Lipogenesis in interscapular brown adipose tissue of virgin, pregnant and lactating rats

THE EFFECTS OF INTRAGASTRIC FEEDING*

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Lipogenesis in brown adipose tissue of virgin rats increased 8–10-fold after intragastric feeding with glucose or medium-chain triacylglycerol, and this increase was prevented by short-term insulin deficiency. Brown adipose tissue increased in weight during pregnancy, regressed during lactation and hypertrophied again on weaning; the rate of lipogenesis paralleled these changes. Glucose did not increase brown-adipose-tissue lipogenesis at mid-lactation.

Brown adipose tissue is the main site of cold-induced non-shivering thermogenesis (Foster & Frydman, 1979). Prolonged cold exposure causes a severalfold increase in brown-adipose-tissue lipogenesis (Trayhurn, 1979; Rath *et al.*, 1979), which is related to the temperature difference to which the animal is exposed (Rath *et al.*, 1979). By analogy with cold-induced thermogenesis, dietary-induced thermogenesis is defined as the increased heat production caused by over-eating for long periods (Himms-Hagen, 1979). Brown adipose tissue hypertrophies in rats that become hyperphagic when given palatable foods, suggesting that it may also be a site of dietary-induced thermogenesis (Rothwell & Stock, 1979). However, the immediate effect of feeding on brown-adipose-tissue lipogenesis has not been studied in normal rats, and high rates of brown-adipose-tissue lipogenesis have only been described in cold-adapted animals (McCormack & Denton, 1977; Trayhurn, 1979; Rath *et al.*, 1979). McCormack & Denton (1977) were first to describe the importance of brown adipose tissue as a site of lipogenesis after cold adaptation and to show that the rate of lipogenesis *in vivo* was sensitive to the plasma insulin concentration. In the present study we investigated the effects of intragastric feeding on lipogenesis *in vivo* in brown and white adipose tissue and liver of virgin, pregnant and lactating rats. We studied pregnant and lactating rats because, although both are hyperphagic, pregnancy is associated with fat deposition, but the converse may occur during lactation (Smith & Walsh, 1976).

* Dedicated to Sir Hans Krebs for his 80th birthday.

Experimental

Albino Wistar rats were fed *ad libitum* on Oxoid breeding diet (Oxoid, London S.E.1, U.K.). Virgin rats weighed 220–260 g and pregnant rats (7–14 days) weighed 330–370 g. At birth the litters were culled to ten pups. The early lactating rats (1–2 days *post partum*) weighed 260–320 g and the mid-lactating rats (10–14 days *post partum*) 320–380 g. ‘Weaned’ rats (340–390 g) refers to mid-lactating rats which had their litters removed 24 h before the experiment. All experiments were started between 09:00 and 10:00 h. Glucose and fructose (2 mmol/100 g body wt., 2 M solution) and medium-chain triacylglycerol (a mixture of C₆ to C₁₂ fatty acids with at least 95% C₈ and C₁₀; Cow & Gate Ltd., Trowbridge, Wilts., U.K.) and long-chain triacylglycerol (trioleoylglycerol; British Drug Houses, Poole, Dorset, U.K.) were administered (1 ml/100 g body wt.) intragastrically through a plastic tube. Insulin (2 units; ‘Isophane Insulin Injection’, Nordisk Insulinlaboratorium, Copenhagen, Denmark) and noradrenaline (0.2 mg, as the acid tartrate containing sodium metabisulphite; ‘Levophed’; Winthrop Laboratories, Surbiton, Surrey, U.K.) were injected subcutaneously. Short-term diabetes was induced with streptozotocin (5 mg/100 g body wt.; in 0.01 M-sodium citrate buffer, pH 4.5; Upjohn Co., Kalamazoo, MI, U.S.A.) injected intravenously 90 min before intragastric feeding (Schein *et al.*, 1971). Rates of lipogenesis *in vivo* were measured by the incorporation of ³H₂O into lipid (Stansbie *et al.*, 1976). ³H₂O (4 mCi; 0.4 ml) was injected intraperitoneally 60–90 min after feeding. The rats were

