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ORIGINAL ARTICLE

# Adaptative response of nitrogen metabolism in early endotoxemia: role of ornithine aminotransferase

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**Abstract** Arginine (Arg) and glutamine (Gln) utilization is greatly increased during catabolic stress. While the supply of both amino acids has been advocated in this situation, arginine administration is possibly associated with deleterious effects. From a metabolic point of view, these two amino acids are reciprocal precursors via ornithine aminotransferase (OAT). We hypothesized that OAT plays a key role in the interconversion between Arg and Gln. To test this hypothesis, we evaluated the influence of OAT activity in a model of septic shock induced by intraperitoneal injection of lipopolysaccharide (LPS) in wild-type (WT) and transgenic mice overexpressing OAT (OAT) in the liver, kidney and intestine, i.e. the three main organs of OAT expression. Plasma and tissue amino acid concentrations and tissue OAT expression and activity were measured. Five hours after LPS injection, WT and OAT mice showed a similar response to LPS in terms of inflammatory cytokine production and protein catabolism, suggesting that the interconversion between Arg and Gln through this pathway remains limited. Endotoxemia led to a significant decrease in plasma Orn levels and an increase in liver Orn levels. Of note, Orn levels were always lower in OAT mice. While only plasma Arg and Gln remained unaffected by LPS treatment, hepatic Gln was significantly increased without any difference between the two genotypes. In this model of early endotoxemia, arginine and glutamine maintained their metabolic homeostasis. Our results show an inhibition of OAT activity and expression in the liver following LPS treatment. These data highlight the importance of OAT in ornithine metabolism, especially in the liver, and suggest a post-transcriptional regulation of OAT by LPS in the liver.

**Keywords** Arginine · Ornithine · Glutamine · Amino acid metabolism · Endotoxemia · Stress

**Abbreviations**

| | |
|---|---|
| OAT | Ornithine aminotransferase |
| Gln | Glutamine |
| Arg | Arginine |
| LPS | Lipopolysaccharide |
| WT | Wild type |
| mOAT | Murine OAT |
| hOAT | Human OAT |

## Introduction

The metabolic response to stress involves a major mobilization of protein stores. However, alterations in arginine (Arg) and glutamine (Gln) availability represent a threat for patient survival, as loss of lean body mass correlates with morbidity and mortality (Windsor and Hill 1988).

Arginine and glutamine play a central role in catabolic situations since they exert many actions on protein metabolism and immunological function. Glutamine, besides its essential role in inter-organ nitrogen transport, is the preferential substrate for rapidly dividing cells like endothelial cells, fibroblasts and lymphocytes (Curi et al. 2005), and its

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availability may be a crucial factor in maintaining the integrity of endothelial cells and immune system function (Cunha et al. 2003). Arginine is also involved in the immune response, especially as a substrate for nitric oxide synthesis (Barbul et al. 1984), and also in the regulation of protein synthesis, particularly by stimulating growth hormone and insulin secretions (Knopf et al. 1966). Therefore, glutamine and arginine play a central role in the metabolic response to stress, during which they are considered as conditionally essential amino acids. Furthermore, low plasma arginine and glutamine concentrations are correlated with increased mortality in septic animals, and with a worse outcome in septic patients (Oudemans-van Straaten et al. 2001). Thus, the maintenance of nitrogen homeostasis in early stage of endotoxemia constitutes a major target in septic patients in order to prevent an imbalance that could lead to multi-organ failure syndrome.

In the early events of sepsis, nitrogen homeostasis is maintained while amino acid metabolism undergoes major modifications in order to adapt to increasing nitrogen demand. However, the exact regulatory mechanism remains unknown. It is therefore essential to gain further insight into the early mechanisms underlying the regulation of arginine and glutamine homeostasis in order to better manage nutritional support in septic conditions.

Glutamine and arginine share a common metabolic pathway in which the enzyme ornithine aminotransferase (OAT, L-ornithine 2-oxo acid aminotransferase; EC 2.6.1.13) could play a pivotal role.

OAT is a nuclear-encoded mitochondrial matrix enzyme (Peraino et al. 1969) that catalyzes the interconversion of ornithine and glutamate semialdehyde (Fig. 1). It is mostly found in the liver, kidney and small intestine (Herzfeld and Knox 1968), where its expression and activity are regulated in a tissue-specific manner by protein supply and glucagon in the liver and intestine (Matsuzawa et al. 1994; Boon et al. 1999) and by sex hormones in the kidney (Mueckler et al. 1984). The reaction is directed towards glutamate semialdehyde synthesis and thus towards glutamine in the liver and kidney, and towards ornithine synthesis in the small intestine. Therefore, OAT can play a role in the synthesis of arginine or glutamine, two amino acids that play important roles in nitrogen homeostasis: arginine as a powerful activator of ureagenesis (Kawamoto et al. 1982) and glutamine a key inter-organ nitrogen transporter.

While the importance of OAT enzyme seems limited for nitrogen homeostasis in physiological situations, where arginine and glutamine are easily available, it might be limiting in catabolic situations, where the latter's availability is known to be reduced (e.g. sepsis, trauma) by the increase in arginine and glutamine demand (Luiking et al. 2004).

