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ORIGINAL ARTICLE

Underestimated contribution of skeletal muscle in ornithine metabolism during mouse postnatal development

Benjamin Ladeuix · Claude Duchamp · Olivier Levillain

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Abstract Ornithine aminotransferase (L-ornithine 2-oxo-acid aminotransferase, OAT) is widely expressed in organs, but studies in mice have focused primarily on the intestine, kidney and liver because of the high OAT-specific activity in these tissues. This study aimed to investigate OAT activity in adult mouse tissues to assess the potential contribution to ornithine metabolism and to determine OAT control during postnatal development. OAT activity was widely distributed in mouse tissues. Sexual dimorphism was observed for most tissues in adults, with greater activity in females than in males. The contribution of skeletal muscles to total OAT activity (34 % in males and 27 % in females) was the greatest (50 %) of the investigated tissues in pre-weaned mice and was similar to that of the liver in adults. OAT activity was found to be regulated in a tissue-specific manner during postnatal development in parallel with large changes in the plasma testosterone and corticosterone levels. After weaning, OAT activity markedly increased in the liver but dropped in the skeletal muscle and adipose tissue. Anticipating weaning for 3 days led to an earlier reduction of OAT activity in skeletal muscles. Orchidectomy in adults decreased OAT activity in the liver but increased it in skeletal muscle and adipose tissue. We concluded that the contribution of skeletal muscle to mouse ornithine metabolism may have been underestimated. The regulation of OAT in skeletal muscles differs from that in the liver. The present findings suggest important and tissue-specific metabolic roles for OAT during postnatal development in mice.

Keywords Orchidectomy · Testosterone · Liver · Skeletal muscle · Weaning · Postnatal

B. Ladeuix · O. Levillain
Institut de Biologie et Chimie des Protéines, Université de Lyon, Université Lyon 1, Lyon, France

B. Ladeuix · C. Duchamp (✉)
UMR5023, Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés, Ecole Nationale des Travaux Publics de l'Etat, Centre National de la Recherche Scientifique, Université de Lyon, Université Lyon 1, 6 rue Raphaël Dubois, 69622 Villeurbanne, France
e-mail: claude.duchamp@univ-lyon1.fr

Introduction

In addition to their role as the building blocks of proteins and polypeptides, amino acids regulate key cellular processes and metabolic pathways that are necessary for maintenance, growth, reproduction and immunity (Kim et al. 2007; review in Wu et al. 2009). Among them, glutamate and proline are of interest because of their implication in cellular communication, the extracellular matrix and neoglucogenesis (Kim et al. 2007; Wu et al. 2013). These amino acids can be formed through the catalytic activity of ornithine aminotransferase (L-ornithine 2-oxo-acid aminotransferase, OAT, EC 2.6.1.13), an enzyme that is widely expressed throughout all kingdoms (bacteria, fungi, plants and animals) (Scher and Vogel 1957; Lu and Mazelis 1975; Nada et al. 2010) and links the production of amino acids to synthesise proteins and glucose to produce energy. L-ornithine, the substrate of OAT, is indeed known to play a pivotal role in several metabolic pathways as a precursor for L-proline, L-glutamate, L-glutamine, L-citrulline and L-arginine biosynthesis.

OAT catalyses the reversible transamination of L-ornithine with α-ketoglutarate, thus leading to the production of one molecule of glutamate and one molecule of glutamate-γ-semialdehyde, which is spontaneously converted into

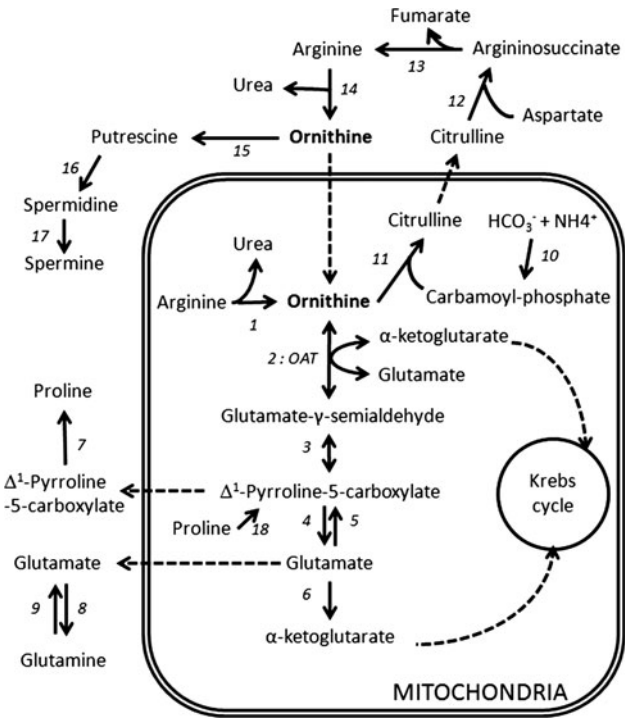

Fig. 1 Simplified metabolism of ornithine. *1* Arginase II, *2* ornithine aminotransferase (*OAT*), *3* spontaneous conversion, *4* pyrroline-5-carboxylate dehydrogenase, *5* pyrroline-5-carboxylate synthase, *6* glutamate dehydrogenase, *7* pyrroline-5-carboxylate reductase, *8* glutamine synthase, *9* glutaminase, *10* carbamyl-phosphate synthase I, *11* ornithine carbamyl transferase, *12* argininosuccinate synthase, *13* argininosuccinase, *14* arginase I, *15* ornithine decarboxylase, *16* spermidine synthase, *17* spermine synthase, *18* proline oxidase. *Dotted lines* amino acids exchanges

