Inter-Organ Relationships between Glucose, Lactate and Amino Acids in Rats Fed on High-Carbohydrate or High-Protein Diets

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1. Inter-organ relationships between glucose, lactate and amino acids were studied by determination of plasma concentrations in different blood vessels of anaesthetized rats fed on either a high-carbohydrate diet [13% (w/w) casein, 79% (w/w) starch] or a high-protein diet [50% (w/w) casein, 42% (w/w) starch]. The period of food intake was limited (09:00–17:00h), and blood was collected 4h after the start of this period (13:00h). 2. Glucose absorption was considerable only in rats fed on a high-carbohydrate diet. Portal-vein–artery differences in plasma lactate concentration were higher in rats fed on this diet, but not proportional to glucose absorption. Aspartate, glutamate and glutamine were apparently converted into alanine, but when dietary protein intake was high, a net absorption of glutamine occurred. 3. The liver removed glucose from the blood in rats fed on a high-carbohydrate diet, but glucose was released into the blood in rats fed on the high-protein diet, probably as a result of gluconeogenesis. Lactate uptake was very low when amino acid availability was high. 4. In rats on a high-protein diet, increased uptake of amino acids, except for ornithine, was associated with a rise in portal-vein plasma concentrations, and in many cases with a decrease in hepatic concentrations. 5. Hepatic concentrations of pyruvate and 2-oxoglutarate decreased without a concomitant change in the concentrations of lactate and malate in rats fed on the high-protein diet, in spite of an increased supply of pyruvate precursors (e.g. alanine, serine, glycine), suggesting increased pyruvate transport into mitochondria. 6. High postprandial concentrations of plasma glucose and lactate resulted in high uptakes of these metabolites in peripheral tissues of rats on both diets. Glutamine was released peripherally in both cases, whereas alanine was taken up in rats fed on a high-carbohydrate diet, but released when the amino acid supply increased. 7. It is concluded that: the small intestine is the main site of lactate production, and the peripheral tissues are the main site for lactate utilization; during increased ureogenesis in fed rats, lactate is poorly utilized by the liver; the gut is the main site of alanine production in rats fed on a high-carbohydrate diet and the liver utilizes most of the alanine introduced into the portal-vein plasma in both cases.

Lactate and amino acids are the main substrates for gluconeogenesis in the rat, alanine being the predominant gluconeogenic amino acid (McDonald *et al.*, 1976). A glucose/lactate cycle (Cori) and a glucose/alanine cycle have previously been described (Mallette *et al.*, 1969; Felig, 1973). Production of alanine and glutamine has mainly been studied in non-splanchnic tissues, although the digestive tract plays an important part in the metabolism of these amino acids, particularly during the postprandial period (Yamamoto *et al.*, 1974; Windmueller & Spaeth, 1977).

Overall studies of the interorgan relationships can obviously only be carried out *in vivo*, and observations in the splanchnic bed are especially relevant. This approach is particularly important for an appreciation of the function of the liver, as present understanding of hepatic metabolism has been gained mainly from investigations *in vitro* (perfused livers, isolated hepatocytes), frequently with starved animals or poorly specified nutritional conditions. McDonald *et al.* (1976) have emphasized that investigations *in vivo* in which arteriovenous differences across an organ are measured provide only qualitative information about metabolite fluxes, because of the difficulties of measuring blood flows in the rat. Moreover, the fate of the metabolites removed by an organ cannot be unequivocally determined by these methods. However, it is possible to compare the uptake and release of different metabolites and, in the splanchnic bed, to estimate the percentage uptake of substrates originating from the digestive tract. By using similar techniques, Aikawa *et al.* (1973), Yamamoto *et al.* (1974) and McDonald *et al.* (1976) have shown the importance of the interorgan relationships in the metabolism of lactate and amino acids.

The purpose of the present work was to compare these relations in rats fed on high-carbohydrate or high-protein diets. The results of the present study suggest that, with an abundant supply of amino acids accompanied probably by very active gluconeogenesis, lactate is poorly utilized by liver, and the gut is the main site of lactate and alanine production in fed rats.

Materials and Methods

Reagents

Chemicals were obtained from Prolabo, Paris, France, or Merck, Darmstadt, Germany. Enzymes and cofactors were purchased from Boehringer, Mannheim, Germany, or Sigma, St. Louis, MO, U.S.A.

