Improved nitrogen metabolism in rats fed on lipid-rich liquid diets

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N metabolism was studied in young rats fed on lipid-rich, isonitrogenous, purified liquid diets, a convenient and easy technique for inducing voluntary overfeeding of energy and lipids under controlled nutritional conditions. Overfed rats showed a marked N retention at the expense of a reduced production of urea. The capacities of isolated hepatocytes to synthesize urea and glucose from added precursors were greatly diminished. The activities of the urea cycle enzymes and several enzymes involved in the availability of NH_3 for this pathway were concomitantly reduced in overfed animals. Therefore, our results showed an improved N metabolism in overfed rats promoted by the overfeeding of lipids that could be due to an enhanced biosynthetic utilization and a reduced catabolism of amino acids. In addition, the versatile and accurate technique for inducing overfeeding in young rats used in the present study could have many advantages for nutritional studies.

Overfeeding: Liquid diet: Nitrogen metabolism: Urea cycle

The sensitivity of N balance to changes in energy intake has been demonstrated in numerous studies. It is well known that when diet provides adequate amounts of protein the addition of energy-yielding nutrients (either carbohydrate or fat) results in a linear improvement in N balance in humans and animals (Munro, 1951, 1964, 1978; Inoue *et al.* 1973; Garza *et al.* 1976; Reeds *et al.* 1981). However, the underlying biochemical mechanism whereby energy intake above requirements affects N metabolism in long-term studies remains obscure.

In a previous paper (Barber *et al.* 1985) we reported an increased N retention in cafeteria-fed rats, a model of voluntary hyperphagia of fat-rich food in which animals were offered a variety of palatable supermarket foods (Rothwell & Stock, 1979). This finding was in agreement with the protein-sparing effect of fat. Our experimental results with this model suggested that the marked N retention was due to a decrease in amino acid catabolism and in urea production by the liver (Barber *et al.* 1985, 1987). The cafeteria-feeding regimen has contributed substantially to the understanding of energy balance regulation (Rothwell & Stock, 1981); however, it has been criticized because it is difficult to exert adequate control over the diet composition (Moore, 1987).

Studies on the metabolic adaptations induced by dietary manipulations require strict control of the nutritional composition of the diets to obtain clear results. Most studies performed in overfed animals have been carried out using diets which do not provide the established nutritional requirements (National Research Council, 1978). Frequently protein intake is below requirement levels in high-fat and high-sucrose diets. In cafeteria diets most of the components of the food are unknown. In this sense it is important to ensure that, apart from the subject of the study, the intake of each component of the diet, including micronutrients is maintained at a constant level.

The present work was designed to study the protein-sparing effect of fat on both N balance and amino acid catabolism by using a novel feeding technique developed as an easy model of voluntary intake of lipid-rich, isonitrogenous liquid diets. The liquid diets were prepared with products of precisely known composition to allow accurate control of each individual component. Advantages of this overfeeding-induction technique and possible applications for other nutritional studies are discussed.

After a brief physiological characterization of our overfeeding model, we studied the total N and N compound balances. The increased N retention at the expense of reduced urinary excretion of urea led to our interest in exploring the processes involved in hepatic amino acid catabolism. Thus, we evaluated the capacity of isolated hepatocytes to synthesize urea and glucose. Indeed, we found a diminished capacity to produce urea and glucose in the hepatocytes isolated from overfed rats. In addition we studied the activities of the enzymes of the urea cycle and those involved in general amino acid metabolism and glucose production by the liver. The enhanced N retention, diminished urea production, inhibited glucose formation and reduced activities of key enzymes, mainly those concerning urea synthesis, promoted in rats by overfeeding on high-energy, lipid-rich, isonitrogenous liquid diets represented improved N metabolism, as discussed.

MATERIALS AND METHODS

Experimental design and diets

Male Wistar rats (Panlab S.L., Barcelona, Spain) were housed in groups of four or five animals per cage after weaning. They were fed *ad lib* on a commercial solid non-purified diet (Panlab S.L.) and tap water under controlled conditions of light (12 h light–12 h dark, lights on at 08.00 hours) and temperature ($22 \pm 1°$). When rats reached weights of 160–180 g they were placed in individual cages and randomly divided in two groups: control and overfed. The control group was fed on the purified liquid diet C (control diet) for 21 d. The overfed group was fed on two high-energy, lipid-rich, isonitrogenous liquid diets: H^1 and H^2.

