Effect of a Low-Protein Diet on the Incorporation of Amino Acids into Rat Serum Lipoproteins

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Triglycerides accumulate in the liver of the rat after the administration of a wide variety of chemical agents, and in certain nutritional deficiencies produced experimentally. Impairment of the synthesis, or secretion from the liver, of the serum low-density lipoproteins has been shown to cause the accumulation of the triglycerides in several of these situations. Diminished incorporation of amino acids into the lipoproteins has been shown to occur after the administration of carbon tetrachloride (Seakins & Robinson, 1963), ethionine (Robinson & Harris, 1961), puromycin (Robinson & Seakins, 1962) and orotic acid (Roheim *et al.*, 1966) and in choline deficiency (Mookerjee, 1968). It has been suggested that this mechanism may also account for the fatty liver in protein-depleted rats (Best *et al.*, 1955). The present study provides some direct evidence for this. The point is of some importance because fatty liver is a prominent feature, and may sometimes be a cause of death, in human protein-energy (protein-calorie) malnutrition (Waterlow, 1948; Waterlow & Alleyne, 1971).

Materials and methods

Locally bred albino rats, weighing 65–80 g, were fed *ad lib.* on a 6%-casein diet (Waterlow & Stephen, 1966). There was a consistent rise in total liver content of lipids, which reached about three times the normal value after 10 days of feeding, and all the increase in lipid was triglyceride. Age-matched controls were fed on a stock diet (Purina).

On the 10th. day of feeding on the test diet, the rats, after being kept without food overnight, were either injected via the tail vein with DL-[1-¹⁴C]leucine (30–50 Ci/mol) or given a constant infusion of [U-¹⁴C]glycine (10 Ci/mol), as in the method described by Garlick (1969). Both amino acids (from The Radiochemical Centre, Amersham, Bucks., U.K.) were dissolved in 0.9% NaCl and each rat received 10 μ Ci/100 g body wt. At 1.5 h after the injection, or after 4 h infusion, the specific radioactivities of serum lipoproteins and of liver protein or of free glycine in liver and serum were measured. The serum was collected from clotted blood by centrifugation at 2500 rev./min in an MSE Mistral 2L centri-

fuge for 20 min at 4°C. Serum lipoproteins were isolated and purified as described previously (Seakins & Robinson, 1963). Liver protein was purified by the method of Lombardi & Oler (1967). Glycine, in protein-free extracts of liver and serum, was measured by the method of Alexander *et al.* (1945), scaled down tenfold. All proteins were finally dissolved in 1 M-NaOH, from which solution portions were taken for the determination of protein (Lowry *et al.*, 1951) and radioactivity (Lombardi & Oler, 1967). Glycine was separated from the extracts of liver and serum by paper chromatography, eluted from the chromatogram and its radioactivity counted in the same system as that used for the proteins. A Beckman LS150 counter was used and quench corrections were made by the channels-ratio method.

Results

It has been shown previously (Waterlow & Stephen, 1966; Haider & Tarver, 1969) and it was confirmed in this study that, after the injection of a labelled amino acid, the liver protein is more highly labelled in the depleted rats than in the controls. This is due to the changes induced by the low-protein diet in the amino acid precursor pool. To allow for this the lipoprotein specific radioactivity was related to the liver specific radioactivity and the relative specific radioactivities were compared in the two groups after the injection of leucine.

When the lipoproteins were fractionated into two groups, namely lipoproteins $d < 1.019$ and lipoproteins $d 1.019$ – 1.063 , the relative specific radioactivities of both fractions were significantly lower in the depleted rats ($P < 0.05$; Student's *t* test). The greater decrease was in the $d < 1.019$ fraction (Table 1).

This finding was confirmed by a constant-infusion technique. After a 4 h infusion of labelled glycine, the specific radioactivity of free glycine in both the liver and serum was higher in the depleted rats than in the controls ($P < 0.05$), but there was no significant difference between the two groups in the specific radioactivity of the lipoproteins $d < 1.019$ (Table 2). The ratio

$$\frac{\text{Product specific radioactivity}}{\text{Precursor specific radioactivity}}$$

i.e.

$$\frac{\text{Lipoprotein specific radioactivity}}{\text{Free liver glycine specific radioactivity}}$$

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Table 1. *Incorporation of DL-[1-¹⁴C]leucine into liver protein and serum low-density lipoprotein fractions of protein-depleted and control rats*

Rats were injected intravenously with DL-[1-¹⁴C]leucine (10 μ Ci/100 g body wt.). Each rat was killed 1.5 h after the injection. Lipoprotein fractions were prepared by ultracentrifugation. Specific radioactivity is expressed as c.p.m./mg of protein and the values shown are means $\pm$ S.E.M. There were four rats in each group.

