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Concomitant increase in hepatic triacylglycerol biosynthesis and cytosolic fatty-acid-binding-protein content after feeding rats with a cholestyramine-containing diet

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Cholestyramine feeding of rats increased the rate of palmitate and glycerol incorporation into triacylglycerols of isolated hepatocytes. Concomitantly an increase of fatty-acid binding by hepatic cytosolic proteins was observed, which could be attributed to an elevation of the content of the fatty-acid-binding protein (M_r 12000). The involvement of this protein in cholesterol, bile-acid and triacylglycerol metabolism is discussed.

Induction of bile acid synthesis by administration of bile-salt sequestrants or by biliary drainage is accompanied by an increase in the plasma content and hepatic synthesis of triacylglycerols and very-low-density lipoproteins (Nestel & Grundy, 1976; Witztum *et al.*, 1976; Adler *et al.*, 1978; Angelin *et al.*, 1978). Previously we observed that feeding rats with a diet containing cholestyramine results in an increased rate of palmitate incorporation into triacylglycerols of isolated hepatocytes (Kempen *et al.*, 1982).

In the present paper we wish to document in more detail this effect of cholestyramine on triacylglycerol synthesis and to consider the possibility that this effect is mediated by a rise in the cytosolic content of the FABP or Z-protein in the liver. The reasons that led us to this assumption are as follows.

(1) FABP stimulates hepatic triacylglycerol synthesis in cell-free systems (O'Doherty & Kuksis, 1975; Burnett *et al.*, 1979).

(2) There is a positive correlation between the hepatic content of FABP and the rate of fatty acid uptake or utilization for triacylglycerol synthesis in the liver in several conditions involving hormonal, dietary and pharmacological manipulations (Fleischner *et al.*, 1975; Mishkin *et al.*, 1975; Renaud *et al.*, 1978; Burnett *et al.*, 1979; Ockner *et al.*, 1979, 1980).

(3) Evidence has come forth that FABP is very similar to, if not identical with, SCP_1 in physical and molecular properties and in tissue distribution (Billheimer & Gaylor, 1980; Dempsey *et al.*, 1981; Ockner *et al.*, 1982). This latter protein stimulates a number of microsomal activities involved in cholesterol synthesis from squalene (Dempsey, 1974; Gaylor & Delwiche, 1976; Srikantaiah *et al.*, 1976), but it can also bind fatty acids and stimulate enzymes catalysing fatty-acid activation and esterification (Daum & Dempsey, 1979, 1980).

At the time our studies were started, we were not familiar with any work on the modulation of the hepatic SCP content. Since the rate of cholesterol synthesis is severalfold increased by cholestyramine (Kempen *et al.*, 1983), it seemed plausible to us that the SCP content could be elevated as the result of this treatment. Indeed, Morin *et al.* (1982) reported that a rise in SCP activities takes place in liver cytosol of cholestyramine-fed rats. In the present paper a similar increase of cytosolic FABP activity will be demonstrated.

Abbreviations used: FABP, fatty-acid-binding protein; SCP, sterol carrier protein.

Experimental

Materials

All radiolabelled compounds were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.; Lipidex 1000 was from Packard Instrument Co., Downers Grove, IL, U.S.A.; fatty-acid-free bovine serum albumin and calibration proteins were from Sigma, St. Louis, MO, U.S.A.

Triacylglycerol synthesis

Male Wistar rats (175–200g) were obtained from the Centraal Proefdierenbedrijf TNO, Zeist, The Netherlands. The rats were fed *ad libitum* on a control or a cholestyramine-containing diet, prepared exactly as described previously (Kempen *et al.*,

1982). Hepatocytes were isolated as described by Kempen (1980) and Kempen *et al.* (1982) and incubated in dilute suspensions in Krebs–Henseleit (1982) buffer, pH 7.4, containing 2% (w/v) fatty-acid-free bovine serum albumin and various concentrations of [U-^{14}C]palmitate. In some experiments 2mM-[1(3)-^{3}H]glycerol was present in addition to 0.2mM-palmitate. The rate of triacylglycerol synthesis was calculated as the incorporation of radiolabel into the triacylglycerol fraction between 10 and 30min of incubation, divided by 3 times the mean specific radioactivity of the free-fatty-acid fraction (Kempen *et al.*, 1982), or by that of glycerol 3-phosphate prevailing during this period. The specific radioactivity of glycerol 3-phosphate was determined as described by Pikkukangas *et al.* (1982).