We hypothesized that OAT, through its position at the crossroads between arginine and glutamine synthesis, could be involved in the adaptative response in early endotoxemia. We investigated this hypothesis using a model of early endotoxemia generated in mice by lipopolysaccharide (LPS) intraperitoneal injection. In this model the animals present sepsis-like symptoms with similarities to pathophysiological responses in patients with sepsis, and offers the most reliable way to reproduce the metabolic disturbances observed in sepsis-like conditions (Schlegel et al. 1999) while arginine and glutamine homeostasis are maintained. We also tested the response to endotoxin challenge in a model of transgenic mice overexpressing OAT in the liver, kidney and intestine. Indeed, in physiological conditions, OAT overexpression has only limited metabolic consequences, most probably because of compensatory mechanisms ensuring amino acid homeostasis. However, OAT overexpression could bring a metabolic advantage in the response to stress. The role of OAT in the response to stress was analyzed in terms of cytokine secretion, OAT expression and activity, and plasma and tissue amino acid levels.

## Materials and methods

### Chemicals

All chemicals were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France), Virbac (Carros, France), Pfizer (Paris, France), Invitrogen (Cergy-Pontoise, France) and Bio-Rad (Marne-la-Coquette, France), except where otherwise indicated.

### Animals and feeding conditions

Animal care followed French and European Community guidelines for the protection of animals used for experimental and other scientific purposes, and our protocol was approved by the Ile-de-France Regional Ethics Committee (No. 03-02). The mice were kept in standard cages and given ad libitum access to a standard chow (M20, Dietex, Saint-Gratien, France).

### Generation of transgenic mice

The EAB-9K-OAT transgene was produced by inserting the blunted NotI-SalI fragment of the human OAT cDNA at the ECORV site into the plasmid construct (SPORT-6) containing the chimeric EAB-9K promoter construct, which is known to allow transgene expression in the liver, intestine and kidney (Cadoret et al. 2001). The chimeric EAB-9K promoter contains the 4.9 kbp regulatory sequences (from −4,580 to +365) of the rat Calbindin-D9K gene (Colnot et al. 1998) linked to the EAB enhancer of the rat aldolase B gene (Gregori et al. 2002). In situ