	Specific radioactivity (c.p.m./mg)			Lipoprotein relative specific radioactivity	
	Liver	Serum lipoproteins <i>d</i> < 1.019	Serum lipoproteins <i>d</i> 1.019–1.063	Lipoproteins <i>d</i> < 1.019/liver	Lipoproteins <i>d</i> 1.019–1.063/liver
Depleted rats	2218 $\pm$ 222	7580 $\pm$ 290	11 706 $\pm$ 1051	3.17 $\pm$ 0.15	5.45 $\pm$ 0.69
Controls	999 $\pm$ 60	8342 $\pm$ 383	7591 $\pm$ 548	8.43 $\pm$ 0.89	7.63 $\pm$ 0.99

Table 2. *Incorporation of [U-¹⁴C]glycine into serum lipoproteins *d* < 1.019 of protein-depleted and control rats during a constant infusion*

Rats were infused with a solution of [U-¹⁴C]glycine at a constant rate of 0.5 ml/h for 4 h, each rat receiving 10 μ Ci/100 g body wt. The specific radioactivities of serum lipoproteins *d* < 1.019 and free glycine in the serum and liver were measured at the end of the infusion. Glycine specific radioactivity is expressed as c.p.m./ μ mol of glycine, lipoprotein specific radioactivity as c.p.m./mg of protein. The last column shows, in addition, the means $\pm$ S.E.M.

	10 ⁴ $\times$ Specific radioactivity (c.p.m./ μ mol)		Specific radioactivity (c.p.m./mg) Lipoproteins <i>d</i> < 1.019	Lipoprotein specific radioactivity Liver glycine specific radioactivity
	Serum glycine	Liver glycine		
Depleted rats	4.9	2.8	4570	0.16
	3.5	2.0	3840	0.19
	2.8	1.7	4700	0.28
Controls	0.59	0.37	4450	1.20
	2.1	1.5	5900	0.39
	0.94	1.0	3760	0.37

is a measure of the fractional rate of synthesis. This ratio was significantly lower in the depleted rats than in the controls ($P < 0.05$).

Discussion

The present studies show that, in rats that develop a fatty liver when fed on a low-protein diet, there is diminished incorporation of amino acids into the serum low-density lipoproteins. The effect is most marked on the lipoproteins *d* < 1.019. This finding is important in view of the fact that it is this lipoprotein fraction that is the main vehicle for the transport of triglyceride from the liver to the serum (Havel *et al.*, 1962).

The constant-infusion technique is a more accurate way of comparing rates of amino acid incorporation into protein, because in the single-injection type of experiment, even when a correction is made for the specific radioactivity of the free amino acid precursor at the time when the animal is killed, the time-course

of change of specific radioactivity during the interval after injection may be quite different in animals treated in different ways.

The fractional synthesis rate cannot be computed directly from the ratio of specific radioactivities shown in the last column of Table 2. After the injection of [¹⁴C]glycine some of the radioactivity in the protein will be in serine and other amino acids. To calculate the fractional synthesis rate it is necessary to isolate the protein-bound glycine and measure its specific radioactivity. This was not possible with the small amounts of protein available. However, under the conditions of these measurements the fractional rate of synthesis will vary approximately linearly with the ratio of specific radioactivities (Waterlow & Stephen, 1968; Garlick, 1972). Therefore one may conclude from the mean values in Table 2 that the fractional synthesis rate of lipoproteins *d* < 1.019 in the depleted rats was about one-third of that in the controls.

The calculation of absolute synthesis rate requires that the pool size be known. There is evidence for the

presence in serum of an apolipoprotein that can be reutilized for lipoprotein synthesis without synthesis *de novo* (Roheim *et al.*, 1965). It has been suggested that the concentration of this apolipoprotein is greatly diminished in protein-depleted rats; moreover, since the intravenous injection of it into depleted rats with fatty livers causes the release of triglyceride from liver into the serum, its supply appears to be rate-limiting for lipid secretion from the liver under these conditions (Flores *et al.*, 1970). If, then, the size of the apolipoprotein pool is low in the depleted rats, the absolute rate of synthesis will be even more diminished than the fractional rate.

The present data therefore suggest that in rats on a low-protein diet there is impairment of lipoprotein synthesis by the liver, this being most marked in the very-low-density fraction.

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