Biochem. J. (1980) **192**, 361–364
Printed in Great Britain

Rapid inhibition of lipogenesis *in vivo* in lactating rat mammary gland by medium- or long-chain triacylglycerols and partial reversal by insulin

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(*Received 16 June 1980/Accepted 14 July 1980*)

An intragastric load of medium- or long-chain triacylglycerols inhibited lipogenesis in lactating rat mammary gland *in vivo* by 82 or 89% respectively. This inhibition was reversed partially by insulin administration. Long-chain triacylglycerols inhibited hepatic lipogenesis *in vivo* but medium-chain triacylglycerols increased it 2-fold. Glucose utilization *in vitro* by mammary gland acini from triacylglycerol-fed rats was normal.

During lactation, the proportion of fat in the diet does not affect the fat content of milk, but the fat composition of the milk resembles that of the diet when this is rich in fat (Garton, 1963; Coniglio & Bridges, 1966; Grigor & Warren, 1980). High-fat diets given throughout lactation decrease lipogenesis in the mammary gland *in vivo* in both mice (Romsos *et al.*, 1978) and rats (Agius *et al.*, 1980; Grigor & Warren, 1980) and *in vitro* (Coniglio & Bridges, 1966). These studies show that mammary gland lipogenesis is decreased when an increased supply of dietary lipid has been available throughout lactation. However, a key question is whether the gland can respond rapidly to an increase in fat intake. To investigate this point, we studied the effects of a single intragastric load of medium- or long-chain triacylglycerol on the rates of lipogenesis *in vivo* in the mammary gland of the lactating rat that had been fed on normal chow (a high-carbohydrate/low-fat diet) throughout lactation. For comparison, lipogenesis was also measured in liver and brown adipose tissue.

Experimental

Albino Wistar rats were subjected to a 12h light/12h dark cycle (with the light phase from 08:00 to 20:00h) and were fed on a standard diet (Oxoid breeding diet, Oxoid, London SE1, U.K.). At parturition the litters were culled to 10 pups and the lactating rats were used 10–14 days post-partum (body wt. 330–360g). All experiments were started between 09:00 and 10:00h. Long-chain triacylglycerol (glycerol trioleate, BDH, Poole, Dorset, U.K.) and medium-chain triacylglycerol (a mixture of C_6–C_{12} fatty acids with at least 95% C_8 and C_{10}; Cow and Gate, Trowbridge, Wilts., U.K.) were administered intragastrically (1ml/100g body wt.) through a plastic tube after the rat had been anaesthetized with diethyl ether for 3min. Glucose (2mmol/100g body wt., 2M) and glycerol (2ml) were administered similarly. Controls were intubated with saline (154mM-NaCl, 1ml/100g body wt.). Insulin (2 units; Isophane insulin injection; Nordisk Insulinlaboratorium, Copenhagen, Denmark) was injected subcuteneously 40min after intragastric feeding. Rates of lipogenesis *in vivo* were measured by the incorporation of ^3H_2O into lipid after saponification (Stansbie *et al.*, 1976). The rats were left with their litters throughout the experiment and were injected with 4mCi of ^3H_2O (0.4ml) 2h after the intragastric load. They were anaesthetized after 50min (sodium pentobarbital, 60mg/kg body wt.) and dissected at 60min. An arterial blood sample was withdrawn for determination of blood glucose concentration (Slein, 1963) and plasma ^3H_2O specific radioactivity. The inguinal mammary gland, liver and interscapular brown adipose tissue were removed and weighed and the lipid of 1g samples of tissue was saponified and extracted for determination of ^3H_2O incorporation (Stansbie *et al.*, 1976). The rest of the mammary gland was used for the preparation of acini (Robinson & Williamson, 1977). The acini were incubated in Krebs–Henseleit (1932) saline containing D-[1-^{14}C]glucose (5mM) to study glucose removal and formation of lactate and pyruvate and the incorporation of D-[1-^{14}C]glucose into lipid and $^{14}CO_2$ as described previously (Agius *et al.*, 1980). To investigate the effects of medium-chain triacylglycerol *in vitro* on the metabolism of acini from control-fed rats, 2.5% (w/v) bovine serum albumin with or without 4mM-medium-chain triacylglycerol (final concentration) was included in the incubation medium. The medium-chain triacyl-

