Amino acid uptake by liver of genetically obese Zucker rats

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Alanine and glutamine uptake by the liver of 50–52-day-old genetically obese Zucker rats and their lean littermates has been studied. The net uptake *in vivo* of L-alanine is 2-fold higher in the obese animals. No significant change in L-glutamine net balance was found. We also studied the Na⁺-dependent uptake of L-alanine and L-glutamine into plasma-membrane vesicles isolated from either obese- or lean-rat livers. $V_{\max}$ values of both L-alanine and L-glutamine transport were 2-fold higher in those preparations from obese rats. No change in K_m was observed. As suggested by inhibition studies, this seemed to be mediated by an enhancement of the activities of systems A, ASC and N. We conclude that the liver of the obese Zucker rat is extremely efficient in taking up neutral amino acids from the afferent blood, which results in an enhanced net uptake of L-alanine *in vivo*. The changes in transport activities at the plasma-membrane level might contribute to increase amino acid disposal by liver, probably for lipogenic purposes, as recently reported by Terrettaz & Jeanrenaud [Biochem. J. (1990) 270, 803–807].

INTRODUCTION

The Zucker rat has been extensively used as a model for human obesity and other associated metabolic disorders, such as type II non-insulin-dependent diabetes mellitus (Argilés, 1989). During week 4 of life the *fa/fa* Zucker rat develops insulin resistance in brown adipose tissue and skeletal muscle, but not in white adipose tissue (Pénicaud *et al.*, 1987), thus leading to accumulation of fat and changes in the animal morphology. However, no evidence for insulin resistance at the hepatic level has been provided so far, although it might appear later in older animals (Terrettaz & Jeanrenaud, 1983). The liver of the obese Zucker rat shows a net protein accretion when animals are fed *ad libitum*, as compared with their lean littermates. Indeed, this may result in a higher liver mass, irrespective of the known steatosis appearing later (Cleary *et al.*, 1980; Lin, 1985; Martin *et al.*, 1986). This apparent growth can be mediated by both hypertrophic and hyperplastic means, and it has been shown that in 14-month-old obese Zucker rats the liver DNA content is higher than in their lean littermates (Greenwood *et al.*, 1982). This obviously suggests that liver mass will be increasing during the development of the obese phenotype, not only because of protein accretion or lipid accumulation, but also because of hyperplasia.

Physiological situations leading to hyperphagia and liver hypertrophy and hyperplasia, such as gestation and lactation (Remesar *et al.*, 1981), are characterized by a high efficiency of the liver in extracting amino acids from the blood (Casado *et al.*, 1987*a,b*). These features, at least during pregnancy, are also coincident with a state of hyperinsulinaemia and insulin resistance (Leturque *et al.*, 1984).

The aim of the present work was to study whether the liver of obese animals shows a higher efficiency in extracting amino acids from blood and whether this could be mediated by stable changes in the transport capacities at the plasma-membrane level.

MATERIALS AND METHODS

Animals

Obese Zucker rats (50–52 days old) were used. Development of the obese phenotype was easily detected at this age by evident changes in the animal morphology. Their lean littermates were

used as controls in all the experiments performed. Rats were purchased from the Laboratory Animal Service of the University of Barcelona, where they had been kept under controlled conditions of temperature (22 ± 2 °C), humidity (40–60 %) and light (12 h on/12 h off). All experiments were carried out 3 h after the beginning of the light cycle (near post-absorptive conditions).

Experiments *in vivo*: amino acid uptake by liver of anaesthetized obese and lean Zucker rats

All experiments were performed on rats that had been previously anaesthetized by an intraperitoneal pentobarbital injection (60 mg/kg body wt.). Amino acid availability, net uptake and relative fractional extraction were calculated as previously reported (Casado *et al.*, 1987*a*) from the measurements on afferent and efferent blood alanine, glutamine and glutamate levels and liver blood flows. This method has been previously validated in our laboratory (Casado *et al.*, 1987*a,b,c*; Pastor-Anglada *et al.*, 1987; Felipe *et al.*, 1991) and involves a rapid sampling of blood reaching and leaving the organ, to be used for estimates of amino acid concentrations. Blood flows were measured by an indicator-dilution method, based on that reported by Katz & Bergman (1969), and previously adapted in our laboratory for small mammals (Casado *et al.*, 1987*a*). Glutamine, glutamate and alanine blood concentrations were measured by enzymic spectrophotometric techniques (Lund, 1986; Williamson, 1986).

