^b	232±43 ^c	240±44 ^c	<0.001 ¹
Glycine	38±3 ^c	224±33 ^a	145±8 ^b	145±14 ^b	<0.001
Glutamic acid	25±3 ^b	52±4 ^a	30±5 ^b	36±3 ^b	<0.001
Citrulline	57±3 ^a	28±3 ^b	282±16 ^c	35±4 ^b	<0.001 ¹
Threonine	467±20 ^a	178±12 ^b	172±15 ^b	157±15 ^b	<0.001
Alanine	972±121	913±144	680±102	803±113	0.41
Proline	924±68 ^a	385±25 ^b	322±21 ^b	354±25 ^b	<0.001 ¹
Lysine	1142±77 ^a	1148±59 ^a	772±60 ^b	1003±85 ^a	<0.01
Cysteine	39±3 ^a	27±3 ^b	23±2 ^b	21±2 ^b	<0.001
Methionine	102±5 ^a	64±2 ^b	62±1 ^b	64±2 ^b	<0.001 ¹
Valine	230±11 ^a	103±8 ^b	111±16 ^b	93±7 ^b	<0.001
Isoleucine	64±4 ^a	35±6 ^b	36±9 ^b	28±4 ^b	0.001
Leucine	134±8 ^a	67±9 ^b	66±15 ^b	54±6 ^b	<0.001
Phenylalanine	66±2 ^b	73±2 ^a	65±2 ^a	63±1 ^a	0.01
Tryptophane	79±6 ^a	29±2 ^b	39±4 ^c	28±2 ^b	<0.001 ¹

Data are means ± SEM of 6-8 dams, means in a row with superscripts without a common letter differ, *P*<0.05 ; the letter ‘a’ denotes the highest value within the row.

¹Difference assessed using Kruskal-Wallis test.

TABLE 3. Plasma amino acid concentrations at GD21 in fetuses born to dams fed 20% protein diet (NP) , low protein diet alone (LP), or supplemented with citrulline (LP+CIT) or Arginine (LP+ARG) throughout gestation.

	<i>Group</i>				<i>P</i>
	NP	LP	LP+CIT	LP+ARG	
	<i>μmol/L</i>				
Histidine	153±8 ^a	30±2 ^b	36±7 ^b	29±3 ^b	<0.001 ¹
Taurine	527±15 ^a	529±35 ^a	382±13 ^b	435±19 ^b	<0.001 ¹
Serine	463±12 ^b	701±55 ^a	619±28 ^a	619±19 ^a	<0.001 ¹
Glutamine	1383±56 ^b	1917±124 ^a	1274±46 ^b	1419±57 ^b	<0.001 ¹
Arginine	180±12 ^{a,b}	152±16 ^a	308±34 ^c	239±28 ^b	0.007 ¹
Glycine	321±13 ^c	583±29 ^a	488±18 ^b	509±33 ^{a,b}	<0.001
Glutamic acid	173±11	164±8	143±8	161±11	0.30
Citrulline	43±2 ^b	42±4 ^b	250±119 ^a	45±3 ^b	<0.001 ¹
Threonine	644±33 ^a	260±23 ^b	242±29 ^b	192±15 ^b	<0.001
Alanine	880±86	888±92	652±78	931±85	0.26
Proline	1396±121 ^a	642±50 ^b	580±50 ^b	524±37 ^b	<0.001 ¹
Lysine	1657±74	1891±112	1183±69	1150±85	<0.001
Cysteine	46±3 ^a	22±2 ^b	20±1 ^b	19±1 ^b	<0.001
Tyrosine	200±17	178±22	183±24	186±11	0.89
Methionine	243±18 ^a	138±6 ^b	138±7 ^b	137±6 ^b	<0.001 ¹
Valine	627±20 ^a	304±19 ^b	319±26 ^b	259±18 ^b	<0.001
Isoleucine	178±5 ^a	116±12 ^b	120±14 ^b	95±11 ^b	<0.001
Leucine	424±14 ^a	250±20 ^b	241±19 ^b	204±13 ^b	<0.001
Phenylalanine	243±9 ^b	281±14 ^a	268±1 ^{a,b}	230±9 ^{b,c}	0.01
Tryptophane	108±3 ^a	107±8 ^{a,b}	131±10 ^b	100±7 ^a	0.04

¹Differenced analyzed using Kruskal-Wallis test

FIGURE LEGENDS

Fig 1. Quantification by RT-PCR of the gene expression of amino acid transporters SNAT1, SNAT2, SNAT4, LAT1, LAT2 and 4hFC mRNA in placentas collected at GD21 of rats fed 20% protein diet (NP), 4% low protein diet (LP), low protein diet plus citrulline (LP+CIT) or low protein diet plus arginine (LP+ARG). GAPDH and Ywhaz served as the internal control. Results are expressed as a percentage of the C group (n = 6 pools of 4 placentas /group).