Preparation of delipidated rat liver cytosol

A 4g piece of the left major lobe was obtained, after anaesthesizing the rat with diethyl ether and perfusing the liver for 2min with ice-cold buffer A. This buffer contained 100mM-sucrose, 50mM-KCl, 30mM-Na_2EDTA and 50mM-potassium phosphate (pH 7.2) and was perfused via a catheter in the portal vein through the liver *in situ*, after severing the vena cava inferior. The liver fragment was forced through a steel mesh, and the resulting pulp was homogenized in a Dounce homogenizer in 3 vol. of ice-cold buffer A. The homogenate was centrifuged for 15min at 10000*g* and 4°C. The supernatant was collected (without the fatty cake at the meniscus) and centrifuged for 60min at 105000*g* in the MSE 6 × 14 swing-out rotor at 4°C. The resulting supernatant (cytosol) was stored at −20°C.

For delipidation, 4ml of the cytosol, containing about 30mg of protein, was subjected to chromatography on a column of Lipidex 1000 (1cm × 15cm), equilibrated with 10mM-potassium phosphate buffer, pH 7.4, at 37°C. Elution was performed with the same buffer (flow rate 20ml/h). All protein was present in the void volume. Further details of this procedure are described elsewhere (Glatz & Veerkamp, 1983). Protein content was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Assay of fatty-acid binding

The binding of [1-^{14}C]palmitate to cytosolic protein was measured with a recently developed assay procedure in which Lipidex 1000 is employed at 0°C as adsorber of unbound palmitate (Glatz & Veerkamp, 1983).

Results

Triacylglycerol synthesis in hepatocytes

Feeding rats with a cholestyramine-containing diet results in an increased capacity for triacylglycerol synthesis in their hepatocytes, as is evident from the incorporation of ^{14}C-labelled palmitate added in concentrations between 0.2 and 1.0mM (Fig. 1). Furthermore, in incubations with 2mM-[^{3}H]glycerol and 0.2mM-palmitate, incorporation of glycerol in triacylglycerols also was stimulated about 2-fold by feeding the resin [0.94 ± 0.32 and 1.90 ± 0.24 nmol · h^{-1} · (mg dry wt.)$^{-1}$ in hepatocytes of three control and three cholestyramine-fed rats, respectively; $P < 0.05$]. This indicates that the effect on the incorporation of [^{14}C]palmitate really represents a stimulation of triacylglycerol synthesis, and not only an increased turnover of fatty acyl moieties in pre-existent triacylglycerol molecules.

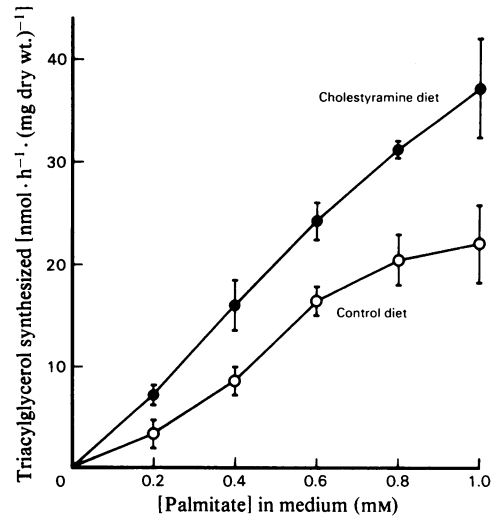

Fig. 1. *Triacylglycerol synthesis measured at different concentrations of [U-^{14}C]palmitate by hepatocytes obtained from control* (○) *or cholestyramine-fed* (●) *rats*

Values represent means ± S.D. for three experiments for both nutritional conditions.