Liver DNA and protein and glutaminase and glutamine synthetase activities

Another set of animals was used in order to measure liver DNA (Burton, 1956) and protein (Peterson, 1979), by classical and well-validated spectrophotometric methods. Phosphate-dependent glutaminase and glutamine synthetase activities were also determined as described by Joseph & McGivan (1978) and Arola *et al.* (1981) respectively, as reported in Casado *et al.* (1988).

Preparation of plasma-membrane vesicles

A third set of animals was used to study the kinetic parameters of amino acid transport across the plasma membrane. Plasma-membrane vesicles from rat liver were purified by a Percoll-

Abbreviation used: MeAIB, *N*-methylaminoisobutyrate.

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density-gradient method, as previously described by Pastor-Anglada *et al.* (1987). These preparations were assayed for protein (Peterson, 1979) and other enzyme markers to assess the degree of purification of plasma-membrane fractions and the contamination by other subcellular membranes. 5'-Nucleotidase (EC 3.1.3.5) activity was used as a plasma-membrane marker and was measured as described by Aronson & Touster (1974) by determining the amount of P_i liberated during the incubation time by using the vanadomolybdate reagent of Lin & Morales (1977). Glucose-6-phosphatase (EC 3.1.3.9), *N*-acetyl- β -glucosaminidase (EC 3.2.1.30) and succinate dehydrogenase (EC 1.3.99.1), used as markers of endoplasmic reticulum, lysosomal and mitochondrial membranes respectively, were measured as described by Baginski *et al.* (1974), Carroll (1978) and Bonner (1955) respectively.

Uptake measurements

Amino acid transport into the inner vesicular space was measured by a filtration procedure adapted from Sips *et al.* (1980) and described extensively in Pastor-Anglada *et al.* (1987). Basically, the incubation medium consisted of 0.25 M-sucrose, 0.2 mM- $CaCl_2$, 10 mM- $MgCl_2$, 10 mM-Hepes/KOH, pH 7.4, 100 mM-NaSCN or -KSCN and the amino acid of interest, whether L-[2,3- 3H]alanine or L-[G- 3H]glutamine (Amersham), at different concentrations depending on the characteristics of each experiment and monitoring and adapting the specific radioactivity of the substrate to the aim of every specific assay. Thus the dependence of uptake rates on substrate concentration was measured as a function of increasing concentrations of the amino acid but corrected with sucrose to keep constant the osmolarity of the medium. Other details of uptake measurements are discussed in the Results section.

Calculation of the kinetic parameters

Kinetic constants of the Na^+ -dependent uptake of alanine and glutamine were derived mathematically from the experimental data by regression analysis using Enzfitter software (Elsevier Biosoft, Cambridge, U.K.). Kinetic analysis was always done in triplicate on plasma-membrane fractions from four single purifications. This means that the results are means $\pm$ S.E.M. of the kinetic parameters calculated for every rat liver.

RESULTS

Animals

Body and liver weights as well as protein and DNA content of lean and obese Zucker rats are shown in Table 1. Obese animals showed a higher body weight and liver mass than their lean littermates. The increase in liver weight might be partially due to net protein accretion. No hyperplasia seems to occur at this stage, as deduced from the unaltered DNA levels observed in obese rats as compared with their lean littermates.

