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The hepatic amino acid system A transport activity, is up-regulated in obese Zucker rats

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The utilization of L-alanine by liver is dependent on amino acid uptake from blood. This uptake, mainly mediated by the A transport system, may be regulated by different nutritional and physiologic conditions. The regulation of this transport system by diets with different protein content was tested in lean and obese Zucker rats. High-protein (HP) and low-protein (LP) diets led to changes in the rats' growth patterns, especially in lean animals. However, homeostasis was relatively well maintained, as seen in plasma values, in spite of the increased urea production in the HP groups and increased triacylglycerides in the LP groups. The obese animals took up L-alanine at a higher rate than the lean animals. Obesity led to the emergence of a high-affinity component (K_M approximately 0.1–0.2 mM) in the transport system, which was not dependent on the protein content of the diet. This component has a 10-fold increase in affinity for L-alanine, but with an approximately 3- to 5-fold reduction in maximal velocity of transport. (J. Nutr. Biochem. 10:716–722, 1999)

Keywords: Zucker rats; hyperproteic diet; hypoproteic diet; L-alanine transport; isolated membrane vesicles; liver

Introduction

Obese Zucker rats are currently used as a model for human obesity because they demonstrate hyperphagia, hyperinsulinemia, and an efficient energy storage system.[1] They also have a more impaired thermogenic system[2] and lower muscular mass and protein content[3] than their lean counterparts, in contrast with the comparable total body protein shown by 60-day-old obese Zucker rats.[4,5] Amino acid metabolism in lean and obese animals differs, because in obese rats the catabolic utilization of individual amino acids increases.[6] The livers of obese animals vary in their capacity to manage nitrogen metabolism: Differences in ammonia uptake,[7] as well as individual amino acid uptake and regulation of specific catabolic enzymes, have been described.[8,9] The hyperphagia of these animals has been considered a key factor in increasing energy availability.[10] This specific feeding pattern and many other metabolic adaptations (such as insulin resistance and increased lipid deposition) are the consequences of their leptin receptors failing to function.[11] Thus, the considerable liver hypertrophy described in these animals[12] may influence the amino acid metabolism and, in consequence, affect their uptake rates. Thus, maintenance of K_M values and an increase in the V_{max} of different transport systems has been reported.[13] However, there is no information available regarding the pattern followed by these systems in the presence of variable chronic amino acid availability in the diet, in spite of the known tendency of diets that have a protein imbalance (high- or low-protein content diets) to decrease both food intake and growth,[14] which implies changes in protein metabolism.

This study sought to determine the influence of a chronic modification of amino acid availability in the diet on the modification of the Na^+-dependent L-alanine uptake pattern in liver, especially the possible up-regulation of the A system, in both lean and obese animals.

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Methods and materials

Animals and diets

Thirty-day-old male lean (Fa/?) and obese (fa/fa) Zucker rats were kept under controlled conditions (temperature 21–22°C; lights on from 8:00 AM to 8:00 PM; humidity 65–75%). The rats were bred at the Animal Service of the University of Barcelona from heterozygous stock obtained from Harlan (Gannat, France). The rats, which had ad libitum access to food and water, were fed for 30 days with either a standard diet (reference diet; RD), a high-protein content (HP) diet, or a low-protein content (LP) diet, and had free access to tap water. The diets were purchased from B&K (Sant Vicent del Horts, Spain) and Harlan Tekland (Madison, WI USA) and had the following composition. The RD diet (B&K) was composed of 2.5% lipid, 18% protein, and 67% digestible carbohydrate (21% of protein-derived energy); the HP diet (Harlan) was composed of 2.8% lipid, 34.5% protein, and 53% digestible carbohydrate (36% of protein-derived energy); and the LP diet (Harlan) was composed of 2.85% lipid, 9.2% protein, and 78% digestible carbohydrate (9.7% of protein-derived energy). All the diets were isocaloric, with a mean gross energy content of 16 kJ/g. Animals were maintained in individual cages and the daily energy consumption was measured.

