Regulation of lipogenesis and of non-saponifiable-lipid synthesis *in vivo* at birth and after prolonged starvation in the newborn rat

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The rate of lipogenesis in the liver was increased by glucose injection at birth, mediated by the insulin secretion. In addition, glucagon decreased the rates of lipogenesis and non-saponifiable-lipid synthesis after birth. These rates decreased after prolonged starvation in the newborn rat. Tri-iodothyronine injection increased the rates of lipogenesis enhanced in response to glucose administration after prolonged starvation in liver and brown adipose tissue. Dexamethasone, however, increased the rates of lipogenesis enhanced in response to glucose in liver and prevented the increase in the rates of lipogenesis in brown adipose tissue.

The rate of lipogenesis in foetal rat liver decreases during the last 2 days of gestation (Lorenzo *et al.*, 1981). These changes are concurrent with the accumulation of glycogen in foetal liver (Devos & Hers, 1974) and do not seem to correlate with plasma insulin concentration (Benito *et al.*, 1982). In addition, we have reported the existence of an inverse relationship between lipogenesis and glycogen synthesis in foetal-rat liver during late gestation, which was also observed when glycogen synthesis was enhanced by treatment with dexamethasone (Benito *et al.*, 1982). Thus the potential glucose made available by the maternal blood supply rather than plasma insulin seems to be the main factor involved in controlling foetal-liver lipogenesis, as suggested by Lorenzo *et al.* (1983). However, glucose administration to the mother increases foetal-liver lipogenesis and plasma insulin concentration at 22 days of gestation (Lorenzo *et al.*, 1983). These results seem to suggest that the regulation of liver lipogenesis by insulin described in adult rats also works on foetal lipogenesis before birth. In addition, the rate of lipogenesis in foetal brown adipose tissue increases during late gestation, decreasing sharply after birth (Pillay & Bailey,

1982a), suggesting that in this tissue a direct relationship between fatty acid synthesis and plasma insulin concentration seems to occur. On the other hand, the rate of non-saponifiable-lipid synthesis decreases parallel to fatty acid synthesis in the foetal liver during the last 2 days of gestation (Pillay & Bailey, 1982a). Concurrently, the 3-hydroxy-3-methylglutaryl-CoA reductase activity increased in the foetal liver (McNamara *et al.*, 1972). Thus these results seem to suggest that glucose availability may be limiting the rate of non-saponifiable-lipid synthesis in the liver, as it did for lipogenesis during late gestation. However, although some results suggested a link between the change to a high-carbohydrate content diet and the increase in the 3-hydroxy-3-methylglutaryl-CoA reductase activity during the suckling–weaning transition (McNamara *et al.*, 1972), no evidence has been found for a direct effect of glucose administration on the rates of non-saponifiable-lipid synthesis during the foetal–neonatal transition in the rat. Thus the first aim of the present work was to study the short-term regulation by glucose, insulin secretion and glucagon of lipogenesis and non-saponifiable-lipid synthesis at birth in the rat.

On the other hand, much work has been done on the influence of the suckling and weaning period on lipid synthesis in the rat (Benito *et al.*, 1979; Pillay & Bailey, 1982b). There is no evidence, however, about the pre-suckling period, where the influence of maternal milk on lipid synthesis in the

Abbreviation used: T₃, 3,3',5-L-tri-iodothyronine.

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newborn may be precluded. In addition, the prolonged starvation allowed us to perform experiments on the precocious effect of long-term hormones such as T_3 and dexamethasone on lipogenesis in the newborn rat. Accordingly, the second aim of the present work was to study the long-term regulation of lipogenesis by T_3 and dexamethasone in starved newborn rats previously given glucose.

Experimental

Albino Wistar pregnant rats (300–350 g) fed on a stock laboratory diet were killed for the experiments between 09:00 and 10:00 h. Conception was assumed by the presence of spermatozoa in the vagina, and gestational age was confirmed by the foetal weight. On day 22 of gestation foetuses were delivered by rapid hysterectomy. Newborn rats (5.2–5.5 g) belonging to the same litter were injected intraperitoneally, immediately after birth, with either 0.9% NaCl or glucose (36 mg/rat) or glucose plus mannoheptulose (15 mg/rat) or glucagon (0.5 μ g/rat).