Diet C was formulated to meet the National Research Council (1978) recommendations and those of the American Institute of Nutrition (1977) and adapted to liquid form on the basis given by Lieber & De Carli (1982, 1986). The composition (g/l) of diet C was: casein (vitamin-free) 52·0, DL-methionine 0·8, dextrin 162·7, maize oil 13·0, AIN-76A vitamin mix 2·6, AIN-76 mineral mix 9·1, choline chloride 0·3, cellulose powder 10·0, xanthan gum 2·0 and distilled deionized water to 1 l. Sucrose, originally used as the main carbohydrate source, was omitted in order to avoid the induction of an excessive intake of energy (Ramirez, 1987) and replaced by an isoenergetic quantity of dextrin (Reeves, 1989). The energy density of the diet was 4·18 MJ/l (1000 kcal/l) and its distribution (% of energy) was: protein 22, carbohydrate 66, lipid 12. Energy values of diet ingredients (kJ/g) were based on published data: casein 17·86, maize oil 37·45, dextrin 16·57. Daily, each animal was offered fresh diet in an open-bottomed graduated cylinder at a pre-fixed volume that allowed the same intake of energy and protein as that of animals fed on pelleted standard diet (i.e. 2·7–3·0 g protein/24 h and 200–230 kJ/24 h).

The H^1 diet was designed for rapid overfeeding of the animals by increasing the lipid content to obtain a 40% increment in energy with respect to diet C: 57·7 g maize oil/l and 5·86 MJ/l (1400 kcal/l). This diet was given to the rats for 10 d. Afterwards it was replaced by diet H^2 which was designed to have a 25% increment in energy density: 41·0 g maize oil/l and 5·23 MJ/l (1250 kcal/l). Rats were offered this diet for a further 11 d. Concentrations of all other components in diets H^1 and H^2 were maintained at the same level with respect to diet C. This was done in order to ensure the same intake and

nutritional quality of all nutrients (except lipids) with respect to diet C. Each day each animal received fresh diet at the same volume as the control group: diet H^1 between days 1 and 10 and diet H^2 between days 11 and 21. The replacement of diet H^1 by diet H^2 at day 11 was done in order to avoid the decrease in voluntary intake of the first diet shown by the animals beyond this limit.

Vitamin (AIN-76A) mix, mineral (AIN-76) mix and casein (micropulverized, vitamin-free) were purchased from ICN Biomedicals, High Wycombe, Bucks. Pure maize oil without additions and other compounds used for the formulation of diets were obtained from Sigma Chemical Co., St Louis, MO, USA.

Samples

During the last 48 h of the dietary treatment the animals were placed in individual metabolic cages for collection of urine and faeces. The urine samples were collected every 24 h into 25 ml test-tubes containing 0·5 ml 6 M-HCl. Faeces were collected every 24 h and homogenized with water in a motor-driven Teflon-glass homogenizer at adequate dilutions for N content assay. Blood was collected in heparinized tubes from the retroorbital plexus under light diethyl ether anaesthesia.

Rats were killed by decapitation after the 21 d dietary treatment, between 10.00 and 12.00 hours to minimize diurnal variations. Livers were rapidly excised, weighed, frozen between two aluminum blocks pre-cooled in liquid N_2 and conserved at $-30°$ until analyses were done. Selected fat pads were excised and weighed. For isolation of hepatocytes, rats were starved for 24 h and anaesthetized with Pentothal (60 mg/kg body weight in 9 g NaCl/l) before the procedure.

Isolation of hepatocytes

The preparation of isolated hepatocytes approximated to the method of Berry & Friend (1969). Cells (15–20 mg wet weight/ml) were incubated for 40 min in Krebs-Henseleit bicarbonate buffer containing 3 mM-Ca^{2+} and appropriate additives (at 10 mM of each one) for urea synthesis (none, alanine, ammonia plus ornithine, ammonia plus ornithine plus lactate) and for glucose synthesis (fructose, glycerol, pyruvate, lactate and alanine). Urea and glucose synthesis were constant over the incubation period. Viability was assessed routinely by the trypan-blue exclusion. In all cases more than 85% of the cells excluded the colourant.

