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Influence of dietary arginine on sexual dimorphism of arginine metabolism in mice

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Abstract

We have studied the influence of dietary arginine on tissue arginine content, and arginine metabolism in CD1 mice. Dietary arginine restriction produced by feeding mice with a low arginine diet (0.06%) produced a marked decrease in arginine concentrations in the plasma, skeletal muscle and kidney of female mice (72%, 67% and 54%, respectively) while in male mice the decreases were smaller (58% in blood and 18% in the skeletal muscle). This diet abolished not only the sexual dimorphism in arginine content observed in mice fed with the diet containing 1% arginine, but also reduced renal activities of arginase and nitric oxide synthase in the female mice and ornithine decarboxylase and the decarboxylation of arginine in the male mice. Urinary putrescine excretion was dramatically reduced by arginine restriction in the male mice whereas orotic acid excretion increased about 30 fold in both sexes; urea and creatinine excretion did not change. Taken together our results indicate that dietary arginine plays a relevant role in the maintenance of the sexual dimorphism in arginine content and arginine metabolism in CD1 mice, and that this may have physiological significance because of the important effects that arginine-derived products exert on a variety of cellular processes.

Keywords: L-arginine; Ornithine decarboxylase; Arginase; Nitric oxide synthase; Arginine decarboxylation; Sexual dimorphism; Mouse kidney

1. Introduction

The amino acid L-arginine is involved in multiple metabolic reactions in mammalian tissues. Apart from its classic and well known implication in the detoxification of ammonia via the Krebs-Henseleit cycle and in protein synthesis [1,2], more recent studies have demonstrated that L-arginine participates in many other important reactions such as nitric oxide (NO) synthesis and agmatine or creatine formation [2]. NO and agmatine are products formed by the action of nitric oxide synthase (NOS) and arginine decarboxylase (ADC), respectively, which play relevant roles in mammalian physiology and recently have attracted considerable pharmacological interest [3–8]. The kidney plays a central role in arginine metabolism, not only because most known enzymatic reactions involving L-arginine as substrate take place in renal tissue but because this organ is essential for arginine synthesis in many animal species including the human [2,9–12] (Fig. 1). Consequently, the rodent kidney offers a useful model for studying arginine metabolism and physiology.

The role of dietary arginine and endogenous synthesis in the regulation of arginine homeostasis appears to be dependent on several factors including differences in species, age and synthesis of citrulline by the small intestine [2,12–14]. On the other hand, there is a marked renal sexual dimorphism in rodents, especially in mice were the male kidney differs from the female not only in size but in many cellular and molecular properties [15,16]. Among the renal enzymes, gender-differences are particularly remarkable in the case of ornithine decarboxylase (ODC), which is a key rate limiting enzyme in the synthesis of putrescine, a diamine that serves as a precursor of the polyamines spermidine and spermine [17]. These ubiquitous cations participate in many processes such as cell growth and differentiation and also in death [18,19]. The role of the very high ODC activity found in the murine male kidney is controversial: while some studies have postulated that renal ODC activity mediates kidney hypertrophy [20], others have shown that the inhibition of renal ODC by α-difluoromethylornithine, a potent and irreversible inhibitor of ODC, does not alter the effect