Purification of plasma membranes

On day 30 of treatment, the animals were decapitated, their livers were immediately excised, and samples of blood were collected in heparinized tubes. All experiments took place at the beginning of the light cycle. Partial purification of plasma membrane vesicles was performed by a Percoll density gradient centrifugation described previously[15] and based on previous reports.[16] Protein level in homogenate and membrane preparations was determined.[17] The activity of 5′-nucleotidase (EC 3.1.3.5) was used as a plasma membrane marker and was measured in both homogenate and membrane preparations.[18] The level of contamination was assessed by measuring the activities of three enzyme markers of membranes belonging to other subcellular organelles as follows: cytochrome oxidase (EC 1.9.3.1)[19] as a mitochondrial marker, β-N-acetylglucosaminidase (EC 3.2.1.30)[20] as a lysosomal marker and glucose-6-phosphatase (EC 3.1.3.9)[21] as an endoplasmic reticulum marker. Assays performed on both homogenate and plasma membrane preparations enabled the recoveries and enrichments of all enzyme markers used to be calculated. No attempt was made to differentiate the plasmatic and canalicular membranes.

Transport assays

L-[2,3]-^{3}H-alanine transport by liver plasma membrane vesicle preparations was measured using a filtration procedure described previously.[15] Briefly, plasma membrane preparations were mixed with transport medium to give the following final concentrations: 0.25 M sucrose, 0.2 mM $CaCl_2$, 10 mM HEPES/KOH (pH 7.4), 10 mM $MgCl_2$, either 100 mM NaSCN or KSCN, and L-alanine at different concentrations depending on the characteristics of the experiment and adapting the specific radioactivity to each specific assay. Thus, the dependence of uptake rates on alanine concentration was measured as a function of increasing concentrations of amino acid, but corrected with sucrose to keep the osmolarity of the medium constant. At the desired time (5 sec), the transport reaction was stopped by adding 1 mL of ice-cold stop solution [0.25 M sucrose, 0.2 mM $CaCl_2$, 10 mM HEPES/KOH (pH 7.4), 10 mM $MgCl_2$, 100 mM NaCl], and the whole volume was rapidly filtered through a nitrocellulose filter (0.45 μm pore size; Schleicher and Schüll, Dassel, Germany) and washed with 4 mL of ice-cold stop solution. Radioactivity was counted on the filters. In all studies, the nonspecific binding to the membrane vesicle preparations was subtracted.

In the time-course uptake determinations (overshoot), a fixed concentration of 2 mM L-alanine was used. In the inhibition assays, the concentration of L-alanine was fixed to 1 mM and the various amino acids that can cause inhibition were added to the medium of assay at a 20 mM concentration. The time of assay was 5 seconds.

Plasma analytical determinations

Aliquots of blood were centrifuged and samples of plasma were obtained. Plasma parameters were determined by using standard procedures: glucose (MPR Kit, Boehringer Mannheim, Mannheim, Germany); urea (B-035 kit, Menarini, Firenze, Italy); creatinine (555 kit, Sigma, St. Louis, MO USA); and triacylglycerol (GPO liquolor, Human, Taunsstein, Germany). Alanine was measured by means of a high performance liquid chromatography (HPLC) system (Kontron, Milan, Italy) after precolumn derivatization with phenylisothiocyanate.[22]

Calculations and statistics

The experimental data were processed and fitted using the Fig P program (Fig P Software Corporation, Durham, NC USA), and the decomposition of decay pattern of Eadie-Hofstee plot was performed following the indications of Rosenthal[23] and Paul et al.[24]

All data are presented as group mean values ± SEM, derived from triplicate assays from six animals in each group. The differences caused by diet and by strain were determined by two-way analysis of variance (ANOVA), with a 95% confidence level for the *F*-test. Differences caused by diet were assessed using one-way ANOVA and the Duncan test.

Results

After 30 days of dietary treatment, obese animals had ingested more energy than lean rats: Specifically, lean animals ingested 7.75 ± 0.66 MJ (RD group), 7.12 ± 0.57 MJ (HP group), and 4.89 ± 0.36 MJ (LP diet), and the obese animals ingested 9.12 ± 0.79 MJ (RD group), 9.28 ± 0.81 MJ (HP group), and 10.15 ± 0.84 MJ (LP group). This difference in feeding pattern was not maintained in the efficiency of deposition: Animals in the lean groups expended 47 kJ for each gram deposited in the RD group, and the HP and LP groups expended 38 kJ/g and 53 kJ/g, respectively; obese animals expended 36 kJ/g , 44 kJ/g, and 48 kJ/g for RD, HP, and LP groups, respectively.