Δ^1-pyrroline-5-carboxylate (Fig. 1) (Boutry et al. 2012; Yao et al. 2012; Wu et al. 2013). The latter molecule can be metabolised further into a second molecule of glutamate or a molecule of proline. In the reverse direction, the transamination of glutamate with Δ^1-pyrroline-5-carboxylate to produce L-ornithine and α-ketoglutarate is catalysed by OAT. In that way, OAT represents an anaplerotic reaction that can replenish components of the Krebs cycle by synthesising α-ketoglutarate through the transamination of glutamate (Boutry et al. 2012; Yao et al. 2012; Wu et al. 2013).

OAT is expressed in many tissues in rats (Herzfeld and Knox 1968; Herzfeld and Raper 1976; Alonso and Rubio 1989; Kumar et al. 2009), but functionally, the reversible reaction catalysed by OAT is rather specialised for ornithine synthesis in the intestine and for ornithine catabolism in other organs (Alonso and Rubio 1989; Matsuzawa et al. 1994). OAT expression has been less widely studied in mouse tissues, with studies focusing only on the liver, kidney and intestine (Lim et al. 1998; Yu et al. 2003; Levillain et al. 2005, 2007; Ventura et al. 2009) that exhibited the highest specific OAT activities. Other tissues have not been studied. This relative disinterest could be misleading, as the low specific activity in large tissues could result in a higher contribution to whole body ornithine metabolism.

During perinatal development, OAT plays an essential role because the absence of OAT expression in mice is lethal shortly after birth (Wang et al. 1995). Because lethality can be rescued through short-term arginine supplementation (Wang et al. 1995), it was postulated that arginine, an essential amino acid in young mammals (Wu et al. 2009, 2013), cannot be synthesised from ornithine in the gut of $OAT^{-/-}$ mice. Therefore, the availability of arginine to the hepatic urea cycle and tissue synthesis of proteins and nitric oxide is reduced (Kerwin and Heller 1994; Wang et al. 1995). The modulation of OAT activity to produce or spare arginine would therefore be of utmost importance in the early postnatal period, when arginine availability is rather limited due to its small quantity in mother's milk and when insufficient endogenous synthesis occurs to fulfil the arginine requirements and achieve maximal growth (Visek 1986; Davis et al. 1993; Reyes et al. 1994; Cynober and Coudray-Lucas 1995). Tissue OAT expression gradually increased during postnatal development in rat and mouse kidneys and in the rat liver, with large changes occurring at puberty with the onset of sexual hormone secretion (Herzfeld and Knox 1968; Volpe et al. 1969; Levillain et al. 2007). 17β-Estradiol is known to upregulate OAT expression in the rat kidney (Herzfeld and Knox 1968; Lyons and Pitot 1977; Mueckler and Pitot 1983; Mueckler et al. 1984; Levillain et al. 2004), while testosterone downregulates OAT expression in the mouse kidney (Levillain et al. 2005). However, the ontogenic profile of OAT activity in the liver and kidney does not apply to all tissues. For instance, intestinal OAT activity was very high at birth but markedly reduced at weaning (Wang et al. 1995). Because no information on OAT expression in other tissues is available during mouse postnatal development, it cannot be inferred that the ontogenic pattern and endocrine regulation is similar in all tissues.

The aim of this study was (i) to investigate OAT expression and activity in a variety of mouse tissues to determine their respective potential contribution to ornithine catabolism, and (ii) to analyse OAT activity during postnatal development in different mouse tissues and investigate potential regulatory factors.

Materials and methods

Animals

In a first series of experiments, adult male (35–40 g) and female (30–32 g) Swiss mice obtained from Janvier (Le

Genest-Saint-Isle, France) were used. Animals were given free access to tap water and standard laboratory food (2018 Teklad Global, 18 % Protein Rodent Diet, Harlan, Gannat, France).

In a second series of experiments, adult male and female Swiss mice from Janvier were mated (1 male for 2 females). As soon as the first signs of pregnancy were observed, females were reared in individual boxes. Due to the high protein need during gestation and lactation (Millican et al. 1987), females were given free access to tap water and a protein-enriched diet (20 % Protein SPPS, Safe, Vénissieux, France). Pups were killed at postnatal days 5, 10, 15, 18, 20, 22, 25, 27, 30, 35, 40 and 45. Pups were classically weaned at postnatal day 21, but in a parallel experiment, some pups were weaned earlier at postnatal day 18 (anticipated weaning) and killed at 19, 20 or 21 days old. After removal from their mother, pups were given free access to standard laboratory food. To minimise the potential influence of different genetic backgrounds, reproducers were from the same line used in other experiments, and pups from different litters were used to constitute age groups. The number of pups per litter was normalised to nine soon after birth and a total of 24 litters was used.

In a third series of experiments, adult male Swiss mice from Janvier were bought either intact or castrated and killed 18 days after surgery.