Analytical determinations

The following metabolites were measured: urea (Nuzum & Snodgrass, 1976), creatinine (Naranayan & Appleton, 1980) and glucose (Bergmeyer & Bernt, 1974*a*). Analysis of N content was by Kjeldahl digestion and Nessler's reaction as modified by Minari & Zilversmit (1963). Total lipids were assayed as described by Frings *et al.* (1972). Triacylglycerols (Nägele *et al.* 1984), total cholesterol (Siedel *et al.* 1983) and cholesterol bound to high-density lipoproteins (HDL; Warnick *et al.* 1979) were measured by routine enzymic methods.

Enzyme assays

The following glucose-metabolism-related enzymes were assayed: glucose-6-phosphatase (*EC* 3.1.3.9; Baginski *et al.* 1974), fructose-1,6-biphosphatase (*EC* 3.1.3.11; Opie & Newsholme, 1967), phosphoenolpyruvate carboxykinase (*EC* 4.1.1.32; Shimazu & Ogasawara, 1975), pyruvate carboxylase (*EC* 6.4.1.1; McClure *et al.* 1971), and pyruvate

kinase (*EC* 2.7.1.40; Gutmann & Bernt, 1974). Urea-cycle enzyme activities were measured in liver homogenates as follows: carbamoylphosphate synthetase I (*EC* 6.3.4.16) and ornithine carbamoyltransferase (*EC* 2.1.3.3) as the rate of citrulline production, argininosuccinate synthetase (*EC* 6.3.4.5), argininosuccinate lyase (*EC* 4.3.2.1) and arginase (*EC* 3.5.3.1) as the rate of urea production (Schimke, 1962; Barber *et al.* 1985, 1987). Hepatic aspartate aminotransferase (*EC* 2.6.1.1; Bergmeyer & Bernt, 1974*b*), alanine aminotransferase (*EC* 2.6.1.2; Bergmeyer & Bernt, 1974*c*) and glutamate dehydrogenase (*EC* 1.4.1.3; Grisolía *et al.* 1964) were measured in liver by kinetic NADH-coupled methods. Carbamoylphosphate synthetase I was measured by competitive indirect enzyme-linked immunosorbent assay (ELISA) with carbamoylphosphate synthetase I polyclonal antibodies prepared by standard methods (a kind gift from Dr J. Timoneda). The assay conditions approximated to those established previously by Cerdá *et al.* (1988).

Presentation of results

Results in the table are presented as the mean value for each set of animals. Variability was estimated by the standard error of the mean. Responses in the control and overfed groups were subjected to one-way analyses of variance and significance tests were carried out at the 5% level. Differences between groups are expressed as the percentage change in the overfed group relative to the control group.

RESULTS AND DISCUSSION

Model of overfeeding on liquid diets

The first objective of the present work was the development of a new model of controlled overfeeding in young rats suitable for studies on metabolic responses to dietary manipulations. We selected the liquid-diet form, an easy and convenient technique in which the modification and the adjustment of the level of each nutrient becomes simple and accurate. An important premise in our experimental design was the isolation of only one nutritional factor for manipulation; the amount of lipid, avoiding alterations in the intake of other nutrients, particularly protein. In this sense our diets allowed us to feed the animals at the same level of protein, carbohydrates, vitamins and minerals, all except the amount of lipid and consequently of energy. Moreover we did not introduce modifications in the nutritional quality of diets, i.e. composition of amino acids or fatty acids. In addition it was necessary that diets were voluntarily accepted by the animals, in order to avoid stressful actions such as forced tube feeding, and that they were given for a convenient period to study the adaptive changes in N metabolism.

Table 1 shows the nutritional treatment of both groups of animals, control and overfed. The overfed group received 32% more energy than the control group in the whole dietary-treatment period. During the first period (days 1–10) the overfed rats ingested an energy excess of 37% with the aid of the energy-rich diet H^1. In the second period, in which diet H^2 was offered, the energy excess was 26%, adequate for sustaining the overfeeding until day 21. Lipid intakes of the overfed group through the two periods exhibited more marked differences, these being 4·3 and 3·2 times the intake of the control group in the first and second periods respectively. The overall change was 3·7-fold. Importantly, protein, carbohydrate and micronutrient intakes were the same, as revealed by the unchanged diet-volume intake.