Lean animals fed the LP diet weighed less at the end of the study than the other groups. Obese animals weighed more than their lean counterparts (*Table 1*). Livers of both lean and obese rats weighed less in animals in the LP group, but when the results were expressed as a percentage of body weight, the differences between groups disappeared. Alanine concentration was different between the lean and obese groups, the main differences being that lean animals had higher values for both HP and LP groups. The plasma glucose levels varied little in lean animals, but obese HP and LP groups had lower values than the obese RD group. As expected, the HP group showed the highest urea values, in both lean and obese groups, with a significant difference between lean and obese animals. The obese animals had significantly higher triacylglycerol values than the lean animals, with the LP groups having significantly higher

Table 1 Body and liver weights and plasma parameters of Zucker lean and obese rats fed different protein content diets

	Lean			Obese		
	RD	HP	LP	RD	HP	LP
Body weight (g)	230 ± 5.7[a]	228 ± 4.57[a]	165 ± 5.34[b]	298 ± 8.17[a]	325 ± 11.5[a]	280 ± 11.4[a]
Liver weight (g)	10.9 ± 0.31[a]	10.6 ± 0.22[a]	7.21 ± 0.26[b]	15.8 ± 1.86[a]	15.3 ± 0.62[a]	13.4 ± 0.57[a]
% of body weight	4.74 ± 0.30[a]	4.66 ± 0.06[a]	4.36 ± 0.10[a]	5.31 ± 0.26[a]	4.71 ± 0.06[a]	4.59 ± 0.11[a]
Alanine (μM)	232 ± 18[a]	376 ± 28[b]	492 ± 44[b]	299 ± 25[a]	231 ± 32[a]	330 ± 29[a]
Glucose (mM)	6.87 ± 0.06[a]	7.02 ± 0.13[a]	6.82 ± 0.11[a]	7.99 ± 0.16[a]	6.38 ± 0.15[b]	6.97 ± 0.21[c]
Urea (mM)	5.97 ± 0.44[a]	11.1 ± 0.56[b]	4.78 ± 0.64[a]	9.51 ± 0.38[a]	13.1 ± 0.34[b]	5.01 ± 0.11[c]
Triacylglycerols (mM)	0.74 ± 0.04[a]	0.71 ± 0.18[a]	1.02 ± 0.09[b]	1.53 ± 0.18[a]	1.96 ± 0.11[ab]	2.37 ± 0.34[b]
Creatinine (μM)	86.8 ± 7.98[a]	87.3 ± 6.24[a]	62.8 ± 7.86[b]	81.6 ± 2.53[a]	111 ± 7.52[b]	135 ± 8.62[c]

All the values are the mean ± sem of six animals
Two-way analysis of variance (ANOVA). There were significant differences (95% confidence level) for **diet** (weight, $F = 21.2$; liver weight, $F = 17.3$; alanine, $F = 6.35$; glucose, $F = 7.87$; urea, $F = 133$; and triacylglycerols, $F = 6.32$) and for **strain** (weight, $F = 153$; liver weight, $F = 129$; alanine, $F = 5.21$; urea, $F = 24.9$; triacylglycerols, $F = 74.9$; and creatinine $F = 17.4$).
One-way ANOVA: In each strain, the values with different letters are significantly different ($P < 0.05$) using the Duncan test.
RD–reference diet. HP–high-protein diet. LP–low-protein diet.

triacylglycerol levels than the other groups. The creatinine levels were higher in obese than in lean groups, with the lean LP group showing the biggest differences from the RD group.

The percentage of recovery and relative specific activities (enrichment) of the marker enzymes were not different between groups (data not shown). Thus, the enrichments were 8- to 11-fold for 5′-nucleotidase. These values were higher than those of other markers (glucose-phosphatase, 1.11-–1.25-fold; N-acetyl-β-glucosaminidase, 0.17-–0.35-fold; and succinate dehydrogenase, 0.29-—0.51-fold). These values agreed with previous results from our laboratory.[15] According to these data, all preparations from the experimental groups showed similar low levels of contamination by other types of cell membranes.