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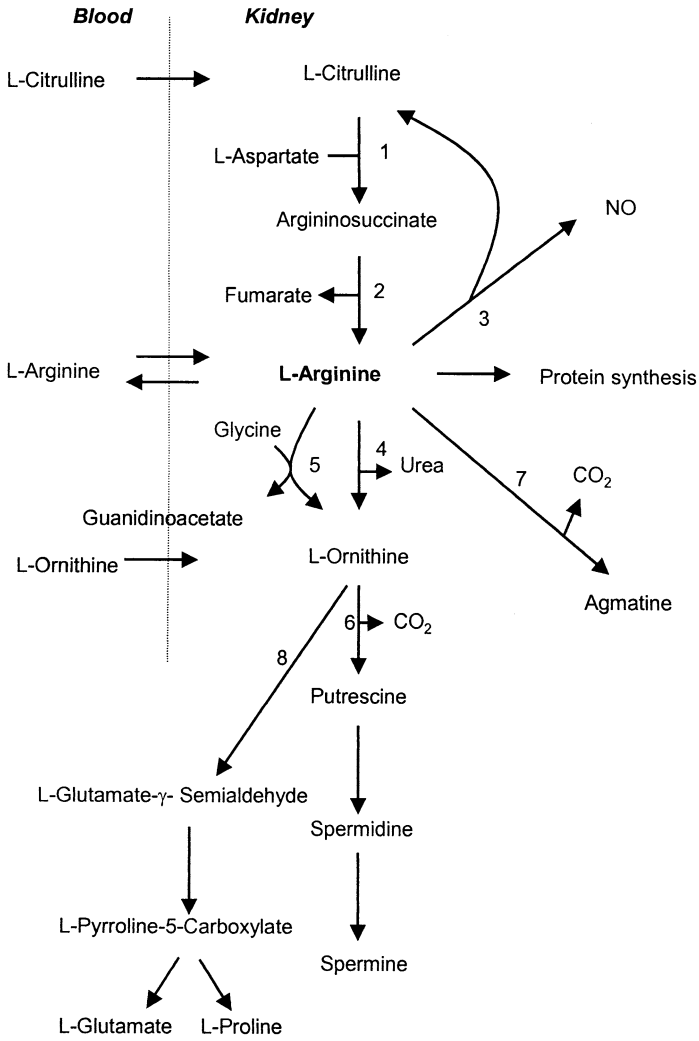

Fig. 1. Arginine metabolic pathway in the kidney. 1) Arginosuccinate synthase (EC 6.3.4.5). 2) Arginosuccinate lyase (EC 4.3.2.1). 3) Nitric oxide synthase (EC 1.14.13.19). 4) Arginase (EC 3.5.3.1). 5) Arginine:glycine amidinotransferase (2.1.4.1). 6) Ornithine decarboxylase (EC 4.1.1.17). 7) Arginine decarboxylase (EC 4.1.1.19). 8) Ornithine aminotransferase (EC 2.6.1.13).

of androgens on kidney hypertrophy [21,22]. Moreover, although it is clear that the male kidney has a high consumption of ornithine due to the elevated ODC activity, there are no data about the influence of this pathway on arginine renal content, nor how this affects arginine supply to the other tissues. Nevertheless while renal arginine metabolism has been studied in the different parts of the nephrone using microdissecting techniques [23–26], quantitative data on arginine metabolism in the intact kidney are scarce.

The aim of the present work was to examine the influence of dietary arginine on both the renal sexual dimorphism and the metabolism of arginine in mice. We have studied a) the influence of gender on arginine levels in several murine tissues, b) the differences in the *in vitro* enzymatic activities of the renal enzymes related to L-arginine metabolism and c) the influence of dietary arginine on these parameters. Our findings reveal the existence of a clear sexual dimorphism in arginine content and metabolism in mice, especially in the kidney, and that this is affected by dietary arginine. The results may have physiological interest because of the important relevance of L-arginine in mammalian metabolism.

2. Methods and Materials

2.1. Animals and treatments

Adult Swiss CD1 mice were used in all experiments. All procedures were in accordance with institutionally approved and current animal care guidelines. Animals were randomly divided in three groups that were fed under three different schedules: a) the first group received standard chow (SC); b) to study the effects of dietary arginine restriction a second group was fed with a semisynthetic arginine-deficient diet (ADD); c) a third group received ADD supplemented with

1% arginine (ADD + Arg). Body weight gain and arginine levels measured in mice fed during 20 days with ADD diet supplemented with 1% arginine in the drinking water were indistinguishable from those obtained in mice fed with the standard chow. All animals were maintained at 22°C ambient temperature and 50% relative humidity under controlled 12h light-dark cycles.

For urine analysis, mice were placed in metabolic cages and urine was collected daily. Urine samples were centrifuged at 5000 × g for 10 min and the supernatants were frozen at −70°C until analysed. Blood samples were collected under light ether anaesthesia by cardiac puncture. Plasma was obtained by centrifugation at 4°C and was kept frozen at −70°C. Mice were killed by cervical dislocation under ether anaesthesia and kidneys and other tissues were quickly removed, weighed and processed.

