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Evidence for a Reciprocal Relationship between Lipogenesis and Ketogenesis in Hepatocytes from Fed Virgin and Lactating Rats

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Lipogenesis is increased in hepatocytes from fed lactating rats compared with virgin rats. Inhibition of lipogenesis with 5-(tetradecyloxy)-2-furoic acid resulted in increased ketogenesis from endogenous substrate, but not from oleate. Dihydroxyacetone increased lipogenesis and esterification of [1-^{14}C]oleate and decreased ketogenesis; these changes were reversed by the inhibitor. The reciprocal relationship between lipogenesis and ketogenesis in hepatocytes from fed rats may be due to alterations in [malonyl-CoA] [McGarry, Mannaerts & Foster (1977) *J. Clin. Invest.* **60**, 265–270; Cook, King & Veech (1978) *J. Biol. Chem.* **253**, 2529–2531], but this mechanism is not considered to be sufficient to explain the increased ketogenesis in starvation completely.

It has been reported that malonyl-CoA, a key intermediate in the synthesis of lipid *de novo*, inhibits the activity of carnitine acyltransferase I (EC 2.3.1.21) in rat liver mitochondria (McGarry *et al.*, 1977). This has led these authors to propose that the function of malonyl-CoA is to act as both a precursor for fatty acid synthesis and a suppressor of fatty acid oxidation. In this way, it might provide a regulatory link between lipogenesis and ketogenesis and play an important role in the intrahepatic disposal of long-chain fatty acids between the pathways of esterification and oxidation (for a review see Williamson & Whitelaw, 1978). If this postulate is correct there should be an inverse relationship between lipogenesis (and esterification) and ketogenesis. Indeed, it is well established in the rat that, on transition from the fed to the starved state, the rate of lipogenesis and esterification decreases and that of ketogenesis increases. A crucial question, however, is whether such a correlation also exists in the fed state.

If a regulatory link exists between lipogenesis and ketogenesis, inhibition of the former should result in an increased rate of ketone-body synthesis and decreased esterification. To test this point we have examined the effects of 5-(tetradecyloxy)-2-furoic acid, an inhibitor of the transmitochondrial membrane transport of citrate (Ribereau-Gayon, 1976), on the metabolism of hepatocytes from fed virgin and lactating rats. This compound has been shown to decrease lipogenesis in hepatocytes from fed rats (Panek *et al.*, 1977) and in mammary-gland acini from lactating rats (Robinson & Williamson, 1977). Hepatocytes from lactating rats were chosen because they have a lower rate of ketogenesis compared with hepatocytes from virgin rats (Whitelaw & Williamson, 1977), and studies with liver slices suggest they may have a higher rate of lipogenesis (Smith, 1973). While this work was in progress, evidence was presented that there is a reciprocal relationship between [malonyl-CoA] and the rates of ketogenesis in hepatocytes from meal-fed rats (Cook *et al.*, 1978) which are known to have high rates of lipogenesis (Harris, 1975).

Experimental

Female rats of the Wistar strain were used. Virgin rats weighed between 180 and 250g and lactating rats between 260 and 320g. The litter size was eight to twelve pups and the period of lactation was 12–16 days. The rats were fed *ad libitum* on Oxoid breeding diet for rats and mice (Oxoid Ltd., London S.E.1, U.K.). Rats were anaesthetized with Nembutal (60mg/kg body wt.; solution in 0.9% NaCl).

All enzymes and coenzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. 5-(Tetradecyloxy)-2-furoic acid was a gift from Dr. A. Richardson (Merrell National Laboratories, Lockland Station, Cincinnati, OH, U.S.A.).

[1-^{14}C]Oleate, [1-^{14}C]acetate and $^{3}H_2O$ were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Isolated hepatocytes were prepared essentially by the method of Berry & Friend (1969) as modified by Krebs *et al.* (1974).