Table 2 shows the percentages of inhibition of L-alanine uptake caused by different amino acids: The inhibitory effect of different amino acids at saturable concentrations on the uptake of 1 mM L-alanine. The inhibition of sodium-dependent uptake was determined as:

$$I = 100 - ([V_i^{Na} - V_i^{K}]/[N^{Na} - V^{K}]) \times 100 \qquad (1)$$

where I represents the percentage of inhibition caused, V^{Na} and V^{K} represent the uptake rate in the presence and absence of sodium, respectively, and V_i^{Na} and V_i^{K} represent the uptake rates measured in the presence of the competing amino acid. As expected, the inhibition of L-alanine uptake alone showed the highest values of inhibition, without significant variations between the lean and obese animals. The inhibition caused by methyl-aminoisobutyric acid (MeAIB) and Leu was higher in obese than in lean animals. Dietary treatment caused significant differences in the inhibition caused by MeAIB and Leu in lean animals and by MeAIB and Leu in obese animals.

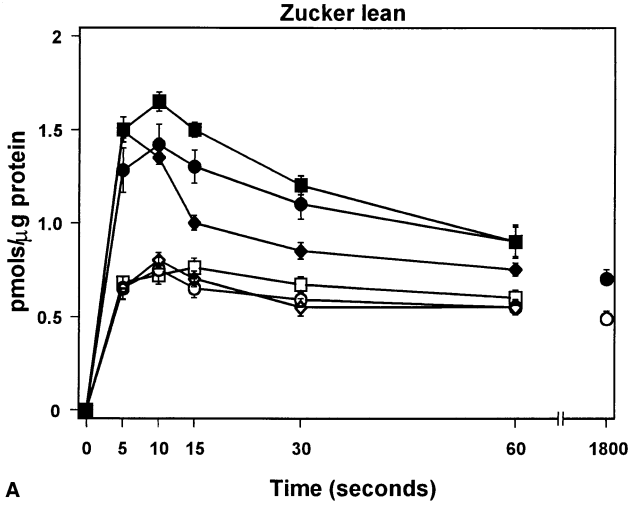
Figure 1 shows the L-alanine uptake responses in a time-dependent manner. As can be seen in the lean animals (*Figure 1A*), a higher uptake was observed in a Na^+-medium than in a K^+-medium, attaining the highest uptake rate at 5 seconds. The obese animals (*Figure 1B*) showed the same pattern as lean animals, but attained a higher response than lean animals.

L-alanine uptake followed the same pattern in all groups. Thus, the total transport in Na^+-rich medium was higher than the linear diffusion (plus the possible Na^+-independent transport) in the presence of K^+, and the deduced saturable Na^+-dependent transport was the result of the difference between total transport and diffusion (plus the possible Na^+-independent transport). *Figure 2* shows the Michaelis-

Table 2 Inhibition caused by various amino acids on L-alanine uptake by isolated liver membrane vesicles from lean and obese Zucker rats fed different protein content diets

	Lean			Obese		
	RD	HP	LP	RD	HP	LP
Alanine	86.5 ± 2.03[a]	90.1 ± 2.63[a]	91.1 ± 2.95[a]	93.6 ± 1.72[a]	88.4 ± 2.57[a]	85.3 ± 4.33[a]
MeAIB	74.3 ± 4.97[a]	67.6 ± 2.75[ab]	66.5 ± 1.25[b]	73.8 ± 2.54[a]	90.6 ± 3.08[b]	91.2 ± 3.12[b]
Leucine	40.4 ± 3.45[a]	44.6 ± 4.3[a]	61.6 ± 3.12[b]	65.9 ± 5.95[a]	59.8 ± 3.07[a]	50.5 ± 7.94[b]
Glutamine	49.9 ± 6.07[a]	44.1 ± 4.96[a]	65.2 ± 4.44[a]	55.9 ± 9.18[a]	64.1 ± 3.85[a]	61.5 ± 7.08[a]
Cysteine	61.1 ± 6.82[a]	54.6 ± 3.79[a]	53.7 ± 4.16[a]	60.4 ± 4.58[a]	51.4 ± 5.69[a]	72.6 ± 7.56[a]
Proline	62.6 ± 3.76[a]	70.9 ± 3.95[a]	66.6 ± 4.75[a]	61.5 ± 4.56[a]	70.2 ± 4.85[a]	72.4 ± 6.83[a]

Results indicate the percent of inhibition in the uptake. All the values are the mean ± SEM of triplicates from six animals.
Two-way analysis of variance (ANOVA). There were significant differences (95% confidence level) for **diet** (glutamine, $F = 5.24$) and for **strain** (leucine, $F = 7.24$; MeAIB 8,24).
One-way ANOVA: In each strain, values with different letters are significantly different ($P < 0.05$) using the Duncan test.
RD–reference diet. HP–high-protein diet. LP–low-protein diet. MeAIB–methyl-aminoisobutyric acid.



