

# Influence of dietary arginine on the anabolic effects of androgens

A Cremades<sup>1</sup>, C Ruzafa, F Monserrat, A J López-Contreras and R Peñafiel

<sup>1</sup>Department of Biochemistry and Molecular Biology and Department of Pharmacology, Faculty of Medicine, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain

(Requests for offprints should be addressed to R Peñafiel; Email address: rapegar@um.es)

## Abstract

Feeding mice an arginine-deficient diet decreased plasma concentrations of arginine, citrulline and ornithine in the females and arginine in the males, abolishing the sexual dimorphic pattern of these amino acids found in mice fed the standard diet. In addition, the restriction of dietary arginine produced a marked decrease in body and renal weights as well as in the activity of renal ornithine decarboxylase, decreases that were gender dependent since they were observed exclusively in males. The fact that these changes were not associated with the decrease in the circulating levels of testosterone and that the dietary arginine restriction prevented the body weight gain induced by testosterone treatment of female mice fed the standard diet indicates that dietary arginine is required for

the anabolic action of androgens. Moreover, under certain conditions that could compromise the renal synthesis of arginine, as in the compensatory renal hypertrophy that follows unilateral nephrectomy, the myotrophic effect of testosterone was transiently impaired. The results also revealed that arginine deficiency produced an opposite effect in the expression of IGF-I and IGF-binding protein 1 in the liver and kidney. Taken together, our results indicate that dietary arginine may be relevant to the anabolic action of testosterone, and suggest that this effect may be mediated by changes in the insulin-like growth factor system.

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## Introduction

Androgens are responsible for the induction of the male phenotype and for the regulation of the growth and differentiation of several extragenital tissues (Bardin & Catterall 1981). Most androgen actions are mediated through the androgen receptor (AR) belonging to the superfamily of nuclear receptors. Upon binding to *cis*-acting DNA elements, androgen-AR complexes regulate the transcription of androgen-responsive genes (Brinkman *et al.* 1999, Verhoeven & Swinnen 1999, Kadi *et al.* 2000, Sheffield-Moore 2000). Many lines of evidence indicate that different hormones and growth factors, such as growth hormone (Jansson *et al.* 1985, Veldhuis & Iranmanesh 1996, Niermann & Watson 1999), catecholamines (Hannan *et al.* 1991) and insulin-like growth factors (Hobbs *et al.* 1993, Niermann & Watson 1999, Gayan-Ramirez *et al.* 2000), may modulate androgen action, probably by affecting the phosphorylation of AR or by interacting with different coactivators or corepressors (Urban *et al.* 1995, Verhoeven & Swinnen 1999, Sheffield-Moore 2000, Kuhn 2002).

Apart from the role of hormonal and genetic factors in the control of growth, nutritional signals, and among these the amino acids, also play an important role in the

regulation of genes involved in the control of growth (Fafournoux *et al.* 2000). L-Arginine is an important amino acid that participates in multiple biochemical processes in mammals. In addition to its implication in the urea cycle and protein synthesis, it serves as a precursor for the synthesis of amino acids, nitric oxide, polyamines, creatine, agmatine and other guanidino compounds (Barbul 1986, Morris 1992, Reyes *et al.* 1994, Wu & Morris 1998, Wu & Meininger 2002). In most mammalian species, arginine is not considered an essential amino acid for healthy adults, since it can be synthesized in the tubular cells of the kidney from the citrulline generated in the small intestine (Rose *et al.* 1954, Visek 1986, Dhanakoti *et al.* 1990). However, arginine may be considered a conditionally essential amino acid for adult animals under certain stressful conditions, and for young and growing animals, when the endogenous synthesis is inadequate to accomplish arginine requirements (Visek 1986, Reyes *et al.* 1994, Cynober *et al.* 1995). In addition, it has been reported that supplemental dietary arginine may have beneficial effects since it enhances wound healing and angiogenesis, improves protein anabolism, stimulates the immune functions and ameliorates cardiovascular disorders (Adjei *et al.* 1995, Cui *et al.* 1999, Shi *et al.* 2000, Boger & Bode-Boger 2001).