The incubation procedure and measurements of esterification of [1-^{14}C]oleate and conversion of [1-^{14}C]acetate into lipid were as described by Whitelaw & Williamson (1977). Lipogenesis was measured with $^{3}H_2O$ by the method described by Harris (1975). The following metabolites were determined in the neutralized $HClO_4$ extracts by enzymic methods:

glucose (Slein, 1963), L-lactate (Hohorst *et al.*, 1959), acetoacetate and D-3-hydroxybutyrate (Williamson *et al.*, 1962). Measurements of radioactivity were carried out as described by Williamson *et al.* (1975).

The rates of ketogenesis, lipogenesis and esterification were calculated from the graphical plots of the 20, 40 and 60min values; these were usually linear.

Results and Discussion

The rates of lipogenesis from endogenous substrates measured with ^3H_2O were 2-fold higher in hepatocytes from lactating rats than in hepatocytes from virgin rats (Table 1). Addition of dihydroxyacetone (5mM), which was found by Harris (1975) to be the best substrate for lipogenesis in hepatocytes from meal-fed rats, increased the rate by 55% in hepatocytes from virgin rats and by 70% in hepatocytes from lactating rats. A 2-fold difference in the rate of [1-^{14}C]acetate (2mM) conversion into lipid was also observed between hepatocytes from virgin and lactating rats (results not shown). It has previously been reported that the hepatic activity of certain lipogenic enzymes and the rate of incorporation of [1-^{14}C]acetate into lipid in liver slices is higher in lactating rats (Smith, 1973) and the present results confirm this finding in isolated hepatocytes. Higher rates of lipogenesis have been observed in hepatocytes from male (Panek *et al.*, 1977) and female (Harris, 1975; Mapes, 1977) rats which have been meal-fed with a low-fat high-carbohydrate diet, but in the present experiments we did not wish to interfere with the normal dietary regime of the rats. It is well established that lactating rats have a higher food intake than virgin rats (Cole & Hart, 1938; Fell *et al.*, 1963) and this may play a role in the increased rate of lipogenesis.

As expected, oleate (1mM) decreased both the rate of endogenous lipogenesis (Table 1; Mayes & Topping, 1974) and that in the presence of dihydroxyacetone. In confirmation of the results of Panek *et al.* (1977), 5-(tetradecyloxy)-2-furoic acid inhibited the endogenous rate of lipogenesis by at least 80% in hepatocytes from both virgin and lactating rats. The inhibition may be due to decreased transport of citrate from the mitochondria (Ribereau-Gayon, 1976) or alternatively to inhibition of acetyl-CoA carboxylase (Kariya & Wille, 1978). There is no evidence at present that the inhibitor directly affects enzymes in the pathways of esterification or ketogenesis.

The rates of ketogenesis from endogenous substrates and in the presence of oleate were lower in the hepatocytes from lactating rats (see also Whitelaw & Williamson, 1977). Addition of 5-(tetradecyloxy)-2-furoic acid significantly increased the rate of endogenous ketogenesis in hepatocytes from both virgin (2-fold higher) and lactating rats (3-fold higher), but did not affect the rate with oleate (Table 1).

Dihydroxyacetone decreased the rate of ketogenesis when oleate was present; the absolute decrease was similar with hepatocytes from lactating rats (0.26μmol/min per g wet wt.) to that with hepatocytes from virgin rats (0.22μmol/min per g wet wt.). 5-(Tetradecyloxy)-2-furoic acid significantly increased the rate of ketogenesis from oleate plus dihydroxyacetone in hepatocytes from lactating rats, and there was a tendency for a similar effect in hepatocytes from virgin rats. Thus inhibition of lipogenesis resulted in increased ketogenesis from both endogenous substrates and from oleate plus dihydroxyacetone, and this was particularly marked with hepatocytes from lactating rats, where the rates of lipogenesis were higher. When lipogenesis was already partially inhibited, as in incubations with oleate alone, there was no further increase in ketogenesis on addition of 5-(tetradecyloxy)-2-furoic acid, even though a further inhibition of lipogenesis occurred.

Dihydroxyacetone increased the esterification of [1-^{14}C]oleate by 36% in hepatocytes from virgin rats and by 70% in hepatocytes from lactating rats. 5-(Tetradecyloxy)-2-furoic acid did not affect esterification of oleate in hepatocytes from either type of rat, but it suppressed the increased rate of esterification observed in the presence of dihydroxyacetone.