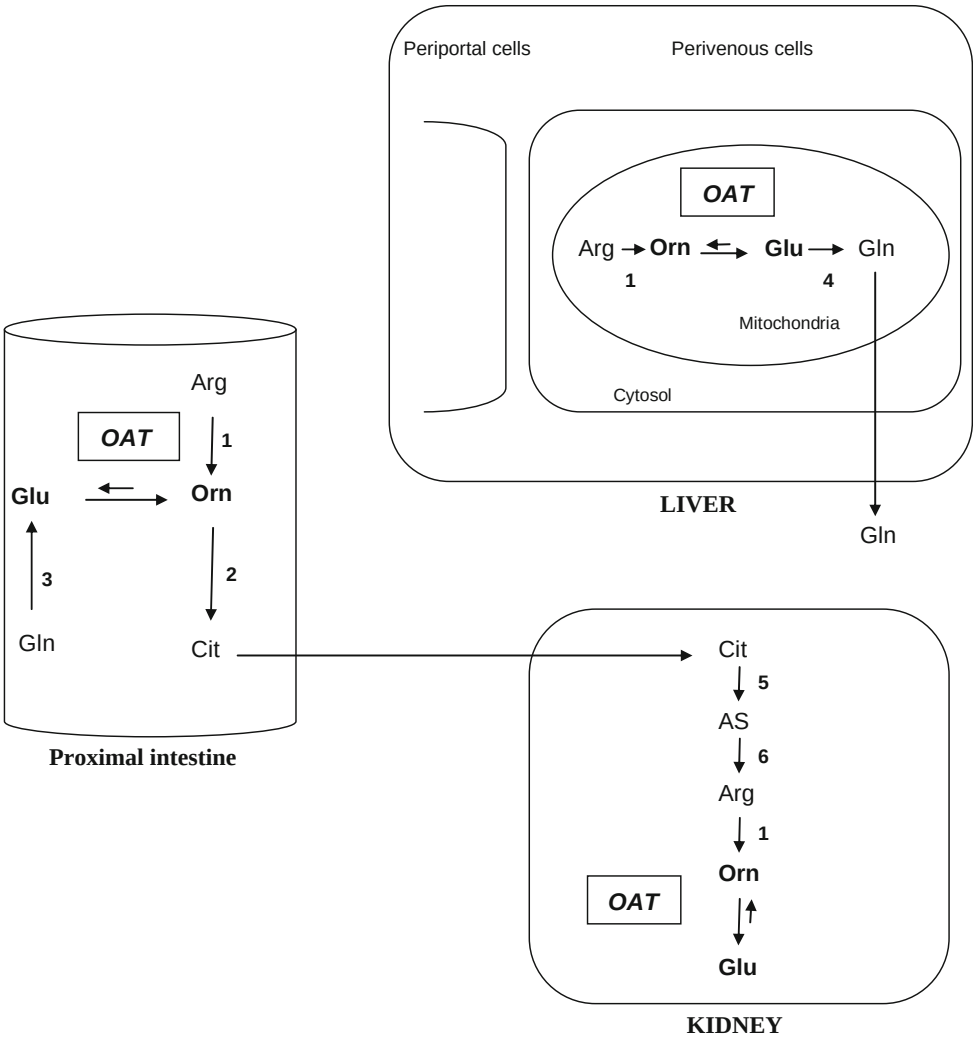


**Fig. 1** Overview of the OAT pathway. This is a theoretical scheme corresponding to physiological conditions. *Arg* arginine, *Gln* glutamine, *Glu* glutamate, *GSA* glutamate semialdehyde, *Cit* citrulline, *Orn* ornithine, *AS* argininosuccinate, *1* arginase, *2* ornithine transcarbamylase, *3* glutaminase, *4* glutamine synthase, *5* argininosuccinate synthase, *6* argininosuccinate lyase

hybridization experiments showed that in the liver of wild-type mice, OAT expression was localized only in perivenous cells while its expression was extended towards the periportal region in transgenic mice (Ventura et al. 2009). Human OAT cDNA containing the 1,320 bp open reading frame (ORF) of the human OAT gene was obtained from an IMAGE clone kindly provided by the RZPD (German Resource Center for Genome Research). Transgenic mice (EAB-9K-OAT) were obtained by microinjecting fertilized (C57/BPXDBA) F1 mouse eggs with the EAB-9K-OAT hybrid DNA construct. Transgenic mice were identified by southern blotting (data not shown). While three different transgenic lines were identified, only one of them showed high transgene expression level and significant modifications in amino acid plasma and tissue levels (Ventura et al. 2009). The experiments were therefore performed in only this transgenic line. The founder produced one transgenic line that was then genotyped by PCR using the forward primers 5′-GAGGGTAGGGGGATCGG and reverse primer 5′-CAATGCCCTTGGTTGACAGC. The study was conducted on heterozygous mice, using wild-type (WT) littermates as controls. We worked with heterozygous transgenic mice because OAT is regulated at least in part at the translational level (Levillain et al. 2007) and we clearly observed with our transgenic lines that there was no linear relationship between OAT mRNA and OAT activity (Ventura et al. 2009).

## Experimental design

The animals were submitted to LPS challenge according to a well-validated model of septic shock (Hallemeesch et al. 2002). A preliminary study was performed with transgenic and WT mice that had received an intraperitoneal injection of 10 mg/kg body weight of lipopolysaccharide (LPS from *Escherichia coli*: 0127:B8, Sigma–Aldrich) and were sacrificed after 2, 5 or 8 h ($n = 2$–6 per group). Fifteen wild-type (WT) C57/BL6 mice and 15 C57/BL6 transgenic mice (OAT) were randomized into four groups ($n = 7$–8 per group) according to genotype and treatment. Animals

received an intraperitoneal injection of 10 mg/kg body weight of LPS or an equivalent volume of NaCl. After the injection, food was removed and mice were left free access to water (Hallemeesch et al. 2002). Five hours later, the animals were anesthetized by intraperitoneal injection of 10 ml/kg of a solution of ketamine (100 mg/ml) and medetomodin (1 mg/ml) (Domitor®) and killed by cervical dislocation. Blood was removed by intracardiac puncture by a first operator. Part of the blood samples was put in glass tubes, allowed to clot at room temperature and centrifuged at 4°C (30 mins, 4,000 rpm), and the sera were kept at −80°C until cytokine measurement. The rest of the blood was collected into heparinized tubes (Starstedt, Orsay, France) for amino acid quantitation. Blood samples were immediately centrifuged for 10 mins at 4°C (5,000 rpm) and the plasma was deproteinized with sulfosalicylic acid (30 mg/ml). After a 10 mins incubation period at 4°C, the samples were centrifuged for a further 10 mins at 4°C (5,000 rpm), and supernatants were collected, frozen in liquid nitrogen and stored at −80°C until amino acid determination. Quickly after blood removal, a second operator performed a laparotomy, and promptly removed 10 cm of proximal jejunum from each animal. Jejunal section samples were washed with ice-cold saline solution flushed through the lumen. Three sections of proximal jejunum were collected and stored at −80°C. Simultaneously, a third operator collected three samples of the liver that were weighed and stored at −80°C for quantitation of tissue amino acids, OAT mRNA and OAT activity, respectively. The same operator also removed both kidneys, which were then weighed and frozen at −80°C to measure OAT mRNA expression and OAT activity.

### Cytokine assays

Serum IL-6 and TNFα were quantified by sandwich ELISA using commercially available kits (R&D System, Lille, France). Preliminary tests with several sample dilutions showed that the samples contained high concentrations of IL-6 and TNFα; therefore, they were diluted to one-fourth for TNFα and one hundredth for IL-6 in assay diluent before analysis. Results are expressed in picograms per milliliter.