All animals were housed in a controlled environment maintained at 25 ± 1 °C and 45 % humidity with a 12-h dark cycle (lights on at 7h00). Animal care complied with French regulations for the protection of animals used for experimental and other scientific purposes and with European Community regulations (Council of Europe N°123, Strasbourg, 1985). The authors were given authorisation by the "Direction Départementale de la Protection du Patrimoine" (n° 69-33 and 69266391) and the local Animal Care Committee to use animals for these experiments (protocol n° BH 2009-15).

Tissue sampling

Mice were anesthetised (i.p.) using 0.1 mL/30 g BW of pentobarbital sodium (Nembutal 6 %, Clin Midy, Paris, France) that was diluted 1:4 in 0.9 % NaCl solution for pups and diluted 1:2 for adult mice. After laparotomy, blood was collected from the abdominal aorta, stored on ice during the rest of the dissection and then centrifuged for 15 min at 21,000*g* to collect plasma. The abdominal aorta was cut to remove the blood from all tissues. The following organs were rapidly harvested: liver, heart, white adipose tissue (WAT), brown adipose tissue (BAT), skeletal muscles (*Gastrocnemius*, *Biceps surae*, *Quadriceps femoris*), whole intestine, testes, ovaries and the brain subdivided into three portions: (1) hemispheres, septum, thalamus, hypothalamus, hippocampus; (2) cerebellum, midbrain; and (3) brainstem. To simplify the terminology, the term "hemisphere" was used for the first subdivision, and the term "cerebellum" was used for the second subdivision. Pieces of intestine were also sampled along the digestive tract. Tissues were weighed rapidly and frozen in liquid nitrogen. Plasma and tissues were kept at −80 °C.

Determination of tissue OAT activity

To determine OAT activity, tissues were homogenised at 4 °C with a Turrax homogeniser in a buffer composed of 0.33 M sucrose, 5 mM HEPES, 1 mM EGTA, 1 mM DTT, and 0.5 % Triton X-100 (pH 7). Homogenates were frozen/thawed three times and centrifuged at 21,000*g* for 10 min at 4 °C. OAT activity was determined as previously described (Peraino and Pitot 1963; Herzfeld and Knox 1968). Briefly, supernatants were incubated with a buffer composed of 75 mM potassium phosphate pH 7.5, 20 mM L-ornithine, 0.45 mM pyridoxal phosphate, 5 mM α-aminobenzaldehyde, and 3.75 mM α-keto-glutarate at 37 °C in a dark room. Blanks were free of α-keto-glutarate. The reaction was stopped by adding 40 % trichloroacetic acid (TCA). Samples were centrifuged at 21,000*g* for 3 min at 4 °C, and absorbance was measured on the clear supernatant at 440 nm on a Hitachi U-1100 spectrophotometer (Meylan, France). The specificity of the reaction catalysed by OAT was verified by the addition of gabaculine (100 µM final), a specific inhibitor of OAT (Jung and Seiler 1978) that strongly inhibits the enzymatic reaction in tissue extracts (83–98 %). The protein concentration of tissue supernatants was determined using the Bradford method.

Biochemical characteristics of OAT

The OAT kinetic parameters were determined by measuring the initial speed of reaction at increasing concentrations of ornithine (0, 5, 7.5, 10, 12.5, 15 and 20 mM) and a fixed α-keto-glutarate concentration. Kinetic parameters (K_m, V_{max}) were determined by the direct linear plot of Eisenthal Cornish-Bowden (Eisenthal and Cornish-Bowden 1974). K_m was expressed in mM and V_{max} was expressed in nmol pyrroline-5-carboxylate (P5C) per minute per mg soluble protein.

Determination of corticosterone and testosterone plasma concentrations

The corticosterone concentration was determined by radioimmunoassay (RIA) (Filipski et al. 2002). Testosterone was measured by RIA after extraction by an organic

solvent and partition chromatography of the plasma samples (Déchaud et al. 1989).

Calculations and statistical analyses

OAT activity was expressed in nmol P5C per minute per mg soluble protein, in nmol P5C per min per mg tissue, or in nmol P5C per min per organ using the molar extinction coefficient of P5C (Herzfeld and Knox 1968). The results were expressed as the mean ± SE. Multiple comparisons were made using an ANOVA with the Bonferroni Dunnett test when appropriate. Comparisons between the means were made using Student's *t* test or the Mann–Whitney *U* test. A significance level of 95 % was used. Statistical analyses were performed using the software Statview.

