Amino acid metabolism and protein synthesis in lactating rats fed on a liquid diet

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1. Amino acid metabolism was studied in control virgin rats, lactating rats and virgin rats protein-pair-fed with the lactating rats (high-protein virgin rats). 2. Urinary excretion of nitrogen and urea was higher in lactating than in control virgin rats, and in high-protein virgin rats it was higher than in lactating rats. 3. The activities of urea-cycle enzymes (units/g) were higher in high-protein virgin than in lactating rats, except for arginase. In lactating rats the activities of carbamoyl-phosphate synthase, ornithine carbamoyltransferase and argininosuccinate synthase were lower than in control virgin rats. When the liver size is considered, the activities in lactating rats were similar to those in high-protein virgin rats, except for arginase. 4. *N*-Acetylglutamate content was higher in high-protein virgin rats than in the other two groups. 5. The rate of urea synthesis from precursors by isolated hepatocytes was higher in high-protein virgin rats than in the other two groups. 6. The flooding-dose method (*L*-[4-³H]phenylalanine) for measuring protein synthesis was used. The absolute synthesis rates of mammary gland, liver and small-intestinal mucosa were higher in lactating rats than in the other two groups, and in high-protein virgin rats than in control virgin rats. 7. These results show that the increased needs for amino acids during lactation are met by hyperphagia and by a nitrogen-sparing mechanism.

INTRODUCTION

Lactation in the rat is associated with hyperphagia and widespread changes in the metabolism of the different tissues in order to provide a constant supply of nutrients to the mammary gland (Bauman & Currie, 1980). One of the potential signals for the redistribution of glucose and lipid to the gland is an increase in the glucagon/insulin ratio. This rise happens because the lactating mammary gland is a highly insulin-sensitive tissue and is responsible for the low values of plasma insulin during lactation (Burnol *et al.*, 1983). Furthermore, the lactating mammary gland has no receptors for glucagon (Robson *et al.*, 1984).

The net amino acid uptake by mammary gland of fed lactating rats is 15 mmol/day (Viña *et al.*, 1987). The major fate of these amino acids is the synthesis of milk proteins. The rest of the amino acids appear in the milk as free compounds, and can be used for the synthesis of small peptides, and those that yield acetyl-CoA in their degradation are used for lipid synthesis (Viña & Williamson, 1981).

Protein synthesis has been measured by the phenylalanine flooding method in lactating mouse, and the synthesis was increased in liver, gastrointestinal tract and mammary gland (Millican *et al.*, 1987). The uptake of amino acids by the liver during lactation has been also reported (Casado *et al.*, 1987), and it has been shown that the high uptake of amino acids by mammary gland does not affect hepatic availability when the lactating group was compared with normal fed virgin rats.

The aim of our work is to study changes in amino acid metabolism in different tissues during lactation to assure a correct supply of amino acids to the lactating mammary gland. We have studied total nitrogen excretion, urea synthesis in isolated hepatocytes, urea-cycle enzyme activities, liver *N*-acetylglutamate content, protein synthesis (flooding method) in different tissues, hepatic amino acid content and amino acid uptake by several tissues in control virgin rats, in lactating rats and in virgin rats protein-pair-fed with the lactating rats (high-protein virgin rats).

The N balance in control virgin and lactating rats was similar despite the high uptake of amino acid by the mammary gland; this is due to the hyperphagia of the lactating rats and/or to a nitrogen-sparing mechanism. Therefore, in order to study this problem we have introduced a group of virgin rats that have the same protein intake as lactating rats to dissociate the effects of lactation from those of the protein intake.

We have found that, during lactation, hyperphagia is not accompanied by the increase in ureogenesis and in urinary N excretion found in virgin rats protein-pair-fed with the lactating rats, and this may support the existence of a nitrogen-sparing mechanism.

MATERIALS AND METHODS

Rats

Female Wistar rats were housed individually in cages and given a commercial laboratory diet and tap water *ad libitum* until they reached the age of 2–3 months. Afterwards a liquid diet was used.