anaesthetized 50 min later (sodium pentobarbital, 60 mg/kg body wt.) and dissected at 60 min. An arterial blood sample was withdrawn for determination of blood glucose concentration (Slein, 1963) and plasma $^3\text{H}_2\text{O}$ specific radioactivity. Interscapular brown adipose tissue, parametrial white adipose tissue and the liver were removed and weighed and the saponified lipids extracted (Stansbie *et al.*, 1976). Care was taken to dissect away any white adipose tissue adhering to the interscapular brown adipose tissue; this process took 1–2 min. The percentage fat content (after saponification) of each tissue was determined and the rates of lipogenesis were expressed as μmol of $^3\text{H}_2\text{O}$ incorporated into lipid/h per g fat-free wet wt. (Table 1). This enables comparison of rates between tissues with a different fat content.

Results and discussion

Effects of intragastric feeding

In virgin rats that were fed on glucose the rate of lipogenesis in liver was increased by 135% and in white adipose tissue by 160%, but in brown adipose tissue it was increased 8-fold (Table 1). Administration of insulin had a similar effect, despite the resulting hypoglycaemia (blood glucose concentration $2.31 \pm 0.49 \text{ mM}$), whereas fructose had little effect. An oral load of medium-chain triacylglycerol resulted in a similar response to that of glucose, but long-chain triacylglycerol caused no significant change (Table 1). Medium-chain triacylglycerol, have been reported to stimulate insulin secretion (Yeh & Zee, 1976), and an effect of insulin on brown-adipose-tissue lipogenesis has been shown in cold-adapted rats (McCormack & Denton, 1977).

Short-term treatment (2.5 h) with streptozotocin in the present experiments resulted in marked hyperglycaemia (blood glucose concentration $15.5 \pm 0.92 \text{ mM}$) and presumably insulin deficiency (Schein *et al.*, 1971). In these animals, feeding with glucose and medium-chain triacylglycerol did not increase lipogenesis in brown adipose tissue, but insulin did (Table 1).

In animals fed *ad libitum*, brown-adipose-tissue lipogenesis was 3–4 times that in white adipose tissue and liver, but after feeding with glucose or medium-chain triacylglycerol it was 11-fold higher. The rates of lipogenesis (expressed per g wet wt.) in brown adipose tissue of the virgin animals fed *ad libitum* are in agreement with previous data for 'warm-acclimated' rats, whereas the elevated rates observed after feeding with glucose or medium-chain triacylglycerol are comparable with the rates observed in rats that had been exposed to 4°C for 3–4 weeks (McCormack & Denton, 1977; Trayhurn, 1979), which indicates that prior exposure to cold is not a prerequisite for high rates of lipogenesis in brown adipose tissue.

Effects of noradrenaline on brown adipose tissue

Noradrenaline has a thermogenic effect in brown adipose tissue, and its concentration is elevated during cold exposure in the rat (Depocas & Behrens, 1977) and also after a meal in man (Shetty *et al.*, 1979). We therefore investigated its effect on lipogenesis in brown adipose tissue. Noradrenaline decreased the percentage fat content of brown adipose tissue to $25.3 \pm 2.4\%$ (cf. Table 2), which might suggest an increase in fatty acid oxidation. It did not affect the rate of lipogenesis in the virgin rats fed *ad libitum*, but it prevented the increase in $^3\text{H}_2\text{O}$

Table 1. Rates of lipogenesis *in vivo* in brown and white adipose tissue and liver of virgin rats after intragastric feeding or hormonal treatment

For experimental details see the text. Rates of lipogenesis are expressed as μmol of $^3\text{H}_2\text{O}$ incorporated into lipid/h per g fat-free wet wt. The values are means $\pm$ S.D., with the numbers of rats shown in parentheses. Values that are significantly different by the Student's *t* test from the control rats are shown: * $P < 0.05$; ** $P < 0.005$.