Animals and diets

Male Sherman rats, weighing 180–220 g, were fed on diets containing (%, w/w): high-carbohydrate diet, wheat starch (79), casein (91 % protein; 13), corn oil (2), salt mixture (5), vitamin (1); high-protein diet, wheat starch (42), casein (50), the other ingredients being in the same proportions for the two isocaloric diets.

The salt and vitamin mixtures were from U.A.R., Villemoisson/Orge, France.

The animals received food from 09:00 to 17:00 h, and lighting was from 21:00 to 09:00 h. The daily food intake after 10 days of adaptation did not significantly differ between the two groups of animals (20.8 ± 0.7 and 19.0 ± 1.5 g of dry matter/day for high-carbohydrate and high-protein diet respectively).

Tissue samples

Rats that had been on one of the diets for 10–14 days were anaesthetized with Nembutal (40 mg/kg) 4 h after the beginning of the daily food intake. After laparotomy, 0.8–1.0 ml of blood was withdrawn into a heparinized syringe from the portal vein, then the aorta, or a hepatic vein (inside the left hepatic lobe), then the portal vein, or an iliac vein, then the aorta. The plasma was deproteinized with 2 vol. of 0.6M-$HClO_4$. Portions of liver were frozen within a few seconds of excision in clamps that had been cooled in liquid N_2 (Wollenberger et al., 1960) and immediately crushed in 0.4M-$HClO_4$ (1 g of tissue/7 ml).

After centrifugation ($8000g$, 1 min), the $HClO_4$ supernatant was neutralized with K_2CO_3 to avoid glutamine hydrolysis and the extracts were kept below $-20°C$.

Determination of metabolites

The following metabolites were determined by enzymic methods: glycogen (Keppler & Decker, 1974), pyruvate (Czok & Lamprecht, 1974), lactate (Gutmann & Wahlefeld, 1974a), alanine (Williamson, 1974), ammonia (Kun & Kearney, 1974), urea (Gutmann & Bergmeyer, 1974), 2-oxoglutarate (Bergmeyer & Bernt, 1974), malate (Gutmann & Wahlefeld, 1974b), glutamine (Lund, 1974), aceto-acetate (Mellanby & Williamson, 1974), 3-hydroxy-butyrate (Williamson & Mellanby, 1974), glucose (Bergmeyer et al., 1974). Non-esterified fatty acids were determined by the method of Fahlolt et al. (1973), modified by including a preliminary extraction of non-esterified fatty acids by acetone and, after evaporation, the use of a solvent mixture of chloroform/heptane (4:3, v/v).

To measure amino acid concentration, plasma was deproteinized with 4 % (w/v) sulphosalicylic acid immediately before analysis; the hepatic extracts were adjusted to pH 2.2 with sulphosalicylic acid. Amino acids were then determined with three lithium buffers: 0.20 M, pH 2.58 (70 min); 0.40 M, pH 3.00 (110 min); 1.66 M, pH 3.58 (120 min). The temperature was 34°C up to 70 min, then 68°C; the buffer flow rate was 36 ml/h. An LKB model 4101 amino acid analyser (LKB, Cambridge, U.K.) fitted with a 6 mm × 350 mm column containing Aminex A9 resin was used.

The significance of difference between means was assessed by Student's t test.

Results

Plasma metabolite concentrations in the aorta (Table 1)

Plasma glucose and lactate concentrations were slightly, but not significantly, higher in rats fed on the high-carbohydrate diet than in those fed on the high-protein diet (glucose 8.55 and 7.77 mM, lactate 2.34 and 2.02 mM respectively). Under similar sampling conditions, but with rats fed on a diet of seed cake plus cereal (20 % crude protein; 50 % starch; 15 % various carbohydrates; 5 % crude fibre) ad libitum, about 6.5 mM-glucose and about 1.5 mM-lactate were found in the plasma. In spite of the large difference in amino acid supply, only very slight variations in the plasma concentrations of most of the amino acids occurred, except for glycine, which decreased, and proline, lysine and branched-chain amino acids, which increased, on the high-protein diet.

Digestive tract (Table 2)

Since the duration of food intake was limited (09:00–17:00 h), at the time of sampling (13:00 h), the absorption was very high.

Table 1. *Arterial concentrations of plasma glucose, lactate and amino acids*

For experimental details see the text. Values are means $\pm$S.E.M. for ten rats for glucose, lactate and urea, and means$\pm$S.E.M. for three experiments with seven rats each for amino acids. The statistical significance of the variation with diet is indicated by * ($P<0.05$) and † ($P<0.01$).