FABP content of liver cytosol

The binding of palmitate to delipidated rat liver cytosolic proteins was studied as a function of the total palmitate concentration. As reported previously (Glatz & Veerkamp, 1983), the binding isotherm, analysed by the method of Scatchard (1949), is consistent with the presence on the cytosolic proteins of a single class of saturable binding sites for palmitate. Fig. 2 shows the binding isotherms for the liver cytosol preparations from a control and a cholestyramine-fed rat. It is clear from Fig. 2 and from the collective data of similar experiments (Table 1) that the cytosol of cholestyramine-fed rats contains a significantly higher capacity for palmitate binding per μg of protein than does the cytosol from control rats. With both types of cytosol

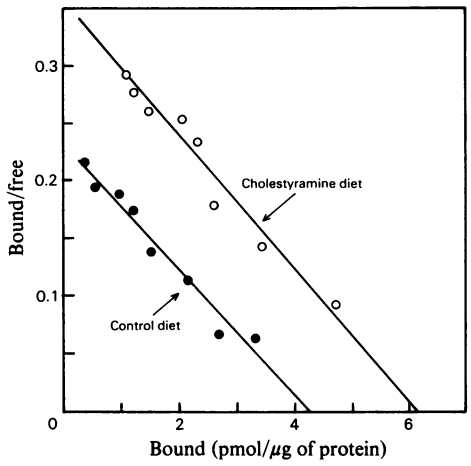

Fig. 2. *Scatchard analyses of the binding of palmitate by the delipidated cytosol from control (●) and cholestyramine-fed (○) rats*

Cytosolic protein (23 μg) was incubated with 0.1–3 μM-[1-^{14}C]palmitate, and, after equilibration, protein-bound and unbound palmitate were separated by the use of Lipidex 1000 at 0°C (Glatz & Veerkamp, 1983). The results of representative experiments are shown.

Table 1. *Palmitate binding to delipidated liver cytosolic proteins from control and cholestyramine-fed rats*

Data are derived from Scatchard (1949) analysis of individual binding isotherms, as described by Glatz & Veerkamp (1983), with 20–30 μg of cytosolic protein per assay. They represent means ± S.D. for the number of separate preparations (*n*) given, and are compared by the unpaired *t* test. Abbreviations used: $B_{max.}$, maximum binding; NS, not significant.

Diet	*n*	Apparent K_d (μM)	$B_{max.}$ (pmol/μg of protein)
Control diet	5	1.01 ± 0.16	4.39 ± 0.26
Cholestyramine diet	7	1.06 ± 0.22	6.03 ± 0.67
	P ...	NS	<0.001

preparations, however, half-maximal saturation is reached at the same palmitate concentrations (1 μM). These findings suggest that the liver cytosol of cholestyramine-fed rats contains 1.5-fold more binding sites per mg of protein, but on the same molecular species.

When the liver cytosolic proteins were fractionated by chromatography on Sephadex G-75, the major fatty-acid-binding fraction was eluted at the same position with both control and cholestyramine-fed rats (Fig. 3). This fraction corresponds to M_r

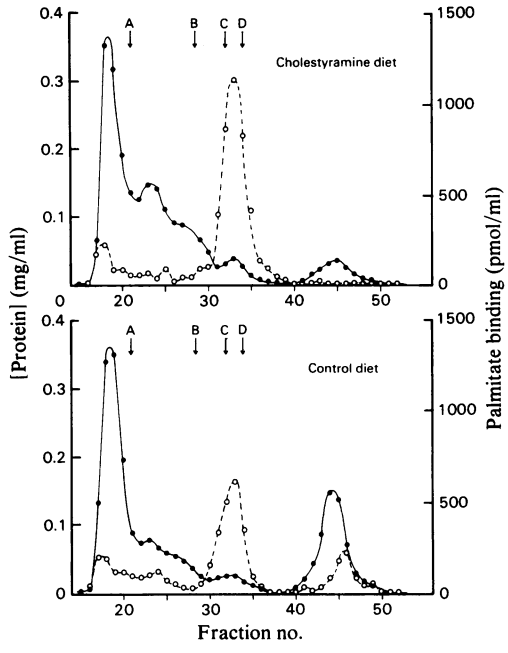

Fig. 3. *Gel filtration on a column (1.6 cm × 85 cm) of Sephadex G-75 of 10 mg of liver cytosolic protein from control and cholestyramine-fed rats*