Results

OAT activity in adult male and female tissues

OAT activity was detected in all investigated tissues. When expressed in I.U. (nmol P5C/min/mg prot), the highest OAT activity was observed in the intestine (Fig. 2a). OAT activity was lower in the kidney, liver, ovary and heart and much lower in other tissues. As expected, a much higher hepatic and renal OAT activity was observed in the females than in the males. A similar sexual dimorphism was also observed in the skeletal muscle, WAT and brain stem ($P < 0.05$–0.002), but not in the intestine. Given that the protein concentration differed between male and female tissues, OAT activity was expressed in nmol P5C/min/mg tissue (Fig. 2b). Using this expression, the intestine was still the tissue with the highest OAT activity, followed by the kidneys, liver, heart and ovary, while other tissues had a much lower activity. Sexual dimorphism of OAT activity was still observed in this unit for a number of tissues, including the intestine ($P < 0.05$). Because of the differences in the tissue masses, the total OAT activity per organ was calculated to estimate the individual potential contribution to animal ornithine metabolism (Fig. 2c). The total OAT activity of these 11 organs was 3,725 nmol P5C/min in females and only 3,113 nmol P5C/min in males. In females, the liver was the main potential contributor to animal ornithine metabolism (34 % of total P5C production), followed by skeletal muscles (27 %), kidney (21 %) and intestine (10 %). The contribution of the other organs was very weak (4–0.2 % of the total). In males, skeletal muscles and liver were the main contributors to ornithine metabolism in almost equal proportions (34 and 32 % of P5C production in muscles and liver, respectively), while the intestine contribution was lower (15 %) and the kidney contribution to ornithine metabolism was weak (9 %).

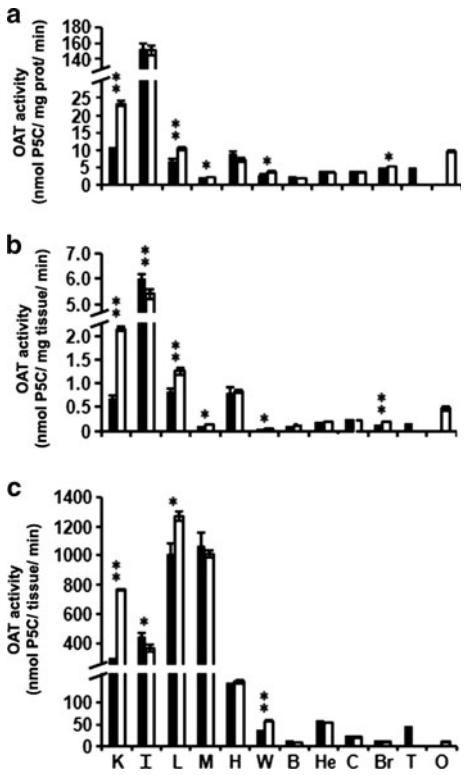

Fig. 2 OAT activity in adult male and female mouse tissues. OAT activity was measured in kidney (*K*), liver (*L*), skeletal muscle (*gastrocnemius*) (*M*), heart (*H*), white adipose tissue (*W*), brown adipose tissue (*B*), hemispheres (*He*), cerebellum (*Ce*), brainstem (*Br*), testis (*T*) and ovaries (*O*). **a** OAT activity expressed in nmol P5C/min/mg prot; **b** OAT activity expressed in nmol P5C/min/mg tissue; **c** total OAT activity per organ expressed in nmol P5C/min/tissue. For the total activity in skeletal muscles, data from average values determined in the *Biceps surae*, *Gastrocnemius* and *Quadriceps femoris* were extrapolated to whole body muscle mass (45 % of body mass in males and 40 % in females). Solid bars: males, open bars: females. The results are expressed as the mean ± SE, $n = 9$ mice for each tissue and sex. Significant differences were tested using the Mann–Whitney *U* test. Male vs. female: $^{*}P < 0.05$ and $^{**}P < 0.002$

Other male tissues contributed little to the total OAT activity (4–0.3 %). Sexual dimorphism in favour of females was still observed per organ in the kidney, liver and WAT ($P < 0.05$–0.002).

OAT activity in male and female mouse tissues during postnatal development

Based on their relative high potential contribution to total OAT activity in adulthood, liver, skeletal muscles and WAT were further investigated during postnatal development. Hepatic OAT activity, as expressed per mg protein (Fig. 3a), varied with age and sex (ANOVA 2, $F_{age} = 67$ $F_{sex} = 55$, $P < 0.0001$). OAT activity in the male and female liver slowly rose between 5 and 20 days of age (+100 %, $F = 21$ in males and 16 in females, $P < 0.001$). Between 20 and

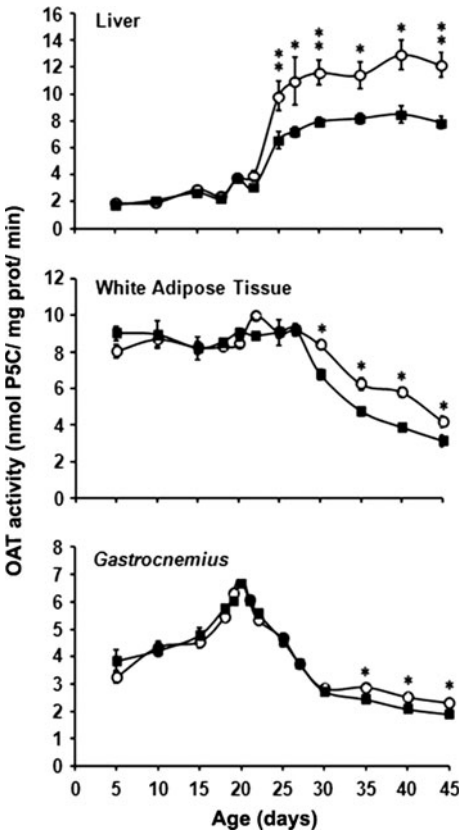

Fig. 3 OAT activity in male or female liver, white adipose tissue and skeletal muscle (*gastrocnemius*) during postnatal development. *Black square* male, *open circle* female. The results are expressed as the mean ± SE, $n = 6$ mice for each tissue, age and sex. Significant differences were tested using an ANOVA followed by the Bonferroni Dunnett test and Student's t test. Male vs. female: $^{*}P < 0.05$ and $^{**}P < 0.01$. *Error bars* that are not visible were covered by the *symbols*