After the first injection, newborn rats were injected with 0.3 mCi of 3H_2O (The Radiochemical Centre, Amersham, Bucks., U.K.). The newborn rats were maintained, without feeding, in an incubator (Tectron; Selecta, Barcelona, Spain) at 37°C and 70% relative humidity for 1 h. At 1 h 15 min after delivery, the animals were killed by decapitation, exsanguinated by using heparinized capillary pipettes, and their plasma was separated by centrifugation. Then samples of liver (300 mg), brain (160 mg) and brown adipose tissue (100 mg) were taken, and added to 1 ml of 30% (w/v) KOH,

and the non-saponifiable lipid and fatty acids extracted by the method of Stansbie *et al.* (1976). Alternatively, newborn rats were injected intraperitoneally at birth with 0.9% NaCl, or T_3 [20 μ g/rat, dissolved in 0.9% NaCl/20 mM-NaOH (1:1, v/v)], or dexamethasone (80 μ g/rat). Then the animals were maintained in an incubator as mentioned above, and after prolonged starvation (15 h) were injected with 0.3 mCi of 3H_2O containing glucose (36 mg/rat) during 1 h. At 16 h after delivery, without feeding, the animals were killed and used for the experiments as indicated above.

Plasma insulin was measured by radioimmunoassay by the method of Hales & Randle (1963). Neutralized $HClO_4$ extracts were used to determine glucose (Krebs *et al.*, 1963, 1964). Liver glycogen was determined, in samples obtained by freeze-clamping in liquid N_2 , as glucose after enzymic hydrolysis as described by Keppler & Decker (1974).

Results and discussion

Rates of lipogenesis and non-saponifiable-lipid synthesis in the newborn rat at birth

The rates of lipogenesis in liver and brown adipose tissue in the newborn rat at birth were the same as those in foetuses on day 22 of gestation (Lorenzo *et al.*, 1981; Pillay & Bailey, 1982a; Table 1). In addition, the rate of lipogenesis in brown adipose tissue in the newborn rat at birth appears to be 4-fold higher than that in the liver (Table 1). These results are in agreement with the decreased rate of lipogenesis in the foetal liver during late gestation (Lorenzo *et al.*, 1981) and the increased

Table 1. Rates of lipogenesis and non-saponifiable-lipid synthesis, and plasma glucose and insulin concentrations and liver glycogen in newborn rats at birth

For details see the Experimental section. The results are means $\pm$ S.E.M. ($n = 6-20$). Rates of lipogenesis and non-saponifiable-lipid synthesis are expressed as μ mol of 3H_2O incorporated into saponifiable and non-saponifiable fraction/h per g wet wt. respectively. Values that are significantly different from those for control newborn rats are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Treatment ...	Control (0.9% NaCl)	Glucose	Glucose + mannoheptulose	Glucagon
Lipogenesis					
Liver		9.7 $\pm$ 0.6	12.7 $\pm$ 0.4***	9.3 $\pm$ 0.3	8.4 $\pm$ 0.2*
Brain		6.1 $\pm$ 0.3	4.6 $\pm$ 0.2***	4.0 $\pm$ 0.2***	5.4 $\pm$ 0.2*
Brown adipose		40.3 $\pm$ 4.4	43.7 $\pm$ 2.7	42.0 $\pm$ 1.4	27.3 $\pm$ 2.4***
Non-saponifiable-lipid synthesis					
Liver		6.9 $\pm$ 0.4	7.1 $\pm$ 0.6	6.7 $\pm$ 0.4	5.1 $\pm$ 0.2***
Brain		4.1 $\pm$ 0.2	4.1 $\pm$ 0.2	4.7 $\pm$ 0.2	3.2 $\pm$ 0.1***
Brown adipose		8.4 $\pm$ 0.7	10.5 $\pm$ 1.4	10.0 $\pm$ 0.8	5.4 $\pm$ 0.6**
Neonatal plasma					
Glucose (μ mol/ml)		2.0 $\pm$ 0.2	12.2 $\pm$ 0.1	13.2 $\pm$ 0.1	3.0 $\pm$ 0.2**
Insulin (μ units/ml)		29.1 $\pm$ 2.5	65.8 $\pm$ 10.5	18.4 $\pm$ 3.0	—
Liver glycogen (μ mol/g wet wt.)		481 $\pm$ 24	521 $\pm$ 15	504 $\pm$ 14	421 $\pm$ 13*

