Effect of Dietary Protein Depletion on the Galactose Elimination Capacity in Intact Rats[1]

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ABSTRACT The galactose elimination capacity (GEC) of rats fed low protein liquid diets ad libitum with 5% or 0.5% of the energy as protein was compared with that of a control group receiving a similar diet with 25% of the energy as protein. The daily dietary energy intake was almost identical in the three groups. The rats fed the 5% protein diet showed slightly higher GEC than the control group. In the rats receiving the 0.5% protein diet, GEC was significantly decreased to approximately half of the control values and the hepatic protein content was reduced to the same extent. Body weight and liver weight showed reductions to approximately 75% of control values. Triglyceride content in the livers was increased five times in the protein depleted rats. The results indicate that GEC in rats deprived of dietary protein is determined by the amount of hepatic protein. J. Nutr. *106:* 1492–1496, 1976.

INDEXING KEY WORDS liver function · protein · galactose

Protein malnutrition has been claimed to be a cause of hepatic dysfunction, the most important clinical example of this being kwashiorkhor (1). The role of malnutrition as a contributory factor in liver disease of the alcoholic is still debated (2, 3). In experimental animals, and particularly in rats, a protein deficient, but otherwise adequate diet has been shown to cause changes in the function and composition of the liver. Changes in liver function have been demonstrated concerning the oxidation of ethanol (4), protein synthesis (5), urea synthesis (6), and metabolism of fatty acids (7). Changes in liver composition have been shown regarding lipids (8), liver proteins (9), and nuclear DNA content (10).

The purpose of the present study was to quantitate alterations in the liver function of the rat during protein depletion. As a measure of the liver function, the galactose elimination capacity (GEC), which appears to be a useful indication of the "functioning liver mass" (11), was chosen. The great majority of a presented galactose load is metabolized in the liver and converted to major glucose metabolites via UDP-galactose; that is the form in which galactose is incorporated in glyco- and lipoproteins but which does not take part in the net conversion of galactose. Thus, the galactose elimination capacity is not directly involved in the metabolism of either proteins or lipids, which may both be modified by protein depletion per se.

MATERIALS AND METHODS

Female Wistar rats weighing from 160 to 203 g were fed one of three liquid diets for 21 days: A) Control diet, containing 25% of the energy as protein, 25% as fat, and 50% as glucose (23 rats). B) Protein depleted diet, containing 0.5% of the energy as protein, 25% as fat, and 74.5% as glucose (21 rats). C) Protein poor diet, containing 5% of the energy as protein, 25% as fat, and 70% as glucose (4 rats). The diets were prepared as described pre-

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TABLE 1

Energy intake and change in body weight of female rats fed liquid diets ad libitum with either 25% (A), 0.5% (B), or 5% (C) of the energy as protein for 21 days

	A Control	B	C
	25% protein $N = 5$	0.5% Protein $N = 5$	5% Protein $N = 4$
Energy intake (kcal/day)	70 ± 3[1]	78 ± 3	81 ± 5
Change in body weight (g/day)	0.6 ± 0.4	−1.4 ± 0.2[2]	1.5 ± 0.3

[1] Mean ± SEM. [2] Significantly different from group A by Student's *t*-test ($P < 0.001$).

viously (12).[2] All diets contained 1.0 kcal/ml and were fed ad libitum. The rats had free access to a cake consisting of a balanced mineral mixture (13).

The body weight and the energy intake of five rats of group A, five of group B, and four of group C, kept in metabolic cages was measured daily. The daily change in weight was estimated as the slope of the linear regression of weight on time. The rest of the rats were kept in stock rat cages and weighed once a week.

The galactose elimination capacity (GEC) was determined (14) in 18 rats of group A, in 16 rats of group B, and in the four rats of group C. The non-fasting rats were anesthetized with intraperitoneal injection of thiopental sodium[3] (100 mg/kg body weight to group A and C, 75% of this dose was given to group B to obtain the same level of anaesthesia). They were tracheotomized, intubated, and nephrectomized. Galactose was administered via a catheter in a jugular vein as a single injection, [2 mmoles/kg body weight in order to ensure saturated, not concentration dependent, galactose metabolism (14)] followed by a continuous infusion during 62 minutes, (2.1 μmoles/minute for groups A and C, and 1.1 μmoles/minute for group B. These infusion rates were found to maintain approximately constant galactose concentration in pilot experiments). Following an equilibration period of 20 minutes, blood was drawn from a catheter in the carotid artery with an interval of 7 minutes for enzymatic analysis of galactose (15).

The galactose elimination capacity (GEC) was estimated as

$$GEC = I - 0.4 \cdot \text{Body weight} \cdot dc/dt$$

Where I is the infusion rate of galactose, 0.4; body weight the volume of distribution for galactose (14), and dc/dt the slope of the linear regression of the arterial galactose concentration on time. The infusion rate was adjusted to equal the elimination rate, so that the mean of dc/dt was not significantly different from zero. Mean galactose concentration during infusion showed no systematic distribution in the groups, and the range was from 2 to 4 mM, ensuring that the galactose elimination was not concentration dependent, due to full saturation of enzyme systems (14).

The protein concentration in liver tissue was measured by the UV method (16). The liver concentration of triglycerides was determined by enzymatic analysis of glycerol after hydrolysis (17). Results were compared by Student's *t*-test (23).

RESULTS

The protein content of the diets did not significantly influence the energy intake of the rats kept in metabolic cages (table 1). Of these, group B lost weight during the feeding period, whereas group C had a slightly, but not significantly, higher daily gain in weight than the rats in group A.