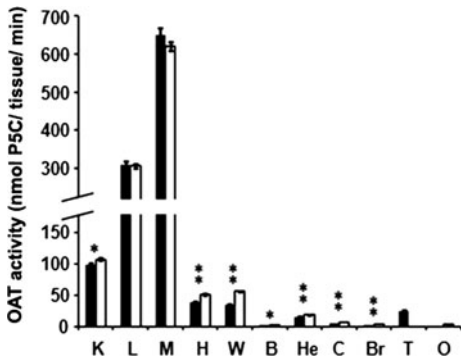

Fig. 4 Total tissue OAT activity in male or female mice at 20 days of age. Activity is expressed in nmol P5C/min/tissue. Activity was analysed in kidney (*K*), liver (*L*), skeletal muscle (*M*, data extrapolated to whole body muscle mass from average values determined in *Biceps surae*, *Gastrocnemius* and *Quadriceps femoris*), heart (*H*), white adipose tissue (*W*), brown adipose tissue (*B*), hemispheres (*He*), cerebellum (*C*), brainstem (*Br*), testis (*T*) and ovaries (*O*). *Black bars* male, *open bars* female. The results are expressed as the mean ± SE, $n = 9$ mice. Significant differences were tested using the Mann–Whitney U test. Male vs. female: $^{*}P < 0.05$ and $^{**}P < 0.002$

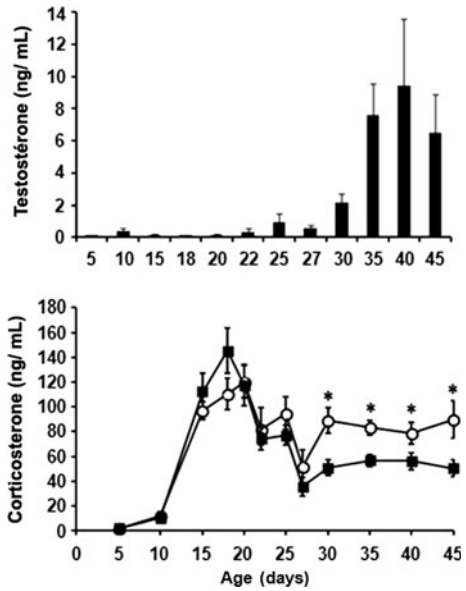

Fig. 5 Plasma testosterone and corticosterone levels during postnatal development. *Black bars* or *black squares* males, *open circles* females. The results are expressed as the mean ± SE; $n = 6$–13. Significant differences were tested using an ANOVA and the Mann–Whitney U tests. Male vs. female: $^{*}P < 0.05$. *Error bars* that are not visible were covered by the *symbols*

25 days of age (i.e. around the time of weaning), OAT activity markedly increased and then plateaued by 45 days of age at 2.6 times (males) and 3.2 times (females) higher than before weaning (Bonferroni Dunnett test, d20 vs. d45, $P < 0.0001$). Sexual dimorphism of hepatic OAT activity in favour of the female appeared from 22 days of age. WAT OAT activity during postnatal development also varied with age and sex (ANOVA 2, $F_{sex} = 47.1$, $F_{age} = 122$, $P < 0.0001$) (Fig. 3b). WAT OAT activity in both males and females was high and stable from postnatal day 5 to day 27 and no differences between sexes were observed. OAT activity suddenly dropped at 27 days of age, and it was 2.2-fold (males) and 3-fold (females) lower at 45 days of age (Bonferroni Dunnett test, d27 vs. d45, $P < 0.0001$). A sexual dimorphism in favour of females appeared at day 30 (Student's t test, male vs. female, $P < 0.003$). Postnatal changes in skeletal muscle OAT activity differed from that in liver (Fig. 3c). OAT activity in both males and females gradually increased after birth and peaked by day 20 (+75 and +117 % in males and females, respectively, $P < 0.001$). After weaning, muscle OAT activity markedly dropped, and the values were 3.6-fold (males) and threefold (females) lower at 45 than at 20 days of age. From 35 days of age, sexual dimorphism in favour of females was observed (Mann–Whitney U test, male vs. female, $P < 0.03$).

Because tissue OAT activity markedly changed during postnatal development, total OAT activity was calculated

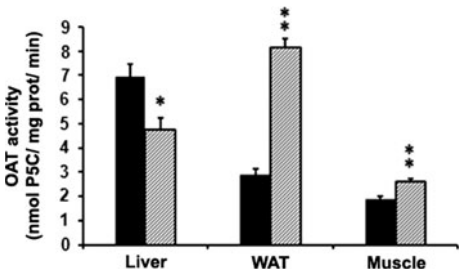

Fig. 6 Effect of orchidectomy on OAT activity in liver, white adipose tissue (*WAT*) and skeletal muscle (*gastrocnemius*) of adult male mice. *Black bars* control mice, *hatched bars* orchidectomised mice. Mice were killed 18 days after orchidectomy. The results are expressed as the mean ± SE; $n = 8$. Significant differences were tested using Student's t test. Control vs. orchidectomy: $*P < 0.01$, $**P < 0.001$

at 20 days of age, i.e. just before weaning. By 20 days of age, the skeletal muscles showed the highest total OAT activity and contributed to 50 % of the potential ornithine catabolism of the 10 organs studied (Fig. 4). The potential contributions of the liver and kidney (25 and 9 % of total, respectively) to total OAT activity were lower. The potential contributions of other tissues were very weak.