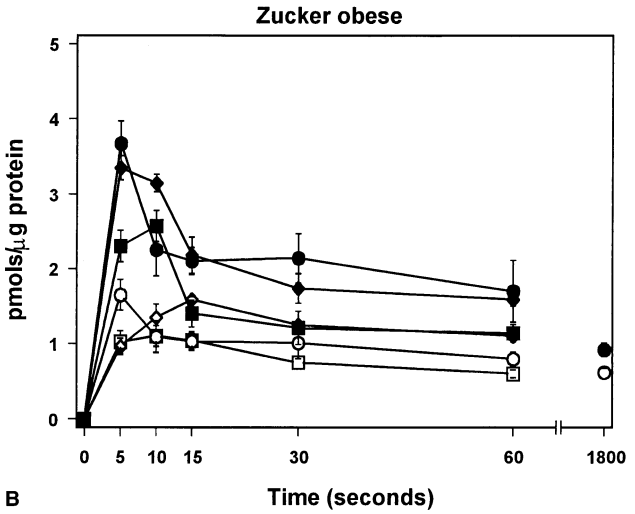
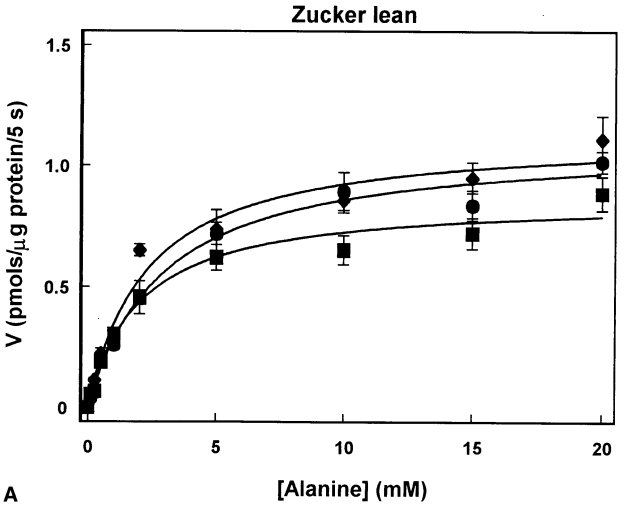


Figure 1 The uptake of L-alanine by membrane vesicles over time is shown. *(A)* The pattern of lean rats in a Na^+-rich medium (solid symbols) and in a K^+-rich medium. *(B)* The pattern of obese rats. ●○, RD group; ■□, HP group; ◆ ◇, LP group.



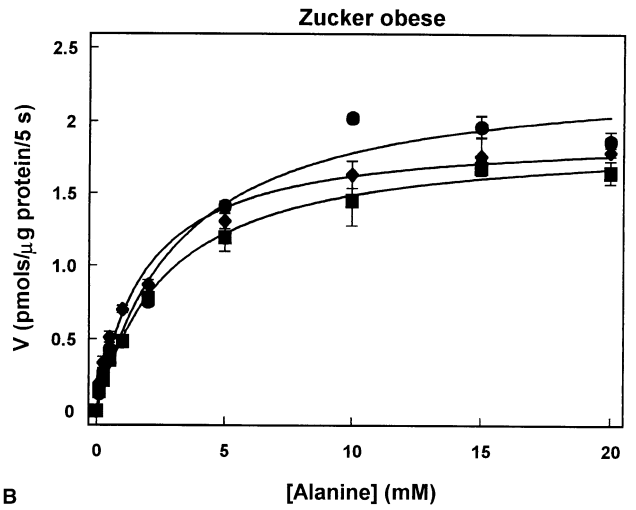


Figure 2 The Michaelis-Menten kinetics of Na^+-dependent L-alanine uptake in liver plasma membrane vesicles from lean rats *(A)* and obese rats *(B)*. ●, RD group; ■, HP group; ◆, LP group.

Menten representation of Na^+-dependent pattern followed by the lean groups (*Figure 2A*) and the obese groups (*Figure 2B*). All groups show a saturable pattern, with the obese groups having higher values than the lean groups.