2.2. Diets

SC) Standard chow UAR A03 was obtained from Panlab (Barcelona, Spain) and contained 1.23 g arginine /kg and a mean content of 23.5% proteins, 49.8% carbohydrates, 5% lipids, 4% cellulose and 5.7% minerals. ADD) Arginine-deficient semi-synthetic diet obtained from ICN Pharmaceuticals (Costa Mesa, CA) contained 0.06% arginine, 43.69% dextrin type II, 21.47% sucrose, 16.21% amino acid mix (excluding arginine), 10% corn oil, 5% salt mix, 0.2% choline chloride, 1.09% sodium acetate, 0.05% sodium chloride, 1.25% calcium carbonate, 1.04% potassium phosphate and ICN vitamin diet fortification mixture. ADD + Arg) ADD diet supplemented with 1% arginine.

2.3. In vitro studies using renal slices

Renal arginine synthesis from citrulline and putrescine formation from ornithine were studied in renal slices from male and female mice incubated at 37°C in a Krebs-Henseleit based medium [26] supplemented with the appropriate amino acid. After extraction, kidneys were rinsed with cold saline and cut into slices about 2mm thick. Renal slices were incubated at 37°C in a medium (in a proportion 1:4 w/v) containing 137 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 0.8 mM $MgSO_4$, 1 mM $CaCl_2$, 0.33 mM Na_2HPO_4, 20 mM Hepes pH 7, 5 mM glucose, 1 mM pyruvate, 2 mM glutamate, 45% manitol and 6% dextran, and bubbled with oxygen with gentle agitation. Renal slices and medium samples were withdrawn at intervals of 0, 1, 2 and 3 hours at the levels of amino acids and polyamines were determined.

2.4. Enzymatic measurements.

Tissues were homogenized with the aid of a Polytron homogenizer in buffer A (25 mM Tris pH 7.2, 2 mM dithiothreitol, 0.1 mM pyridoxal phosphate, 0.1 mM EDTA, 0.25M sucrose) for assaying ODC, ADC, arginase and OAT or in buffer B (20 mM Hepes pH 7.2, 1 mM dithiothreitol, 0.1 mM pyridoxal phosphate, 0.1 mM EDTA, 0.25 M sucrose) for NOS activity. Both buffers contained proteinase inhibitors (0.5 mM phenylmethylsulfonylfluoride and 10 mM benzamidine). Mitochondrial and cytosolic fractions were obtained by differential centrifugation following standard procedures [27]. ODC activity was determined according to published methods [22,28] by measuring $^{14}CO_2$ release from 0.4 mM L-[1-^{14}C]-ornithine (specific activity 4.2 mCi/mmol, Moravek Biochemicals Inc, USA). ADC activity was also measured by a radiometric method based on $^{14}CO_2$ formation from 0.3 mM L-[1-^{14}C]-arginine (5.5 mCi/mmol, ARC Inc, St. Louis, MO). In some experiments, kidneys were homogenized in 5mM Tris pH 8.0 containing 0.05 mM pyridoxal phosphate, 0.8 mM magnesium sulfate and 0.25M sucrose. Arginase activity was determined using a coupled enzymatic assay based in the measurement of the rate of urea formation from 0.3 mM L-[^{14}C-guanidino]-arginine (3.43 mCi/mmol, NEN, Boston, MA) calculated from $^{14}CO_2$ formation after urease treatment [29]. Nitric oxide synthase was measured as described previously [30] by determining ^{3}H-citrulline formation from ^{3}H]-arginine. In brief, 50μl of extract was mixed with 50μl of L-[2,3,4,5-^{3}H]-arginine (53 Ci/mmol, ARC Inc, St Louis, MO) and 200μl of 50 mM Hepes pH 7.4 containing 1 mM NADPH, 1 mM EDTA, 1.25 mM calcium chloride, 1 mM dithiothreitol, 0.4 mM L-arginine and 10 μg/ml calmodulin. After incubation at 22°C for 1h, 4 ml of 20mM Hepes pH 5.5 containing 2 mM EDTA was added and the mixture was applied to a column of Dowex AG 50WX-8(Na^+). Tritiated citrulline was eluted by washing the column with deionized water and radioactivity was measured by liquid scintillation. Activity was calculated after substracting the counts given by the corresponding blank. Ornithine aminotransferase assay was carried out as described previously [31]: incubation at 37°C for 20 min 100 μl of crude extract with 150 μl of potassium pyrophosphate (50mM) containing 100 mM L-ornithine, 35 mM 2-oxoglutarate, and 8μg/ml pyridoxal phosphate. The reaction was stopped by addition of 0.25 ml of 10% trichloroacetic acid and 0.5 ml of a 5% solution of o-aminobenzaldehyde in 95% ethanol. After 10 min incubation at 37°C, the absorption of the reaction mixture was read at 440 nm (ϵ = 2700 M^{-1} cm^{-1}).