As we have shown in Table 1, the final nutritional treatment of the animals was in accordance with all our experimental premises. The liquid-diet technique provided an easy method for measuring the exact intake of each nutrient and facilitated the dietary

Table 1. *Diet, energy and major-nutrient intakes of control and overfed rats*†
(Values are means with their standard errors for twelve animals)

Treatment group...	Control‡		Overfed		Percentage change	Statistical significance of change
	Mean	SEM	Mean	SEM		
Diet intake						
Daily (ml/24 h)	53·1	1·0	52·8	1·4	−1	NS
Total (ml)	1106	2	1105	21	−0	NS
Energy intake						
Daily (kJ/24 h)						
First period	218	4	299	8	+37	*
Second period	226	2	285	6	+26	*
Total (MJ)	4·63	0·06	6·09	0·11	+32	*
Protein intake						
Daily (g/24 h)	2·80	0·05	2·79	0·07	−1	NS
Total (g)	58·4	0·8	57·9	0·8	−1	NS
Lipid intake						
Daily (g/24 h)						
First period	0·678	0·014	2·93	0·08	+332	*
Second period	0·703	0·008	2·24	0·05	+219	*
Total (g)	14·59	0·15	53·6	1·0	+267	*
Carbohydrate intake						
Daily (g/24 h)	8·83	0·14	8·8	0·2	−1	NS
Total (g)	184	2	184	3	−0	NS

NS, not significant.
* $P < 0·05$.
† For details of diets and procedures, see pp. 362–363.
‡ The control group was fed on liquid diet C (4·18 MJ/l) for 21 d. The overfed group was fed on liquid diet H^1 (5·86 MJ/l) for 10 d and liquid diet H^2 (5·23 MJ/l) for the remaining 11 d. Nutrient intakes that were different between the two consecutive periods (days 1–10 and days 11–21, as energy and lipid intakes) are given separately.

manipulations component by component (day by day if necessary) under strict nutritional control. Indeed, the proposed model of overfeeding is just one possibility for this versatile technique.

Characterization of the overfed animals

Overfed rats showed an increase in total body weight (20% change relative to the final weight of the control group) accompanied by a marked development of the adipose tissue mass, as shown in Table 2. Weights of three important adipose tissue pads measured in this study were increased in the overfed animals. The retroperitoneal zone showed the greatest deposition of fat in absolute terms (+1·35 g). However, the relative change was greater in the pericardial zone (approximately twofold). Values in Table 2 also show the contribution of each pad to body weight, pointing out an impaired adipose tissue:body weight development. The liver also showed a large increment in its lipid content, both absolute mass and tissue concentration. It is notable that the increment in liver weight in the overfed group (19%) had important consequences in the subsequent study of hepatic metabolism, although it was in proportion to the increased size of these animals.

It is noteworthy that fat gain was significant in overfed animals as a consequence of the energy excess ingested in spite of the use of maize oil and dextrin in the formulation of the diets. These components have been reported to result in low fat gains compared with sucrose and other fats commonly used (Baltzell & Berdanier, 1985; McCargar *et al.* 1989*a*).

Table 2. *Physiological variables and plasma lipids of control and overfed rats*†

(Values are means with their standard errors for twelve animals for body and adipose-tissue weights and for six animals for other variables)

Treatment group...	Control		Overfed		Percentage change	Statistical significance of change
	Mean	SEM	Mean	SEM		
Body wt						
Initial (g)	170·9	1·2	171·4	1·9	+0	NS
Final (g)	228	3	275	6	+20	*
Adipose tissue mass						
Epididymal						
Total (g)	2·12	0·09	2·88	0·11	+36	*
Relative (%)	0·93	0·04	1·05	0·04	+13	*
Retroperitoneal						
Total (g)	1·65	0·17	3·0	0·3	+84	*
Relative (%)	0·72	0·08	1·11	0·10	+54	*
Pericardial						
Total (g)	0·283	0·010	0·59	0·03	+107	*
Relative (%)	0·124	0·003	0·213	0·008	+72	*
Liver						
Weight (g)	9·0	0·3	10·7	0·5	+18	*
Total lipids						
Concentration (mg/g)	60·5	1·8	102	4	+69	*
Total (mg)	495	25	976	54	+97	*
Plasma						
Total lipids (mg/l)	4330	190	4480	80	+3	NS
Triacylglycerols (mg/l)	2020	100	2050	70	+1	NS
Total cholesterol (mg/l)	2050	60	2170	30	+6	NS
HDL-cholesterol (mg/l)	490	20	427	17	−12	NS

NS, not significant; HDL, high-density lipoprotein.