Plasma testosterone and corticosterone levels during postnatal development

The plasma testosterone concentration was very low during the first 25 days of life and then rose slowly from day 25 to 35 (Fig. 5), where it peaked. The plasma corticosterone concentration was very low during the first 10 days of life and then rose markedly to peak at 18 days of age (Fig. 5). Weaning was associated with a drop in corticosterone that stabilised by 30 days of age to levels that were lower in males than in females (53 ± 1 vs. 85 ± 2 ng/mL; $P < 0.05$).

Effect of orchidectomy on OAT activity

Among the potential factors controlling the postnatal ontogeny of OAT in liver, WAT and skeletal muscles, sexual maturation occurring at puberty (between 25 and 30 days of age in mice) was of primary interest. A group of 45-day-old males were surgically orchidectomised and killed 18 days after surgery, a period sufficient to reduce endogenous testosterone to undetectable levels (Lin et al. 2010). Orchidectomy (Fig. 6) significantly reduced OAT activity in the liver (−31 %, $P < 0.008$) but increased that in WAT (+182 %, $P < 0.0001$) and skeletal muscles (+38 %, $P < 0.0001$). Orchidectomy restored WAT OAT activity to the level measured before puberty and increased skeletal muscle OAT activity to the level measured in adult female mice.

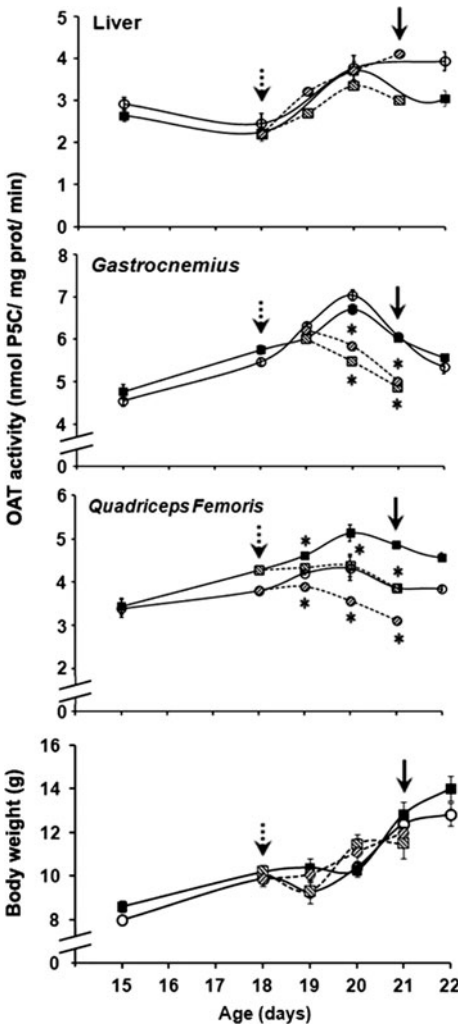

Fig. 7 Effect of anticipated weaning on tissue OAT activity and body weight in male or female mice during postnatal development. *Black squares* males, *open circles* females. *Solid line* control mice (weaned at 21 days of age, *solid arrow*); *dotted line* and *hatched symbols*: anticipated wean (weaned mice at 18 days of age, *dot arrow*). The results are expressed as the mean ± SE; $n = 6$. Significant differences were tested using an ANOVA and Mann–Whitney U tests. Control vs. anticipated wean: $*P < 0.05$. *Error bars* that are not visible were covered by the *symbols*

Effect of early weaning on liver and muscle OAT activity

Because our results indicated marked postnatal changes in tissue OAT activity associated with the weaning period, we investigated whether weaning precocity influenced liver and skeletal muscle OAT activity by weaning pups at the age of 18 days instead of 21 days (Fig. 7). The results indicated that anticipated weaning did not affect male or female liver OAT activity during this ontogenic period. In contrast, in both males and females, anticipating the time of weaning induced a temporal shift in the drop of muscle OAT activity that occurred earlier in mice weaned at

Table 1 OAT kinetic parameters in tissues of adult mice

	Males	Females	*P* value
V_{max}			
Liver	7.70 ± 0.06	9.98 ± 0.15	0.049
White adipose tissue	3.01 ± 0.02	4.63 ± 0.06	0.014
Gastrocnemius muscle	2.33 ± 0.11	2.80 ± 0.12	0.014
K_m			
Liver	2.79 ± 0.04	4.94 ± 0.17	0.049
White adipose tissue	2.36 ± 0.08	5.49 ± 0.04	0.014
Gastrocnemius muscle	4.24 ± 0.04	4.51 ± 0.29	0.027

Values were determined by the Eisenthal Cornish-Bowden representation and are expressed in mM of ornithine for K_m and nmol pyrroline-5-carboxylate (*P5C*) per minute per mg soluble proteins for V_{max}. The results are expressed as mean ± SE; $n = 5$. Statistical differences between males and females were tested using Mann–Whitney *U* tests

18 days than in those weaned at 21 days of age (Fig. 7). This protocol did not significantly affect body weight of growing pups.