Homeostasis of L-arginine in plasma is regulated by dietary arginine intake, protein turnover, transport, arginine synthesis and catabolism (Barbul 1986, Morris 2002). The relative importance of these processes may be dependent on different factors such as age, animal species or dietary salt intake (Castillo *et al.* 1994, Kitiyakara *et al.* 2001). Thus, while endogenous synthesis of arginine plays an important role in regulating arginine homeostasis in neonatal and growing pigs (Wu & Knabe 1995), dietary arginine intake appears to be the main regulator of arginine levels in adult humans (Castillo *et al.* 1994). In rodents, it has been reported that feeding adult animals an arginine-deficient diet decreased arginine concentration in plasma (Gross *et al.* 1991). In a recent study, we found that there is a marked gender dimorphism in the levels of arginine in plasma, kidney and skeletal muscle, female mice having higher levels than males (Ruzafa *et al.* 2003). Moreover, the restriction of dietary arginine produced a marked decrease of arginine in plasma and tissues that almost abolished the sexual dimorphism found in the levels of this amino acid. This dietary restriction also affected the activities of enzymes related to the metabolism of arginine and ornithine that are regulated by sex hormones, suggesting the existence of some interaction between dietary arginine and hormone action (Ruzafa *et al.* 2003).

In the present study, we have investigated the influence of dietary arginine in the metabolic action of testosterone in mice, a species in which testosterone is the major determinant of the gender dimorphism found in extragenital tissues (Koenig *et al.* 1980, Bardin & Catterall 1981). We have studied the influence of dietary arginine in renal size, renal ornithine decarboxylase activity and body weight gain, since these parameters are clear indicators of the anabolic action of testosterone in extragenital tissues (Berger & Watson 1989). Moreover, we have also studied the influence of conditions in which renal arginine synthesis may be compromised, as in the case of recovery after unilateral nephrectomy, in the action of androgens.

## Materials and Methods

### Animals and treatments

Male and female Swiss CD1 mice (Harlan Interfauna Ibérica, Barcelona, Spain) weighing 35–40 (males) and 26–30 g (females) were bred in our animal facilities. They were housed at  $22 \pm 1$  °C ambient temperature and 50% relative humidity under a 12-h light-dark cycle (lights on at 0800 h) in stainless-steel cages. They were divided into different groups that received commercial rodent solid diets and water *ad libitum*. The standard chow (A04; Panlab, Barcelona, Spain) contained 17% proteins, 3% fat, 59% carbohydrate, 12% moisture, 4% fiber, 1% vitamins and 4% minerals, with a calorific content of about 2900 kcal/kg. The L-arginine, L-lysine and L-methionine contents of the diet were 0.98%, 0.85% and 0.31%

respectively. The arginine-deficient diet (ICN Biomedicals, Aurora, OH, USA) contained 46.4% sucrose, 23.1% cornstarch, 10% corn oil, 0.5% AIN 76 vitamin mix, 5% AIN 76 mineral mix, 0.2% choline bitartrate, 0.26% L-histidine free base, 0.53% L-isoleucine, 0.71% L-leucine, 1.16% L-lysine HCl, 0.53% L-methionine, 0.75% L-phenylalanine, 0.53% L-threonine, 0.53% L-valine, 2.25% L-glutamic acid, 0.75% glycine, 0.11% L-tryptophan, 1.26% L-alanine, 0.66% L-aspartic acid, 0.81% L-proline, 0.75% L-cysteine free base, 1.26% L-serine, 0.81% L-tyrosine and 1.01% L-asparagine anhydrous. For arginine supplementation, animals were fed the arginine-deficient diet and L-arginine (in the hydrochloride form) in the drinking water. Arginine solutions were changed daily. The pH of the aqueous solutions with 0, 1 and 5% arginine were 7.2, 7.1 and 6.8 respectively.

Nephrectomy was performed under ether anesthesia in adult female mice. The right kidney was removed and weighed immediately after surgery. Mice were killed 2–4 weeks after nephrectomy, and the remaining kidney was removed and weighed. Adult female mice were treated with testosterone propionate (100 mg/kg) and killed after 2 weeks of treatment. Testosterone propionate, purchased from Sigma, was dissolved in olive oil and injected subcutaneously every other day. Growth hormone (10 µg) was injected subcutaneously every 12 h for 20 days.

Animals were killed by cervical dislocation under ether anesthesia, and tissues were quickly removed, weighed and processed. Procedures involving animals and their care were conducted in conformity with institutional guidelines that comply with national (RD 223/1988) and international laws and policies (EEC Council Directive 86/009; *NIH Guide for the Care and Use of Laboratory Animals*, NIH publication no. 85–23 1985).