### Amino acid measurements

Frozen tissues were homogenized using an Ultra-Turrax (Ika-Labortechnik, Staufen, Germany) homogenizer, in ice-cold 10% trichloroacetic acid containing 0.5 mM EDTA and 200 μM norvaline (internal standard). The acid-soluble fraction containing free amino acids was separated from precipitated proteins by centrifugation for 10 mins at 4°C (2,500*g*). Supernatants were stored at −80°C until amino acid analysis. Amino acid concentrations in tissues and deproteinized plasma were determined by ion-exchange chromatography on an amino acid analyzer (AminoTac JLC-500/V; Jeol, Tokyo, Japan) (Neveux et al. 2004). Quantification of renal amino acid content was not performed, given the high metabolic heterogeneity displayed by the kidney that would not allow a clear interpretation of the results (Silbernagl et al. 1996). We chose to present plasma phenylalanine and branched-chain amino acid (BCAA) concentrations as markers of whole body protein turnover (Darmaun and Cynober 2004) and protein catabolism (Matthews 2007), respectively, and other OAT-related amino acids (i.e. glutamine, glutamate, arginine, ornithine and citrulline). Of note, high arginase activity made it impossible to reliably measure arginine concentration in the liver.

### OAT activity

OAT activity was measured in liver, jejunum and kidney homogenates as described by Herzfeld and Knox (1968). Tissues were homogenized (100 mg/ml) at 4°C in an homogenization buffer (0.33 M sucrose, 5 mM HEPES, 1 mM EGTA, 1 mM DTT, 0.5% Triton X-100, pH 7.4) using an Ultra-Turrax homogenizer. In order to disrupt mitochondria, the homogenates were subjected to three cycles of freezing in liquid nitrogen and thawing at 37°C, and then centrifuged at 600*g* for 10 mins The assays were performed with 25, 50 and 100 μl of liver, kidney or jejunum homogenate supernatants, respectively, that were added to 200 μl of an assay mixture consisting of 75 mM potassium phosphate buffer (pH 7.5), 20 mM Orn, 0.45 mM pyridoxal phosphate, 5 mM *o*-aminobenzaldehyde, and 3.75 mM α-ketoglutarate, and incubated for 15 min at 37°C. A blank without α-ketoglutarate was run in parallel. Quantitation of OAT activity was performed by measuring the condensation product of P5C with *o*-aminobenzaldehyde by spectrophotometry at 440 nm. The amount of P5C produced was calculated from its molar extinction coefficient of $2.71 \times 10^3\ M^{-1}\ cm^{-1}$ (Strecker 1965). Enzyme activity is expressed as nanomoles of P5C formed per minute at 37°C in relation to the amount of tissue protein. Quantitation of tissue proteins was performed using the Biuret method (BC kit, Uptima-Interchim, Montluçon, France). After homogenization and centrifugation as described above, 25 μL of the supernatants (diluted 1/50 in sterile water) were distributed in duplicates in microplates. Two hundred microliters of reagent mix was added to each well, and the plates were incubated for 30 mins at 37°C. Protein concentration was

determined spectrophotometrically at 550 nm. Bovine albumin was used as standard.

### mRNA quantification

Total RNA was extracted from mice tissues using Trizol reagent (Invitrogen, Cergy-Pontoise, France) according to the manufacturer's protocol. Messenger RNAs were analyzed by real-time PCR. Reverse transcription (RT) was performed with 2 μg of total RNA using the Superscript™ First-Strand Synthesis System for RT-PCR (Invitrogen). mRNA quantitation was performed on a SmartCycler System (Cepheid, Sunnyvale, USA) with a 1:100 dilution of the cDNA using the SYBRGreen Kit for SmartCycler (Eurogentec, Angers, France). Data were expressed as the ratio between target gene and internal control (18S). Specific primer sequences were as follows: murine OAT, forward primer 5′-GGGCTCTTGTGAAACTCTGC and reverse primer 5′-AGATGGGTCCGTTTCTCCTT; human OAT, forward primer 5′-AGA -CTGCCTGTAAACTA GCTCGTAAG and reverse primer 5′-ACTGGAGATAG CAGACAA -CGTCCT; 18S: forward primer 5′-GTA ACCCGTTGAACCCCATT and reverse primer 5′-CCA TCCAATCGGTAG.

### Statistics

Results are presented as means ± standard error of the mean (SEM). Statistical analysis was performed with Statview 5.0 software (SAS Institute, Cary, NC) using two-way ANOVA followed by a Newman–Keuls test, and the sort criteria were genotype and treatment, except for hOAT expression, which required a student's *t* test with treatment criteria; $p < 0.05$ was considered as significant.

## Results

### Cytokines

Analysis of the time-course of cytokine response in mice showed LPS increased circulating concentrations of TNFα and IL-6. Both cytokine levels peaked at 2 h after LPS treatment, but TNFα returned to baseline after 5 h while IL-6 remained above measurable limits after 8 h. There were no differences in cytokine concentrations between WT and transgenic mice at 5 h after LPS injection. Moreover, LPS-treated mice displayed visible signs of acute illness around 4 h after LPS injection. Although LPS injection did not induce any mortality in our experiments in WT or transgenic mice, the LPS-treated mice showed lethargy, mild diarrhea, hypothermia and piloerection, these symptoms becoming severe 8 h after the LPS injection (Fig. 2).