2.5. Analytical determinations

For amino acid and polyamine analysis tissues were extracted with 5% perchloric acid (1:5 w/v) using a Polytron homogenizer, and after centrifugation at 10000 × g for 10 min the supernatants were analyzed. For polyamine analysis the dansylation method of Seiler [32] was used. Dansylated polyamines were separated by HPLC, using a Lichrosorb 10-RP-18 column (4.6 × 250 mm) and acetonitrile:water mixtures (running from 70:30 to 96:4 ratio during 25 min of analysis) as mobile phase. 1,6-Hexanediamine was used as internal standard. Detection of the derivatives was carried out using a fluorescence detector with a 340 nm excitation

Table 1
Influence of gender and dietary arginine on the arginine content of mice tissues

Tissue	Male		Female	
	SC	ADD	SC	ADD
Plasma (μM)	106 ± 14	44 ± 4[a]	147 ± 17[d]	41 ± 5[a]
Skeletal muscle (μmol/kg)	367 ± 37	300 ± 24[c]	728 ± 220[e]	244 ± 36[b]
Kidney (μmol/kg)	263 ± 88	238 ± 104	440 ± 79[e]	202 ± 85[c]

Results are given as the mean ± SD of five animals per group. SC: standard chow; ADD: arginine deficient diet, 20 days. Statistical significance: [a] $p < 0.001$, [b] $p < 0.01$, [c] $p < 0.05$ vs SC; [d] $p < 0.001$, [e] $p < 0.05$ vs control male.

filter and a 435 nm emission filter. Amino acids were isolated by ion exchange chromatography, using an amino acid autoanalyzer (Chromaspeck, Rank Hilger) and detected by fluorometry after reaction with o-phthaldialdehyde.

Creatinine was determined by a colorimetric method based on Jaffe's reaction. Urine was diluted with 25 mM hydrochloric acid to a final volume of 0.8 ml and 1.3 ml 14 mM picric acid and 0.4 ml 1.4 M NaOH were added. After incubation at 37°C for 15 min the absorbance at 500 nm was read and compared with standard creatinine solutions. Urea was measured using the Endpoint Bun kit (Sigma Diagnostics) following the manufacturer's instructions. A colorimetric method [33] was used for the measurement of orotic acid excretion. Diluted urine (0.5 ml) was mixed with 0.4 ml of 0.4M citrate pH 2.5 and 0.1 ml of saturated bromine solution and incubated at 40°C for 5 min. After adding 0.2 ml of 5% ascorbic acid and incubating for 5 min, 0.4 ml of p-dimethylaminobenzaldehyde was added and incubated for a further 10 min period. After extraction with butylacetate (0.8 ml), the absorbance was read at 458 nm.

2.6. Statistical analysis

The data are presented as means ± SD. Statistical analysis was performed using either the Student's t test or analysis of variance followed by the Newman-Keuls test. P values < 0.05 were considered statistically significant.

3. Results

The analysis of arginine in blood and several tissues of mice (brain, liver, kidney, pancreas, heart, lung and skeletal muscle) showed that only in blood, kidney and skeletal muscle was there a sexual dimorphism in arginine content. The arginine concentrations in blood, kidney and skeletal muscle of male mice were 72%, 60% and 50% of the values found in the corresponding tissues of female mice (Table 1). Feeding switch from the standard diet containing about 1% arginine to a low arginine content diet (0.06%) produced a marked reduction in the arginine values in blood, kidney and skeletal muscle. These changes were more marked in females than in males, leading essentially to the disappearance of the sexual dimorphism in the arginine content (Table 1). Supplementation of the low arginine diet with 1% arginine in the drinking water restored the arginine values (data not shown). Further supplementation of the diet with arginine (up to 5%) produced a significant increase in arginine content, especially in the skeletal muscle, reaching values about 2-3-fold higher than those found in mice fed with the standard diet.