Alanine and glutamine liver balances in obese rats

The data regarding alanine and glutamine availabilities to the liver and balances are shown in Table 2. Blood flows used to calculate these parameters were: hepatic vein, 24.7 ± 1.8 and 29.3 ± 1.3 ml/min, and portal vein, 14.1 ± 1.1 and 15.1 ± 1.4 ml/min, for lean and obese Zucker rats respectively. Alanine levels in the portal vein are higher in obese than in lean animals; this results in an increased availability of this amino acid to the liver. The net hepatic balance is also greater in the obese Zucker rats, as is the fractional extraction, which is double that found in their lean littermates. However, no changes in either liver glutamine availability or uptake were found in the obese pheno-

Table 1. Body and liver weights and hepatic protein and DNA contents

Body and liver weights are means $\pm$ S.E.M. of measurements made on all the animals used in the different sets of experiments performed (16–20). Protein and DNA contents were determined in 8 lean and 8 obese Zucker rats. Statistics: obese versus lean, $**P < 0.01$, $***P < 0.001$ (Student's *t* test).

	Lean	Obese
Body wt. (g)	186 ± 5	$243 \pm 6^{***}$
Liver wt. (g)	8.8 ± 0.3	$12.0 \pm 0.5^{***}$
(g/100 g body wt.)	5.0 ± 0.4	5.1 ± 0.3
Liver protein (mg)	1852 ± 79	$2318 \pm 119^{**}$
(mg/g of liver)	208 ± 10	189 ± 14
DNA (mg/liver)	13.8 ± 0.6	15.8 ± 1.2

Table 2. L-Alanine and L-glutamine concentrations in blood and net uptake by liver of lean and obese Zucker rats

L-Alanine and L-glutamine net uptake and fractional extraction rates were calculated from the afferent and efferent blood concentrations of these amino acids. The results are means $\pm$ S.E.M. of observations made in eight animals from each physiological condition. Statistics: obese versus lean, $*P < 0.05$, $**P < 0.01$ (Student's *t* test).

	Lean	Obese
L-Alanine		
Concn. (μM)		
Hepatic vein	311 ± 13	325 ± 7
Portal vein	391 ± 49	$537 \pm 29^*$
Aorta	329 ± 5	370 ± 24
Availability ($\mu mol/min$)	9.0 ± 0.7	$13.4 \pm 0.5^{**}$
Balance ($\mu mol/min$)	1.33 ± 0.6	$3.84 \pm 0.4^*$
Fractional extraction (%)	13.9 ± 5.4	$28.4 \pm 2.2^*$
L-Glutamine		
Concn. (μM)		
Hepatic vein	475 ± 46	391 ± 18
Portal vein	355 ± 31	403 ± 25
Aorta	502 ± 23	432 ± 24
Availability ($\mu mol/min$)	10.7 ± 1.4	12.3 ± 0.2
Balance ($\mu mol/min$)	-1.93 ± 1.30	1.50 ± 1.10
Fractional extraction (%)	-26.5 ± 16.2	10.3 ± 8.3

type. The same pattern was found for glutamate balance (results not shown). However, a certain tendency to retain glutamine and glutamate by the liver of obese animals was found.

Characterization of the plasma-membrane fractions

Before the analysis of alanine transport into our plasma-membrane preparations, we performed a characterization of our plasma-membrane fractions, on the basis of their contamination by other subcellular membranes and the presence of an osmotic-sensitive space and, hence, the apparent vesicular volume. Our procedure yielded a high enrichment (7.7 ± 1.3 - and 8.1 ± 1.0 -fold for lean and obese rats respectively) and recovery (23 ± 6 and $24 \pm 5\%$ for lean and obese rats respectively) of 5'-nucleotidase activity and very low enrichments of *N*-acetyl- β -glucosaminidase

and succinate dehydrogenase (results not shown). Some enrichment in glucose-6-phosphatase was also observed, but it was extremely low (about 1.3–1.4-fold) and in any case equal in both experimental groups, lean and obese. Furthermore, our plasma-membrane fractions were vesiculated, because we were able to detect an osmotic-sensitive space in our preparations, as deduced from the evidence that osmolarity of the medium has an inverse relationship with intravesicular alanine content at equilibrium (results not shown). Preparations from both lean and obese animals behave in a similar way in relation to the changing osmolarity; thus equal values of unspecific binding can be calculated from the intercept with the x axis. Furthermore, from the alanine content at the equilibrium in iso-osmotic conditions, we could derive the apparent vesicular volume, which was 228 ± 9 and 219 ± 13 nl/mg of protein ($n = 4$) for the lean- and obese-rat preparations respectively. Thus our plasma-membrane preparations from lean- and obese-rat livers do not differ in their physico-chemical properties.