	Arterial concentration (mM)	
	High-carbohydrate diet	High-protein diet
Glucose	8.55±0.23	7.77±0.40
Lactate	2.34±0.10	2.02±0.08
Alanine	0.77±0.08	0.83±0.05
Glutamine	0.59±0.06	0.65±0.04
Glutamate	0.05±0.02	0.09±0.01
Aspartate	0.02±0.005	0.02±0.005
Asparagine	0.16±0.02	0.14±0.02
Glycine	0.18±0.01*	0.13±0.01
Proline	0.58±0.02†	0.92±0.05
Serine	0.33±0.04	0.32±0.03
Threonine	0.47±0.04	0.47±0.02
Valine	0.29±0.02†	0.77±0.04
Leucine	0.18±0.02†	0.53±0.03
Isoleucine	0.11±0.02†	0.30±0.02
Tyrosine	0.10±0.02	0.13±0.01
Phenylalanine	0.08±0.01	0.10±0.01
Methionine	0.14±0.02	0.11±0.01
Lysine	0.68±0.05*	0.85±0.05
Histidine	0.09±0.02	0.11±0.02
Citrulline	0.10±0.02	0.10±0.02
Ornithine	0.06±0.01	0.06±0.01
Arginine	0.10±0.01	0.13±0.01
Urea	4.20±0.12†	12.30±0.34

Table 2. *Portal-vein–aorta differences in the concentrations of plasma glucose, lactate, amino acids and NH_3*

Amino acid composition of casein is given. Concentrations of glutamine and asparagine have been calculated from the data of Yamamoto *et al.* (1974). For experimental details see the text. Values are means$\pm$S.E.M. for ten rats for glucose, lactate and NH_3, and means$\pm$S.E.M. for three experiments with seven rats each for amino acids. The statistical significance of the variation with diet is indicated by * ($P<0.05$) and † ($P<0.01$).

	Portal-vein–artery differences (mM)		Casein composition (μmol/g)
	High-carbohydrate diet	High-protein diet	
Glucose	5.04±0.52†	0.98±0.35	—
Lactate	0.88±0.07*	0.57±0.09	—
Alanine	0.52±0.03†	1.26±0.06	422
Glutamine	0.01±0.02†	0.46±0.06	675
Glutamate	0.02±0.01†	0.20±0.05	792
Aspartate	0.01±0.005	0.04±0.01	170
Asparagine	0.10±0.01†	0.38±0.06	330
Glycine	0.11±0.02†	0.35±0.01	244
Proline	0.27±0.03†	0.98±0.17	789
Serine	0.14±0.02†	0.58±0.02	478
Threonine	0.10±0.02†	0.47±0.05	322
Valine	0.10±0.01†	0.39±0.04	578
Leucine	0.11±0.01†	0.45±0.02	689
Isoleucine	0.09±0.01†	0.30±0.01	389
Tyrosine	0.05±0.01†	0.15±0.02	278
Phenylalanine	0.04±0.01†	0.17±0.01	278
Methionine	0.06±0.01†	0.14±0.01	211
Lysine	0.17±0.03†	0.73±0.06	489
Histidine	0.03±0.01†	0.14±0.01	189
Citrulline	0.02±0.02	0.08±0.04	—
Ornithine	−0.01±0.01*	0.04±0.01	—
Arginine	0.04±0.01†	0.14±0.01	189
NH_3	0.07±0.03*	0.17±0.02	—

Glucose and lactate. Glucose absorption, as suggested by arteriovenous differences, was very high in rats fed on the high-carbohydrate diet and much less in those on the high-protein diet, which still contained 42% (w/w) of starch.

Lactate release was higher in the high-carbohydrate-fed rats; the increase in glucose in intestinal cells appears to be responsible. This lactate release was, however, far from being proportional to the quantity of glucose appearing in the portal vein; evidently the proportion of glucose metabolized to lactate increases as glucose absorption is decreased.

Amino acids. The molar fraction of alanine (cf. total amino acids) in the portal-vein plasma substantially exceeded the alanine fraction of casein, especially in rats on the high-carbohydrate diet, whereas the aspartate, glutamate and glutamine fractions were considerably lower than in casein. In fact, for both diets the increase in alanine concentration (portal-vein-aorta difference compared with casein) was almost exactly equal to the decrease in aspartate+glutamate+glutamine. It therefore

seems that the metabolism of aspartate, glutamate and glutamine in the intestine leads to the formation of equivalent amounts of alanine. The fractional recovery of glutamate and glutamine in portal blood (cf. casein) was higher in rats on the high-protein diet, presumably because the enzymic capacity for their metabolism in the intestine is exceeded. It would explain the finding that the arteriovenous difference for alanine does not increase to the same extent as the protein supply in the diet. The data indicate the same high degree of intestinal metabolism of aspartate on both diets. Asparagine seems to be poorly metabolized: absorption was proportional to protein intake and its fractional recovery in portal blood was close to the fraction in casein. The absorption of most of the other amino acids appeared to be both proportional to the dietary protein and to their respective percentages in casein. It is impossible, however, to draw quantitative

conclusions about intestinal utilization or respective speeds of absorption of amino acids without kinetic data and blood-flow measurements.