rate of lipogenesis in brown adipose tissue at that time (Pillay & Bailey, 1982a). Treatment with glucose at birth increased rates of lipogenesis in neonatal liver, as previously seen *in situ* in foetuses on days 21 or 22 of gestation (Lorenzo *et al.*, 1983). Treatment with glucose plus mannoheptulose, however, did not produce any significant effect on the rate of lipogenesis in the neonatal liver (Table 1). These results indicate that the effect of glucose on neonatal-liver lipogenesis is exerted through its capacity to induce release rather than by itself (Table 1), and confirm the previous suggestion that the regulation of liver lipogenesis by insulin observed in adult rats works on foetal-liver lipogenesis before birth (Lorenzo *et al.*, 1983). Conversely, glucose administration to the newborn immediately after birth did not produce any significant effect on the rate of lipogenesis in brown adipose tissue, as it did in virgin and lactating rats (Agius & Williamson, 1980). These results seem to indicate that glucose availability does not limit the rate of lipogenesis *in vivo* in brown adipose tissue as it does in foetal liver. In addition, glucose administration to the newborn rat increased the plasma insulin concentration (Table 2), which in fact stimulated lipid synthesis *in vivo* in brown adipose tissue from cold-adapted adult rats (McCormack & Denton, 1977). Thus the rate of lipogenesis observed in brown adipose tissue seems to be maximal in the rat at birth. The rate of lipogenesis from the brain shows its lowest values as compared with liver and brown adipose tissue in the newborn rat (Table 1). Moreover,

glucose administration decreased the rate of fatty acid synthesis in the rat at birth (Table 1). These results may be explained by considering the possibility that glucose administration may be decreasing lactate oxidation by the neonatal brain *in vivo*, which in fact has been suggested to be the main precursor in the energy supply to the brain during the first 2 h of the neonatal period in the rat (Arizmendi & Medina, 1983). If so, the glucose supply due to the high respiratory activity of the foetal brain required for neuronal function (Hamburger & Hyden, 1963) increases its ATP/ADP ratio, which inhibits the glycolytic flux (very low in the newborn rat; Hotherhall *et al.*, 1979) at the level of phosphofructokinase (Reijnierse *et al.*, 1975), the rate-limiting enzyme in the neonatal brain, as it does in muscle in the adult. Thus glucose administration to the newborn rat may result in less acetyl-CoA being available for fatty acid synthesis in the brain.

On the other hand, the rate of non-saponifiable-lipid synthesis in the liver is not affected by the treatment with glucose immediately after birth in the newborn rat (Table 1). Considering that non-saponifiable-lipid synthesis decreased parallel to fatty acid synthesis during late foetal development (Pillay & Bailey, 1982a), it could be expected that glucose availability may be limiting both pathways, in late foetal life. Conversely, our results show that glucose supply to the newborn, which is hypoglycaemic at birth (Table 1), did not increase the incorporation of ³H₂O into non-saponifiable lipid as it did into fatty acid synthesis (Table 1).

Table 2. Rates of lipogenesis and non-saponifiable-lipid synthesis, and plasma glucose and insulin concentrations and liver glycogen in the starved newborn rats

For details see the Experimental section. The results are means $\pm$ S.E.M. ($n = 6-20$). Rates of lipogenesis and non-saponifiable-lipid synthesis are expressed as μmol of ³H₂O incorporated into saponifiable and non-saponifiable fraction/h per g wet wt. respectively. Values that are significantly different from those for control newborn rats at birth (* etc.), or from values for newborn rats starved for 15 h, then injected with glucose (1 h), and treated with NaCl ($\dagger$ etc.), are indicated by: *, $\dagger P < 0.05$; **, $\dagger\dagger P < 0.01$; ***, $\dagger\dagger\dagger P < 0.001$.