Figure 3 shows two samples of the linearization of saturable Na^+-dependent transport. *Figure 3A* shows the Eadie-Hofstee plot of the lean HP group. In this case, as in all other lean groups, the linear representation correlated well ($r > 0.90$). Conversely, the same linearization in the obese groups had a hyperbolic pattern ($r > 0.93$), as can be seen in *Figure 3B*, which shows the plot of the obese LP group. The obese groups all had this pattern, which indicates the presence of a complex transport system, as indicated by the hyperbolic pattern. We then separated the curve into two linear components. Thus, in *Figure 3B*, the deduced linear components (a low-affinity/high-capacity component and a high-affinity/low-capacity component) also are shown.

Table 3 lists the values of the kinetic parameters of alanine transport, derived from either the Michaelis-Menten plot or Eadie-Hofstee plot. The values for lean groups are identical in both types of representation, with the HP group having lower values than the RD group in the maximal velocity of uptake (V_{max}) and in affinity (K_M). The lean LP group showed no variation in maximal uptake or in the affinity from the RD group. The values in the Michaelis-Menten plot showed a general increased uptake in the obese groups than in the lean groups, and tended to maintain their affinity (K_M) except in the HP group, which showed a decrease (increased value). The kinetic parameters of the obese groups are more complex, because the hyperbolic pattern of Eadie-Hofstee plot showed two different linear components: The first showed low capacity (velocity) and high affinity (low K_M values), whereas the second showed high capacity and low affinity. Thus, the first component

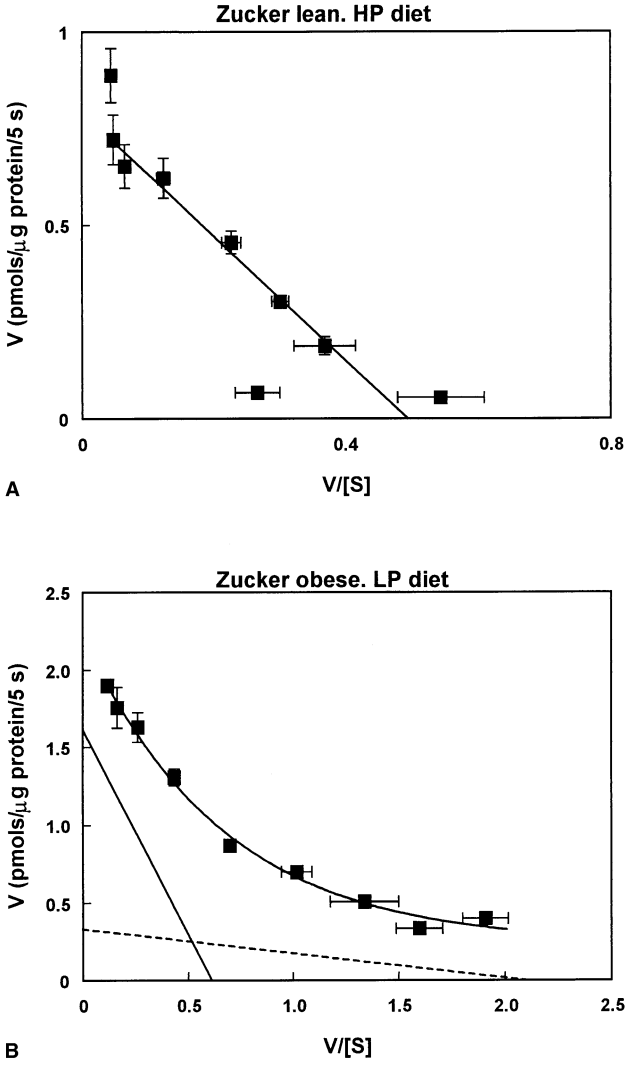


Figure 3 Effect of L-alanine concentration on L-alanine uptake into plasma membrane vesicles from livers of lean rats fed high-protein (HP) diet *(A)* and obese rats fed low-protein (LP) diet *(B)*. In obese animals, plots are broken down into a low-affinity/high-capacity component (solid line) and a high-affinity/low-capacity component (dashed line). The values were plotted according the values obtained after analyzing the data, as described in the text.

showed a significant increase in velocity in the LP group and a maintained affinity. These kinetic parameters are not in the same range as those of lean animals. The second component did not show variations in capacity, and the values are in the same range as those derived from Michaelis-Menten and were higher than those shown for lean animals. This component showed lower affinity in obese than in lean animals, although in the LP group it was significantly lower than in the RD group.