Hepatic metabolite concentrations (Tables 3 and 4)

Hepatic glycogen concentrations were higher in rats fed on the high-carbohydrate diet. Glucose concentrations were similar in rats fed on either diet, as were lactate concentrations. The pyruvate concentration decreased, the higher the dietary protein intake, which corresponds to an increase in the lactate/pyruvate ratio (and hence in the cytosolic [NADH]/[NAD$^+$] ratio). Malate concentrations were similar, but 2-oxoglutarate concentration was lower in rats on the high-protein diet, perhaps because of increased transaminase activity. This decrease in 2-oxoglutarate concentration coincides with an increase in glutamate concentration in rats on the high-protein diet, whereas glutamine, aspartate, alanine, glycine, serine and threonine decreased. With this diet, branched-chain amino acid and lysine concentrations increased markedly, because of a

higher hepatic uptake. On the other hand, ornithine increased, although its hepatic uptake was not markedly modified, and perhaps was the result of more active ureogenesis.

Table 3. *Concentrations of metabolites in the liver*

Values given in μmol/g fresh wt. of tissue, except for glycogen (mg/g), are means ± S.E.M. for ten rats. N.S., Not significant.

| | Metabolic concentration (mM) | | |
	High-carbohydrate diet	High-protein diet	P
Glycogen	48.0 ± 2.10	30.4 ± 3.9	<0.001
Glucose	7.48 ± 0.32	7.43 ± 0.36	N.S.
Lactate	1.10 ± 0.09	0.97 ± 0.07	N.S.
Pyruvate	0.30 ± 0.01	0.15 ± 0.01	<0.001
[Lactate]/ [pyruvate]	3.67	6.46	
Malate	0.31 ± 0.04	0.32 ± 0.03	N.S.
2-Oxoglutarate	0.46 ± 0.06	0.28 ± 0.04	<0.02

Table 4. *Uptake or release of glucose, lactate, amino acids and NH₃ by the liver*

Portal plasma concentrations and portal–hepatic-vein differences are expressed as mM and hepatic concentrations as μmol/g fresh wt. of tissue. For experimental details and calculation of percentage of hepatic uptake see the text. Values are means ± S.E.M. for ten rats for glucose, lactate, and NH₃ and means ± S.E.M. for three experiments with seven rats each for amino acids. ‡ Amino acids present in the liver in very low concentration. The statistical significance of the variation with diet for portal–hepatic-vein differences and for liver concentrations is indicated by * (P < 0.05) and † (P < 0.01).