### Amino acids

LPS treatment resulted in significantly higher plasma phenylalanine, BCAA and urea concentrations compared to the saline groups (Table 1).

Endotoxemia was responsible for an increase in plasma glutamate and citrulline while plasma ornithine decreased. However, plasma arginine and glutamine levels were not affected by LPS treatment. OAT overexpression did not modify plasma amino acid levels, although the lower ornithine concentration in transgenic mice nearly reached significance ($p = 0.055$).

In the liver, LPS treatment induced a strong increase in glutamine, glutamate, ornithine, citrulline and urea content. In OAT-overexpressing mice, hepatic ornithine concentration was significantly lower compared to wild-type mice, whatever the treatment.

In the jejunum, endotoxemia led to a decrease in arginine concentration. OAT overexpression decreased ornithine levels in the jejunum but had no further impact on the intestinal response to LPS treatment.

### OAT expression and activity

In the liver, OAT overexpression resulted in an increase in OAT activity. LPS treatment had an inhibitory effect on hepatic OAT activity in both WT and OAT transgenic mice. This inhibitory effect of LPS treatment was also

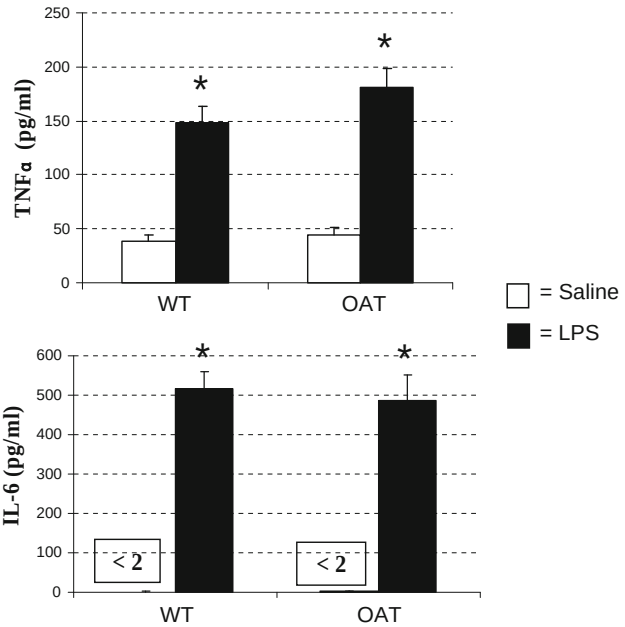


**Fig. 2** Time-course of murine cytokine response after LPS injection. Results are presented as means ± SEM and expressed as pg/ml or ng/ml

**Table 1** Effect of LPS treatment and OAT overexpression on concentrations of selected amino acids in plasma, liver and jejunum

| | Genotype | | *p* | |
|---|---|---|---|---|
| | WT | OAT | T | G |
| *Plasma* | | | | |
| Phenylalanine | | | | |
| Saline | 62 ± 3 | 55 ± 2 | **<0.0001** | 0.701 |
| LPS | 80 ± 4* | 85 ± 8* | | |
| BCAA | | | | |
| Saline | 425 ± 14 | 369 ± 23 | **0.0009** | 0.588 |
| LPS | 541 ± 40 | 566 ± 85* | | |
| Glutamate | | | | |
| Saline | 9 ± 1 | 8 ± 1 | **0.006** | 0.760 |
| LPS | 14 ± 2 | 14. ± 3 | | |
| Ornithine | | | | |
| Saline | 29 ± 1 | 26 ± 1 | **0.009** | 0.055 |
| LPS | 26 ± 1 | 23 ± 2 | | |
| Citrulline | | | | |
| Saline | 76 ± 4 | 86 ± 2 | **0.0004** | 0.188 |
| LPS | 95 ± 1* | 96 ± 5 | | |
| Arginine | | | | |
| Saline | 83 ± 3 | 86 ± 4 | 0.116 | 0.728 |
| LPS | 79 ± 5 | 78 ± 4 | | |
| Glutamine | | | | |
| Saline | 522 ± 41 | 520 ± 45 | 0.143 | 0.583 |
| LPS | 439 ± 33 | 479 ± 35 | | |
| *Liver* | | | | |
| Glutamine | | | | |
| Saline | 2,750 ± 114 | 2,801 ± 88 | **0.003** | 0.750 |
| LPS | 3,134 ± 132* | 3,187 ± 150* | | |
| Glutamate | | | | |
| Saline | 829 ± 106 | 954 ± 98 | **<0.0001** | 0.707 |
| LPS | 1,739 ± 132* | 1,708 ± 133* | | |
| Ornithine | | | | |
| Saline | 231 ± 14 | 149 ± 15 $^{\#}$ | **<0.0001** | **<0.0001** |
| LPS | 341 ± 19* | 264 ± 22*$^{\#}$ | | |
| Citrulline | | | | |
| Saline | 63 ± 4 | 56 ± 4 | **<0.0001** | 0.151 |
| LPS | 87 ± 5* | 80 ± 5* | | |
| *Jejunum* | | | | |
| Arginine | | | | |
| Saline | 227 ± 30 | 165 ± 22 | **0.016** | 0.607 |
| LPS | 151 ± 15* | 134 ± 9 | | |
| Glutamine | | | | |
| Saline | 1,036 ± 69 | 921 ± 106 | 0.504 | 0.543 |
| LPS | 1,019 ± 60 | 1,048 ± 55 | | |
| Glutamate | | | | |
| Saline | 2,077 ± 154 | 1,832 ± 145 | 0.351 | 0.113 |
| LPS | 2,202 ± 173 | 1,979 ± 121 | | |
| Ornithine | | | | |
| Saline | 53 ± 3 | 35 ± 2 $^{\#}$ | 0.545 | **0.005** |
| LPS | 42 ± 3 | 43 ± 3 | | |
| Citrulline | | | | |
| Saline | 165 ± 15 | 150 ± 10 | 0.083 | 0.445 |
| LPS | 190 ± 18 | 181 ± 16 | | |

