Short Communications

Regulation of Hepatic Lipogenesis by Plasma Free Fatty Acids: Simultaneous Studies on Lipoprotein Secretion, Cholesterol Synthesis, Ketogenesis and Gluconeogenesis

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(Received 27 December 1973)

1. An inverse logarithmic relationship was found between lipogenesis and the concentration of serum free fatty acids perfusing the rat liver.
2. Increased concentrations of serum free fatty acids did not alter cholesterol synthesis, triglyceride secretion or increase gluconeogenesis.
3. No direct relationship was found between ketogenesis and either lipogenesis or gluconeogenesis.

Hepatic fatty acid synthesis (lipogenesis) is markedly depressed in starved, diabetic or fat-fed animals (Masoro, 1962). This may be brought about by long-term changes in total amounts of the lipogenic enzymes and by acute alterations of enzyme activity by metabolic modifiers (Lowenstein, 1972). A candidate for the role of principal physiological modifier is the plasma free fatty acid acting directly or through its metabolic product, long-chain acyl-CoA. In all the three metabolic states mentioned above, where lipogenesis is depressed, plasma free fatty acids are increased. However, because of the complexities of interpretation *in vivo* this does not provide evidence for a direct inhibitory effect of increased plasma free fatty acids on hepatic lipogenesis. Porter & Long (1958) showed that long-chain acyl-CoA inhibited fatty acid synthesis in a soluble pigeon liver system. Subsequently it was shown that acetyl-CoA carboxylase (EC 6.4.1.2), a key enzyme in the pathway of fatty acid synthesis, was inhibited by long-chain acyl-CoA (Tubbs & Garland, 1963; Bortz & Lynen, 1963). The physiological relevance of these findings was questioned when Dorsey & Porter (1968) and Pande & Mead (1968) suggested that the inhibitory action of free fatty acids and long-chain acyl-CoA could be attributed to their non-specific detergent properties. Recently it has been demonstrated that free fatty acids in the presence of albumin inhibit fatty acid synthesis promoted by lactate and fructose in isolated hepatocytes prepared from neonatal chicks (Goodridge, 1973).

As yet there is no unequivocal evidence that lipogenesis in the intact liver, whether *in vivo* or when perfused *in vitro*, is inhibited directly by elevated concentrations of free fatty acids within the physiological range. The present communication provides

this evidence and additional information on the relationships between lipogenesis on the one hand and lipoprotein secretion, cholesterol synthesis, ketogenesis and gluconeogenesis on the other.

Experimental

Livers from fed male Wistar albino rats (340–360 g) were perfused for 90 min with 140 ml of whole defibrinated rat blood by the method of Mayes & Felts (1966). All livers were perfused at a constant flow rate of 12 ml/min and a pO_2 of 90–100 mmHg (12–13 kPa). Various quantities of oleic acid [Sigma (London) Chemical Co., London S.W.6, U.K.] complexed with crystalline bovine serum albumin (Armour Pharmaceutical Co., Eastbourne, U.K.) were infused, first as a rapid priming infusion and then as a slower infusion at constant rate. Perfusate free fatty acids were stabilized at concentrations which spanned the physiological range 0.34–1.57 μ mol/ml of serum. The addition of albumin to the perfusate resulted in a molar ratio of albumin:free fatty acid slightly greater than normal. Thus effects ascribed to the presence of free fatty acids are unlikely to be due to the surface-active properties of uncomplexed free fatty acids.