The rats used in the different experimental groups were virgin rats and rats in their first lactation. The lactating rats had between 8 and 10 pups and were used at day 14 of lactation, which is considered to be the peak of lactation. The virgin rats were divided in two groups: control and high-protein.

The lactating and control groups were fed with the liquid diet for 2 weeks. The high-protein group was protein-pair-fed with the lactating rats by increasing the protein content of the diet. This was done in order to maintain a constant intake of the other nutrients and to avoid a stress to the rat. They also had the diet for 2 weeks (Table 1).

All rats were maintained on a 12 h light/12 h dark cycle and under controlled conditions of temperature. All experiments were performed between 10:00 and 12:00 h to minimize diurnal variations.

Rats were anaesthetized with Pentothal (60 mg/kg body wt. in 0.9% NaCl). For the protein-synthesis study rats were anaes-

Abbreviations used: FSR, fractional (protein) synthesis rate; ASR, absolute synthesis rate.

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thetized with diethyl ether. We have used this anaesthetic because it has been reported that protein synthesis is not affected by ether (Sampson *et al.*, 1984).

For the collection of urine and faeces, animals were placed for 24 h in individual metabolic cages. The urine samples were collected into 25 ml test tubes containing 1 ml of 6 M-HCl. Faeces were collected and homogenized with water in a Potter-Elvehjem homogenizer and diluted to 200 ml. These measurements were performed twice on each animal. To avoid contamination of the maternal urine and faeces with those excreted by the pups, we wrapped the low part of the pup's body with cotton and an elastic band.

Liquid diet

Diets were formulated to meet the National Research Council-National Academy of Sciences (U.S.A.) (1978) recommendations. The composition of the control diet (% of energy) was 22 % from protein, 12 % from lipid and 66 % from carbohydrate.

The composition of the control diet (g/l) was: vitamin-free casein 54.5, DL-methionine 0.8, corn oil 3.4, olive oil 10.1, sucrose 131.2, dextrin 39.4, vitamin mix 2.6, mineral mix 9.2, choline chloride 0.4, xanthan gum 2.0, cellulose powder 10.0, and distilled deionized water. All diets were made daily.

Vitamin (AIN-76A) mix, mineral (AIN-76) mix and casein were purchased from ICN Biomedicals, High Wycombe, Bucks., U.K. Other compounds used for the diet were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Preparation of isolated hepatocytes

This was done by the method of Berry & Friend (1969) as modified by Romero & Viña (1983). Cells (15–20 mg wet wt/ml) were incubated (40 min) in Krebs-Henseleit (1932) bicarbonate buffer containing 3 mM-Ca²⁺. Urea synthesis was constant over the 40 min incubation period. Viability was assessed routinely by the Trypan-Blue-exclusion method as described by Baur *et al.* (1975). In all cases more than 85 % of the cells excluded Trypan Blue.

Determination of metabolites

N-Acetylglutamate. N-Acetylglutamate in HClO₄ tissue extracts was first separated from glutamate by ion exchange. It was then deacylated with aminoacylase, and the resulting glutamate, after adsorption to and elution from an AG 50 column, was quantified by a fast-h.p.l.c. method using pre-column derivative formation with *o*-phthalaldehyde, separation in a C₁₈ reverse-phase column, and fluorescence detection (Alonso & Rubio, 1985).

Creatinine. This metabolite was measured as described by Yatzidis (1974).

Urea. We used the method of Nuzum & Snodgrass (1976).

Tissue protein content. This was measured by the method of Lowry *et al.* (1951).

Analysis of N content

This was done in triplicate by the Nessler method as modified by Minari & Zilversmit (1963).

Amino acid determination.

For blood and liver amino acid analysis see Barber *et al.* (1985). Blood was collected in heparinized syringes from the femoral vein, hepatic vein (inside the left hepatic lobe), portal vein and the aorta in the three experimental groups. In the lactating group blood was also collected from the pudic-epigastric vein.