Results are expressed as μmol/L plasma or nmol/g tissue for amino acids, and presented as means ± SEM ($n = 7$–8 per group). ANOVA effects are presented with the $p$ values, with significant $p$ values in bold type

*T* treatment, *G* genotype, *BCAA* branched-chain amino acids

$^{\#}$ $p < 0.05$ versus WT, * $p < 0.05$ versus saline

found for murine endogenous OAT expression and human OAT transgene expression in the liver (Fig. 3).

Endotoxemia did not significantly affect OAT expression and activity in the jejunum (mOAT expression: WT-Sal 0.9 ± 0.1 vs. WT-LPS 0.6 ± 0.1 and OAT-Sal 0.7 ± 0.1 vs. OAT-LPS 0.7 ± 0.2 arbitrary units, OAT activity: WT-Sal 11.3 ± 1.0 vs. WT-LPS 10.0 ± 0.7 and OAT-Sal 12.9 ± 1.3 vs. OAT-LPS 11.4 ± 0.9 in nmol $min^{-1}$ $\mu g^{-1}$ of protein) or in the kidney (mOAT expression: WT-Sal 0.7 ± 0.1 vs. WT-LPS 0.6 ± 0.1 and OAT-Sal 0.6 ± 0.1 vs. OAT-LPS 0.4 ± 0.1 arbitrary units, OAT activity: WT-Sal 8.3 ± 0.9 vs. WT-LPS 7.3 ± 0.9 and OAT-Sal 10.3 ± 1.5 vs. OAT-LPS 9.9 ± 1.2 in nmol $min^{-1}$ $\mu g^{-1}$ of protein).

## Discussion

This study investigated the metabolic response to stress, and more specifically the importance of OAT expression, when amino acid homeostasis is challenged. We observed that the maintenance of arginine and glutamine homeostasis in endotoxemic mice was associated with modifications in arginine and glutamine metabolism in both intestine and liver. Arginine and glutamine metabolites, and especially ornithine, have been shown to play an important role in the adaptative mechanisms of the metabolic response to stress. Inhibiting OAT activity and expression in the liver resulted in a sparing of hepatic ornithine secondary to LPS treatment. Moreover, OAT overexpression led to a decrease in hepatic ornithine levels, highlighting the essential role of OAT in maintaining ornithine homeostasis in the liver.

In the present mouse model of early endotoxemia, LPS injection created an inflammatory state illustrated by a