Fatty acid and cholesterol synthesis was measured by following the incorporation of 3H in 3H_2O into hepatic and perfusate total fatty acid and total cholesterol. For this 5 mCi of 3H_2O (The Radiochemical Centre, Amersham, Bucks., U.K.) in 1.0 ml of 0.9% NaCl was added to the bulk of the perfusate simultaneously with the commencement of infusion of the free fatty acid. The technique and assumptions used in calculation were similar to those of Windmueller & Spaeth (1966). The methods of sampling the liver and blood and of extraction for analysis of radioactive lipids have been described

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Table 1. *Effect of variations in concentration of serum free fatty acid on lipid and carbohydrate metabolism of perfused livers from fed rats*

Livers were perfused for 90 min by methods described in the text. The free fatty acid concentration of the perfusate was raised by an infusion of an albumin-oleate complex. Determinations carried out at 5, 30, 60 and 90 min showed that the concentration was stable throughout this period. The other concentrations and rates refer to those attained in the steady state. In the calculation of the rate of fatty acid synthesis the assumption has been made that the average molecule of fatty acid synthesized has 31.7 carbon-bound hydrogen atoms, of which 13.3 are ^3H derived from $^3\text{H}_2\text{O}$ (Windmuller & Spaeth, 1966). The glycogen concentrations are terminal values. Statistical tests were carried out by using the analysis of variance. Each horizontal line represents one perfusion.

Serum free fatty acids ($\mu\text{mol/ml}$)	Fatty acid synthesis ($\mu\text{mol of fatty acid/h per g}$)	Secretion of fatty acid in VLD lipoproteins ($\mu\text{mol of fatty acid/h per g}$)	Fraction of VLD-lipoprotein fatty acids accounted for by newly synthesized fatty acids (%)		Total cholesterol synthesis (nmol of cholesterol/h per g)	Ketogenesis ($\mu\text{mol of acetate plus 3-hydroxybutyrate/h per g}$)	Perfusate glucose ($\mu\text{mol/ml}$)	Glycogen (g/100 g)	Lactate uptake ($\mu\text{mol/h per g}$)
0.34	6.13	6.61	50.8		80	4.5	9.6	2.37	42.6
0.46	5.45	9.51	21.8		117	4.3	10.0	3.13	34.6
0.46	5.19	—	—		—	4.6	9.6	3.40	45.0
0.75	3.60	8.52	18.4		—	—	11.8	0.52	—
0.81	2.63	7.71	13.7		129	2.2	7.4	0.65	43.8
1.00	2.86	10.15	13.8		104	5.1	8.9	0.94	55.0
1.40	2.34	10.53	5.4		207	11.2	9.4	1.03	57.0
1.51	2.15	6.61	9.1		92	9.5	—	—	35.8
1.57	3.20	8.76	8.3		38	9.8	10.2	1.64	52.7
Correlation with free fatty acid concentration	$P < 0.01$	$P > 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

previously, together with the methods used for determining glycogen, glucose, lactate, triglyceride and free fatty acids (Topping & Mayes, 1972). Blood ketone bodies were determined by the method of Williamson *et al.* (1962). Radioactivity in total fatty acids and cholesterol was determined after saponification and separation by a modification of the methods of Exton *et al.* (1972).

Results and discussion

Lipogenesis. In the absence of infused oleate the free fatty acid concentration stabilized at $0.34 \mu\text{mol/ml}$ of serum and, under these conditions, the rate of total hepatic fatty acid synthesis was $6.13 \mu\text{mol/h per g}$. In other perfusions in which the serum free fatty acids in the steady state were at higher concentrations there was a corresponding fall ($P < 0.01$) in the rate of hepatic lipogenesis (Table 1). The greatest inhibition occurred over the range of free fatty acid concentrations $0.3\text{--}0.8 \mu\text{mol/ml}$, i.e. in the range through which the plasma free fatty acids increase during transition from the fed to the starved state. There was also a significant correlation ($P < 0.05$) between the concentration of hepatic glycogen and the rate of lipogenesis. However, when a multiple regression analysis (Brownlee, 1949) was performed, only the negative regression of lipogenesis on free fatty acid concentration remained statistically significant ($P < 0.025$).