Table 2 shows the values of arginase activity in liver, small intestine and kidney, tissues that are very active in arginine metabolism. In order to compare the activity with those of other enzymes implicated in arginine metabolism, arginase activity was determined at pH 7.2 instead of at its optimum pH. The activity, as expected, was much higher in the liver than in the kidney or small intestine. However, only the renal enzyme showed a clear sexual dimorphism, the female kidney having a significantly higher activity. Renal NOS also presented gender dimorphism, the enzymatic activity in the female kidney being twice as high as in the male kidney (Table 2). The NOS activity was approximately three orders of magnitude lower than the arginase activity in the kidney. Table 2 also shows the values of

Table 2
Influence of gender on the activities of enzymes related to arginine metabolism in mouse tissues

Enzyme	Sex	Liver	Small intestine	Kidney
Arginase (μmol/hg)	Male	13.52 ± 0.64	0.284 ± 0.039	0.037 ± 0.004
	Female	14.23 ± 0.37	0.260 ± 0.031	0.116 ± 0.008[a]
NOS (pmol/hg)	Male	n.d.	n.d.	5.30 ± 0.20
	Female	n.d.	n.d.	13.55 ± 0.61[a]
OAT (μmol/hg)	Male	32.62 ± 3.16	93.95 ± 6.12	48.92 ± 1.83
	Female	34.77 ± 10.22	95.90 ± 2.70	95.94 ± 2.06[a]

Results are the mean values ± SD from 4 animals per group and are expressed as μmol or pmol of substrate transformed per hour and g of wet tissue. [a] $p < 0.001$ vs male; n.d.: not determined.

Table 3
Influence of gender on renal ODC activity and renal and urinary polyamine content

	Male	Female
ODC activity (μmol/hg)	4.98 ± 0.62	0.12 ± 0.03[a]
Putrescine (nmol/g)	143 ± 15	36 ± 5[a]
Spermidine (nmol/g)	395 ± 101	390 ± 40
Spermine (nmol/g)	631 ± 119	692 ± 57
Urinary putrescine (mM)	4.05 ± 0.41	0.25 ± 0.08[a]

Results are the means ± SD from 5 animals per group. ODC activity is expressed as μmol CO_2 released per hour and g of wet tissue.
[a] $p < 0.001$ vs male.

ornithine aminotransferase (OAT) activity in liver, small intestine and kidney. Only the renal enzyme presented sexual dimorphism; again the activity in the female was higher than in the male. In contrast to these findings, renal ODC activity as reported by others was dramatically higher in the males than in females (Table 3). This sexual dimorphism was also reflected in the renal putrescine content and in the concentration of putrescine in the urine that was 16-fold higher in males. Urinary putrescine represents about 7.4% of the arginine ingested by the male mice and 0.15% in the female. The values were calculated from the amount of pellet ingested and the arginine content of the diet (54 μmol arginine/g pellet). Table 4 compares the rates of arginine and ornithine decarboxylation by renal extracts from male and female kidney. The rate of arginine decarboxylation by the renal extracts was higher in males than in females; in both sexes the values were lower than those measured for ornithine decarboxylation. While in male mouse arginine decarboxylating activity was cytosolic in the female the activity was located mainly in the non-soluble fraction. Arginine decarboxylation was not affected by 1 mM difluoromethylarginine, an irreversible inhibitor of bacterial and plant arginine decarboxylases [34].

The influence of gender on the renal ability to synthesize arginine from citrulline was studied by incubating renal slices in physiological medium supplemented with citrulline and measuring the time course of arginine, ornithine and putrescine elevations in the slices and in the medium. Table 5 shows that the calculated amount of arginine produced

Table 4
Influence of gender on arginine and ornithine decarboxylating activity in murine renal extracts

Fraction	Male		Female	
	OD	AD	OD	AD
Crude extract	340 ± 97	12.2 ± 2.4[a]	14.1 ± 3.7[b]	3.32 ± 1.20[a,b]
Cytosolic supernatant	291 ± 68	14.5 ± 3.2[a]	7.4 ± 1.2[b]	0.63 ± 0.04[a,b]

Results are the means ± SD from 3 animals per group and are expressed as nmol CO_2 released per hour and g of wet tissue. OD: ornithine decarboxylating activity; AD: arginine decarboxylating activity.
[a] $p < 0.001$ vs OD, [b] $p < 0.001$ vs male.