* $P < 0·05$.

† For details of diets and procedures, see pp. 362–363.

Table 2 also shows the plasma concentrations of total lipids, triacylglycerols, total cholesterol and HDL-cholesterol. No significant changes were observed in these variables in spite of the large difference in lipid intake between two groups of animals.

Nitrogen compounds

According to our experimental design, N intake was the same in both groups of animals (Table 3). However, N excretion was substantially different: while faecal excretion remained unchanged, the urinary excretion of total N was greatly diminished in overfed animals. Therefore, the daily N retention was greatly enhanced in these animals, at least at the end of the nutritional treatment when the analyses were conducted. This fact was in agreement with the known protein-sparing effect of fat (Munro, 1951, 1978; Reeds *et al.* 1981; Barber *et al.* 1985; McCargar *et al.* 1989*a*, *b*). The difference in urinary N excretion correlated with the decrease in urinary urea excretion shown by overfed rats at the same time. Furthermore, the urinary total N:urea-N value was the same in both groups. The concentration of creatinine in plasma and the daily creatinine excretion were similar in control and overfed groups. Thus, the renal clearance of this metabolite was not significantly affected by the quantitative differences in the diets.

The quantity of N retained (72 mg/24 h) correlated well with the increment in body

Table 3. *Nitrogen balance and major nitrogen-containing compounds in serum and urine of control and overfed rats*†

(Values are means with their standard errors for six animals)

Treatment group...	Control		Overfed		Percentage change	Statistical significance of change
	Mean	SEM	Mean	SEM		
N						
Intake (mg N/24 h)	450	4	441	6	−2	NS
Excretion						
Urine (mg N/24 h)	233	10	150	9	−37	*
Faeces (mg N/24 h)	14	3	15	3	+5	NS
Balance (mg N/24 h)	203	3	275	7	+35	*
Urea						
Urine (mmol/24 h)	7·0	0·4	4·4	0·2	−37	*
Urinary urea-N: total N (%)	83·5	1·7	81	2	−3	NS
Plasma (μmol/ml)	7·2	0·3	6·8	0·3	−5	NS
Creatinine						
Urine (μmol/24 h)	57	5	51	7	−11	NS
Plasma (nmol/ml)	27·9	1·3	27·0	1·5	−3	NS
Clearance (ml/min)	1·41	0·08	1·29	0·10	−8	NS

NS, not significant.
* $P < 0.05$.
† For details of diets and procedures, see pp. 362–363.

weight shown by the overfed rats, assuming that this N was used to form protein mass (16% N) and the protein content of the whole body did not change (18% of protein in the whole rat). Obviously, N retention increased progressively throughout the dietary treatment. Therefore, the calculated value of 53 g was greater than the real one of 47 g. The main factor responsible for the N imbalance was the reduced urinary output of N, parallel to a diminished urea excretion (Table 3). The unaltered creatinine balance and the maintenance of the same plasma urea level discounted possible alterations in renal function that could block urea excretion. In this sense, the reduced urinary urea excretion could be due to reduced urea formation.

Urea and glucose synthesis by isolated hepatocytes

In order to obtain information about the alterations in N metabolism observed as a consequence of the overfeeding of lipid, we studied the capacity of hepatocytes to synthesize urea and glucose from several precursors. Table 4 shows that urea synthesis was greatly diminished in the hepatocytes isolated from overfed animals. This reduced capacity for urea production was manifested in the absence of exogenous precursors (−25%), in the presence of NH_3 plus ornithine (−57%), by adding lactate to both compounds (−36%), the combination that promoted the maximal urea production, and in the presence of alanine (−32%), the main gluconeogenic amino acid.