### Amino acid analysis

Blood was collected by cardiac puncture under light ether anesthesia. After centrifugation, plasma was collected and treated with 5% trichloroacetic acid (1:5 w/v). After centrifugation at 10 000 g for 10 min, the supernatants were analyzed. Amino acids were isolated by ion-exchange chromatography with an amino-acid autoanalyzer (Chromaspeck; Rank Hilger Analytical, Westwood, Kent, UK) and detected by fluorometry after reaction with *o*-phthalaldehyde (Sigma). Norleucine was used as internal standard. The system was calibrated with 0.01 mM amino-acid standard solution, including physiological acidic, neutral and basic amino acids (Sigma). Amino-acid concentrations in plasma were calculated and expressed as mM.

### Enzymatic measurements

For enzyme determination, kidneys were homogenized with the aid of a Polytron homogenizer in buffer

**Table 1** Effect of dietary arginine restriction on body and renal weights. Animals were fed an arginine-deficient diet (AD) for 20 days. Results are the means  $\pm$  s.d. Number of animals per group: 12. Relative kidney weight represents the ratio between the weight of a pair of kidneys (mg) and the body weight (g)

Treatment	Body weight (g)		Body weight gain (g)	Kidney weight (mg)	Relative kidney weight (mg/g)
	Initial	Final			
Control male	40.7 $\pm$ 2.8	41.2 $\pm$ 2.2	0.36 $\pm$ 0.50	332 $\pm$ 35	16.3 $\pm$ 1.7
AD male	42.0 $\pm$ 1.7	38.7 $\pm$ 3.1 <sup>a</sup>	-3.2 $\pm$ 0.7 <sup>b</sup>	260 $\pm$ 43 <sup>b</sup>	13.2 $\pm$ 1.6 <sup>b</sup>
Control female	29.4 $\pm$ 2.1	29.5 $\pm$ 2.0	0.1 $\pm$ 0.1	170 $\pm$ 23	10.9 $\pm$ 2.3
AD female	27.5 $\pm$ 1.2	27.6 $\pm$ 0.9	0.8 $\pm$ 0.6	159 $\pm$ 10	11.8 $\pm$ 0

<sup>a</sup> $P < 0.01$  vs initial weight.

<sup>b</sup> $P < 0.001$  vs control male.

containing 25 mM Tris (pH 7.2), 2 mM dithiothreitol, 0.1 mM pyridoxal phosphate, 0.1 mM EDTA and 0.25 M sucrose. The extract was centrifuged at 20 000 *g* for 20 min, and ornithine decarboxylase (ODC) activity was determined in the supernatant. ODC activity was determined basically as described by Russell and Snyder (1968) by measuring <sup>14</sup>CO<sub>2</sub> release from L-[1-<sup>14</sup>C] ornithine. The incubation mixture contained 20 mM Tris (pH 7.2), 0.1 mM pyridoxal phosphate, 0.1 mM EDTA, 2 mM dithiothreitol and 0.4 mM L-[1-<sup>14</sup>C] ornithine (New England Nuclear, Boston, MA, USA) (specific activity 2.6 Ci/M) in a total volume of 62.5  $\mu$ l. The samples were incubated at 37 °C for 30 min, and the reaction was stopped by adding 0.5 ml of 2 M citric acid. Activity was expressed as  $\mu$ mol CO<sub>2</sub> produced per hour and per gram of wet weight.

#### Testosterone analysis

Plasma was obtained by centrifugation at 4 °C and was kept frozen at -70 °C until analysis. Testosterone was determined by ELISA, using Enzymun-test kits supplied by Boehringer Mannheim Immunodiagnosics (Mannheim, Germany).

#### RT-PCR

Total RNA was extracted from tissues with the RNAqueous-Midi Kit (Ambion, Austin, TX, USA) and purified according to the manufacturer's specifications. Total RNA was reverse transcribed, using oligo(dT)<sub>18</sub> as primer and (M-MLV) reverse transcriptase (Ambion). Products were amplified by means of Taq polymerase (Sigma), using specific primers pairs within the linear range for each gene product. Amplified products were resolved by electrophoresis in 2% agarose gels containing 40 mM Tris/acetate and 1 mM EDTA (pH 8.0) in a horizontal slab gel apparatus using 1 TAE buffer. The gel was stained with ethidium bromide (0.2  $\mu$ g/ml for 15 min), followed by destaining in water, photographed by