When the data were plotted on logarithmic coordinates the regression between the rate of lipogenesis and the free fatty acid concentration reached the highest level of significance ($P < 0.001$), which is indicative, most likely, of the kinetics of the underlying mechanism. The rates of lipogenesis found are similar to those obtained by other investigators both *in vivo* (Lowenstein, 1971) and in the perfused liver (Windmueller & Spaeth, 1966, 1967). It may also be inferred from the present data that lipogenesis is not completely suppressed by free fatty acids at the higher physiological concentrations. Thus at a free fatty acid concentration of $0.8 \mu\text{mol/ml}$ of serum, which is comparable with the concentration found in starved rats, there was a synthesis of $3.5 \mu\text{mol}$ of fatty acid/h per g. As livers from starved rats have a rate of fatty acid synthesis of less than 2% of that of non-starved controls (Windmueller & Spaeth, 1966), present results indicate that additional factors, e.g. hormonal balance and enzyme adaptation (Lowenstein, 1972), are responsible for the very low rates of lipogenesis in livers of starved animals.

Secretion of lipoproteins. The rate of secretion of triglycerides by the perfused liver has been correlated both with the serum free fatty acid concentration (Gidez *et al.*, 1962) and with the rate of hepatic lipogenesis (Windmueller & Spaeth, 1967). The present results (Table 1) did not show any significant

change in the rate of secretion of VLD-lipoprotein* fatty acids with increased concentrations of serum free fatty acids. The fraction of VLD-lipoprotein fatty acids accounted for by newly synthesized fatty acids fell from approx. 50% to 5% as the serum free fatty acid concentration was increased and indicates that the extra contribution of free fatty acids towards VLD-lipoprotein secretion is compensated by a corresponding decrease in the contribution from hepatic lipogenesis.

Cholesterol synthesis. The results show that the rate of cholesterol synthesis did not change significantly with increase in concentration of free fatty acids (Table 1). It is implied that the decrease in cholesterol synthesis in livers of starved animals (Van Bruggen *et al.*, 1952) cannot be attributed to the increase of plasma free fatty acids.

Ketogenesis. Stadie (1958) proposed that a decrease in utilization of acetyl-CoA in fatty acid synthesis was a direct cause of ketogenesis. However, in fat-fed rats there was an absence of a relation between lipogenesis and ketogenesis (Mayes, 1959). Lately the concept of a direct inverse relationship has been revived (McGarry & Foster, 1971). Present results (Table 1) indicate that under conditions of increasing free fatty acid concentration lipogenesis may be halved before there is any rise in the rate of ketogenesis, and it is concluded again that there is no causal relationship between lipogenesis and ketogenesis. Similar conclusions have been made by Barth *et al.* (1973).

Gluconeogenesis. There are conflicting views as to whether plasma free fatty acids stimulate hepatic gluconeogenesis (e.g. Williamson *et al.*, 1966; Exton & Park, 1967). Previous results (Mayes, 1970) indicated that over the greater range of physiological concentrations free fatty acids did not play a direct role in the short-term regulation of gluconeogenesis from either amino acids or lactate. Results reported in Table 1 support this conclusion in demonstrating no significant change in the rate of lactate uptake over a wide range of serum free fatty acid concentrations. There was no correlation between ketogenesis and the rate of lactate uptake.

Conclusions. The present studies demonstrate that plasma free fatty acids are regulators of lipogenesis in the intact liver. However, the results provide no information on the precise mechanism involved. It is possible that long-chain acyl-CoA inhibits acetyl-CoA carboxylase or the transport of citrate from mitochondria (Halperin *et al.*, 1972). Equally, it may be that pyruvate dehydrogenase, a key enzyme on the pathway of lipogenesis from carbohydrate, is regulatory. Attention is drawn to the inverse relationship between serum free fatty

* Abbreviation: VLD lipoprotein, very-low-density lipoprotein ($d < 1.006$).

acids and the proportion of active to inactive pyruvate dehydrogenase in the liver (Wieland *et al.*, 1972). When the results obtained by these authors are plotted logarithmically, they may be virtually superimposed on our own logarithmic plot of lipogenesis against the serum free fatty acid concentration.

We are grateful for the able assistance of Mr. R. Gardner and Mr. A. Mangalji and for support from the Medical Research Council.

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