Alanine uptake into plasma-membrane vesicles

The time course of alanine transport into plasma-membrane vesicles from either lean or obese Zucker rats is shown in Fig. 1(a). All measurements were made under zero-*trans* conditions, with either 100 mM-NaSCN or 100 mM-KSCN in the medium. Neither of these thiocyanates was present in the intravesicular space. L-Alanine uptake was clearly concentrative in the Na⁺ medium, but the overshoot reached a higher level in those preparations from obese (4–5-fold) than in those from lean (2–3-fold) -rat livers. Uptake in the K⁺ medium was identical for both experimental conditions. Kinetic measurements of alanine transport were made in either Na⁺ or K⁺ medium in a range of alanine concentrations of 0.1–20 mM. In the absence of Na⁺ (K⁺ medium), the uptake rates showed a linear relationship with the substrate concentrations (Fig. 1b), giving identical K_d values for both experimental groups (0.153 s^{-1}). This component of transport might be assumed to be simple diffusion across the plasma membrane, because no saturability was observed. After subtracting this component from the measurements made in the Na⁺ medium, we obtained a saturable component, which was further analysed for $V_{\max}$ and K_m values (Fig. 1b). The Na⁺-dependent component of L-alanine transport across the plasma membrane showed a higher $V_{\max}$ in those preparations from obese rats than in those from their lean littermates (0.57 ± 0.05 and $1.01 \pm 0.08 \text{ pmol/5 s per } \mu\text{g}$ of protein for lean- and obese-rat preparations respectively; $P < 0.01$). No changes in K_m were observed (2.1 ± 0.5 and $2.0 \pm 0.4 \text{ mM}$ for lean and obese-rat preparations respectively). To characterize further the role of both systems A and ASC in mediating the enhancement of L-alanine transport in rat liver, we used the amino acid analogue N-methylaminoisobutyric acid (MeAIB) to inhibit selectively the A component. This was done up to 5 mM-alanine. We did not feel confident with the data obtained beyond this substrate concentration, because in order to obtain effective inhibition of the A system we were forced to use high amounts of MeAIB, which seriously affected the osmotic environment of the vesicles even if sucrose was added as a control to compensate for MeAIB levels. The results that we obtained are shown in Table 3. Basically, our data suggest that both systems A and ASC are responsible for the enhancement of Na⁺-dependent L-alanine uptake by the liver of obese Zucker rats.

Glutamine uptake into plasma-membrane vesicles

To analyse whether the obese phenotype, affecting the activities of systems A and ASC, could also modify other Na⁺-dependent transport systems relevant to liver metabolism, we undertook the

Fig. 1. L-Alanine transport into plasma-membrane vesicles from rat liver

(a) The time course of 0.2 mM-L-alanine uptake was monitored on pooled plasma-membrane vesicles from four individual preparations from either obese (○, □) or lean (●, ■) rats. The measurements were done in triplicate either in a KSCN (□, ■) or in a NaSCN (○, ●) medium. (b) L-Alanine uptake as a function of substrate concentration was monitored on plasma-membrane vesicles from four individual preparations from either obese (○, □) or lean (●, ■) rats. Vesicles were incubated in the presence of increasing concentrations of L-alanine, in either a KSCN (□, ■) or a NaSCN (○, ●) medium. The substrate-concentration-dependence of the Na⁺-dependent L-alanine uptake is shown in the inset (△, obese; ▲, lean). The S.E.M. values were not greater than 13% of the means of 4 observations at each point.