0306-3283/80/100361-04$01.50/1

glycerol was mixed with the albumin solution and the mixture was subjected to ultrasonication for 2min. It was then added to the Krebs–Henseleit saline to give the final concentrations indicated above.

Results and discussion

Experiments in vivo

A single intragastric load of medium-chain triacylglycerols (about 6mmol) inhibited lipogenesis in lactating mammary gland *in vivo* by 82% compared with the control rate, whereas long-chain triacylglycerol (about 4mmol) inhibited lipogenesis by 89% (Table 1). Low concentrations of glycerol inhibit uptake of glucose and hence lipogenesis in mammary gland acini *in vitro* (Robinson & Williamson, 1977); however, a load of glycerol (25mmol; blood concentration about 3mM) did not inhibit mammary gland lipogenesis *in vivo* (Table 1). Glucose feeding or insulin administration had no effect on mammary gland lipogenesis in the control rat but they restored the lipogenic rate to 40–60% ($P<0.0025$) of the control rate in the long-chain triacylglycerol-fed rat and to 75% ($P<0.0025$) of the control in the medium-chain triacylglycerol-fed rat. The failure of insulin to reverse the rate of lipogenesis completely in the triacylglycerol-fed rats may not be a consequence of the accompanying hypoglycaemia (blood glucose concentration, 1.41 ± 0.21mM) because insulin treatment of saline-fed and glucose-fed rats resulted in a similar degree of hypoglycaemia (blood glucose concentration, 1.87 ± 1.10mM) but this did not inhibit lipogenesis (Table 1). Glucose-feeding resulted in a 3-fold increase in plasma insulin concentration (results not shown) and this may explain the increase in mammary gland lipogenesis in the triacylglycerol-fed rats that were given glucose.

Long-chain triacylglycerol feeding also inhibited hepatic lipogenesis (45%) but medium-chain triacylglycerol increased it 2-fold (Table 1). The different effects of medium-chain triacylglycerol feeding on lipogenesis in mammary gland and liver are not surprising, since medium-chain fatty acid synthesis occurs in lactating rat mammary gland (Dils & Popjak, 1962) but probably to a lesser extent in liver. Medium-chain fatty acids can be absorbed directly into the portal blood and oxidized in the liver, unlike long-chain triacylglycerols which are transported via the lymphatics and bypass the hepatic–portal system (Senior, 1968). Long-term feeding with medium-chain triacylglycerols, in non-lactating rats, resulted in inhibition of hepatic lipogenesis from glucose *in vitro* (Wiley & Leveille, 1973) but increased incorporation of acetate into lipid both *in vivo* and *in vitro* (Kritchevsky & Tepper, 1965). We have shown that in virgin rats a single intragastric load of medium-chain triacylglycerol (or glucose) increases hepatic lipogenesis 2-fold and brown-adipose-tissue lipogenesis 10-fold (Agius & Williamson, 1980). In the present study, brown-adipose-tissue lipogenesis in lactating rats was not significantly increased by either medium-chain triacylglycerol or glucose; however, both substrates increased hepatic lipogenesis (Table 1).

Experiments in vitro

The inhibition *in vivo* of mammary gland lipogenesis that occurs after long-term 'cafeteria' feeding (Agius *et al.*, 1980) or on starvation (24h) (Robinson *et al.*, 1978) is still present in mammary gland

Table 1. *Rates of lipogenesis in vivo in mammary gland, liver, and brown adipose tissue in lactating rats after triacylglycerol feeding*

For experimental details see the text. The values are means ± S.D. for the number of rats shown in parentheses. Values that are significantly different by the Student *t* test from the control rats are shown: $*P<0.05$; $**P<0.005$.