| | High-carbohydrate diet | | | | High-protein diet | | | |
	Portal vein	Liver	Portal–hepatic-vein differences	Hepatic uptake(%)	Portal vein	Liver	Portal–hepatic-vein differences	Hepatic uptake(%)
Glucose	13.59 ± 0.65	—	3.10 ± 0.40	13, 2	8.75 ± 0.50	—	−0.90 ± 0.25†	
Lactate	3.22 ± 0.12	—	0.48 ± 0.12	7.3	2.59 ± 0.09	—	0.10 ± 0.10*	2.8
Alanine	1.29 ± 0.05	2.36 ± 0.15	0.44 ± 0.04	25	2.09 ± 0.01	1.72 ± 0.11*	1.22 ± 0.07†	49
Glutamine	0.60 ± 0.07	4.13 ± 0.38	−0.10 ± 0.02	—	1.11 ± 0.07	3.29 ± 0.12	0.44 ± 0.04†	31
Glutamate	0.07 ± 0.02	1.69 ± 0.11	0.00 ± 0.02	0	0.29 ± 0.05	2.53 ± 0.12†	0.15 ± 0.03*	39
Aspartate	0.03 ± 0.01	1.63 ± 0.15	—	—	0.06 ± 0.01	1.06 ± 0.17	0.03 ± 0.01	50
Asparagine	0.26 ± 0.03	‡	0.10 ± 0.01	31	0.52 ± 0.07	‡	0.34 ± 0.05*	56
Glycine	0.29 ± 0.01	1.70 ± 0.16	0.09 ± 0.01	25	0.48 ± 0.01	0.58 ± 0.06†	0.30 ± 0.01†	52
Proline	0.85 ± 0.09	‡	0.17 ± 0.04	12	1.90 ± 0.13	0.70 ± 0.05	0.65 ± 0.15*	22
Serine	0.47 ± 0.03	0.64 ± 0.05	0.09 ± 0.01	11	0.90 ± 0.04	0.41 ± 0.04*	0.53 ± 0.03†	49
Threonine	0.57 ± 0.04	0.40 ± 0.04	0.06 ± 0.02	6	0.94 ± 0.06	0.31 ± 0.03	0.43 ± 0.08†	36
Valine	0.39 ± 0.03	0.22 ± 0.03	0.05 ± 0.02	6	1.16 ± 0.08	0.61 ± 0.09*	0.24 ± 0.06*	12
Leucine	0.29 ± 0.01	0.18 ± 0.01	0.06 ± 0.02	11	0.98 ± 0.04	0.44 ± 0.03†	0.26 ± 0.07*	15
Isoleucine	0.20 ± 0.01	0.11 ± 0.01	0.04 ± 0.02	8	0.60 ± 0.03	0.26 ± 0.03*	0.14 ± 0.02*	17
Tyrosine	0.15 ± 0.02	0.06 ± 0.01	0.04 ± 0.01	19	0.28 ± 0.01	0.11 ± 0.04	0.11 ± 0.02*	28
Phenylalanine	0.12 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	17	0.27 ± 0.01	0.10 ± 0.04	0.11 ± 0.02*	26
Methionine	0.20 ± 0.02	0.05 ± 0.01	0.03 ± 0.02	6	0.25 ± 0.01	0.07 ± 0.02	0.10 ± 0.01*	28
Lysine	0.85 ± 0.04	0.95 ± 0.15	0.10 ± 0.02	6	1.58 ± 0.09	1.50 ± 0.12*	0.54 ± 0.02†	24
Histidine	0.12 ± 0.02	0.29 ± 0.05	0.02 ± 0.005	10	0.25 ± 0.01	0.28 ± 0.01	0.14 ± 0.01†	50
Citrulline	0.12 ± 0.02	0.05 ± 0.01	—	—	0.22 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	37
Ornithine	0.05 ± 0.01	0.13 ± 0.02	−0.07 ± 0.02	—	0.10 ± 0.01	0.30 ± 0.02†	−0.06 ± 0.01	—
Arginine	0.14 ± 0.02	0.04 ± 0.01	—	—	0.27 ± 0.02	0.08 ± 0.01*	0.16 ± 0.05	46
NH₃	0.12 ± 0.03	0.70 ± 0.07	0.09 ± 0.02	77	0.22 ± 0.04	1.05 ± 0.06†	0.20 ± 0.03†	89

Liver balance of metabolites

The hepatic-artery supply must be taken into account in order to calculate metabolite uptake across the liver. Portal blood flow has been assumed to represent 70% of the total hepatic blood flow (Greenway & Stark, 1971). Fluctuations in the percentage of hepatic blood flow in the hepatic artery between 20 and 40% do not markedly modify the balance of most of the metabolites, except those showing very high portal-vein–artery differences. The hepatic percentage uptake was calculated in the following way:

$$\frac{[0.3\,(\text{aorta}-\text{hepatic vein}) + 0.7\,(\text{portal vein}-\text{hepatic vein})] \times 100}{[0.3\,(\text{aorta})+0.7\,(\text{portal vein})]}$$

Glucose (Tables 3 and 4). There was a net uptake (13.2%) of glucose in rats on the high-carbohydrate diet. The fact that this occurred in the presence of high glycogen concentrations (48.0mg/g of liver) shows that anaesthesia and sampling do not lead to glycogenolysis. Liver released glucose into the plasma of rats on the high-protein diet, probably as a result of active gluconeogenesis. The released glucose would not represent the total amount of gluconeogenesis, since the end product of this pathway can be glycogen; however, as suggested by the lower glycogen concentrations (30.4mg/g of liver) in rats on the high-protein diet, this pathway is probably inefficient.