State of rats Treatment	...	At birth	Starved (8h)	Starved (16h)	Starved (15h), then injected with glucose (1h)		
					NaCl	T ₃	Dexamethasone
Lipogenesis							
Liver		9.7±0.6	5.8±0.2***	2.3±0.1***	5.1±0.4	7.4±0.6††	7.3±0.6††
Brain		6.1±0.3	8.2±0.6**	3.3±0.2***	6.2±0.4	6.8±0.5	4.4±0.3†††
Brown adipose		40.3±4.4	9.0±0.8***	3.6±0.6***	5.7±0.4	9.7±0.9†††	3.4±0.3†††
Non-saponifiable-lipid synthesis							
Liver		6.9±0.4	4.7±0.6*	1.3±0.1***	1.8±0.2	—	—
Brain		4.1±0.2	4.2±0.8	2.3±0.4**	2.8±0.1	—	—
Brown adipose		8.4±0.7	6.9±0.6*	1.3±0.1***	1.9±0.3	—	—
Neonatal plasma							
Glucose (μmol/ml)		2.0±0.2	1.8±0.2	1.4±0.2*	12.8±1.2	11.8±1.3	12.4±1.2
Insulin (μunits/ml)		29±3	15±2*	7±0.7*	30±6	38±6	42±6
Liver glycogen (μmol/g wet wt.)		481±24	256±21**	57±4***	83±6	80±6	90±7

These results may be explained by the fact that 3-hydroxy-3-methylglutaryl-CoA reductase in the liver has been found to reach its maximal activity before birth, falling sharply *post partum* (McNamara *et al.*, 1972). The liver 3-hydroxy-3-methylglutaryl-CoA reductase, which has been suggested not to be the rate-limiting step for cholesterol synthesis in foetal liver (Pillay & Bailey, 1982a), may be controlling the flux of cholesterol synthesis immediately after birth, as suggested in the adult rat (Geelen *et al.*, 1980). On the other hand, the administration of glucose did not produce any significant increment in the rate of non-saponifiable-lipid synthesis in brown adipose tissue in the same circumstances (Table 1). These results are in agreement with those obtained above on the rate of fatty acid synthesis in brown adipose tissue and seem to suggest that its rate becomes maximal at birth.

Glucagon administration immediately after birth decreased the liver glycogen concentration in the newborn rat (Table 1), as previously reported (Martin *et al.*, 1981). In addition, administration of glucagon decreased the observed rates of lipogenesis in the liver, brain and brown adipose tissue in the control newborn rat (Table 1). The inhibition of fatty acid synthesis in the tissues studied by glucagon treatment is concurrent with the increase in the plasma glucagon/insulin ratio in the rat at birth (Martin *et al.*, 1981), and suggests that glucagon action on fatty acid synthesis by adult rats *in vivo* (Klain & Weiser, 1973) is operating on the foetal tissues immediately after birth. Concurrently, the rates of non-saponifiable-lipid synthesis are inhibited by glucagon in the liver, brain and brown adipose tissue (Table 1). Whether glucagon affects the rates of fatty acid and cholesterol synthesis in the newborn rat through a common regulatory point before the acetyl-CoA step or through different points after the cytosolic branchpoint at acetyl-CoA into both pathways remains to be established.

Rates of lipogenesis and non-saponifiable-lipid synthesis after prolonged starvation in the newborn rat

The rates of lipogenesis decreased significantly in the liver and brown adipose tissue after 8 h and 16 h of prolonged starvation as compared with those values in newborn rats at birth (Table 2). In addition, the rates of non-saponifiable-lipid synthesis decreased parallel to fatty acid synthesis in the liver and brown adipose tissue in the same experimental period. These changes in the flux of lipid synthesis are concurrent with the fall in liver glycogen content and plasma insulin concentration (Table 2). Conversely, no differences were observed in the rates of saponifiable and non-saponifiable lipid synthesis during the first 8 h of

food deprivation in the neonatal brain (Table 2), suggesting that the glucose supply from the depletion of the glycogen stored in the liver is mainly utilized by the brain, independently of plasma insulin concentration, as an alternative substrate to lactate after the first 2 h of neonatal life. Afterwards the fatty acid and non-saponifiable-lipid synthesis decreased between 8 and 16 h of starvation. The glucose injection after 15 h of starvation increased the rates of lipogenesis observed in the liver, brain and brown adipose tissue as compared with those from starved newborn rats (Table 2). The increased rates of fatty acid synthesis by glucose were parallel to an enhanced plasma insulin concentration (Table 2). These results suggest that the inhibition observed as a consequence of prolonged starvation is partially due to decreased glucose supply for lipid synthesis relative to very low plasma insulin concentration. The rates of non-saponifiable-lipid synthesis in the tissues studied, however, were not affected by glucose injection into newborns after prolonged starvation (Table 2). Thus the rates of non-saponifiable-lipid synthesis after starvation seem to be limited by the enzymic capacity rather than by glucose availability, as suggested above for fatty acid synthesis.