analysis of N-system activity in our plasma-membrane preparations. Thus we studied L-glutamine transport, because we and others have previously shown that L-glutamine uptake into plasma-membrane vesicles from rat liver mainly occurs through system N (Leonardi *et al.*, 1988; Ruiz *et al.*, 1990). When monitoring the time course in a Na⁺ medium of L-glutamine uptake into the plasma-membrane preparations from both ex-

Table 3. MeAIB-sensitive and MeAIB-insensitive Na⁺-dependent L-alanine uptake into plasma-membrane vesicles from lean and obese Zucker rats

Plasma-membrane vesicles from rat liver were purified as described in the Materials and methods section. The preparations were incubated in the presence of different L-alanine concentrations, in the presence or absence of saturating concentrations of MeAIB. The results (pmol/5 s per mg of protein) are means $\pm$ S.E.M. of observations made in triplicate on four independent vesicle preparations from either lean or obese Zucker rats. Statistics: obese versus lean, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Student's t test).

[L-Alanine] (mM)	Na ⁺ -dependent L-alanine uptake		MeAIB-sensitive uptake		MeAIB-insensitive uptake	
	Lean	Obese	Lean	Obese	Lean	Obese
0.1	34 $\pm$ 5	64 $\pm$ 4**	26 $\pm$ 5	50 $\pm$ 5*	8 $\pm$ 3	18 $\pm$ 2*
0.25	58 $\pm$ 9	149 $\pm$ 14**	34 $\pm$ 4	105 $\pm$ 11***	24 $\pm$ 6	44 $\pm$ 4*
0.5	154 $\pm$ 33	241 $\pm$ 6*	94 $\pm$ 32	149 $\pm$ 7	60 $\pm$ 15	92 $\pm$ 7
1	178 $\pm$ 32	351 $\pm$ 30**	130 $\pm$ 23	173 $\pm$ 5	48 $\pm$ 19	178 $\pm$ 28**
2	231 $\pm$ 60	453 $\pm$ 41*	134 $\pm$ 33	220 $\pm$ 19	97 $\pm$ 50	233 $\pm$ 24*
5	419 $\pm$ 64	764 $\pm$ 80*	232 $\pm$ 68	495 $\pm$ 91	187 $\pm$ 25	269 $\pm$ 87

perimental conditions, we observed that this was also concentrative (Fig. 2a) and the overshoot was higher in plasma-membrane vesicles from obese rats than in those from their lean littermates. As for alanine, we analysed the substrate-dependency of glutamine transport in either K⁺ or Na⁺ medium, in a range of substrate concentrations of 0.1–5 mM. In the K⁺ medium, uptake showed a linear relationship to L-glutamine concentrations in the incubation medium (Fig. 2b). This gave a K_d of about 0.14 s⁻¹, which was equal for both experimental conditions. This component of transport could probably correspond to simple diffusion. When we subtracted this from the uptake rates measured in a Na⁺ medium, we obtained a saturable component of transport (Fig. 2b) which was further analysed for kinetic parameters. In full coincidence with what we reported above for the Na⁺-dependent L-alanine-transport capacities, the V_{max} of the Na⁺-dependent component of L-glutamine transport was 2-fold higher in those preparations from obese animals than in those from their lean littermates (0.25 $\pm$ 0.01 versus 0.12 $\pm$ 0.02 pmol/5 s per μ g of protein; $P < 0.01$). No changes in K_m were observed (0.5 $\pm$ 0.1 versus 0.7 $\pm$ 0.2 mM for obese- and lean-rat preparations respectively). Unfortunately, we were unable to use concentrations greater than 5 mM, because the contribution of the non-saturable component to the overall glutamine uptake was too high and no reliable calculations of the Na⁺-dependent agency could be done. Indeed, at 5 mM-glutamine, about 90% of the uptake occurs through the non-saturable component.