Discussion

The chronic dietary treatment produced a marked fall in the growth rate of the lean animals treated with the LP diet, a situation that has been described[25] and that represents an adaptation of these animals to a decreased availability of amino acids. However, it is interesting to note that although these animals' growth rates decreased, they maintained a good nutritional state, as shown by the maintenance of relative liver weight and the plasma values of glucose and urea. The low creatinine values are consistent with a low muscular catabolic rate and with the lower protein turnover described in these animals.[26] Conversely, the animals treated with HP diet showed, as a particular trend, higher plasma urea values, as expected from the increased catabolic utilization of amino acids due to their increased liver availability.[27] Furthermore, the efficiency of growth was similar (30–50 kJ/g) in the different groups; thus, the possible alteration caused by the possible hyperphagia in the obese group was minimized.

In obese animals, the variations were more pronounced because the animals maintained on the LP diet grew at the same rate as those fed the RD, whereas the animals fed the HP diet grew at a higher rate. The pattern followed by HP obese animals contrasted with the lower growth pattern followed by hyperprotein-fed lean animals,[27] due to increased energy utilization to avoid excessive amino acid concentrations. Furthermore, the catabolic use of amino acid was enhanced in this group, because their plasma urea levels are higher than the usually high levels shown by obese animals fed a standard diet.[28]

The maintenance of the relative weight of the liver does not justify the hepatomegaly described in obese animals,[12] where the increase in protein content (but not DNA) can support a hyperplasic process.[13] The results suggest that the variation in the amount of protein in the diet does not affect either the normal pattern of growth of the liver in Zucker fa/fa rats or the normal metabolic pattern derived from these livers, because their ability to synthesize urea seems normal. Furthermore, the enhanced plasma triacylglycerol levels in the obese rats given a LP diet, similar to lean animals, could be a consequence of an enhanced capacity of the liver to produce triacylglycerols from carbohydrates, which are in excess in obese animals.

The lack of significant differences in the enrichment values of enzymatic markers, either in lean or obese groups, indicates that the preparations obtained are fully comparable with those reported previously.[29] Furthermore, all groups show a comparable pattern of L-alanine uptake over time. Thus, variations in the kinetic parameters are caused only by the modifications generated by the adaptation to different protein content in the diet. The fact that all the lean groups have nearly the same values for affinity and capacity with both the Michaelis-Menten kinetic and the Eadie-Hofstee plot indicates the suitability of this saturable transport. Furthermore, the uptake data obtained in lean and obese animals fed the RD are in agreement with previously described results.[13]

Alanine is taken up from the bloodstream by different transport systems, although the A system predominates.[30] The values obtained in the inhibition assays of this study confirm the role of the A system as main transport entity, in spite of the possible participation of other systems. Substrate availability and the passage through the membrane are the critical steps in the development of hepatocytes; thus, amino acid uptake has been considered the limiting factor for amino acid utilization[31,32] and depends not only on the

Table 3 Kinetic parameters: V_{max} and K_M values for Na^+-dependent L-alanine uptake by isolated liver membrane vesicles from animals fed different protein content diets

	Lean			Obese		
	RD	HP	LP	RD	HP	LP
V_{max} (pmols/μg protein/5 s)						
Michaelis-Menten	1.11 ± 0.05[a]	0.86 ± 0.04[b]	1.14 ± 0.06[a]	2.36 ± 0.16[a]	1.89 ± 0.05[b]	1.92 ± 0.07[b]
Eadie-Hosfstee	1.05 ± 0.22[a]	0.79 ± 0.08[a]	1.08 ± 0.09[a]			
Component 1				0.19 ± 0.02[a]	0.22 ± 0.03[a]	0.33 ± 0.02[b]
Component 2				2.32 ± 0.31[a]	2.00 ± 0.19[a]	1.62 ± 0.21[b]
K_M (mM)						
Michaelis-Menten	2.88 ± 0.42[a]	1.97 ± 0.37[b]	2.28 ± 0.48[a]	3.30 ± 0.73[a]	2.76 ± 0.23[a]	1.87 ± 0.28[b]
Eadie-Hofstee	2.69 ± 0.40[a]	1.59 ± 0.29[b]	2.05 ± 0.29[ab]			
Component 1				0.12 ± 0.01[a]	0.13 ± 0.02[a]	0.16 ± 0.02[a]
Component 2				4.79 ± 0.38[a]	5.00 ± 0.44[a]	2.63 ± 0.28[b]