Table 5
Influence of gender on arginine synthesis in renal slices in response to exogenous citrulline

Sex	Arginine	Ornithine	Putrescine	Σ (Arg + Orn + Put)
Male	385	25	24	434
Female	391	54	1	446

Results are given as nmol produced per hour and g of wet tissue and are the means of two experiments. They were calculated from the increments measured in the concentrations of arginine, ornithine and putrescine in the renal slices and in the medium in time-course experiments after addition of 4 mM citrulline to the incubation mixture.

was similar in the two sexes. As expected the amount of putrescine produced in the slices from males was higher than in females. According to these data the amount of arginine synthesized by the mouse kidney corresponded to 2.5% of the dietary arginine uptake. In the same system the formation of putrescine in response to 4 mM ornithine was 32 nmol/hg in the female renal slices and 449 nmol/hg in the male. The ratio of putrescine formation between the two sexes was similar in renal slices to that found in urine.

Fig. 2 shows that dietary arginine affects the activity of enzymes related to arginine metabolism and that this effect was gender-dependent. Renal ODC and arginase decreased in male mice fed with an arginine deficient diet (Fig. 2A and 2B). In contrast, NOS activity only decreased in the kidney of female mice (Fig. 2C). OAT was not affected by the treatment (Fig. 2D). In Table 6 it can be seen that urinary urea and creatinine excretion levels were similar in the male and the female, and that dietary exclusion of arginine did not affect the concentrations of urea and creatinine in urine, but it produced a dramatic decrease in putrescine excretion specifically in the male. A marked increase in the concentration of orotic acid in the urine was also noticed in both male and female mice after dietary arginine restriction, in agreement with a previous report [35].

6. Discussion

Our results show that there is a marked sexual dimorphism in the arginine content of tissues from CD1 mice. This effect could be the consequence of gender differences in the uptake and/or anabolism or catabolism of arginine in rodent tissues. Thus, measurement of both tissue enzyme activities and excretion of nitrogen-containing compounds derived from arginine may provide some information on the fate of arginine and its relationship with whole-body arginine metabolism. Our data indicate that several renal enzymes related to arginine/ornithine metabolism exhibit a sexual dimorphic pattern. Although the existence of murine renal sexual dimorphism in ODC, arginase and OAT is already known, its physiological significance has not been completely understood [36–38]. The higher consumption of ornithine by ODC in the kidney of male mice may partially