The capacity of hepatocytes to synthesize glucose was evaluated from single additions of several precursors (alanine, lactate, pyruvate, glycerol and fructose), each one capable of taking part in diverse metabolic processes or being incorporated into the gluconeogenic pathway at different levels. As shown in Table 4, the hepatocytes isolated from the overfed

Table 4. *Urea and glucose synthesis from given precursors by isolated rat hepatocytes*†
(Values are means with their standard errors for six animals)

Treatment group...	Control		Overfed		Percentage change	Statistical significance of change
Additive	Mean	SEM	Mean	SEM		
Urea synthesis (μmol/min per g wet wt)						
None	0·087	0·004	0·065	0·002	−25	*
NH_4Cl + ornithine	0·67	0·04	0·28	0·02	−57	*
NH_4Cl + ornithine + lactate	2·7	0·2	1·71	0·11	−36	*
Alanine	0·28	0·03	0·192	0·012	−32	*
Glucose synthesis (μmol/min per g wet wt)						
Alanine	0·35	0·02	0·230	0·016	−35	*
Lactate	0·67	0·04	0·45	0·02	−33	*
Pyruvate	0·68	0·04	0·34	0·04	−50	*
Glycerol	0·56	0·04	1·48	0·05	−14	NS
Fructose	2·21	0·05	1·44	0·14	−35	*

NS, not significant.
* $P < 0·05$.
† For details of diets and procedures, see pp. 362–363.

rats exhibited diminished production of glucose from all precursors, a more marked reduction from pyruvate (−50% compared with controls) and negligible change from glycerol (−14%, statistically non-significant).

Glucose-metabolism enzymes

Table 5 shows the activities of five enzymes involved in the control of gluconeogenesis, the main pathway for reutilization of deaminated C skeletons of amino acids (Hers & Hue, 1983). Little change was observed in the activities of the three gluconeogenic enzymes measured in the liver of the animals: fructose-1,6-biphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase (expressed per unit liver weight). However, when liver size was considered the activities were higher in the overfed group. On the other hand, the activities of glucose-6-phosphatase, the enzyme that allows glucose to be released by the liver, and that of pyruvate kinase, an important enzyme in the control of gluconeogenic–glycolytic flux (Pilkis *et al.* 1988), were diminished per unit liver weight: however, the differences were not significant when activities were expressed on a total liver basis. Since the maximal enzyme activities did not change so much in the overfed animals, the modifications in gluconeogenesis shown by the isolated hepatocytes could be attributable to an altered precursor availability (Kinney & Elwyn, 1983) derived from an overall shift in hepatic energy metabolism toward lipogenesis induced by the overfeeding (Drewry *et al.* 1988).

Urea cycle and amino acid catabolic enzymes

To explain the changes observed in urea excretion and hepatic urea production from precursors we measured the activity of the five enzymes involved in urea synthesis. Table 6 shows these values. We found that the five enzyme activities were lower in the liver of the overfed animals than in the control ones when expressed as units/g liver. The most important activity reductions were shown by carbamoylphosphate synthetase I (−36%) and argininosuccinate synthetase (−57%), both of them important enzymes in the

Table 5. *Glucose-metabolism-related enzyme activities† in the liver of control and overfed rats‡*

(Values are means with standard errors for six animals)

Treatment group...	Control		Overfed		Percentage change	Statistical significance of change
	Mean	SEM	Mean	SEM		
Glucose-6-phosphatase						
Activity (U/g)	4·0	0·3	1·38	0·14	−65	*
Total activity (U)	32·5	1·8	12·9	1·0	−60	*
Fructose-1,6-biphosphatase (*EC* 3.1.3.11)						
Activity (U/g)	1·74	0·07	1·79	0·06	+3	NS
Total activity (U)	14·2	0·5	17·0	0·8	+20	*
Phosphoenolpyruvate carboxykinase						
Activity (U/g)	0·36	0·03	0·38	0·03	+6	NS
Total activity (U)	2·9	0·2	3·6	0·3	+23	NS
Pyruvate carboxylase (*EC* 6.4.1.1)						
Activity (U/g)	78·6	1·9	86	4	+9	NS
Total activity (U)	639	8	815	8	+28	*
Pyruvate kinase (*EC* 2.7.1.40)						
Activity (U/g)	40·5	1·9	30·0	1·5	−26	*
Total activity (U)	331	23	283	4	−15	NS

NS, not significant.