All the values are the mean ± SEM of triplicates from six animals. Component 1 (high affinity and low capacity) and component 2 (low affinity and high capacity) are values deduced from Eadie-Hofstee plot.
Two-way analysis of variance (ANOVA): There were significant differences (95% confidence level) for **diet** ($F = 4.24$) and **strain** ($F = 7.24$) for Michaelis-Menten V_{max} values and for **diet** ($F = 4.97$) and **strain** ($F = 4.69$) for K_M values.
One-way ANOVA: In each strain, the values with different letters are significantly different ($P < 0.05$) using the Duncan test.
RD–reference diet. HP–high-protein diet. LP–low-protein diet.

activity of the transport entities but on blood amino acid availability. The fact that amino acid concentration is maintained in a narrow range of concentrations in plasma of rats given diets of variable protein content,[33] which is confirmed by the increase detected, suggests that the availability of alanine is not a factor limiting the rate of uptake, but that it will be the chronic effect of the dietary treatment that causes the possible differences in uptake.

The A system is located in the basolateral and canalicular membranes of hepatocytes,[34] is regulated by several hormones,[35] and is sensitive to amino acid deprivation (adaptive regulation).[36] It also is regulated chronically in different metabolic situations such as pregnancy and lactation.[35] Any interference by protein malnutrition with the ability of insulin and glucagon to up-regulate the A system[37] is not detectable in lean animals, because the LP group does not vary the maximal uptake rate in spite of the slight increase in affinity. The possible participation of the ASC transport system in the L-alanine uptake in lean rats seems unlikely, because the inhibition capacity of MeAIB was markedly lower than had been shown in previous reports.[13] The increase in protein in the diet leads to an increase in the amino acid flux to liver,[32] which allows a generalized increase in the uptake by the liver under in vivo conditions[38] as a consequence of the coordinate interaction of uptake and cellular metabolism. In this case, the general increase in plasma alanine concentration in lean HP rats increases the availability of alanine to liver, which leads to an increased affinity to transporter that is counterbalanced by the decrease in the capacity of the system. The increase in the alanine (and other amino acids) uptake was responsible for greater urea production in these animals. This adaptation contrasts with the lack of response in LP animals that have higher plasma alanine levels and may be higher alanine availability than the RD group. This slow amino acid catabolism does not imply a limited supply for lipoprotein synthesis, because the increased triacylglycerol levels must be produced in the liver and released into very low density lipoprotein (VLDL), with the corresponding apoprotein fraction.

The livers of obese Zucker rats were able to take up twice as much alanine from the bloodstream as the livers of lean rats,[8] in part due to the increased availability of alanine. The same ability has been shown in in vitro conditions, because isolated vesicles show the same pattern.[13] Nitrogen (amino acid) metabolism in the livers of obese animals is noticeably different than in livers of lean animals. Thus, obese animals take up less ammonium,[7] which can be related to an increased mRNA of carbamoyl-P-synthetase,[9] perhaps as a response to increased levels of insulin. However, the greater V_{max} of the Na^+-dependent alanine transport in obese animals (by a factor of 2 versus lean animals), in accordance with published data,[13] suggests that this transport mediates the increased uptake shown in vivo. The inhibition data suggests that most mediated transport is due to the A system, although ASC can play a similar role in the obese animals, especially in rats fed a no standard protein content diet.

This up-regulation of alanine transport is associated with the emergence of a high-affinity component of the transport, similar to what has been described in other assays with hyperproteic diets.[39] Most striking is that the emergence of this transport component seems unrelated to the presence of excess protein in the diet, because it appears in all obese groups, including the RD group. Emergence of this component has not been previously described in obese Zucker rats. Thus, the HP group is able to take up and oxidize a greater amount of amino acids, as can be deduced from the high urea levels in plasma, in spite of their possible utilization for lipid synthesis[40] and in spite of the impaired release of VLDL by Zucker rats fed a hyperproteic diet.[41]

These data lead us to conclude that an increase in protein in the diet elicits an increase in alanine utilization by the liver by decreasing the affinity of the transport system. In obese rats, there is an up-regulation of system A (the emergence of a high-affinity component) as the result of the

development of obesity. The ingestion of different amounts of protein in the diet slightly modulates this emergence.

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