State of rats		Brown adipose tissue	White adipose tissue	Liver
Control (fed <i>ad libitum</i>)	(6)	21.0 ± 6.6	5.9 ± 3.3	7.4 ± 3.6
+ glucose	(6)	$165 \pm 53^{**}$	$15.3 \pm 11.0^*$	$17.4 \pm 8.8^*$
+ fructose	(3)	$30.3 \pm 5.0^*$	6.2 ± 6.5	7.8 ± 3.9
+ medium-chain triacylglycerol	(6)	$217 \pm 109^{**}$	$19.9 \pm 13.8^*$	$18.7 \pm 6.6^{**}$
+ long-chain triacylglycerol	(3)	27.9 ± 2.3	5.6 ± 1.1	4.9 ± 1.0
+ insulin	(5)	$145 \pm 52^{**}$	17.1 ± 16.7	$26.9 \pm 13.9^{**}$
Diabetic (fed <i>ad libitum</i>)	(4)	13.3 ± 6.3	3.1 ± 1.5	4.3 ± 1.7
+ glucose	(3)	14.7 ± 12.0	5.0 ± 1.8	5.7 ± 1.5
+ medium-chain triacylglycerol	(3)	28.4 ± 16.5	5.2 ± 4.2	9.1 ± 3.3
+ insulin	(3)	$185 \pm 52^{**}$	14.5 ± 17.9	6.6 ± 3.4
Control + noradrenaline	(6)	23.1 ± 12.8	4.0 ± 1.7	7.1 ± 2.8
Control + glucose + noradrenaline	(3)	21.5 ± 16.0	6.0 ± 1.8	8.4 ± 2.6
Control + glucose + insulin + noradrenaline	(4)	$9.1 \pm 7.9^*$	4.7 ± 3.3	5.5 ± 0.7

incorporation into lipid after glucose feeding even when the rats were injected with insulin (Table 1). This may be because the newly synthesized fatty acids are oxidized at a fast rate, or because noradrenaline may stimulate fatty acid oxidation but inhibit fatty acid synthesis.

Effects of physiological state

The above results indicate that the rate of lipogenesis in brown adipose tissue responds dramatically to intragastric feeding. It was therefore decided to study brown-adipose-tissue lipogenesis in two hyperphagic situations, namely pregnancy and lactation. The fat-free weight of interscapular brown adipose tissue increased by 65% ($P < 0.01$) during pregnancy, but declined by 24% ($P < 0.01$) at birth. It showed little change from birth to mid-lactation, when the weight was not significantly different from the pre-mating value, but it increased by 50% ($P < 0.005$) when the pups were removed for 24 h (Table 2). The percentage fat content also increased during pregnancy and declined at birth ($P < 0.005$), but did not change on weaning (Table 2).

The rate of brown-adipose-tissue lipogenesis in the animals fed *ad libitum* increased 5-fold during pregnancy ($P < 0.01$). It decreased at birth ($P < 0.05$) and decreased further at mid-lactation ($P < 0.025$), but increased 6-fold on weaning ($P < 0.005$) (Table 2). These changes parallel the hypertrophy and regression of the tissue and the plasma insulin concentration, which increases during pregnancy, declines during lactation (Flint *et al.*, 1979) and increases again on premature weaning (Agius *et al.*, 1979). Glucose feeding did not significantly increase the already high rate of lipogenesis in brown adipose tissue of pregnant rats, and this may be due to the hyperphagia and relative hyperinsulinaemia compared with the virgin rat. Early-lactating rats showed a 160% increase in lipogenesis in brown adipose tissue in response to glucose. This increase was considerably diminished compared with that of virgin rats, and at mid-lactation there was no increase in lipogenesis after glucose feeding (Table 2). Insulin administration does, however, increase brown-adipose-tissue lipogenesis in lactating rats (Agius & Williamson, 1980).

The alterations in lipogenic rate in brown adipose tissue do not parallel the changes in hepatic lipogenesis during lactation (Table 2). Thus hepatic lipogenesis increased 2-fold ($P < 0.0025$) at mid-lactation relative to the virgin rat, whereas the rate in brown adipose tissue decreased by 57% ($P < 0.0025$). If the changes in lipogenesis in brown adipose tissue and liver are expressed on a total-weight basis the results are even more striking (Table 2). During pregnancy, total interscapular-brown-adipose-tissue lipogenesis was 10-fold higher than in the virgin. After birth (within 48 h), both total

Table 2. Effects of pregnancy and lactation on the rates of lipogenesis in brown adipose tissue and liver of the rat

For experimental details see the text. Brown-adipose-tissue weights are expressed in mg and rates of lipogenesis as μmol of $^3\text{H}_2\text{O}$ incorporated into lipid/h per g fat-free wet wt. or per total fat-free wet wt. The values for the total tissue lipogenesis refer to the control state. The values are means $\pm$ S.D., with the numbers of rats shown in parentheses. Values that are significantly different by the Student's *t* test from the appropriate value for the virgin rats are shown: * $P < 0.05$; ** $P < 0.005$.