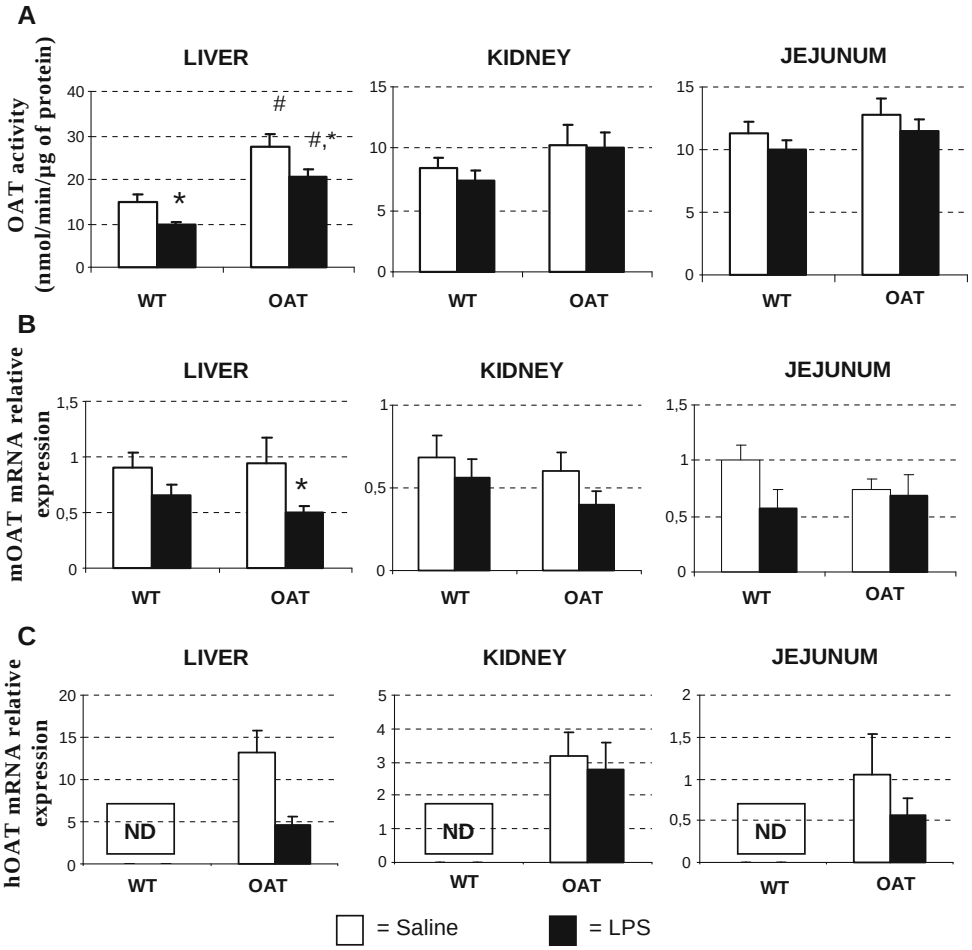


**Fig. 3** Effect of LPS treatment on OAT activity and expression in the liver. OAT activity (nmol/min/µg of protein) was measured in the liver of transgenic and wild-type mice 5 h after LPS or saline injection. Murine OAT (mOAT) and human OAT (hOAT) expressions were measured in the liver of transgenic and wild-type mice 5 h after LPS or saline injection. mRNAs were measured by RT-PCR, and the results (arbitrary units) are expressed as the ratio to the amount of 18S in each sample. Results are presented as means ± SEM. *ND* nondetectable,*$p < 0.05$ versus saline. #$p < 0.05$ versus WT

sharp increase in cytokine secretions after 2 h, which is in agreement with a previous work (Chang and Bistrian 1998). Since cytokines are important mediators in the metabolic response to stress, we chose to investigate the early adaptative mechanism of amino acid metabolism 5 h after LPS injection. Indeed, the LPS dose used in this study was sub-lethal 8 h after the injection, and circulating inflammatory cytokine levels peaked at 5 h post-injection. Furthermore, at the 5 h time-point, Hallemeesch et al. (2002) did not observe great alterations in plasma amino acid concentrations in LPS-treated mice. Since our working hypothesis was to test whether OAT overexpression disrupts Arg/Gln homeostasis, we opted to work with this time-point for our study.

The metabolic response to inflammation was illustrated by high plasma BCAA and phenylalanine concentrations, mimicking the severe catabolic state observed in septic patients. Indeed, these two amino acids are markers of whole body protein turnover (Darmaun and Cynober 2004) and protein catabolism, respectively.

As expected, despite the catabolic state and increasing demand, arginine and glutamine homeostasis were preserved after the early phase of LPS treatment, as their plasma levels remained unchanged. Indeed, plasma concentrations are the final result of rates of appearance and disappearance of amino acids, and therefore reflect whether homeostasis is maintained or not (Cynober 2002). However, LPS-treated mice displayed significant modifications in tissue arginine and glutamine concentrations together with marked changes in their intermediary metabolites (i.e. glutamate, ornithine and citrulline) levels in plasma, indicating changes in their metabolism.

In the jejunum, LPS treatment resulted in a modification of the de novo citrulline synthesis pathway. Indeed, whereas under basal conditions citrulline is mainly synthesized from glutamine (Moinard and Cynober 2007), under endotoxemic conditions, the reduction in glutaminase activity in the gut reported by Souba et al. (1990) suggests that citrulline production is ensured by arginine via arginase activation. Accordingly, we observed a decrease in arginine concentration in the jejunum in response to LPS treatment, whereas glutamine and citrulline concentrations remained unchanged. These results account for an increase in arginine utilization and glutamine sparing. Overexpression of OAT did not result in an altered intestinal response to stress, leading to the

conclusion that OAT seems to be of only limited importance in this organ.

We observed that glutamine and glutamate concentrations were higher in the liver of endotoxemic mice. In periportal hepatocytes, glutamine may therefore provide carbons for energy and gluconeogenesis and nitrogen for ureagenesis, in order to support ammonia detoxification and acid base homeostasis. The stimulation of ureagenesis is further illustrated by the increase in hepatic urea concentration in LPS-treated animals. However, the increase in hepatic glutamine concentration cannot fully account for the doubling in hepatic glutamate concentration secondary to endotoxin challenge. This marked rise in glutamate levels can be related to the stimulation of glutaminase activity in periportal hepatocytes that has previously been shown in a model of septic rats (Ewart et al. 1995). This idea is further supported by previous reports of enhanced hepatic release of glutamate in a model of hyperdynamic septic pigs (Bruins et al. 2003). This regulation could constitute a nitrogen-sparing mechanism for de novo muscle glutamine synthesis. Furthermore, the OAT-mediated reaction is oriented towards glutamate synthesis in the liver. However, in the present study we observed a decrease in hepatic OAT activity after LPS treatment. This inhibition may be a protective mechanism allowing ornithine sparing for other metabolic pathways such as polyamine and/or proline synthesis.