Enzymes of glutamine metabolism

In an attempt to determine whether the enhanced glutamine-transport activity correlates with an induction of glutamine metabolism, we measured glutaminase and glutamine synthetase activities. Both enzymes showed a significant induction in obese rats, but only when the activities were expressed on a total-liver-weight basis: glutamine synthetase, 207 $\pm$ 8 and 270 $\pm$ 23 μ mol/min for lean and obese rats respectively, $P < 0.05$ ($n = 8$); glutaminase, 7.6 $\pm$ 1.5 and 14.4 $\pm$ 1.6 μ mol/min for lean and obese rats respectively, $P < 0.01$ ($n = 8$). Nevertheless, the ratio glutaminase/glutamine synthetase was not significantly altered in the obese phenotype.

DISCUSSION

Amino acid uptake by liver

In this work, we show that the obese Zucker rats can take up appreciable amounts of alanine from the afferent circulation. It

is clear from our results that this increase in net alanine uptake cannot be explained solely by changes in substrate availability, but more importantly by marked changes in the fractional extraction rates. This parameter can be understood as an index of the liver's ability to take up this amino acid irrespective of its blood concentration. As for glutamine, it is not surprising that we failed to detect any significant change in glutamine balance across the liver of obese animals. In fact, in most physiological situations so far studied, glutamine balance is rarely different from zero (Aikawa *et al.*, 1973; Révész *et al.*, 1983; Casado *et al.*, 1987a,b). This might be mainly attributable to two facts. First, a likely low fractional extraction rate might be enough to provide glutamine to the liver cells, because the afferent concentrations of this amino acid are the highest of all the amino acids. Second, the occurrence of a glutamine intercellular cycle in the liver, based on the known zonation of the glutamine-metabolizing enzymes (Haussinger, 1983, 1987, 1989), might explain the lack of a net hepatic balance of this amino acid, which obviously does not mean that a unidirectional influx of glutamine is not taking place at the same time. In fact, acceleration of glutamine metabolism in the liver might be recognized by measuring glutaminase/glutamine synthetase ratios. As suggested by Haussinger (1987) and recently reviewed by Meijer *et al.* (1990), a change in this ratio could determine the net glutamine balance, as reported previously for suckling rats (Casado *et al.*, 1988). However, an altered value accompanied by similar increases in both activities could be interpreted as an index of a high rate of glutamine cycling in the liver. In the obese Zucker rats the potential capacity for glutamine metabolism does not seem to be markedly increased, because glutaminase activity is significantly enhanced only when expressed on a total-liver-weight basis. However, glutaminase might be differentially activated *in vivo* by availability of ammonia (its allosteric activator) (McGivan & Bradford, 1983; Verhoeven *et al.*, 1983). The metabolic fate of these amino acids is of interest, but beyond the scope of the present contribution. Nevertheless, a recent report by Terrettaz & Jeanrenaud (1990) provides striking evidence supporting an important role for alanine as a lipogenic precursor in liver of obese Zucker rats.

Amino acid uptake by plasma-membrane vesicles from obese and lean Zucker rats

Different physiological situations associated with increased amino acid uptake by liver *in vivo*, such as mid- and late-pregnancy (Casado *et al.*, 1987a; Pastor-Anglada *et al.*, 1987, 1990) and the adaptation to a high-protein diet (Révész *et al.*, 1978), are characterized by stable changes in the Na⁺-dependent

Fig. 2. L-Glutamine transport into plasma-membrane vesicles from rat liver

(a) The time course of 0.2 mM-L-glutamine uptake was monitored on pooled plasma-membrane vesicles from four individual preparations from either obese (○, □) or lean (●, ■) rats. The measurements were done in triplicate either in a KSCN (□, ■) or in a NaSCN (○, ●) medium. (b) L-Glutamine uptake as a function of substrate concentration was monitored on plasma-membrane vesicles from four individual preparations from either obese (○, □) or lean (●, ■) rats. Vesicles were incubated in the presence of increasing concentrations of L-glutamine, in either a KSCN (□, ■) or a NaSCN (○, ●) medium. The substrate-concentration-dependence of the Na⁺-dependent L-glutamine uptake is shown in the inset (△, obese; ▲, lean). The S.E.M. values were not greater than 6% of the means of 4 observations at each point.