Alanine (Table 4). The liver removed 25 and 49% of plasma alanine (high-carbohydrate and high-protein diets respectively). The arterial–hepatic-vein difference in alanine concentration was small, since alanine released by the small intestine was removed by the liver. Alanine was taken up against a concentration gradient in rats on the high-carbohydrate diet (concentration in the portal vein was 1.29mM, against 2.36mM in the liver). The decrease in intrahepatic concentrations (1.72mM) and the increase in portal-vein concentration (2.09mM) favoured uptake in rats fed on a high-protein diet.

Aspartate, glutamate, glutamine and asparagine (Table 4). The actual hepatic uptake of aspartate must be negligible with both diets, and hepatic concentrations are high, which underlines the importance of aspartate in intracellular metabolism, but not in interorgan relationships. Liver removed a high percentage of asparagine in the presence of a favourable gradient, since hepatic concentrations were extremely low. There was no glutamate uptake in rats fed on the high-carbohydrate diet, which agrees with the existence of a highly unfavourable gradient for extraction; in contrast, the increase in glutamate concentration in portal-vein plasma of rats on the high-protein diet permitted a noticeable uptake. Glutamine was released or removed by the liver in rats fed on high-carbohydrate or high-protein diets respectively.

Other amino acids (Table 4). Percentage uptake of glycine showed a similar pattern to that of alanine, but from far lower concentrations in the portal vein. Uptake was effected against a concentration gradient. With the high-protein diet, the gradient was more favourable, because of the decrease in concentrations in the liver and the increase in concentrations in the portal plasma. Serine and threonine uptakes of 11 and 6% in rats fed on the high-carbohydrate diet increased to 49 and 36% respectively in rats fed on the high-protein diet. Percentage uptake of proline was moderately increased, although the absolute value was greatly increased. Uptake of branched-chain amino acids was normally low, but markedly increased in rats fed on the high-protein diet. Uptake of aromatic amino acids, which is appreciable in rats on the high-carbohydrate diet, increased in those on the high-protein diet. All these amino acids were extracted either in the absence of a plasma–liver gradient (serine and threonine with the high-carbohydrate diet) or in the presence of a favourable gradient (serine and threonine with the high-protein diet, branched-chain amino acids with both diets). Lysine uptake was considerable in rats on the high-protein diet, so plasma lysine accumulation remains limited by liver metabolism.

Non-esterified fatty acids and ketone bodies (Table 5). With both diets the hepatic clearance of non-esterified fatty acids from plasma and the release of ketone bodies were extremely low.

Table 5. *Hepatic–portal-vein differences for non-esterified fatty acids and ketone bodies*
Values are means±S.E.M. for ten rats. N.S., Not significant.

	High-carbohydrate diet		High-protein diet		
	Portal vein (mM)	Portal–hepatic-vein difference (mM)	Portal vein (mM)	Portal–hepatic-vein difference (mM)	P
Non-esterified fatty acids	0.27±0.03	0.05±0.02	0.24±0.01	0.03±0.01	N.S.
3-Hydroxybutyrate	0.03±0.01	−0.06±0.02	0.07±0.02	−0.09±0.03	N.S.
Acetoacetate	0.07±0.01	−0.05±0.02	0.10±0.01	−0.02±0.01	N.S.

Table 6. *Iliac-vein–artery difference for glucose, lactate, alanine and glutamine*

Alanine and glutamine have been determined by enzymic methods for ten rats (means±S.E.M.). N.S., Not significant.

Metabolite concentration (mM)

	High-carbohydrate diet	High-protein diet	P
Glucose	−1.06 ± 0.21	−0.94 ± 0.29	N.S.
Lactate	−0.40 ± 0.06	−0.30 ± 0.13	N.S.
Alanine	−0.04 ± 0.01	+0.09 ± 0.01	<0.001
Glutamine	+0.05 ± 0.01	+0.09 ± 0.02	<0.05

Peripheral metabolism

Arteriovenous differences between iliac vein and aorta correspond to the metabolism of various tissues, chiefly muscle, but also adipose tissue and skin. Such measurements made across the hindquarter are not necessarily representative of the whole peripheral metabolism.

Glucose and lactate (Table 6). Large peripheral uptakes of glucose and lactate, corresponding to high plasma concentrations, were similar for the two diets.

Alanine and glutamine (Table 6). Alanine was removed from plasma by the hindquarter tissue of rats fed on a high-carbohydrate diet, in agreement with the findings of McDonald *et al.* (1976). Glutamine was released with both diets, although at a higher rate with the high-protein diet, which also shifts the peripheral alanine balance towards release.