Fig 2. Concentrations of total non-essential amino acids (NEAA; panel A), total essential amino acids (EAA), and branched-chain amino acids determined by LC-MSMS in maternal and fetal plasma in the various groups. Arginine and Citrulline concentrations were not used in the calculation.

Fig 1.

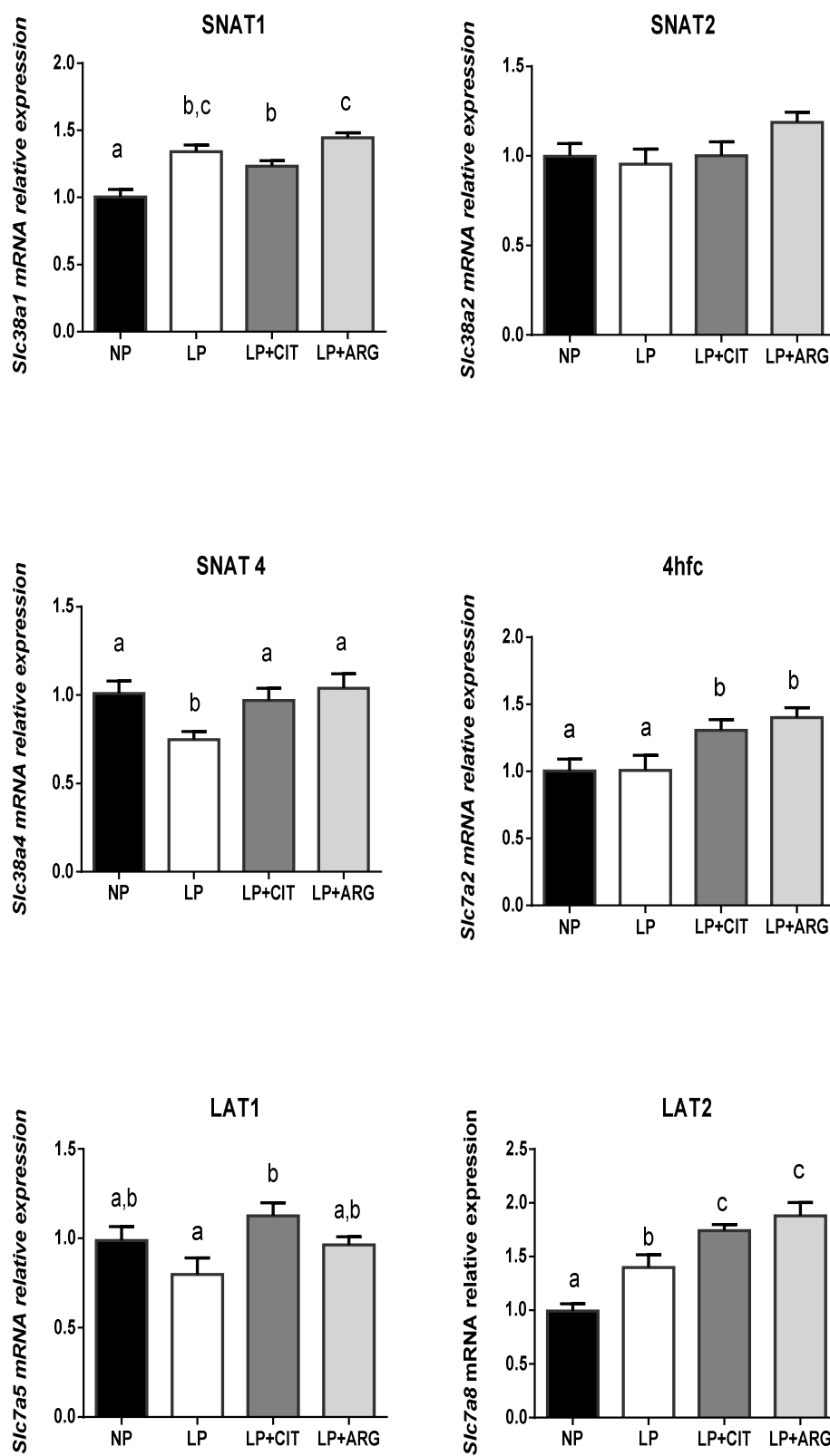


Fig 2.