* $P < 0{\cdot}05$.

† Each enzyme activity was assayed in the appropriate cytosolic, mitochondrial or microsomal fraction of liver homogenate. One unit (U) of enzyme activity was defined as the amount of enzyme required to catalyse the formation of 1 μmol product/min at 25°, except for phosphoenolpyruvate carboxykinase (*EC* 4.1.1.32) and glucose-6-phosphatase (*EC* 3.1.3.9) which were assayed at 37°. Values are expressed as U/total liver and U/g liver to account for the different liver sizes.

‡ For details of diets and procedures, see pp. 362–364.

regulation of the cycle (Schimke, 1962; Saheki *et al.* 1980; Meijer *et al.* 1990). The calculated total activities of these enzymes allowed a more accurate correlation with the *in vivo* data for urea excretion. Total activities of ornithine carbamoyltransferase, argininosuccinate lyase and arginase were unchanged, while total activities of carbamoylphosphate synthetase I and argininosuccinate synthetase were markedly diminished.

A similar concerted reduction in urea-cycle enzyme activities has been described in rats fed on low-protein diets (Schimke, 1962). It is also known that the time-course of change in the urea-cycle enzymes follows quite closely that of the change in the urinary N output after an increase or decrease in protein intake (Das & Waterlow, 1974). Furthermore, similar changes have been reported in rats fed on an energy-rich cafeteria diet (Barber *et al.* 1985, 1987) in accordance with the N-sparing effect of fat.

We place special emphasis on quantities of carbamoylphosphate synthetase I (Table 6). The availability of an immunoassay (ELISA) for this enzyme, the most important enzyme for the control of this metabolic pathway (Meijer *et al.* 1990), allowed us to correlate the changes in the enzyme activity with those in enzyme quantity (Table 6). In overfed rats this enzyme exhibited both an inhibition of activity and a reduction in quantity (−22% per unit liver weight). Although the change in the activity was slightly higher than that in quantity,

Table 6. *Urea-synthesis enzymes,† carbamoylphosphate synthetase I* (EC *6.3.4.16*) *aminotransferases and glutamate dehydrogenase* (EC *1.4.1.3*) *in liver homogenates from control and overfed rats‡*

(Values are means with their standard errors for six animals)

Treatment group...	Control		Overfed		Percentage change	Statistical significance of change
	Mean	SEM	Mean	SEM		
Carbamoylphosphate synthetase I						
Activity (U/g)	5·4	0·3	3·44	0·16	−36	*
Total activity (U)	44	3	32·5	0·8	−26	*
Quantity (mg/g)	8·7	0·3	6·8	0·3	−22	*
Total quantity (mg)	71	3	65	4	−8	NS
Activity:quantity ratio	0·61	0·03	0·511	0·016	−16	NS
Ornithine carbamoyltransferase (*EC* 2.1.3.3)						
Activity (U/g)	227	11	179	10	−21	*
Total activity (U)	1840	68	1710	128	−7	NS
Argininosuccinate synthetase (*EC* 6.3.4.5)						
Activity (U/g)	1·17	0·11	0·50	0·07	−57	*
Total activity (U)	9·5	0·8	4·7	0·7	−50	*
Argininosuccinate lyase (*EC* 4.3.2.1)						
Activity (U/g)	3·68	0·17	2·85	0·03	−23	*
Total Activity (U)	29·9	1·3	27·3	1·4	−9	NS
Arginase (*EC* 3.5.3.1)						
Activity (U/g)	892	47	705	30	−21	*
Total activity (U)	7238	279	6746	485	−7	NS
Aspartate aminotransferase (*EC* 2.6.1.1)						
Activity (U/g)	53	2	49·5	1·8	−6	NS
Total activity (U)	429	15	473	30	+10	NS
Alanine aminotransferase (*EC* 2.6.1.2)						
Activity (U/g)	31	2	13·1	0·9	−56	*
Total activity (U)	250	15	124	6	−50	*
Glutamate dehydrogenase						
Activity (U/g)	9·9	0·5	5·4	0·4	−45	*
Total activity (U)	80	3	52	6	−35	*

NS, not significant.