Arteriovenous differences across the mammary gland of lactating rats were measured as described previously (Viña *et al.*, 1981).

Net uptake of amino acid by a tissue was obtained by multiplying the arteriovenous differences for each amino acid by the blood flow to the tissue. The blood-flow values for the different tissues were taken from Chatwin *et al.* (1969). No significant changes in blood flow have been reported between rats fed on high-protein and standard diets (Rémésy *et al.*, 1988).

Protein-synthesis determination

Fractional rates of protein synthesis (FSRs) were measured *in vivo* by the flooding-dose method described by Garlick *et al.* (1980). Rats were injected through the lateral tail vein with a mixture of L-[4-³H]phenylalanine and unlabelled phenylalanine (150 mM) to give 50 µCi/ml; the amount administered was always 1.0 ml/100 g. At 10 min after administration of the label, rats were killed by cervical dislocation, tissues were rapidly removed and frozen in liquid N₂, and the specific radioactivity of free and protein-bound L-[4-³H]phenylalanine was measured in each tissue [for details see Garlick *et al.* (1980) and Goldspink & Kelly (1984)]. The fractional rate of protein synthesis was calculated as described by those authors. Absolute synthesis rates (ASRs) were calculated as the product of FSR and the tissue protein content.

Enzyme assays

Carbamoyl-phosphate synthase (EC 6.3.4.16) and ornithine carbamoyltransferase (EC 2.1.3.3) activities were measured as the rate of citrulline production. Argininosuccinate synthase (EC 6.3.4.5), argininosuccinate lyase (EC 4.3.2.1) and arginase (EC 3.5.3.1) activities were measured as the rate of urea production (Schimke, 1962; Barber *et al.*, 1987). One unit of enzyme activity is defined as the amount of enzyme which catalyses the formation of 1 µmol of product/h at 37 °C.

Milk production

This was measured as described by Sampson & Jansen (1984). The measurement was done on day 13 of lactation. Our lactating rats fed a liquid diet had a milk production of 30.1 ± 3.0 ml/24 h. This value is similar to that for lactating rats fed on a solid diet.

Statistics

The analyses were conducted by the least-significant-difference test, which consists of two steps. First, an analysis of variance was performed. The null hypothesis was accepted for all numbers of those sets in which *F* was non-significant at the level of *P* < 0.05. Second, the sets of data in which *F* was significant were examined by the modified *t*-statistic at *P* < 0.05.

RESULTS AND DISCUSSION

Body weight and food intake

Table 1 shows that in the three groups the increase between the initial body weight and the final body weight was similar. The energy intake was slightly higher in the high-protein group compared with the control group. This is because the liquid intake was similar in both groups, but the value in kJ/ml was higher in the high-protein diet, owing to the increase in protein content with no changes in the other nutrients. The energy intake of lactating group was significantly higher compared with the other groups (Table 1).

The high-protein group was protein-pair-fed with the lactating rats. The nitrogen intake in these two groups was significantly higher than in the control group.

The lactating rats showed significant increases in liver and small-intestine sizes as compared with the other groups. No

Table 1. Physiological parameters in virgin, lactating and high-protein virgin rats

The results are means $\pm$ S.D. for seven rats. Values that are statistically different from those for virgin rats are shown by * $P < 0.05$. Results from high-protein virgin rats that are statistically different from those for lactating rats are shown by † $P < 0.05$.