Discussion

Intestinal metabolism

It is difficult to obtain positive portal-vein-artery differences for glucose in the rat unless the diet is rich in pure starch or soluble sugars which are absorbed in the small intestine and unless the rats are conditioned to consume their food during a limited daily period. In rats receiving commercial diets we observed only weak glucose absorption (Rémésy *et al.*, 1977); a negative balance has even been reported by McDonald *et al.* (1976). The small intestine is the main site of splanchnic production of lactate, since the metabolic contribution of the caecum and the colon, which are very poorly developed in rats maintained on the diets used, is negligible (Rémésy *et al.*, 1977).

The active metabolism of aspartate, glutamate and glutamine is now a well-known phenomenon (Parsons & Volman-Mitchell, 1974; Windmueller & Spaeth, 1977). In rats fed on a high-protein diet the aspartate aminotransferase activity appears to be sufficient to ensure complete transamination of available aspartate, whereas our findings suggest that alanine aminotransferase activity is limiting for the transamination of free glutamate and glutamate produced by glutamine deamidation, which is a critical step in glutamine metabolism (Windmueller & Spaeth, 1977). Alanine may not be the unique end product of metabolism of acidic amino acids in the rat intestine, since Windmueller & Spaeth (1975) found that glutamine gives rise to CO_2 and various products (lactate, citrulline, proline), although these conversions seem to be of minor importance in our experiments. The appearance of NH_3 in the portal vein is higher in rats fed on the high-protein diet; it possibly originates from intestinal glutamine deamidation.

Hepatic metabolism

The extraction of glucose by the liver of rats fed on a high-carbohydrate diet is dependent on activation of glucose by glucokinase when portal concentrations are high, and on a low activity of glucose 6-phosphatase. The opposite situation occurs with the high-protein diet. With this diet, except for gluconeogenesis, the only possibility for hepatic glucose release would be by glycogenolysis, but by this hypothesis the reconstitution of glycogen stores would have to take place separately from the period of maximum absorption. Moreover, Péret *et al.* (1973) observed postprandial glycogenolysis only after an entirely protein meal of very short duration. Some of the extracted amino acids may be used for the synthesis of cellular or plasma proteins. The liver is probably the main site of 'labile protein' production when amino acid supply is associated with high carbohydrate availability (Munro, 1964) or when a large amino acid supply necessitates a delayed utilization of dietary proteins. This appeared to be the situation in the present study (at least there was no net proteolysis), as release of branched-chain amino acids was not observed. This suggests that any amino acid-induced glucagon secretion (which can engender proteolysis) was balanced by noticeable insulin secretion caused by high plasma glucose (Unger & Orci, 1976).

Portal–hepatic-vein differences in lactate (0.48 mM) and alanine (0.44 mM) concentrations are quite similar in rats fed on the high-carbohydrate diet, although the percentage of alanine uptake is much higher, and gluconeogenesis is probably extremely low. Lactate and alanine taken up in rats fed on a high-carbohydrate diet are likely to be consumed in the tricarboxylate cycle and/or used for lipogenesis.

Only with the high-protein diet, where glucose release was demonstrated, can lactate and alanine utilization in gluconeogenesis be significant. With this diet, portal–hepatic-vein differences in lactate concentration are insignificant. The increased alanine uptake and utilization in rats on the high-protein diet is effected under conditions apparently unfavourable for alanine transamination, except for the rise in alanine aminotransferase activity (Kaplan & Pitot, 1970). Concentrations of alanine and 2-oxoglutarate are decreased, whereas those of glutamate increase. The pyruvate decrease seems to be the main factor, allowing a displacement of the transamination equilibrium in favour of alanine utilization. A decrease in cytosolic pyruvate would be favoured by enhanced transport into mitochondria, particularly under glucagon influence (Titheradge & Coore, 1976). The low uptake of lactate by the liver connected with the high utilization of amino acids remains a puzzling result. The increase in the cytosolic [NADH]/[NAD$^+$] ratio (assuming that this ratio can be accurately measured by the [lactate]/[pyruvate] ratio in crude hepatic extracts) could result in a shift towards a cytosolic redox state unfavourable for lactate oxidation (Krebs et al., 1976). Malate supply to the cytosol is likely to be very high in rats fed on the high-protein diet because of the efflux from mitochondria of C_4 units for gluconeogenesis and ureogenesis directly as malate or as aspartate (then malate via argininosuccinate and fumarate). This NADH-generating pathway could play a part in the alteration of the cytosolic redox state. Malate concentrations in the liver do not suggest this increased availability of malate in the cytosol, perhaps because of the intense oxaloacetate utilization by phosphoenolpyruvate carboxykinase, the activity of which is greatly increased by the high-protein diet (Péret & Chanez, 1976).