Treatment of rats	Tissue ...	Rate of lipogenesis (μmol of ^3H_2O incorporated into lipid/h per g wet wt.)		
		Mammary gland	Liver	Brown adipose tissue
Saline (7)		91.9 ± 20.8	15.4 ± 3.3	5.1 ± 2.5
Long-chain triacylglycerol (7)		9.9 ± 4.3**	8.4 ± 3.8**	4.4 ± 3.0
Long-chain triacylglycerol + glucose (5)		36.7 ± 15.6**	9.7 ± 2.5**	3.8 ± 2.8
Long-chain triacylglycerol + insulin (5)		56.8 ± 10.6**	31.2 ± 19.5*	31.0 ± 18.9**
Medium-chain triacylglycerol (8)		16.8 ± 3.8**	31.0 ± 3.0**	21.3 ± 23.4**
Medium-chain triacylglycerol + glucose (4)		70.1 ± 39.4	29.0 ± 5.7**	13.6 ± 6.5*
Medium-chain triacylglycerol + insulin (4)		67.6 ± 6.9*	33.4 ± 10.6**	42.5 ± 17.9**
Glycerol (4)		97.4 ± 33.8	32.2 ± 16.4*	7.4 ± 3.7
Glucose (7)		105 ± 39.6	23.3 ± 7.8*	6.2 ± 1.8
Insulin (5)		104 ± 7.1	42.4 ± 11.5**	44.6 ± 16.1**
Glucose + insulin		113 ± 26.1	42.9 ± 15.4**	55.1 ± 21.5**

Table 2. *Metabolism of [1-^{14}C]glucose in acini isolated from mammary glands of lactating rats fed intragastrically with medium or long-chain triacylglycerols*

For details see the Experimental section. Where indicated, insulin (30m-i.u./ml) was added to the incubation medium. The results are mean values ± S.D. expressed as μmol/min per 100mg defatted dry wt., with the number of observations in parentheses. Values that are significantly different ($P < 0.05$) from the appropriate control are shown by *.

	Saline-fed (6)		Long-chain triacylglycerol-fed (6)		Medium-chain triacylglycerol-fed (3)		Saline-fed (6)	
Incubation with ...		Insulin		Insulin		Insulin	Albumin	Albumin + medium-chain triacylglycerol
Glucose removal	1.20 ± 0.24	1.33 ± 0.14	1.15 ± 0.26	1.30 ± 0.27	1.37 ± 0.12	1.52 ± 0.30	1.71 ± 0.48	1.24 ± 0.21*
Formation of $^{14}CO_2$	0.54 ± 0.19	0.64 ± 0.17	0.52 ± 0.16	0.62 ± 0.16	0.34 ± 0.07	0.58 ± 0.07	0.38 ± 0.10	0.34 ± 0.04
Incorporation of ^{14}C into lipid	0.48 ± 0.16	0.59 ± 0.08	0.35 ± 0.10	0.44 ± 0.09	0.31 ± 0.02	0.46 ± 0.03	0.40 ± 0.12	0.32 ± 0.07
Lactate formation	0.29 ± 0.05	0.36 ± 0.14	0.49 ± 0.22	0.57 ± 0.20	0.48 ± 0.26	0.57 ± 0.35	0.42 ± 0.37	0.36 ± 0.21
Pyruvate formation	0.05 ± 0.03	0.05 ± 0.03	0.09 ± 0.07	0.10 ± 0.06	0.13 ± 0.07	0.10 ± 0.08	0.08 ± 0.05	0.07 ± 0.02