	Virgin	Lactating	High-protein virgin
Initial body wt. (g)	228 $\pm$ 47	230 $\pm$ 40	215 $\pm$ 33
Final body wt. (g)	251 $\pm$ 45	259 $\pm$ 49	242 $\pm$ 34
Liver wt. (g)	9.7 $\pm$ 1.9	16.9 $\pm$ 2.7*	9.7 $\pm$ 0.8†
Calf muscle wt. (g)	1.53 $\pm$ 0.16	1.38 $\pm$ 0.12	1.60 $\pm$ 0.10†
Small intestine:			
Length (cm)	104 $\pm$ 2	137 $\pm$ 13*	100 $\pm$ 8†
Mucosa (g/cm)	0.028 $\pm$ 0.009	0.069 $\pm$ 0.007*	0.033 $\pm$ 0.008†
Serosa (g/cm)	0.020 $\pm$ 0.004	0.026 $\pm$ 0.003*	0.017 $\pm$ 0.002†
Diet intake:			
ml/24 h	70.2 $\pm$ 6.8	142.1 $\pm$ 11.4*	66.9 $\pm$ 4.1†
Energy intake (kcal/24 h)	70.2 $\pm$ 6.8	142.1 $\pm$ 11.4*	81.2 $\pm$ 5.0*†
(kJ/24 h)	293.4 $\pm$ 28.4	593.9 $\pm$ 47.6*	329.4 $\pm$ 20.9*†
Protein intake (g/24 h)	3.9 $\pm$ 0.4	7.9 $\pm$ 0.6*	7.4 $\pm$ 0.5*
Carbohydrate intake (g/24 h)	12.2 $\pm$ 1.2	24.7 $\pm$ 2.0*	11.6 $\pm$ 0.7†
Lipid intake (g/24 h)	0.95 $\pm$ 0.09	1.92 $\pm$ 0.20*	0.90 $\pm$ 0.06†
Milk production (ml/24 h)	—	30.1 $\pm$ 3.0	—
Weight of pups at 13 days of lactation (g)	—	30.5 $\pm$ 3.2	—

Table 2. Nitrogen balance and concentrations of major nitrogen-containing compounds in serum and urine of the experimental groups

For details see the text. The results are means $\pm$ S.D., with the numbers of observations in parentheses. Values that are statistically different from those for virgin rats are shown by * $P < 0.05$. Results from high-protein virgin rats that are statistically different from those for lactating rats are shown by † $P < 0.05$.

	Virgin	Lactating	High-protein virgin
N intake (mg/24 h)	576 $\pm$ 16 (7)	1168 $\pm$ 96 (7)*	1088 $\pm$ 80 (7)*
N excretion (mg/24 h)			
Urinary	236 $\pm$ 28 (6)	311 $\pm$ 51 (7)*	391 $\pm$ 66 (7)*†
Faecal	32 $\pm$ 13 (4)	55 $\pm$ 25 (4)	49 $\pm$ 21 (4)
Milk	—	525 $\pm$ 93 (4)	—
N balance (mg/24 h)	302 $\pm$ 43 (4)	264 $\pm$ 69 (4)	683 $\pm$ 28 (4)*†
Serum:			
Urea (μ mol/ml)	7.8 $\pm$ 0.7 (7)	10.5 $\pm$ 0.4 (7)*	13.2 $\pm$ 1.8 (5)*†
Creatinine (μ mol/ml)	0.11 $\pm$ 0.01 (4)	0.11 $\pm$ 0.02 (4)	0.19 $\pm$ 0.01 (4)
Urine:			
Urea (mmol/24 h)	6.3 $\pm$ 1.4 (4)	9.0 $\pm$ 1.8 (5)*	12.0 $\pm$ 1.8 (4)*†
Creatinine (mmol/24 h)	0.09 $\pm$ 0.01 (4)	0.10 $\pm$ 0.01 (4)	0.07 $\pm$ 0.01 (4)*†
Creatinine clearance (ml/min)	0.58 $\pm$ 0.08 (4)	0.69 $\pm$ 0.19 (4)	0.45 $\pm$ 0.04 (4)*†

changes were found between the control and the high-protein group.