the enhanced alanine uptake by liver found *in vivo*. This might be the case, given that alanine transport across the plasma membrane of the hepatocyte seems to be a limiting step for intracellular alanine metabolism in the liver (Groen *et al.*, 1982). Effective inhibition of alanine uptake by saturating concentrations of MeAIB might be a good way to study the contribution of both systems A and ASC (Christensen *et al.*, 1965; Kilberg, 1982) to the overall alanine uptake by the liver of the obese animals. Thus we show that both Na⁺-dependent transport systems are contributing to this change, as has been previously reported to occur under the physiological conditions cited above.

The $V_{\max}$ of glutamine transport is increased in the obese animals to the same extent as alanine uptake. Because glutamine transport occurs mainly through system N in our plasma-membrane preparations, we can conclude that the three systems basically involved in neutral amino acid transport by the liver (A, ASC and N) show an enhanced activity in the liver of the obese Zucker rat. This would mean that this organ is extremely efficient in extracting neutral amino acids from the blood. We note that both K_m values are in the range of normal alanine and glutamine blood concentrations, which suggests that availability might also be a key factor determining net uptake of neutral amino acids by the liver *in vivo*. This basal enhancement could contribute to providing amino acids for anabolic purposes, such as protein synthesis (Cleary *et al.*, 1980; Greenwood *et al.*, 1982; the present work) or lipogenesis (Terrettaz & Jeanrenaud, 1990). We speculate on the biochemical causes leading to this co-ordinate increase in the activities of these three systems. Certainly, in most cases, an increase in $V_{\max}$ can correspond to a higher number of carriers inserted in the plasma membrane, especially if measurements are made in plasma-membrane vesicle preparations. However, changes in ionic movements across the membrane cannot be fully discounted, although it would be expected that this should have resulted in a different overshoot pattern in both vesicle populations. This was not the case in our membrane fractions. Regarding the physiological causes of these modifications, hyperinsulinaemia could exert a specific role on system A only, but obesity-associated hyperphagia does not seem to be a key factor determining the other changes, because it has been shown that, in order to enhance system A, ASC and N activities, it is necessary to give a high-protein diet to the animals (Rémésy *et al.*, 1988; Bourdel *et al.*, 1990), otherwise the protein disposal is not sufficiently high to exert any great effect on amino acid transporters. However, interestingly, these three systems, A, ASC and N, are also activated in uncontrolled streptozotocin-induced diabetes (Barber *et al.*, 1982). This pathophysiological model is characterized by net protein accretion when rats are not food-restricted (Almdal & Vilstrup, 1987, 1988). Whether these changes in transport activities rely on an hypertrophic effect on the liver, or whether they have been mediated by genetic programming during the development of the obese phenotype, remains to be established. Indeed, in CHO cells kept in culture, evidence has been provided for a co-ordinate regulation at the gene level of several neutral-amino-acid transport systems (Moffett & Englesberg, 1984; Englesberg & Moffett, 1986). It is not yet known whether this would apply to other cell systems.

Conclusion

The liver of obese Zucker rats develops a high efficiency in extracting amino acids from the afferent blood supply which seems to be, at least partially, mediated by stable changes in system A, ASC and N activities. The activity of these Na⁺-dependent systems results in $V_{\max}$ values for alanine and glutamine transport double those found in their lean littermates. Amino acids entering the hepatocyte might be contributing to

amino acid transport systems at the plasma-membrane level of the hepatocyte (Pastor-Anglada *et al.*, 1987; Felipe *et al.*, 1989; Bourdel *et al.*, 1990). Thus the fact that the $V_{\max}$ of Na⁺-dependent alanine transport in preparations from obese animals was double that found in plasma-membrane fractions obtained from lean rats strongly suggests that this is partially mediating

liver protein accretion as well as to other anabolic processes (such as lipogenesis).

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