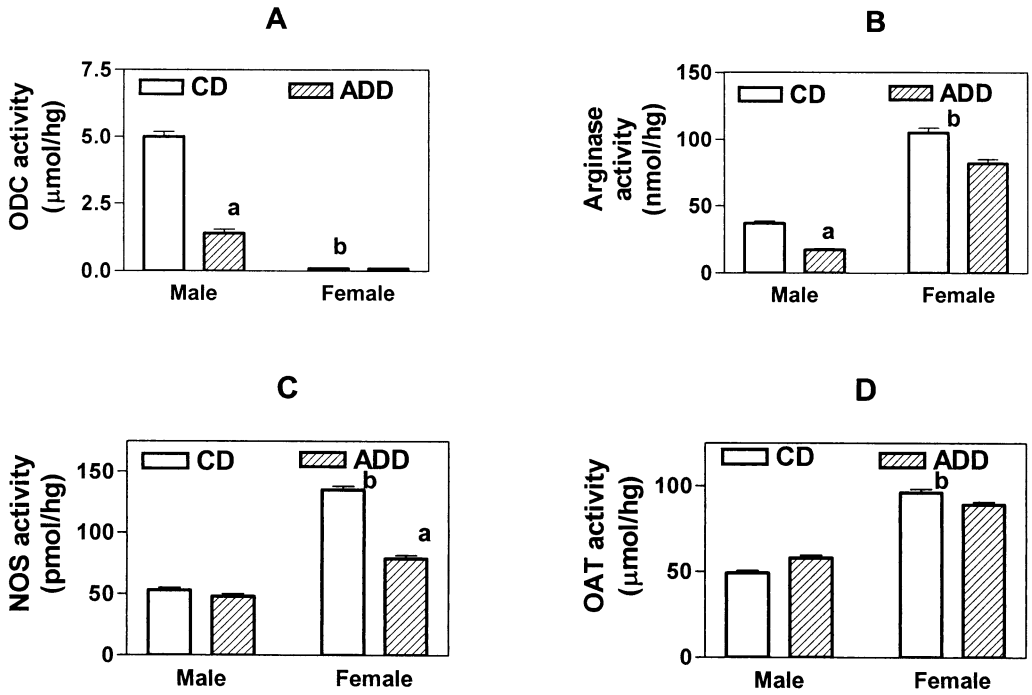

Fig. 2. Effects of dietary arginine on renal activities of enzymes related with arginine metabolism. A) ODC. B) Arginase C) NOS. D) OAT. Results are the means ± SD and are expressed as the amount of substrate transformed per hour and g of wet tissue. a) $p < 0.001$ *vs* control. b) $p < 0.001$ *vs* male.

explain the differences in arginine concentration found between the sexes. In fact, if one accepts that arginine is the main precursor of ornithine, the excretion of putrescine in the urine represents 7.4% of the dietary arginine in the male mouse and 0.15% in the female. This higher utilization of arginine by the male kidney might affect arginine homeostasis and contribute to the gender-dependent dimorphism in plasma and tissue arginine content we observe in mice. The estimated values of arginine degradation in the kidney by decarboxylation of arginine also sustain a higher utilization through this pathway in the kidney of male mice. The existence of ADC in the brain and kidney has been recently demonstrated in the rat [39–41] but to our knowledge the enzyme has not been characterized in mice. Our results do not finally establish that the decarboxylation of arginine by renal extracts is produced by authentic ADC because the simultaneous presence of arginase, ODC and OAT could lead to arginine decarboxylation. However, they indicate that arginine consumption by the kidney is higher in the male than in the female mouse. The identical capacity to synthesize arginine from citrulline showed in our *in vitro* system in association with similar levels of creatinine excretion, suggest that neither renal arginine synthesis nor arginine utilization for creatine synthesis is related to the dimorphism of arginine. On the other hand it appears paradoxical that female mice having higher activities than male in the enzymes implicated in renal arginine catabolism, such as arginase, NOS synthase and OAT, surprisingly showed higher arginine levels.

It is of interest that our study shows that the restriction of dietary arginine affects plasma, muscle and kidney arginine content, but again the decrease observed was gender dependent. In the female mice the reduction in arginine levels were more marked than in the male, leading to abolishment

Table 6
Influence of gender and dietary arginine on the urinary excretion of nitrogen-containing compounds derived from arginine

Metabolite	Male		Female	
	SC	ADD	SC	ADD
Urea (μmol/day)	1240 ± 340	1034 ± 265	1110 ± 120	1098 ± 201
Creatinine (μmol/day)	5.77 ± 1.08	6.76 ± 0.79	5.13 ± 0.15	6.84 ± 0.97
Putrescine (μmol/day)	24.5 ± 2.7	4.6 ± 0.7[a]	0.51 ± 0.34	0.47 ± 0.26
Orotic acid (nmol/day)	0.18 ± 0.03	5.17 ± 0.54[a]	0.10 ± 0.03	3.32 ± 0.43[a]

Results are the means ± SD from 5 animals per group. SC: mice fed with a diet containing 1% arginine for 20 days; ADD: mice fed with an arginine deficient diet for 20 days.

[a] $p < 0.001$ vs SC.

of the sex-related differences in arginine content observed in mice fed with a diet containing 1% arginine. These results indicate that the de novo rate of arginine synthesis does not compensate the supply of exogenous arginine sufficiently to maintain normal arginine levels, especially in the female. Consequently gender may be seen as another factor that regulates arginine homeostasis, at least in the mouse. Moreover, the fact that several enzymes implicated in the metabolism of arginine that show sex dimorphism in mouse kidney were affected by the exogenous arginine supply suggests that dietary arginine may either decrease hormone secretion or interfere with the action of sex steroid hormones. In fact, arginine is known to act as a secretagogue of growth hormone [42] which may participate in the regulation of sex steroid actions. The diminution observed in the activities of enzymes involved in arginine catabolism in the female kidney in response to dietary arginine restriction may be interpreted as part of response mechanisms directed to counteract the decrease in arginine levels. The marked fall produced by the treatment on renal ODC in the male mouse almost abolished putrescine excretion and decreased arginine utilization through this pathway. This may explain, at least in part, the weaker effect of arginine restriction on arginine pools in the male mouse. Independently of the role that the enzyme dimorphism of renal arginine metabolism may have on the maintenance of arginine dimorphism in mice it is likely that other extrarenal processes may contribute to this effect. Among these, arginine uptake by the intestine, liver and skeletal muscle and the utilization of arginine for muscle protein synthesis should be considered [43]. In this regard, it has been reported that increases in the activity of cationic amino acid transporter in the liver, produced by changes in sodium dietary uptake, leads to decreased plasma arginine concentrations [44]. Apart from the influence that the differences in the activities of the enzymes involved in arginine metabolism may have on arginine balance, our results clearly show that both dietary arginine and gender affect the activity of enzymes implicated in the production of certain important physiological mediators such NO, agmatine or polyamines. The implication of these substances in the physio/pathological renal differences observed between sexes [45] remains to be determined.