To gain further insight into the role of OAT in arginine and glutamine homeostasis, we investigated the impact of OAT overexpression on the metabolic response to endotoxemia. OAT overexpression had only limited influence on amino acid metabolism and no incidence at all on the inflammatory response to LPS. However, the measurement of other specific inflammation markers like other cytokines (pro- or anti-inflammatory), eicosanoids, leukotrienes, prostaglandins or acute phase proteins like alpha 2 macroglobulin could bring new insights into differences in the inflammatory status of WT and OAT-LPS-treated mice. Surprisingly, the small intestine of transgenic mice showed a decrease in ornithine concentration despite OAT overexpression. Since this result could be the consequence of modifications in inter-organ amino acid flux, it deserves further study. However, hepatic ornithine concentrations were decreased in OAT-overexpressing mice, showing an inverse relationship between ornithine concentration and OAT activity in the liver, yet OAT overexpression did not modify hepatic glutamine and glutamate concentrations. This result indicates that OAT is not limiting in glutamine synthesis in endotoxemic mice, but it also clearly underlines the essential role of OAT in ornithine catabolism in the liver.

The important role of OAT in the catabolism of arginine and ornithine has already been described in gyrate atrophy, a naturally occurring OAT deficiency characterized by hyperornithinemia, as well as in OAT-null mice (Wang et al. 1995) and during experiments involving chemical inhibitors (Alonso and Rubio, 1989). This may indicate that the OAT-catalyzed reaction favors ornithine consumption at the whole body level. However, OAT expression being extended to the whole liver parenchyma in transgenic mice, it would be interesting to further study the consequence of this overexpression on hepatic metabolism taking into account hepatocyte zonation. Experiments using antegrade and retrograde liver perfusion coupled with gabaculine treatment could allow inhibiting OAT activity selectively in periportal and/or perivenous cells and could be helpful in the understanding of the impact of OAT overexpression in the different cell types.

Interestingly, LPS treatment had an inhibitory effect on endogenous murine OAT. Therefore, it is tempting to speculate whether this regulation is only a serendipitous consequence of LPS-associated perturbations in amino acid metabolism, or if OAT is specifically regulated as its activity would impair the metabolic response to stress. Our results showed that the increase in OAT expression achieved in OAT-overexpressing mice was able to restore OAT activity in LPS-treated mice, although the transgene expression was also submitted to LPS inhibition. However, this increase in OAT activity failed to show dramatic differences in the metabolic and/or inflammatory response to stress.

Glucagon and glucocorticoids are among the regulatory factors of endogenous murine OAT in the liver and are also involved in the response to stress (Brillon et al. 1995; Tessari et al. 1996). Thus, the metabolic response to stress may modulate OAT at both transcriptional and post-transcriptional levels. However, the promoter used for the tissue-specific expression of human OAT includes the calbindin promoter with aldolase B enhancer, two genes that, to our knowledge, do not undergo any modification under inflammation. Nevertheless, hOAT expression decreased in a similar way to mOAT. The regulation of the transgene expression following LPS treatment may have two explanations: (1) the hybrid gene construction might be regulated. Unfortunately, the absence of specific antibody for human OAT did not allow us to establish whether there was any difference at the protein level. Functional genomic analysis for a precise sequencing of the promoter and the transgene would probably help identifying potential regulatory sequences with a common response element sensitive to LPS. (2) This regulation of both endogenous and overexpressed OAT by LPS could indicate that this inhibitory effect acts at the post-transcriptional level, probably through a modulation of messenger stability. This latter hypothesis is supported by the dissociation between OAT expression and activity previously observed in this

model under basal conditions. A kinetic study of LPS effect on endogenous and human OAT transcription, or in vitro mRNA decay experiments would help clarifying this point.

In conclusion, our model of early endotoxemia shows that the maintenance of arginine and glutamine homeostasis demands major modifications in arginine and glutamine metabolism in the splanchnic area (i.e. the liver and intestine). This work is the first to demonstrate an inhibition of liver OAT in the metabolic response to stress. This finding demonstrates the essential role of OAT in glutamine homeostasis by regulating glutamine catabolism in the liver. Moreover, experiments with OAT-overexpressing mice highlighted the importance of OAT in ornithine homeostasis, thus indicating a post-transcriptional regulation of OAT in stress situations. However, it remains unclear whether this lack of metabolic advantages brought by OAT overexpression mice derives from our experimental conditions or if OAT enzyme is indeed not a limiting step in the metabolic response to stress.

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