OAT kinetic parameters in adult mouse organs

The different responses of OAT within tissues prompted us to investigate the potential expression of various OAT isoenzymes with different biochemical characteristics between tissues. The K_m and V_{max} values of OAT were determined in the liver, WAT and skeletal muscles of adult mice using the Eisenthal Cornish-Bowden representation and are summarised in Table 1. The results showed a significant ($P < 0.0001$) effect of both gender and tissue on K_m and V_{max} values. The male liver and WAT K_m values were similar and were significantly lower than the muscle K_m values. In females, the K_m values were higher than in males and similar between tissues. The V_{max} values measured in tissues of male or female mice were in accordance with the activities determined above (Fig. 2) and confirmed a sexual dimorphism.

Discussion

The present paper described the relative expression of OAT in male and female mouse tissues and showed tissue-specific regulations of OAT activity by age, orchidectomy and nutrition.

Unexpected contribution of skeletal muscle in OAT metabolism

The present data confirmed the wide distribution of OAT activity in all the mouse tissues studied, which is in agreement with the OAT expression cartography described in mice (Yu et al. 2003) and in rats (Herzfeld and Knox 1968; Mueckler and Pitot 1983). As classically observed, specific OAT activity per g protein or tissue was the highest in the intestine, followed by the kidney and liver (e.g. Herzfeld and Knox 1968). However, because of tissue mass, the contribution of skeletal muscle to total animal OAT activity was similar to that of the liver in adult mice. In pre-weaned mice, the skeletal muscles were the main contributor (50 %) to the potential ornithine catabolism of the 10 organs studied. The present study emphasised the importance of the unit to compare OAT activity between tissues and indicated that some tissues might have been underestimated in their potential contribution to animal OAT catabolism. This finding could be of importance in physiological situations where arginine may be limited, such as during postnatal development or when energy metabolism is stimulated.

Tissue-specific ontogenic profiles of OAT activity in mice, endocrine and nutritional regulation

Postnatal changes in OAT activity were tissue-specific and occurred during the pre-, peri- and post-weaning periods. In the pre-weaning period, the increase in liver and skeletal muscle OAT activity paralleled the changes in plasma corticosterone levels (Figs. 3, 5), suggesting a role for corticosterone in OAT expression. Thyroid hormones might also be involved because of the postnatal increase in 3,5,3′-triiodo-L-thyronine (T_3) plasma levels (Hadj-Sahraoui et al. 2000) and the positive role exerted by T_3 on OAT expression (Shull et al. 1995). The postnatal development of hepatic OAT was already suggested to be primarily controlled by glucocorticoids and potentiated by thyroxine (Vandewater and Henning 1985).

In the post-weaning period, the large increase in OAT activity in the liver and the decrease in WAT and skeletal muscles occurred in parallel with the onset of testosterone secretion in males, suggesting the direct control of OAT expression by sexual steroids in mice. This finding is in keeping with data in rats showing a link between the large post-weaning rise in liver OAT (Herzfeld and Greengard 1969; Volpe et al. 1969) and the onset of sexual hormone secretion (Levillain et al. 2007; Wu et al. 2010). The potential role of testosterone in the ontogenic profile of OAT in male mice was further supported by the changes in tissue OAT activity following orchidectomy, where liver OAT activity was downregulated (−31 %), skeletal muscle activity was upregulated (+31 %) and adipose tissue was upregulated (+180 %). The present results contrast the lack of effect of testosterone injections on mouse liver OAT (Bulfield and Hall 1981), but the suppression of endogenous testosterone by orchidectomy might be more

efficient than the addition of exogenous testosterone to modify hepatic OAT activity. A negative regulation of OAT by testosterone was already reported in the mouse kidney (Manteuffel-Cymborowska et al. 1995; Levillain et al. 2005, 2007, 2011), and the present data extend these findings to skeletal muscle and WAT. The molecular basis of this tissue-specific regulation of OAT activity is unclear. It is not known whether this regulation relies on different isoenzymic forms in these tissues. The existence of distinct OAT isoenzymes is not supported by observations that hepatic, renal and intestinal OATs, which may undergo divergent responses to various stimuli, share similar physico-chemical, immunochemical and kinetic properties (Sanada et al. 1970; Herzfeld and Raper 1976) or molecular coding sequences (Kobayashi et al. 1989). Differences in K_m between 2 and 4 mM in rat kidney and liver, respectively, were used to support the existence of different isoenzymes (Volpe et al. 1974). The present data on OAT biochemical characteristics showed small differences in the K_m between tissues, similar to those observed between sexes for a given tissue. Furthermore, because the liver and WAT, which exhibited similar K_ms, evolved differently with orchidectomy, it follows that biochemical characteristics could not be used as predictors of the existence of different isoforms with distinct tissue regulation. Interestingly, the higher OAT activity found in the female liver, WAT and skeletal muscles was associated with a lower affinity of the enzyme (Table 1), possibly in relation to aggregation of OAT monomers when OAT expression is increased (Boernke et al. 1981) leading to reduced affinity of the enzyme to its substrates to spare ornithine for use by the urea cycle. Alternatively, the contrasted transcriptional regulation by testosterone might possibly be related to potential differences in tissue co-regulator proteins that are known to affect the transcriptional activity of androgen receptors (Heinlein and Chang 2002) rather than to the differential regulation of still uncharacterised distinct isoenzymes. Estrogens that positively regulate OAT (Herzfeld and Knox 1968; Wu 1979; Mueckler and Pitot 1983) might also be involved in the post-weaning increase in tissue OAT activity, thus leading to the marked sexual dimorphism in OAT activity observed in most tissues (6 of 11) of adult mice, as found in earlier mouse (Levillain et al. 2005, 2007; Ventura et al. 2009) and rat studies (Herzfeld and Knox 1968).