Some aspects of our study related to the complexity of arginine metabolism in the rodent kidney need further consideration. The comparison of different enzymatic activities in kidney extracts may present drawbacks but different studies have in fact demonstrated that the renal enzymes of arginine and ornithine metabolism are not segregated evenly at both cellular and subcellular levels [23–26,46,47]. Moreover, substrate availability may limit product formation. In spite of this there was a good correlation between renal ODC activity and the amount of putrescine present in urine in male mice. These data also agree with the values of putrescine formation found in incubated kidney slices. This suggests that renal ODC activity *in vivo* must be close to that calculated *in vitro* for male kidney extracts (about 5 μmol/hg). Our *in vitro* studies indicate that the rate of arginine formation from citrulline in kidney slices is similar in males and females (about 0.5 μmol/hg) but this value is very low when compared to that found in rats [15,25]. According to our results, the amount of arginine synthesized by the mouse kidney corresponds to 2.5% of the dietary arginine uptake. It is possible that mice may have a reduced renal arginine synthesis in comparison with other mammals such as pigs or rats [2,11,24,48]. Although, comparative studies on arginine synthesizing capacity between rat and mouse nephron segments suggest that the patterns of arginine synthesis in mouse and rat nephrons are similar [24,26], the possibility that arginine synthesis in incubated renal slices may be lower than under *in vivo* conditions cannot be excluded. Regarding polyamine synthesis it is not known whether arginase may control putrescine synthesis by affecting the availability of ornithine. The comparison of putrescine excretion with renal ODC activity and the estimated *in vitro* values of arginase and ODC (about 40 and 5000 nmol/hg, respectively) suggests that other sources for ornithine, different from renal arginase may operate *in vivo*. This is in agreement with previous findings showing that no arginase activity is present in the proximal convoluted tubules of mouse kidney where ODC reaches high levels [25]. An alternative donor of ornithine for putrescine synthesis could be glycine amidinotransferase, the enzyme implicated in the first step of creatine synthesis which produces ornithine from arginine and that colocalizes with ODC in the proximal convoluted tubules [49]. However, the comparison of the values of creatinine and putrescine excretion in the urine of male mice (Table 6) suggests that at least in the male kidney alternative routes for ornithine supply must be involved, including its uptake from plasma. The comparison of the rates of ornithine and arginine decarboxylation by renal extracts from male and female mice indicates that there are differences not only in the absolute activity but in the subcellular localization of arginine decarboxylating activity. While the results found in female kidney agree with a mitochondrial localization of ADC, as has been found in the rat [40,41], the extensive presence of arginine decarboxylating activity in the cytosol of male kidney again raises the possibility that ADC activity may be overestimated in crude preparations. Our results indicate that arginine utilization for NO synthesis in the mouse kidney is low compared with the overall renal arginine catabolism, though owing to the uneven distribution of NOS in the kidney arginine consumption may be relevant in cells having high NOS activity. The fact that renal NOS activity *in vitro* was found to be higher in females than in males (Fig. 2C) requires further work before concluding that the differences in NOS activity between sexes may have physiological relevance [50].

In conclusion, our results demonstrate the existence of a clear sexual dimorphism in arginine and arginine-related enzymes in the mouse, which is dependent on dietary arginine uptake. This could be an important finding considering

current evidence for the beneficial effects that dietary arginine supplementation has in the prevention and treatment of renal and cardiovascular diseases [42,51–54].

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