* $P < 0·05$.

† Enzyme activities were measured in liver homogenates. One unit (U) of enzyme activity was defined as the amount of enzyme required to catalyse the formation of 1 μmol of product/min at 37° for urea-cycle enzymes or 25° for others. Values are expressed as U/total liver and U/g liver to account for the different liver sizes. Quantities of carbamoylphosphate synthetase I were measured by enzyme immunoassay.

‡ For details of diets and procedures, see pp. 362–364.

the difference was not significant. From these results it is possible to disregard inhibitory effects and to conclude that the changes affected mainly the quantity of the enzyme.

Some authors have shown that adaptive changes in the urea-cycle enzyme activities are closely correlated with alterations in the actual amount of the enzymes (Schimke, 1964; Saheki *et al.* 1980; Cerdá *et al.* 1988). For long-term adaptations, changes in the amounts

of the enzymes seem to be more significant in the regulation of this metabolic pathway than changes in intermediate or regulatory metabolites (Waterlow, 1986; Meijer *et al.* 1990).

Table 6 also shows the activities of three important enzymes involved in the catabolism of amino acids and the availability of NH_3 for synthesis of urea. Alanine aminotransferase and glutamate dehydrogenase activities showed a marked reduction in the liver of the overfed rats. However, aspartate aminotransferase activity remained unchanged. The activities of many enzymes of hepatic amino acid metabolism parallel the changes in the urea-cycle enzymes when protein intake is modified (Das & Waterlow, 1974). In this sense, our results correlated well with the N-sparing effect induced by the overfeeding with energy and fat.

Since the most important enzymes involved in the catabolism and elimination of the amino group of the amino acids showed reduced activities in the overfed rats, this could be due to a diminished amino acid degradation as a consequence of the availability of supplementary energy.

CONCLUSIONS

It is well known that the addition of a supplementary source of non-protein energy to an adequate diet improves N balance. However, the underlying biochemical mechanism whereby the energy excess affects N metabolism is still unclear. The large number of dietary manipulations used in this type of work, many of them at first sight inadequate, as discussed, has not aided understanding of these mechanisms.

To characterize the alterations in N metabolism in rats fed on high-energy lipid-rich diets at a constant intake of protein, we have developed a model of voluntary overfeeding based on liquid diets. In our study it was important to adjust precisely the concentration of each nutrient, to isolate only one nutritional factor (the amount of energy from lipid), to measure exactly the amount of each nutrient ingested daily by each animal, to obtain voluntary acceptance of the diet by the animals at least for an adequate period and to avoid stressful actions such as tube feeding. The described procedure has optimally met these requirements. Moreover, in our opinion it has some advantages over semi-synthetic powdered diets commonly used in this type of work.

Our study on N balance has been extended with information about the enzyme systems involved in the elimination of the amino group of amino acids (urea synthesis and related processes) and in the reutilization of the C skeletons (gluconeogenesis). The results reported in the present work seem to indicate reduced amino acid catabolism in overfed growing rats. If this assumption is true, the spared amino acids must be incorporated into tissue protein mass, possibly through changes in protein turnover (Reeds *et al.* 1980, 1981; Waterlow, 1986). As discussed, long-term modifications of urea-cycle and amino acid-catabolism enzymes could depend on the protein breakdown status (Reeds *et al.* 1980; Kinney & Elwyn, 1983; Waterlow, 1986; Meijer *et al.* 1990). However, most of the work in this area of metabolism relates to short-term responses. It still remains a challenge to define the control mechanisms that are most important for long-term adaptations. Further studies are needed to progress in the knowledge of N economy.

Our findings may also have an application in human nutrition. Overfeeding with energy from lipid might be useful in the recovery of malnourished or injured people since it improves N metabolism through an important amino acid-sparing effect (for reviews, see Kinney & Elwyn, 1983; Waterlow, 1986). However, the concomitant fat gain would be disadvantageous.

Finally, we want to point out that as the liquid overfeeding technique has proved to be highly versatile, accurate and easy, this method is probably applicable to a wide variety of

nutritional studies on the metabolic responses to dietary manipulations in addition to those reported in the present paper.

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