UV transillumination by a Gel Doc system camera (Bio-Rad), and the bands were quantified with Multi-Analyst software. Primers (from Sigma) were as follows:

- mouse IGF1 (accession no. AF440694), 8–380: forward 5'-CTTCACATCCTCTCTACCT, reverse 5'-ATTCTGTAGGTCTTGTTTCC
- mouse IGF-binding protein (BP) 1 (accession no. NM\_008341), 581–950: forward 5'-AAAGGCTGCTGTGGTCTCCG, reverse 5'-TCCACTCCATGGGTAGACAC
- mouse IGF1 receptor (R) (accession no. NM\_010513), 3221–3820: forward 5'-AGCCCACCCTGGTCAT catg, reverse 5'-GCTCCATCTCATCCTTGATG.

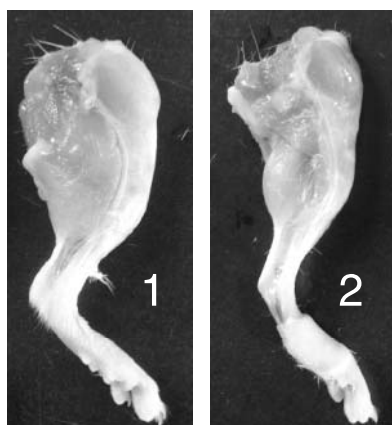
#### Statistical analysis

Results are given as means  $\pm$  s.d. Statistical comparisons were calculated by ANOVA followed by the post-hoc Newman-Keuls multiple range test. The results were considered statistically significant when *P* values were  $< 0.05$ .

#### Results

Feeding mice an arginine-deficient diet for 20 days produced a marked decrease in body and renal weights in the male mice, but the same treatment did not affect these parameters in the female mice (Table 1). This decrease in body weight was mainly associated with the loss of muscle mass, as was evident by comparing the size and weights of the lower legs (Fig. 1). Arginine deficiency also produced a significant decrease of arginine in the plasma of both sexes, while its product ornithine and its precursor citrulline were decreased significantly in the females (Table 2). It must also be noted that the concentrations of the three amino acids studied were higher in control females than in males, confirming previous findings of a sexual dimorphism of arginine in CD1 mice (Ruzafa *et al.* 2003).

Treatment	Weight (mg)
Control (4)	1813±163
Arginine-deficient (4)	1445±187 <sup>a</sup>



**Figure 1** Influence of dietary arginine in lower leg weight and muscle mass of male mice. Lower legs from male mice fed an arginine-deficient diet for 20 days were dissected and compared with those from animals of the same weight fed the standard diet. The weights are the means  $\pm$  s.d. from four animals. (1) Control; (2) arginine-deficient. <sup>a</sup> $P < 0.01$ .

**Table 2** Influence of dietary arginine restriction on plasma arginine, ornithine and citrulline levels. Animals were fed an arginine-deficient diet (AD) for 20 days. Results are the means  $\pm$  s.d. Number of animals per group: 4–6

	Male		Female	
	Control	AD	Control	AD
<b>Amino acid (mM)</b>				
Arginine	0.108 $\pm$ 0.014	0.045 $\pm$ 0.003 <sup>a</sup>	0.152 $\pm$ 0.015 <sup>c</sup>	0.043 $\pm$ 0.005 <sup>a</sup>
Ornithine	0.081 $\pm$ 0.024	0.060 $\pm$ 0.016	0.116 $\pm$ 0.034 <sup>c</sup>	0.058 $\pm$ 0.006 <sup>b</sup>
Citrulline	0.064 $\pm$ 0.016	0.055 $\pm$ 0.012	0.123 $\pm$ 0.015 <sup>c</sup>	0.091 $\pm$ 0.009 <sup>b</sup>

<sup>a</sup> $P < 0.001$  vs control.

<sup>b</sup> $P < 0.05$  vs control.

<sup>c</sup> $P < 0.01$  vs male.

**Table 3** Influence of dietary arginine on the effect of exogenous testosterone on body and renal weights. Mice fed standard, arginine-deficient (AD) or arginine-deficient diet supplemented with 1% arginine or citrulline in drinking water were treated with testosterone propionate (100 mg/kg, every 48 h) for 20 days. Results are the means  $\pm$  s.d. of six animals per group.