acini incubated *in vitro*; we therefore tested whether this was also the case after acute inhibition *in vivo* by intragastric triacylglycerol feeding. The rate of glucose removal by acini prepared from triacylglycerol (medium or long-chain)-fed rats was the same as in acini from control rats (Table 2), even though the rate of lipogenesis *in vivo* was depressed by 82–89% in the triacylglycerol-fed rats. In the acini from the control rats, 15.5 ± 2.4% of the glucose utilized accumulated in the medium as lactate and pyruvate, whereas the proportion of glucose converted to lactate and pyruvate increased to 25.3 ± 4.4% ($P < 0.005$) after long-chain triacylglycerol loading and there was a corresponding decrease in the incorporation of [1-^{14}C]glucose into lipid; however, these changes were not significant. The increased proportion of glucose accumulating as lactate and pyruvate after long-chain triacylglycerol feeding suggests inactivation of pyruvate dehydrogenase that was retained *in vitro*. When acini were incubated with albumin and medium-chain triacylglycerol, the rate of glucose removal was decreased by 27% ($P < 0.05$) (Table 2).

Conclusions

The present experiments indicate that prolonged feeding of a high-fat diet (Coniglio & Bridges, 1966; Romsos *et al.*, 1978; Grigor & Warren, 1980) is not necessary to demonstrate inhibition of mammary gland lipogenesis. The mechanism whereby the mammary gland senses the availability of excess triacylglycerol in blood is not known, nor is the site(s) of inhibition of lipogenesis. However, evidence is accumulating that the short-term regulation of mammary gland lipogenesis is complex and varies in response to the nutritional and hormonal state of the animal in a physiological manner. We have shown that rats fed a 'cafeteria' diet (high in fat) throughout lactation have a depressed rate of mammary gland lipogenesis *in vivo* and that acini from the glands of these rats have decreased rates of glucose utilization, which correlates with the inhibition of lipogenesis *in vivo* (Agius *et al.* 1980). The removal *in vitro* of glucose, and lipogenesis, can be restored to normal by addition of insulin (Agius *et al.*, 1980). In the present study short-term triacylglycerol feeding resulted in decreased lipogenesis *in vivo*, but glucose utilization by the acini *in vitro* was normal. Thus the rapid effects of a triacylglycerol load differ from those of a 'cafeteria' diet given throughout lactation, but resemble those of short-term (2h) insulin deficiency, which decreases mammary gland lipogenesis *in vivo* (Robinson *et al.*, 1978) but does not affect glucose removal by acini *in vitro* (Robinson & Williamson, 1977). However, acini from insulin-deficient rats do accumulate more lactate and pyruvate than do those from rats fed triacyl-

glycerols. The ability of insulin to reverse partially the inhibition of lipogenesis by triacylglycerol loading *in vivo* is further support for the role of this hormone in the short-term regulation of lipogenesis in lactating mammary gland of the rat. The depression of mammary gland lipogenesis in starved (24h) lactating rats and its reversal on short-term (90min) refeeding can be demonstrated *in vitro* (Robinson & Williamson, 1977; Robinson *et al.*, 1978) and may in part be due to changes in the activation state of pyruvate dehydrogenase (Baxter & Coore, 1978; Robinson & Williamson, 1977). Recent work suggests the involvement of another control site in the pathway of lipogenesis from glucose, which is distal to pyruvate dehydrogenase and is sensitive to insulin *in vitro* (M. A. Munday & D. H. Williamson, unpublished work); a likely candidate is acetyl-CoA carboxylase. Whatever the mechanisms, the inhibition of lipogenesis in the lactating mammary gland *in vivo* after triacylglycerol loading is an obvious physiological advantage in that it spares lipogenic substrates (mainly glucose) when there is increased triacylglycerol available in the blood steam.

This work was supported by the Medical Research Council and the U.S. Public Health Service (grant no. AM-11748). L. A. is a Commonwealth Scholar and D. H. W. is a member of the External Staff of the Medical Research Council (U.K.).

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