Elution was performed at room temperature with 10 mM-potassium phosphate buffer, pH 7.4. The fraction volume was 3.7 ml. All fractions were assayed for protein (●) and for palmitate binding (○). For the latter, 50–200 μl samples and 1 μM-[1-^{14}C]palmitate were used. Arrows indicate the elution positions of calibration proteins; A, rat serum albumin; B, chymotrypsinogen; C, horse skeletal-muscle myoglobin; and D, horse heart cytochrome *c*. The recoveries of protein as well as of fatty-acid-binding activity from the column were 85–95%. The results are representative for three independent experiments with each group of rats.

12000–15000 and contains FABP (Ockner *et al.*, 1982). The specific palmitate-binding activity of this fraction was also about 1.5-fold higher in the cytosol of cholestyramine-fed compared with control rats (29–31 and 20–22 pmol/μg of protein respectively). Interestingly the small fatty-acid-binding fraction of M_r 1500–2000 of the cytosol of control rats (Fig. 3), which contains a fatty-acid-binding peptide (Suzue & Marcel, 1975; Rüstow *et al.*, 1979), was absent from the cytosol of cholestyramine-fed rats.

Previously we found evidence that FABP possesses only one fatty-acid-binding site per protein molecule (Glatz & Veerkamp, 1983). FABP accounts for 68 and 81% of the fatty acid binding of our cytosol preparations from control and cholestyramine-fed rats respectively (Fig. 3). Together

with the observation that FABP has an M_r of 12000 (Ockner *et al.*, 1982), it appears from the maximal fatty-acid binding (Table 1) that the cytosolic FABP concentration is $0.68 \times 4.39 \times 12 = 36\,\mu g$/mg of protein in control rats and $0.81 \times 6.03 \times 12 = 58\,\mu g$/mg of protein in cholestyramine-fed rats.

Discussion

In the present study we established that feeding rats with a cholestyramine-containing diet results in an increase of both the rate of triacylglycerol synthesis in hepatocytes and the FABP content of liver cytosol. This correlation corresponds to previous findings that these parameters change in parallel (Fleischner *et al.*, 1975; Mishkin *et al.*, 1975; Renaud *et al.*, 1978; Burnett *et al.*, 1979; Ockner *et al.*, 1979, 1980). When measured at physiologically relevant palmitate concentrations, we found a 2-fold increased triacylglycerol synthesis in hepatocytes from cholestyramine-fed rats (Fig. 1). This rise can only partially be explained by the rise in the cytosolic FABP content, since on cholestyramine treatment the latter increased only 1.5-fold (Table 1). An enhanced activity of the enzyme phosphatidate phosphatase (Angelin *et al.*, 1981) may also be involved in the effect on triacylglycerol synthesis.

Morin *et al.* (1982) showed that feeding of cholestyramine to adult rats increased the activities of hepatic SCP_1 and SCP_2. This correlated with our previous observations that the hepatic rate of cholesterol synthesis (Kempen *et al.*, 1983) and content (Kempen *et al.*, 1982) are elevated by cholestyramine feeding. Since the cytosolic FABP content is analogously modulated by cholestyramine, these results strongly support the idea that SCP_1 and FABP are identical proteins (Billheimer & Gaylor, 1980; Dempsey *et al.*, 1981; Ockner *et al.*, 1982). Our results show that the induction of bile-acid synthesis and, in consequence, of cholesterol synthesis in rat liver by cholestyramine (Kempen *et al.*, 1982, 1983) appears to be accompanied, or partly mediated, by a rise in the hepatic content of this cytosolic protein. We suggest that the observed stimulation of the hepatic triacylglycerol synthesis after cholestyramine treatment is attributable to the same protein modulating fatty acid incorporation into triacylglycerols.

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