Dietary treatment	Body weight (g)		Body weight gain (g)	Kidney weight (mg)	Relative kidney weight (mg/g)
	Initial	Final			
Standard (F)	29.7 $\pm$ 1.6	32.9 $\pm$ 1.5	3.2 $\pm$ 1.2 <sup>a</sup>	265 $\pm$ 15	16.0 $\pm$ 1.7
AD (F)	28.2 $\pm$ 1.3	28.5 $\pm$ 1.6	0.3 $\pm$ 0.7	233 $\pm$ 16	16.3 $\pm$ 1.7
AD+1% Arg (F)	29.7 $\pm$ 1.6	33.4 $\pm$ 1.6	3.4 $\pm$ 1.1 <sup>a</sup>	316 $\pm$ 24	18.7 $\pm$ 1.1
AD+1% Citr (F)	29.8 $\pm$ 0.8	32.7 $\pm$ 1.3	2.9 $\pm$ 1.0 <sup>a</sup>	314 $\pm$ 33	18.7 $\pm$ 2.1
AD+1% Arg (M)	36.4 $\pm$ 2.4	40.6 $\pm$ 1.2	4.1 $\pm$ 0.8 <sup>b</sup>	485 $\pm$ 80	23.9 $\pm$ 2.4 <sup>b</sup>
AD (M)	38.5 $\pm$ 3.0	39.2 $\pm$ 3.5	0.7 $\pm$ 0.6	362 $\pm$ 84	17.7 $\pm$ 2.1

F: female; M: male. Number of animals per group: six. Relative kidney weight represents the ratio between the weight of a pair of kidneys (mg) and the body weight (g).

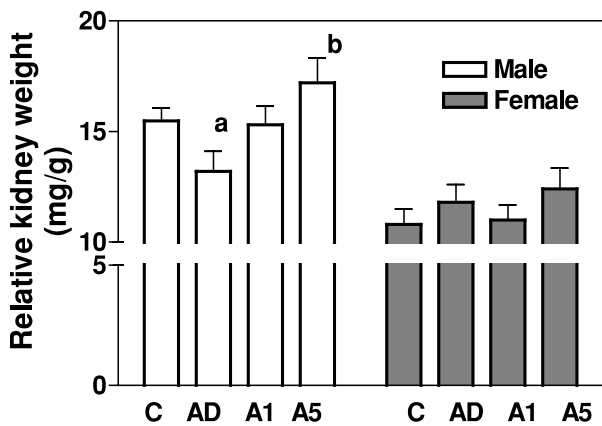
<sup>a</sup> $P < 0.001$  vs AD (F).

<sup>b</sup> $P < 0.001$  vs AD (M).

Moreover, the supplementation of arginine-deficient male mice with 1% arginine in the drinking water for 10 days reversed the changes produced by arginine deficiency, while the daily administration of growth hormone to arginine-deficient male mice during the period of deficiency did not prevent the fall in body and renal weights (data not shown). There were no significant differences in the amount of food consumed by the animals fed the 0%, 1% and 5% arginine diets (5.4  $\pm$  1.3 g/day for male mice and 4.1  $\pm$  1.1 g/day for female mice).

In order to test whether the decrease in kidney and body weights observed in the male mice under dietary arginine-restriction could be related to the diminution of androgens, plasma testosterone levels were measured. No significant variations between control mice (7.1  $\pm$  2.4 ng/ml) and arginine-deficient mice (6.4  $\pm$  2.0 ng/ml) were observed.

The influence of arginine deficiency on the anabolic action of exogenous testosterone was studied by comparing the effect of administration of testosterone propionate on body and kidney weights of female mice fed standard chow and arginine-deficient diet. Table 3 shows that, in animals fed standard chow, testosterone propionate given subcutaneously for 15 days elicited a significant increase in both body and renal weights ( $P < 0.001$ ). However, in