Nitrogen intake and concentrations of major nitrogen compounds in serum and urine

The nitrogen intake was increased by 90% in rats on high-protein diet and by 103% in lactating rats compared with control rats. Urinary N excretion was significantly higher in the high-protein group and in the lactating group compared with the control group. It has been shown in dairy cows that the orotic acid and urea excretion was higher in early lactation (Motyl, 1986). However, our results show that the urinary N excretion in rats was significantly lower in the lactating group than in the high-protein group. These differences in urinary N excretion can be accounted for a decreased excretion of urea (Table 2).

Lactating rats excreted 2.70 mmol of urinary urea/24 h more than the control rats, i.e. 75.6 mg of urinary-urea N, which is in good agreement with the difference in urine N excretion found (75 mg). Lactating rats excreted 3 mmol of urinary urea/24 h less

than the high-protein group, i.e. 84 mg of urinary-urea N. In this case the difference in urinary N excretion found was 80 mg.

Milk N excretion was 525 mg/day. The N balance of the lactating rat and control rats was similar; however, the N balance in the high-protein rats was higher than in the other two groups.

Activity of urea-cycle enzymes

We measured the activities (units/g) of the enzymes of the urea cycle in whole liver homogenates (Table 3), and found that carbamoyl-phosphate synthase, ornithine carbamoyltransferase and argininosuccinate synthase were lower in lactating than in control rats, argininosuccinate lyase was not changed, and arginase was increased. In the high-protein group all the enzyme activities were significantly higher than in the control and lactating groups, except for arginase. It has been shown that the increase in carbamoyl-phosphate synthase I activity in rats fed on a high-protein diet, compared with rats fed on a standard diet, correlates with an increase in the carbamoyl-phosphate synthase

Table 3. Urea-cycle enzyme activities in virgin, lactating and high-protein virgin rats

For details see the text. The results are means $\pm$ S.D. for six rats, expressed as units/g of liver. Values that are statistically different from those for virgin rats are shown by * $P < 0.05$. Results from high-protein virgin rats that are statistically different from those for lactating rats are shown by † $P < 0.05$.

	Virgin	Lactating	High-protein virgin
Carbamoyl-phosphate synthase	322 $\pm$ 46	227 $\pm$ 37*	423 $\pm$ 44*†
Ornithine carbamoyltransferase	8390 $\pm$ 1130	7146 $\pm$ 651*	11328 $\pm$ 2130*†
Argininosuccinate synthase	142 $\pm$ 20	118 $\pm$ 15*	202 $\pm$ 29*†
Argininosuccinate lyase	284 $\pm$ 35	291 $\pm$ 28	459 $\pm$ 48*†
Arginase	58177 $\pm$ 5294	66661 $\pm$ 7648*	61814 $\pm$ 7485

Table 4. Urea synthesis by isolated hepatocytes from virgin, lactating and high-protein virgin rats

For details see the text. The results are means $\pm$ S.D., expressed as μ mol/min per g wet wt. The numbers of rats are shown in parentheses. Values that are statistically different from those for virgin rats are shown by * $P < 0.05$. Results from high-protein virgin rats that are statistically different from those for lactating rats are shown by † $P < 0.05$.

Additions	Virgin (n = 5)	Lactating (n = 4)	High-protein virgin (n = 6)
NH ₄ ⁺ (10 mM) + ornithine (10 mM)	2.88 $\pm$ 0.35	2.65 $\pm$ 0.22	3.39 $\pm$ 0.25*†
NH ₄ ⁺ (10 mM) + ornithine (10 mM) + lactate (10 mM)	3.63 $\pm$ 0.36	3.04 $\pm$ 0.28*	3.80 $\pm$ 0.43†

I content in liver and with a decrease in the half-life of blood urea (Jordá *et al.*, 1988). When liver size is considered, the activities of the urea-cycle enzymes were not changed in the lactating group as compared with the high-protein group, except for arginase.