Increased aspartate aminotransferase activity (Kaplan & Pitot, 1970) and argininosuccinate synthase activity (Schimke, 1962) appear to be responsible for the lower hepatic aspartate concentrations in rats fed on a high-protein diet, in spite of a much higher uptake of asparagine. An increased hepatic uptake is observed with a lower intracellular concentration, particularly with the glucogenic amino acids (alanine, glycine, serine and threonine). The adaptation to a high amino acid supply appears far less efficient for branched-chain amino acids and lysine, since a rise in intracellular concentrations accompanies the increase in hepatic extraction. The impermeability of the cellular membrane of the hepatocytes prevents glutamate release by the liver, in spite of a quite favourable gradient (Hems et al., 1968). This gradient does not prevent glutamate uptake in rats fed on the high-protein diet, although the glutamate increase in the portal vein is limited; perhaps these glutamate exchanges are underestimated

in the absence of measurements in the whole blood. Indeed, glutamate, aspartate, glycine and to a smaller extent proline are more concentrated inside erythrocytes (Blackshear & Alberti, 1975), and Elwyn et al. (1972) suggest the possibility of direct exchanges between tissues and erythrocytes. In rats fed on the high-carbohydrate diet, glycine is more efficiently extracted than serine by the liver, in spite of a more unfavourable plasma-hepatocyte gradient; differences in transport across the hepatocyte membrane could be responsible. This tendency has also been observed by Bloxham (1972) and Yamamoto et al. (1974) with rats fed on a 30%-casein diet, whereas present findings show that, on a 50%-protein diet, percentage uptakes of glycine and serine becomes similar. In starved rats or those on a low-protein diet, hepatic uptake of amino acids, particularly glycine is decreased (Aikawa et al., 1972; Ishikawa, 1977).

The liver removes a large percentage of plasma NH_3 against a considerable gradient and in the presence of appreciable amounts of amino acids, which may be deamidated (glutamine, asparagine) or deaminated.

Supply of energy for gluconeogenesis

The uptake of non-esterified fatty acids is extremely low with both diets, whereas in rats fed on the high-protein diet considerable quantities of amino acids are taken up by the liver. Some amino acids can supply NADH and acetyl-CoA for energy and activation of pyruvate carboxylase (lysine, leucine, isoleucine, tyrosine and phenylalanine) and, our observations show, without a notable release of ketone bodies. The amino acids are probably the largest source of energy for gluconeogenesis and ureogenesis.

Comparison with studies in vitro

Most of the results in vitro (Exton & Park, 1967; Ross et al., 1967; Parrilla & Ayuso-Parrilla, 1976) show that lactate is a better glucogenic precursor than alanine, a finding that may be significant in vivo during starvation. Our results show that when the availability of amino acids is high, hepatic uptake of lactate is very low.

Peripheral metabolism

Our results agree with those of McDonald et al. (1976) showing that the rat peripheral tissues (mainly muscle) are provided with large quantities of pyruvate coming from lactate and glucose uptake. The Cori cycle thus appears to have only a slight importance in rats fed on semi-purified low-lipid diets. The only notable hepatic uptake of lactate observed was in a non-gluconeogenic situation. The intestine appears

to be the principal site of lactate production in fed animals, and lactate can be used for the production of CO_2 or for lipogenesis in numerous tissues, among which the liver is of limited importance. However, in starved rats, the rates of turnover of glucose and lactate decrease and lactate is utilized to a greater extent during gluconeogenesis (Freminet et al., 1975). The glucose/alanine cycle seems not to be very efficient in animals fed on our diets. Alanine is extracted from plasma by the peripheral tissues only to a small extent in rats fed on the high-carbohydrate diet. Moreover, the release of alanine in rats fed on the high-protein diet does not correspond entirely to glucose recycling (Grubb, 1976; Karl et al., 1976). The appreciable release of alanine we observed only with the high-protein diet may well be connected with the increased concentrations of branched-chain amino acids in the arterial plasma (Odessey et al., 1974; Yamamoto et al., 1974).

Glutamine is released in rats on both diets, and so it may represent the most usual form of NH_3 recycling in the peripheral tissues. According to Lund & Watford (1976), however, glutamine release is not measurable in rats fed on a normal diet enriched with glucose. These authors stress that, although the glutamine synthase activity is relatively low in muscle, this tissue, by virtue of its large mass, participates to a large extent in the supply of glutamine to the organism.

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