5-(Tetradecyloxy)-2-furoic acid did not significantly alter the rates of glucose or lactate accumulation when oleate and dihydroxyacetone were present (results not shown).

In the present experiments changes in the rate of lipogenesis were accompanied by reciprocal changes in the rate of ketogenesis, except when oleate was the added substrate. Studies *in vivo* in the rat have shown that hepatic [malonyl-CoA] is related to the rate of lipogenesis (Guynn *et al.*, 1972; Cook *et al.*, 1977). Malonyl-CoA, at near-physiological concentrations, has been shown to be an inhibitor of carnitine acyltransferase I (McGarry *et al.*, 1977) and therefore it is possible that the alterations in the rate of ketogenesis observed in the present experiments in response to alteration in lipogenic rate are in part brought about by changes in [malonyl-CoA]. Thus inhibition of lipogenesis would be expected to decrease [malonyl-CoA], which would relieve inhibition of entry of long-chain fatty acyl-CoA into the mitochondria for oxidation; this in turn would mean less fatty acyl-CoA available for esterification. An increase in lipogenesis would have the opposite effect. Studies with hepatocytes from meal-fed rats have shown that [malonyl-CoA] is inversely related to the rate of ketogenesis (Cook *et al.*, 1978), but rates of lipogenesis or esterification were not measured in these experiments.

Table 1. *Effects of* 5-(*tetradecyloxy*)-2-*furoic acid on lipogenesis, ketogenesis and esterification of* [1-^{14}*C*]*oleate in hepatocytes from fed virgin and lactating rats*
For experimental details see the Experimental section. All results are mean values ± S.E.M.; lipogenesis is expressed as μmol of ^3H_2O incorporated into lipid/min per g wet wt. (20–60 min period), ketogenesis as μmol of ketone bodies formed/min per g wet wt. and esterification as μmol of [1-^{14}C]oleate esterified/min per g wet wt. The numbers of observations are shown in parentheses. Values that are significantly different (Student's *t* test) in the presence of 5-(tetradecyloxy)-2-furoic acid (0.1 mM) from those in its absence are indicated by: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Values for oleate plus dihydroxyacetone that are significantly different from those for oleate alone are indicated by: †$P<0.05$; ††$P<0.001$.

		Lipogenesis		Ketogenesis		Esterification	
Type of rat	Substrate added	Control	+Inhibitor	Control	+Inhibitor	Control	+Inhibitor
Virgin	None	0.045 ± 0.006 (12)	0.009 ± 0.002 (5)***	0.11 ± 0.02 (11)	0.21 ± 0.03 (5)*	—	—
	[1-^{14}C]Oleate (1 mM)	0.017 ± 0.002 (6)	0.009 ± 0.002 (5)	0.84 ± 0.09 (13)	0.82 ± 0.05 (5)	0.14 ± 0.01 (14)	0.12 ± 0.01 (4)
	[1-^{14}C]Oleate+ dihydroxyacetone (5 mM)	0.049 ± 0.012 (4)†	0.014 ± 0.004 (5)**	0.62 ± 0.08 (5)†	0.70 ± 0.03 (5)†	0.19 ± 0.01 (4)††	0.15 ± 0.01 (4)**
	Dihydroxyacetone (5 mM)	0.073 ± 0.015 (6)	—	—	—	—	—
	Acetate (2 mM)	0.050 ± 0.01 (6)	—	—	—	—	—
Lactating (12–16 days)	None	0.097 ± 0.011 (14)	0.013 ± 0.004 (5)***	0.03 ± 0.01 (14)	0.09 ± 0.02 (6)**	—	—
	[1-^{14}C)Oleate (1 mM)	0.046 ± 0.006 (8)	0.017 ± 0.007 (4)*	0.37 ± 0.06 (13)	0.45 ± 0.09 (5)	0.20 ± 0.01 (17)	0.19 ± 0.01 (5)
	[1-^{14}C]Oleate+ dihydroxyacetone (5 mM)	0.077 ± 0.006 (6)††	0.025 ± 0.006 (6)***	0.11 ± 0.02 (6)††	0.27 ± 0.06 (6)*	0.34 ± 0.03 (5)††	0.22 ± 0.01 (5)***
	Dihydroxyacetone (5 mM)	0.17 ± 0.03 (8)	—	—	—	—	—
	Acetate (2 mM)	0.12 ± 0.02 (4)	—	—	—	—	—