***N*-Acetylglutamate content**

Hepatic content (nmol/g of liver) of *N*-acetylglutamate is the relevant parameter for the activity of carbamoyl-phosphate synthase I. It was significantly lower in lactating rats (18.0 $\pm$ 6.3, $n = 5$) than in controls (30.4 $\pm$ 6.1, $n = 5$), and in the high-protein group (51.0 $\pm$ 9.0, $n = 5$) it was significantly higher than in the virgin and lactating groups.

When liver size is considered, the maximum enzyme capacity is similar in the lactating and high-protein groups (see above); however, the fact that the amount of allosteric effector of carbamoyl-phosphate synthase I is higher in the high-protein group than in the lactating group correlates with the higher synthesis and excretion of urea in this group. This is in agreement with the observation that the values found for *N*-acetylglutamate in our experiments are in the range where small changes in this activator induce large modifications in the carbamoyl-phosphate synthase I activity (Alonso *et al.*, 1989). In accordance with these findings, the alanine aminotransferase and phosphoenolpyruvate carboxykinase activities were significantly higher in high-protein rats than in the other two groups (results not shown).

Urea synthesis in isolated hepatocytes

The rate of urea synthesis (expressed as μ mol/min per g wet wt.) from ammonia plus ornithine was similar in lactating and control rats. In high-protein rats the rate was significantly higher than in the other two groups. When L-lactate was added to the incubation medium (ammonia plus ornithine), the rate of urea synthesis was increased in all groups. However, the hepatocytes from lactating rats produced less urea than did hepatocytes from the other two groups (Table 4).

Rates of protein synthesis in lactating mammary gland, liver, jejunum and skeletal muscle

Table 5 shows the values for the fractional synthesis rate (FSR) and absolute synthesis rate (ASR) of mammary gland, liver, jejunum and skeletal muscle of the three different groups. The values of FSR and ASR of lactating mammary gland were similar to values published for rats at peak lactation (Sampson *et al.*, 1986).

The FSRs of livers of the three experimental groups were not significantly different. The ASR of the liver from lactating rats was significantly higher than those from the control and high-protein groups. The ASR for the high-protein group was also significantly higher than that for the control group. However, a previous study in lactating mice showed that both the FSR and ASR were significantly higher in liver of lactating compared with virgin mice (Millican *et al.*, 1987).

The FSR of muscle in lactating rats was similar to that of the control group, but was significantly lower than in the high-protein group.

The FSRs and ASRs of intestinal mucosa in the high-protein and lactating groups were significantly higher than in the control group. The ASR in the lactating group was also significantly higher than in the high-protein group. The FSR and ASR of intestinal serosa in the lactating group were higher than in the other two groups.

All these results show that the protein synthesis (mg/day) in all the tissues, except for muscle, was significantly higher during lactation than in the other two groups. Values for muscle showed a tendency to be lower.

Free amino acid concentrations in liver

Table 6 shows that the free concentrations of all the amino acids in liver were similar in the lactating and in the control groups, except for taurine, L-aspartic acid, L-valine and L-leucine, which were lower in the lactating group. The concentrations of taurine, L-proline, the branched-chain amino acids, L-methionine, L-ornithine and L-lysine were significantly higher in the high-

Table 5. Protein synthesis in different tissues

For details see the text. The results are means $\pm$ S.D., with the numbers of observations in parentheses. Values that are statistically different from those for virgin rats are shown by * $P < 0.05$. Results from high-protein virgin rats that are statistically different from those for lactating rats are shown by † $P < 0.05$.