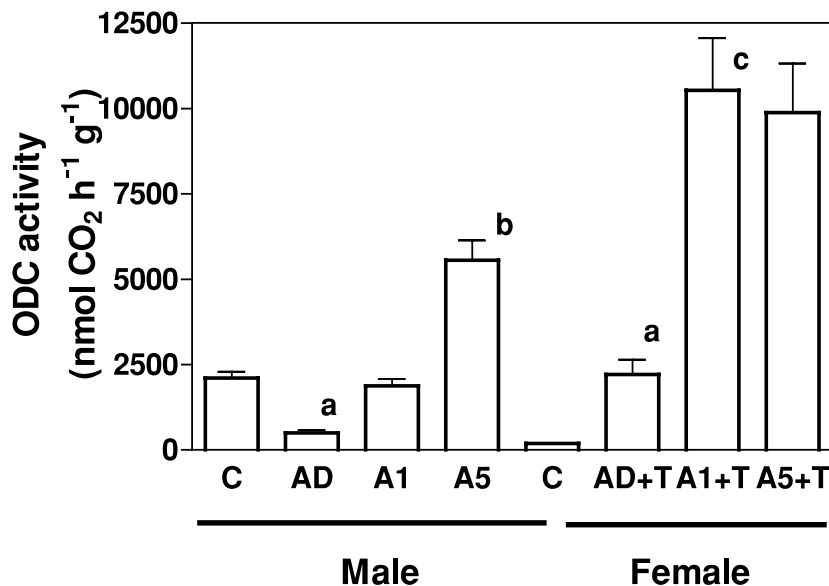
**Figure 2** Influence of dietary arginine on relative renal size. Male and female mice were fed standard diet (C), arginine-deficient diet (AD) or arginine-deficient diet supplemented with 1% or 5% arginine in drinking water (A1 and A5 respectively) for 20 days. Results are the means  $\pm$  s.d. from 4–6 animals per group. Relative kidney weight represents the ratio between the weight of a pair of kidneys (in mg) and the body weight (g). (a)  $P < 0.01$  vs control; (b)  $P < 0.01$  vs control and  $P < 0.001$  vs AD.

mice fed the arginine-deficient diet, the hormone did not affect body weight, although it increased renal mass, as shown in Table 3, which also shows that the concurrent administration of testosterone propionate and 1% arginine or 1% citrulline to arginine-deficient mice produced the

same anabolic effects as those obtained when testosterone was given to mice fed standard chow.

Figure 2 shows the influence of arginine concentration in the diet on relative kidney weight. It can be seen that in male mice there is a direct relationship between the amount of dietary arginine and the renal mass. However, in female mice, the variation of dietary arginine concentration did not significantly affect renal weight. Arginine deprivation also decreased renal ODC activity in the male mouse as well as the induction elicited by exogenous testosterone in the kidney of the female mouse, as shown by Fig. 3, which also shows that arginine supplementation (up to 5%) increased renal ODC activity in the male, while in the female kidney it did not significantly affect the action of testosterone propionate on this enzyme.

Next, we examined the influence of arginine restriction on body weight and renal compensatory hypertrophy induced by unilateral nephrectomy in female mice. Table 4 shows that the increases in the relative renal mass of the remaining kidney after 15 days of nephrectomy in animals fed 1% and 0% arginine were 27% and 19% respectively. The administration of testosterone propionate immediately after nephrectomy produced a higher increase in renal mass in both groups than in nontreated animals, reaching values of 76% and 69% respectively, but it did not affect body weight even in mice fed the standard diet, in contrast to sham operated mice, in which testosterone treatment, for an identical period of time, elicited a considerable increase in body weight. Table 4 also shows that the



**Figure 3** Renal ornithine decarboxylase (ODC) activity of mice under different dietary conditions. Male mice were fed standard diet (C), arginine-deficient diet (AD) or arginine-deficient diet supplemented with 1% or 5% arginine in the drinking water (A1 and A5 respectively) for 20 days, in the absence or presence of testosterone (T). Results are the means  $\pm$  s.d. from 4–6 animals per group. a) and b)  $P < 0.001$  vs control; c)  $P < 0.001$  vs AD+T.



**Table 4** Influence of nephrectomy on the effect of testosterone in body and renal weights in the female mice. Nephrectomized female mice (NE) were fed standard or arginine-deficient diet (AD) for 15 or 30 days and treated with testosterone propionate (100 mg/kg, every 48 h) for 15 days, starting immediately after nephrectomy (NE+T)15, or 15 days after nephrectomy (NE30+T15). Results are the means  $\pm$  s.d. of six animals per group