	Fat-free weight (mg)	% fat	Interscapular brown adipose tissue				Liver		Total tissue Lipogenesis	
			Lipogenesis		Control	Glucose-fed	Control	Glucose-fed	Brown-adipose tissue	Liver
Virgin (6)	144 $\pm$ 44	38.0 $\pm$ 6.5	21.0 $\pm$ 6.6	165 $\pm$ 53.2	7.4 $\pm$ 3.6	17.4 $\pm$ 8.8	15.0 $\pm$ 7.0*	14.7 $\pm$ 7.7	2.7 $\pm$ 0.8	56.9 $\pm$ 28.0
Pregnant (6)	237 $\pm$ 62**	49.9 $\pm$ 10.2**	125 $\pm$ 87*	156 $\pm$ 129	15.0 $\pm$ 7.0*	14.7 $\pm$ 7.7	6.9 $\pm$ 3.8	6.8 $\pm$ 1.7*	27.4 $\pm$ 18.9**	206 $\pm$ 106**
Early-lactating (3)	180 $\pm$ 26*	35.4 $\pm$ 12.4	17.9 $\pm$ 5.0	46.7 $\pm$ 31.8*	15.5 $\pm$ 3.0**	25.0 $\pm$ 8.7	18.9 $\pm$ 6.3**	26.7 $\pm$ 12.2	2.7 $\pm$ 0.7	71.5 $\pm$ 30.3
Mid-lactating (6)	167 $\pm$ 56	41.6 $\pm$ 6.4	9.0 $\pm$ 4.6**	10.7 $\pm$ 2.6**	15.5 $\pm$ 3.0**	25.0 $\pm$ 8.7	15.5 $\pm$ 3.0**	25.0 $\pm$ 8.7	1.5 $\pm$ 0.9*	242 $\pm$ 54**
Weaned (24 h) (6)	250 $\pm$ 82**	42.2 $\pm$ 11.6	53.0 $\pm$ 20.2**	63.9 $\pm$ 36.5**	18.9 $\pm$ 6.3**	26.7 $\pm$ 12.2	18.9 $\pm$ 6.3**	26.7 $\pm$ 12.2	13.7 $\pm$ 10.4*	345 $\pm$ 96**

hepatic lipogenesis and interscapular-brown-adipose-tissue lipogenesis decreased to the pre-mating value. By mid-lactation, brown-adipose-tissue lipogenesis continued to decrease by a further 50% ($P < 0.05$), but hepatic lipogenesis increased by 240% ($P < 0.0025$). On premature weaning, hepatic lipogenesis increased by 40% ($P < 0.025$), whereas brown-adipose-tissue lipogenesis increased 8-fold ($P < 0.01$).

Conclusions

One can only speculate on the physiological significance of the rapid increase in brown-adipose-tissue lipogenesis that occurs after intragastric feeding or insulin treatment, since the fate of the newly synthesized lipid is not known. If the function of lipogenesis in brown adipose tissue were the same as in liver, to produce lipid for export, then it would be expected to follow the same trends as for hepatic lipogenesis, which was not the case, especially during lactation. If, on the other hand, the newly synthesized fatty acids are oxidized *in situ*, then the high rate of lipogenesis after intragastric feeding may be a mechanism for the 'burning off' of substrates, and the failure to observe a similar effect in lactating rats may be an adaptation to conserve lipogenic substrates for the mammary gland. The finding that noradrenaline prevented the incorporation of $^3\text{H}_2\text{O}$ into lipid that is observed after glucose feeding in virgin rats is of interest in view of the report that noradrenaline secretion in man after a meal is greater in obese subjects with subnormal rates of dietary-induced thermogenesis than in lean individuals (Shetty *et al.*, 1979). With the current interest in possible impairment of dietary-induced thermogenesis in relation to obesity, the lactating rat or the diabetic rat may provide a model for studying

defects in brown-adipose-tissue metabolism after feeding.

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