Changes in weight, liver composition, and GEC are given in table 2. The rats in group C had higher initial and final body

[2] Diet

	A	B	C
Protein (g/liter)	62.5	1.25	12.5
Fat (g/liter)	27.8	27.8	27.8
Glucose (g/liter)	102.0	163.5	152.5

Protein, Casein nach Hammersten, Merck, Germany. Fat, corn oil : cod liver oil : peanut oil—0.27 : 0.08 : 0.65. Stabilizer, lecithin, Merck, Germany (10 g/liter diet). Vitamins, ABCDin Ido, Ferrosan, Denmark (1 ml/liter diet).

[3] Pentothal-Natrium, Abbott, Denmark.

TABLE 2

Liver composition and junction in rats fed liquid diets ad libitum with either 25% (A), 0.5% (B), or 5% (C) of the energy as protein for 3 weeks

	A	B		C	
	Control 25% protein	0.5% Protein	*P*	5% Protein	*P*
Initial body weight (g)	177 ± 3 (18)[1]	176 ± 2 (16)	NS	193 ± 1 (4)	<0.005
Final body weight (g)	187 ± 3 (18)	138 ± 2 (16)	<0.005	216 ± 4 (4)	<0.005
Liver weight (g)	6.29 ± 0.21 (18)	4.51 ± 0.13 (16)	<0.005	6.97 ± 0.28 (4)	<0.05
Liver triglycerides (g)	0.04 ± 0.01 (10)	0.18 ± 0.03 (9)	<0.005	—	—
Liver protein (g)	1.32 ± 0.05 (12)	0.70 ± 0.05 (12)	<0.005	—	—
Galactose elim. cap. (μmoles·min^{-1})	2.35 ± 0.14 (18)	1.30 ± 0.08 (16)	<0.005	2.79 ± 0.37 (4)	NS
(μmoles·min^{-1} 100 g^{-1} rat)	1.25 ± 0.07 (18)	0.94 ± 0.07 (16)	<0.005	1.32 ± 0.15 (4)	NS
(μmoles·min^{-1}·g^{-1} liver)	0.37 ± 0.02 (18)	0.29 ± 0.02 (16)	<0.005	0.40 ± 0.04 (4)	NS
(μmoles·min^{-1}·g^{-1} fat free liver)	0.40 ± 0.02 (10)	0.31 ± 0.02 (9)	<0.005	—	—
(μmoles·min^{-1}·g^{-1} liver protein)	1.80 ± 0.12 (12)	2.10 ± 0.18 (12)	NS	—	—

[1] Mean ± SEM. Number of rats in parenthesis. *P*-values from student's *t*-tests against group A. NS—Not significant, $P > 0.05$.

weights and liver weights than those in groups A and B. The GEC showed no significant changes. In group B the rats exhibited the same relative decrease in body weight and liver weight (to 70% of group A values), hence the proportion between body and liver weight was maintained (relative liver weight 3.3% of body weight). Due to a fourfold increase in hepatic triglycerides in group B, the reduction of the fat free liver weight was slightly greater than of the total liver weight (relative amount of fat 0.9% in A and 4.1% in B). Liver protein was reduced to 50% of group A values and the relative protein contents was decreased (from 21% in group A to 15% in group B).

GEC in the group B rats was reduced to approximately 50% of that in the group A. GEC in relation to body weight, liver weight, and fat free liver weight was significantly reduced, whereas GEC in relation to liver protein was not significantly changed.

DISCUSSION

In the present study, dietary protein depletion in rats induced parallel reductions in the galactose elimination capacity (GEC) and in hepatic protein content; accordingly, GEC per gram liver protein remained unchanged. The GEC and liver protein control values were of the same order of magnitude as found by others (14, 18, 19).

The identical intake of energy of the rats in the three groups indicates that the changes found in liver composition and function were not due to reluctance of the rats to eat the diets but rather to lack of protein. The slightly greater gain in weight of the rats fed the 5% protein diet (group C) compared with the control rats (group A) was unexpected, but is in agreement with the findings of Peng (20). A possible explanation is that a moderate protein deficit may be compensated for by a low catabolic rate (21).

The rats fed the 0.5% protein diet (group B) had a higher glucose consumption than the controls. Goresky et al. (22) showed in intact dog livers that the transport of galactose into the hepatocytes may be inhibited by high concentrations

of glucose, but since the capacity of the transport system is very high in relation to the metabolic rate, high glucose concentrations do not readily explain the reduced galactose elimination in the protein depleted rats. This is supported by findings of Keiding.[4] The lower dose of thiopental needed to anesthetize rats fed the 0.5% protein diet (group B) was probably due to reduced relative amount of body fat. Apart from this, no altered tolerance to the anesthetic was observed. Inhibition of galactose break down during barbiturate anaesthesia, as described by Salaspuro (24), was not found.

Feeding rats a diet with 0.5% of the energy as protein (group B) reduced the GEC to about 50% of the control values, whereas rats fed a diet with 5% of the energy as protein (group C) had an insignificant rise in GEC. This effect of a less serious dietary protein deprivation confirms the preliminary results published by Salaspuro (18), who found a small increase in the galactose break down in rats fed an 8% protein diet. It appears that there is no simple dose-effect relation between protein consumption and galactose elimination capacity.

The mean change in GEC in the rats fed the low protein diet (group B) for 3 weeks corresponded to the mean change in hepatic protein content. In contrast to this Goebell and Bode (4) with a similar experimental design found only a slight reduction of hepatic protein, a decrease in liver alcohol dehydrogenase activity to 15% of control values, and an increase in liver phosphofructokinase activity.

It is not clear if these metabolic changes should be considered to be an adaptive depression of hepatic functions or a liver injury. The parallel between galactose elimination capacity and liver protein in the present experiments is in agreement with the concept of the galactose elimination capacity as a measure of the "functioning liver mass" (11).

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