Treatment	Diet	Relative kidney weight (mg/g)		Body weight gain (g)
		1st kidney	2nd kidney	
NE15	Standard	11.3 $\pm$ 1.1	14.4 $\pm$ 2.2 <sup>a</sup>	0.2 $\pm$ 0.6
NE15	AD	11.2 $\pm$ 1.3	13.3 $\pm$ 1.6 <sup>a</sup>	-1.1 $\pm$ 0.8
(NE+T)15	Standard	11.7 $\pm$ 0.8	20.5 $\pm$ 1.5 <sup>a</sup>	0.1 $\pm$ 0.1 <sup>b</sup>
(NE+T)15	AD	11.6 $\pm$ 0.7	19.5 $\pm$ 1.6 <sup>a</sup>	-1.0 $\pm$ 0.5
NE30+T15	Standard	12.0 $\pm$ 0.6	22.5 $\pm$ 2.0 <sup>a</sup>	3.8 $\pm$ 0.5 <sup>c</sup>
NE30+T15	AD	11.9 $\pm$ 0.8	24.3 $\pm$ 1.3 <sup>a</sup>	0.7 $\pm$ 1.0
Sham+T15	Standard	—	16.4 $\pm$ 1.8	3.3 $\pm$ 0.5

<sup>a</sup> $P < 0.001$  vs 1st kidney.

<sup>b</sup> $P < 0.01$  vs sham+T15.

<sup>c</sup> $P < 0.01$  vs NE30+T15 AD.

administration of testosterone 15 days after nephrectomy increased body weight as much as in sham operated animals only when arginine was present in the diet.

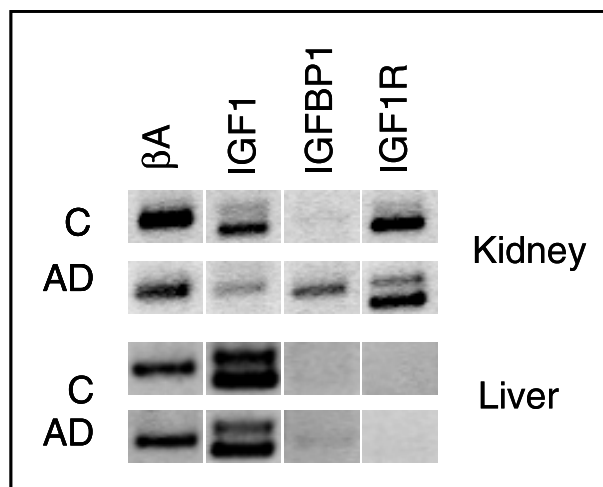
The levels of mRNA of IGF1, IGFBP1 and IGF1R in tissues from control and arginine-deficient male mice were estimated by semiquantitative RT-PCR analysis and compared with those of the constitutive protein  $\beta$ -actin. Figure 4 shows a higher expression of IGF1R in kidney than in liver, while the opposite was true for IGF1. Arginine deficiency produced a small decrease in IGF1 expression in the kidney and a significant increase of

IGFBP1 in liver and kidney. Figure 4 also suggests that this condition may affect the splicing of IGF1R in the kidney.

## Discussion

The precise mechanisms by which gonadal steroids mediate their wide spectrum of effects remain only partially understood. In the kidney, the sexual dimorphism described in many species has been studied most extensively in mice (Bhasin *et al.* 2001, Kuhn 2002). Here, testosterone produces morphologic, cellular and biochemical changes, including increases in the relative renal mass and in the activities of several renal enzymes and proteins such as ornithine decarboxylase, the rate-limiting enzyme of polyamine biosynthesis (Koenig *et al.* 1980, Berger & Watson 1989), but the physiological significance of these changes remains unclear. Moreover, little is known about the influence of sexual renal dimorphism in arginine homeostasis, although the kidney plays an important role in the body synthesis of arginine in mammals (Barbul 1986, Boudy *et al.* 1993, Morris 2002). However, there is ample evidence that skeletal muscle is the major extra-genital site of androgen action and that the increase in muscle mass elicited by testosterone in animals and humans can be related to the direct, indirect, priming and antigluco-corticoid action of androgens on muscle protein metabolism (Danhaive & Rousseau 1986, 1988, Tincello *et al.* 1997, Verhoeven *et al.* 1999, Sheffield-Moore 2000, Sheffield-Moore & Urban 2004).