The treatment with T_3 immediately after birth significantly increased the rates of lipogenesis in response to glucose in the liver and brown adipose tissue as compared with those values for untreated starved newborn rats given glucose (Table 2). In addition, the T_3 injection did not increase the insulin response to glucose observed in the untreated rats (Table 2). These results are in agreement with those obtained on the rates of lipid synthesis in the liver and brown adipose tissue from hyperthyroid young rats (Gnoni *et al.*, 1983). However, it has been suggested that T_3 injection increases the plasma insulin concentration (Hahn & Hassanali, 1982), which is not consistent with our experimental conditions (see Table 2). Thus T_3 injection into the newborn rat after birth may increase the rates of lipogenesis through its capacity to enhance the number of insulin receptors, as suggested by Hahn & Hassanali (1982), or alternatively by a direct effect on glucose transport to the tissue. In addition, it is well established that T_3 injection increases malic enzyme activity in the liver and brown adipose tissue in 4-day-old rats (Hahn & Hassanali, 1982) and in the liver of 14–17-day-old rats (Lockwood *et al.*, 1970), which may account for the increase in NADPH required for lipid synthesis.

Finally, the treatment with dexamethasone at birth increased the rate of response of lipogenesis to glucose as compared with their controls given glucose (Table 2). In addition, the dexamethasone

injection for 16 h did not significantly increase the insulin secretion in response to glucose observed in the untreated newborn rats (Table 2). These results on liver lipogenesis are consistent with those obtained in pregnant rats (Benito *et al.*, 1982), but are not related to a substantial increase in plasma insulin concentration in our experimental conditions. Thus our results seem to indicate that dexamethasone exerts its effect on the fatty acid synthesis in the liver in the neonatal period, in the absence of a significant increase in the insulin secretion. Conversely, the injection of dexamethasone to the newborn rat at birth for 16 h decreased the rates of lipogenesis, enhanced by glucose administration, in the brain and brown adipose tissue as compared with those from untreated newborns (Table 2). These results are in agreement with those observed in the foetal lung at 20–22 days of gestation (Benito *et al.*, 1982), and may be explained by a decrease in glucose utilization by peripheral tissues.

In conclusion, the rates of fatty acid synthesis in the liver seem to be regulated by glucose through its capacity to stimulate insulin secretion at birth and partially after prolonged starvation in the newborn rat. Conversely, the rates of lipogenesis in the brown adipose tissue seem to be maximal at birth and are not regulated by glucose. After prolonged starvation, however, the decreased rates of lipogenesis in brown adipose tissue are stimulated by glucose in the presence of an increased plasma insulin concentration. On the other hand, the rates of non-saponifiable-lipid synthesis do not seem to be regulated by glucose availability at birth or after prolonged starvation. Finally, the glucagon injection inhibited the rates of saponifiable- and non-saponifiable-lipid synthesis in the newborn rat. Thus the short-term regulation of lipogenesis *in vivo* in the liver and brown adipose tissue observed in the adult is operating in the newborn rat immediately after birth. The peak of plasma glucagon concentration described in the newborn rat (Martin *et al.*, 1981), however, seems to be the main factor involved in the regulation of non-saponifiable-lipid synthesis.

The effect of glucose administration on the rates of lipogenesis in the starved newborn rats is enhanced by T_3 injection in the liver and brown adipose tissue. These results suggest that the premature increase in plasma thyroid-hormone concentration induces the same effects as those observed during the suckling–weaning transition, when plasma T_3 concentration increases under physiological conditions, in the rat (Pillay & Bailey, 1982b). In addition, dexamethasone injection enhanced the glucose effect on the rates of lipogenesis in the liver from newborn rats after prolonged starvation. Conversely, dexamethasone

injection prevents the stimulatory effect of glucose on the rates of fatty acid synthesis in the brown adipose tissue under the same experimental conditions. These results suggest that glucocorticoids work effectively on the rates of lipogenesis in the newborn rat after prolonged starvation, with similar results to those seen in the adult (Benito *et al.*, 1982).

Thus the long-term regulation of lipogenesis *in vivo* by precocious enhancement of plasma thyroid-hormone concentration or by treatment with dexamethasone is operating in the newborn, as previously described for the young and adult rats, but in the absence of a significant increase of insulin secretion as a consequence of the hormones' action.

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