	Virgin	Lactating	High-protein virgin
Liver			
Fractional (%/day)	72 $\pm$ 18 (5)	86 $\pm$ 20 (5)	84 $\pm$ 12 (5)
Absolute (mg/day)	956 $\pm$ 240 (5)	2275 $\pm$ 529 (5)*	1402 $\pm$ 186 (5)*†
Calf muscle			
Fractional (%/day)	23 $\pm$ 5 (4)	18 $\pm$ 4 (4)	29 $\pm$ 6 (5)†
Absolute (mg/day)	76 $\pm$ 15 (4)	54 $\pm$ 12 (4)	96 $\pm$ 20 (5)
Small intestine			
Mucosa			
Fractional (%/day)	142 $\pm$ 32 (5)	229 $\pm$ 48 (5)*	208 $\pm$ 17 (3)*
Absolute (mg/day)	594 $\pm$ 133 (5)	2576 $\pm$ 544 (5)*	953 $\pm$ 78 (3)*†
Serosa			
Fractional (%/day)	62 $\pm$ 8 (3)	134 $\pm$ 25 (5)*	64 $\pm$ 17 (5)†
Absolute (mg/day)	213 $\pm$ 28 (3)	869 $\pm$ 162 (5)*	198 $\pm$ 54 (5)†
Mammary gland			
Fractional (%/day)	—	81 $\pm$ 16 (5)	—
Absolute (mg/day)	—	2735 $\pm$ 534 (5)	—

Table 6. Amino acid concentrations in liver from virgin, lactating and high-protein rats

For details see the text. The results are means $\pm$ S.D., expressed as nmol/g of liver. The numbers of rats are shown in parentheses. Values that are statistically different from those for virgin rats are shown by * $P < 0.05$. Results from high-protein virgin rats that are statistically different from those for lactating rats are shown by † $P < 0.05$.

	Virgin	Lactating	High-protein virgin
DL-O-Phosphoserine	336 $\pm$ 31 (5)	257 $\pm$ 84 (5)	294 $\pm$ 61 (6)
Taurine	4167 $\pm$ 1679 (6)	2176 $\pm$ 726 (6)*	6893 $\pm$ 1264 (5)*†
L-Aspartate	3006 $\pm$ 222 (6)	2019 $\pm$ 948 (6)*	3109 $\pm$ 382 (6)†
L-Threonine	1064 $\pm$ 242 (5)	1012 $\pm$ 492 (6)	797 $\pm$ 202 (6)
L-Serine	894 $\pm$ 236 (6)	765 $\pm$ 363 (6)	695 $\pm$ 298 (6)
L-Glutamate	2614 $\pm$ 575 (5)	3712 $\pm$ 1812 (6)	2700 $\pm$ 665 (6)
L-Glutamine	3994 $\pm$ 958 (6)	3434 $\pm$ 515 (5)	3396 $\pm$ 845 (6)
L-Proline	231 $\pm$ 110 (6)	175 $\pm$ 71 (3)	391 $\pm$ 131 (6)*†
Glycine	1863 $\pm$ 333 (6)	1875 $\pm$ 292 (6)	1404 $\pm$ 336 (6)*†
L-Alanine	2918 $\pm$ 194 (5)	3008 $\pm$ 1196 (6)	2399 $\pm$ 50 (5)
L-Valine	240 $\pm$ 19 (5)	177 $\pm$ 38 (6)*	397 $\pm$ 41 (5)*†
L-Cystine	42 $\pm$ 20 (6)	40 $\pm$ 19 (6)	48 $\pm$ 11 (6)
L-Methionine	39 $\pm$ 12 (6)	33 $\pm$ 8 (6)	57 $\pm$ 20 (6)*†
L-Isoleucine	121 $\pm$ 21 (5)	97 $\pm$ 13 (6)	185 $\pm$ 9 (5)*†
L-Leucine	219 $\pm$ 25 (5)	170 $\pm$ 24 (6)*	310 $\pm$ 27 (5)*†
L-Tyrosine	124 $\pm$ 15 (5)	103 $\pm$ 11 (5)	129 $\pm$ 27 (6)
L-Phenylalanine	105 $\pm$ 16 (5)	96 $\pm$ 16 (6)	117 $\pm$ 19 (6)
L-Ornithine	150 $\pm$ 26 (6)	120 $\pm$ 45 (5)	204 $\pm$ 23 (6)*†
L-Lysine	762 $\pm$ 80 (5)	915 $\pm$ 332 (6)	1107 $\pm$ 247 (6)*
L-Histidine	640 $\pm$ 94 (6)	636 $\pm$ 107 (5)	630 $\pm$ 115 (5)
L-Arginine	36 $\pm$ 15 (6)	23 $\pm$ 6 (6)	28 $\pm$ 11 (5)

protein group than in the control group. Similar results were obtained in a previous study (Rémésy *et al.*, 1978). The concentrations of these amino acids, except for L-lysine, were also higher in the high-protein group than in the lactating group. The concentration of L-aspartic acid was significantly lower in the lactating than in the high-protein group.