We recently found that adult female mice had higher plasma arginine levels than males and that restriction of dietary arginine abolished sexual dimorphism in arginine metabolism (Ruzafa *et al.* 2003). The present results



**Figure 4** mRNA levels of IGF1, IGFBP1 and IGF1R in liver and kidney of control and arginine-deficient mice. Male mice were fed standard diet or an arginine-deficient diet (AD) for 20 days. Total RNA was extracted, and RT-PCR was performed as described in Materials and Methods.  $\beta$ A:  $\beta$ -actin.

corroborate and extend our previous findings, since we have found that dietary arginine restriction not only affects plasma arginine concentrations but also reduced the circulating concentration of amino acids directly related to arginine metabolism such as citrulline and ornithine. Interestingly, arginine restriction was associated with a significant decrease in both body and renal weights in males, but not in females. Since the treatment did not decrease the concentration of testosterone in plasma, the effects observed in the male mice are likely to be due to some interaction between arginine and the mechanism of action of testosterone. This possibility is also supported by the finding that renal ornithine decarboxylase, a well-known target of androgen action in the kidney (Berger & Watson 1989), was dramatically decreased in the kidney of male mice fed the arginine-deficient diet. Additionally, the postulated dependence of the extragenital actions of androgens on dietary arginine was also confirmed by the observation that the anabolic effects produced by the treatment of female mice with pharmacologic doses of testosterone were impaired by arginine restriction. Although our study did not determine body composition, we can assume that the changes in body weight associated with testosterone were probably due to changes in muscle mass. This suggestion is also supported by the changes observed in the size of the lower leg.

However, our results also show that dietary arginine supplementation stimulates renal ornithine decarboxylase and kidney hypertrophy in male, but not in female, mice, having a small effect on body weight gain. These findings reveal that while dietary arginine restriction affects the action of testosterone on both muscle and kidney, the effects of arginine supplementation are mainly exerted in the kidney of male mice, suggesting that changes in the concentration of arginine in plasma might be especially relevant to the physiology of the male kidney.

While the studies carried out with intact mice indicate that the manipulation of the arginine content of the diet clearly affects arginine homeostasis and testosterone action, the experiments performed with nephrectomized mice also reveal that the anabolic action of testosterone at the muscular level can be impaired when the endogenous supply of arginine from the kidneys might be compromised as a consequence of the reduction in the renal mass. Our results also show that the refractoriness of muscle mass to testosterone is transitory and limited to the period in which, presumably, the compensatory mechanisms in the remnant kidney, or in peripheral tissues intended to normalize plasma arginine (Boudy *et al.* 1993), are not fully developed.

The molecular mechanisms by which arginine may influence the effects of testosterone on kidney and skeletal mass await to be determined. In fact, the precise mechanisms by which androgens induce renotropic effects and skeletal muscle growth remain unclear, mainly because of the difficulty of isolating their effect from that of the other

hormones and growth factors. Since it is known that IGFs and IGF-BPs are important regulators of testosterone action on skeletal muscle mass (Urban *et al.* 1995, Björntorp 1996, Kuhn 2002, Sheffield-Moore & Urban 2004), that arginine may act as a secretagogue of growth hormone (GH) and insulin in humans and other mammals (Rosenfeld *et al.* 1994, Morimoto *et al.* 2001), that GH and IGF1 can enhance testosterone action in kidney (Niermann 1999), skeletal muscle (Urban *et al.* 1995) and other tissues (Björntorp 1996), and that the induction of IGF-BP1 mRNA and protein is promoted by partial arginine depletion in a range similar to that found in the blood of our arginine-deficient mice (Jousse *et al.* 1998), it is tempting to speculate that arginine might modulate androgen action through a plausible influence on IGFs/IGFBs. Our results showing that arginine deficiency alters the expression of IGF1, IGF-BP1 and IGF1R in the kidney and IGF-BP1 in the liver support this possibility.

Independently of the mechanisms involved in the interaction between arginine and testosterone, our results reveal, for the first time, that the changes in the concentration of arginine in plasma can affect the action of testosterone in muscle and kidney, and that the extent of these effects depends on the circulating levels of the hormone. The present study may have relevance to pathologic conditions associated with decrease in plasma arginine concentration, such as severe burn injury or diabetes (Yu *et al.* 1995, 2001, Pieper & Dondlinger 1997), or to nutritional strategies based on dietary arginine supplementation (Berad *et al.* 2000, Efron & Barbul 2000, Field *et al.* 2000, Preiser *et al.* 2001, Appleton 2002, Oblad *et al.* 2002). Specially, our results suggest that dietary arginine or L-citrulline supplementation may improve the pharmacologic action of synthetic analogs of testosterone used in clinical therapy.

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