Weaning by itself was associated with changes in tissue OAT activity in both males and females. This was observed before puberty, i.e. before the onset of sexual steroid secretion, and may thus involve food transition from weaning. The experimental anticipation of weaning by 3 days induced an early decrease in muscle OAT activity, indicating that skeletal muscle OAT activity was modulated by the transition from a milk diet, rich in lipids (22 %) and relatively poor in proteins (11 %) (Meier et al. 1965; Yajima et al. 2006), to a diet composed of pellets rich in proteins (18 %). The slight decrease in muscle OAT activity observed in normally weaned pups occurred slightly before the pups were actually weaned (between 20 and 21 days). This finding might be related to the observation that pups already eat pellets by 20 days of age, as assessed by traces of pellets in their stomachs. However, liver OAT was not affected by the rise in protein intake induced by early weaning, in contrast with the results of other studies, showing that the protein content of the food modulates rat hepatic OAT activity (Brennan et al. 1970; Mueckler et al. 1983; Matsuzawa et al. 1994; Boon et al. 1999). It could be argued that a very high protein enrichment (up to 70 % of diet) was used to upregulate hepatic OAT activity compared to a diet low (5 %) in proteins. It follows that skeletal muscle OAT was very sensitive to changes in food proteins in young, rapidly growing animals. The higher protein intake after weaning may contribute, at least in part, to the ontogenic drop in muscle OAT and the rise in hepatic OAT activity that were observed in both males and females; however, the molecular basis of such contrasting effects remains to be explored.

Potential physiological significance of a high skeletal muscle OAT activity in pre-weaned mice

The physiological role of OAT in liver, WAT and skeletal muscles during postnatal development is still unclear, but the total activity of these tissues by 20 days of age was far from negligible. Despite its low levels during the first 3 postnatal weeks, hepatic OAT could contribute to (i) the synthesis of glutamate coupled to glutamine synthesis to eliminate the ammonia that is not by the urea cycle and (ii) the production of glutamate for hepatic neoglucogenesis (Mallette et al. 1969) to fuel intense pup growth. In skeletal muscles, the gradual postnatal increase might also provide energy substrates to mitochondria to fuel increased postnatal muscle contractile activity. However, if the enzyme functions in the degradation of ornithine to generate glutamine, the high level of OAT activity in skeletal muscle and its gradual postnatal increase are puzzling at a time when arginine, the substrate of arginases that fuel OAT with ornithine (Fig. 1), is rather limited because of the low amount of arginine in mother's milk and because of possibly insufficient endogenous synthesis for maximal growth (Visek 1986; Reyes et al. 1994; Cynober and Coudray-Lucas 1995). Therefore, it could be postulated that before weaning, the flux in muscle OAT reactions might be in the direction of ornithine synthesis rather than degradation (see Fig. 1). The reaction requires glutamate and proline, which are the major amino acids of mouse milk (Meier et al.

1965). This would spare arginine for use in other processes and in producing ornithine and α-ketoglutarate. Muscle ornithine could fuel ornithine decarboxylase to form polyamines that are potent activators of skeletal muscle growth and development (Lee and Maclean 2011). α-Ketoglutarate could fuel the increased energetic activity and play a role in stimulating cell growth and metabolism (Durán et al. 2012; Yao et al. 2012). As a deamination process, OAT could therefore contribute glutamate into the Krebs cycle in skeletal muscles of pre-weaned mice to fuel energy needs and promote cell growth. After weaning, when arginine is no longer limited, high muscle OAT activity is required and OAT activity drops. In males, secreted testosterone can negatively regulate muscle OAT (present data) but positively regulate ornithine decarboxylase (Lee et al. 2011), thereby further contributing to polyamine production, myoblast proliferation and skeletal muscle growth. Further studies are required to confirm this stimulating hypothesis.

In conclusion, the present data indicated that OAT activity is widely detected in mouse tissues and that a sexual dimorphism is observed for most tissues in adults. Previous studies may have underestimated the contribution of some tissues, such as skeletal muscles, to whole body ornithine metabolism. OAT activity was found to be regulated in a tissue-specific manner during mouse postnatal development in relation to several modulating factors, including sexual steroids, corticosterone and nutrition. These findings suggest important and tissue-specific metabolic roles of OAT during postnatal development in mice.

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Conflict of interest The authors declare that they have no conflict of interest.

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