Amino acid uptake by liver and peripheral tissues

The total amino acid uptake by liver was 16.7 ± 8.0 μ mol/min ($n = 5$) for control rats, 13.8 ± 5.6 μ mol/min ($n = 5$) for high-protein-diet rats, and 34.8 ± 18.2 μ mol/min ($n = 5$) for lactating rats ($P \leq 0.05$ for lactating versus control virgin or high-protein virgin rats).

The rate of hepatic amino acid uptake should not be considered as representative of a 24 h period. This is because the availability of amino acids in the portal vein plays a key role in the amount of amino acid taken up by the liver (Rémésy *et al.*, 1988), and this availability changes through the day. At the time of the experiment (11:00 h) the intake by virgin rats is almost nil, but lactating rats are still eating (Kimura *et al.*, 1970; Munday & Williamson, 1983). Therefore, the fact that hepatic amino acid availability in the lactating rats is higher at the time of the experiment explains the higher uptake of amino acids by their liver as compared with both groups of virgin rats.

All these conditions taken together with the increase in hepatic protein synthesis found in lactating rats (Table 5) can explain the

paradoxical finding of a high uptake of amino acids by liver of lactating rats and a low urea output by these rats.

On the other hand, the fact that the amino acid uptake by the liver in high-protein-diet rats is similar to the value found in control virgin rats and notably lower than in lactating rats could be explained because, as we have mentioned, the experiments were done in post-absorptive animals. It has been shown that glutamine is released by liver to a similar extent by rats fed on a high-carbohydrate diet or a high-protein diet during the post-absorptive state; however, when the portal glutamine was increased, glutamine uptake was higher in rats adapted to the high-protein diet than in rats fed on the high-carbohydrate diet (Rémésy *et al.*, 1988).

The higher amino acid uptake by liver of lactating rats as compared with control virgin rats is in accordance with the fact that glucose output (Burnol *et al.*, 1983), ureogenesis (Tables 2 and 3) and hepatic protein synthesis (Table 5) are increased during lactation.

We have found no significant changes in the release of total amino acids by small intestine in the three experimental groups; however, it was lower in lactating and in high-protein-diet virgin rats than in control rats. Arteriovenous differences across muscle showed a net release of total amino acids in lactating rats and a net uptake in the other two groups. High-protein-diet virgin rats showed a tendency to have a higher uptake as compared with control virgin rats. Amino acid uptake by mammary gland was similar, in our lactating rats fed on the liquid diet, to the value found previously by us (Viña *et al.*, 1987) in lactating rats fed on a solid diet (results not shown).

Physiological implications

Lactating and high-protein-diet virgin rats have a high glucagon/insulin ratio (Burnol *et al.*, 1983; Peret *et al.*, 1981) as compared with control virgin rats. This signal is responsible for the high glucose and urea output by liver of lactating and high-protein rats. However, the *N*-acetylglutamate concentration and urea production were higher in the high-protein group than in the lactating group. This can support the hypothesis that lactating rats spare amino acid from degradation, as compared with virgin rats fed on the same amount of protein. Therefore these amino acids can be used by the mammary gland for protein synthesis, to appear in the milk as free amino acid or as other nitrogen compounds, and by those tissues with increased protein synthesis during lactation. Our results show that the increased needs for amino acids during lactation are met by the hyperphagia and by some nitrogen-sparing mechanism.

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