If it is accepted that effects of modulation of [malonyl-CoA] on the transport of long-chain fatty acyl-CoA into the mitochondria is the explanation for the apparent inverse relationship between lipogenesis and ketogenesis, then the question arises as to how important are these effects for the physiological regulation of ketogenesis. The absolute changes in the rates of ketogenesis observed here are not large compared with those found in hepatocytes from starved rats incubated under similar conditions (Whitelaw & Williamson, 1977). Even if all of the increase in ketogenesis observed on addition of oleate was due to inhibition of lipogenesis, rather than provision of substrate (which is the more likely explanation), this would only represent 54% of the rate of ketogenesis in hepatocytes from starved virgin rats. In addition, the presence of the lipogenic inhibitor did not remove the difference in the rate of ketogenesis between hepatocytes from virgin and lactating rats.

In conclusion, in hepatocytes from fed virgin and lactating rats there appears to be, over a limited range, an inverse relationship between the rates of ketogenesis and lipogenesis. On the basis of the studies of McGarry *et al.* (1977) it is suggested that [malonyl-CoA] may be the regulatory link between the two processes. However, in the present experimental conditions this reciprocal relationship does not play a major role in the regulation of ketogenesis, nor is it likely to explain the large increase in ketogenesis on transition from the fed to the starved state.

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References

Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520

Cole, H. H. & Hart, G. H. (1938) *Am. J. Physiol.* **123**, 589–597

Cook, G. A., Nielsen, R. C., Hawkins, R. A., Mehlman, M. A., Lakshmanan, M. R. & Veech, R. L. (1977) *J. Biol. Chem.* **252**, 4421–4424

Cook, G. A., King, M. T. & Veech, R. L. (1978) *J. Biol. Chem.* **253**, 2529–2531

Fell, B. F., Smith, K. A. & Campbell, R. M. (1963) *J. Pathol. Bacteriol.* **85**, 179–188

Guynn, R. W., Veloso, D. & Veech, R. L. (1972) *J. Biol. Chem.* **247**, 7325–7331

Harris, R. A. (1975) *Arch. Biochem. Biophys.* **169**, 168–180

Hohorst, H. J., Kreutz, F. H. & Bücher, T. (1959) *Biochem. Z.* **332**, 18–46

Kariya, T. & Wille, L. J. (1978) *Biochem. Biophys. Res. Commun.* **80**, 1022–1024

Krebs, H. A., Cornell, N. W., Lund, P. & Hems, R. (1974) *Alfred Benzon Symp.* **6**, 718–743

Mapes, J. P. (1977) *Biochem. J.* **162**, 47–50

Mayes, P. A. & Topping, D. L. (1974) *Biochem. J.* **140**, 111–114

McGarry, J. D., Mannaerts, G. P. & Foster, D. W. (1977) *J. Clin. Invest.* **60**, 265–270

Panek, E., Cook, G. A. & Cornell, N. W. (1977) *Lipids* **12**, 814–818

Ribereau-Gayon, G. (1976) *FEBS Lett.* **62**, 309–312

Robinson, A. M. & Williamson, D. H. (1977) *Biochem. J.* **168**, 465–474

Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed.), pp. 117–123, Academic Press, New York and London

Smith, R. W. (1973) *J. Dairy Res.* **40**, 339–351

Whitelaw, E. & Williamson, D. H. (1977) *Biochem. J.* **164**, 521–528

Williamson, D. H. & Whitelaw, E. (1978) *FEBS Symp. 11th* **42**, 151–160

Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96

Williamson, D. H., McKeown, S. R. & Ilic, V. (1975) *Biochem. J.* **150**, 145–152