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Regulation of lipogenesis *in vivo* by glucose availability and insulin secretion in maternal and foetal tissues during late gestation in the rat

Effect of glucose intubation, streptozotocin-induced diabetes and starvation

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Administration of an oral load of glucose did not change the rate of lipogenesis in maternal liver during late gestation. However, streptozotocin-induced diabetes or starvation decreased maternal liver lipogenesis at 20–22 days of gestation. Glucose intubation, on the other hand, increased foetal lipogenesis at 21–22 days. In addition, maternal starvation decreased foetal lipogenesis and plasma insulin concentration. However, chronic hyperglycaemia induced by streptozotocin administration to the mother did not change foetal liver lipogenesis.

During late pregnancy, the incorporation of maternal lipid into foetal rat tissues is likely to be secondary to lipogenesis *de novo* from maternal carbohydrates (Lorenzo *et al.*, 1981). Thus the increase in plasma lipid observed during the second phase of gestation (Scow *et al.*, 1964; Knoop *et al.*, 1973) results in more glucose being available for the foetus. However, the rate of lipogenesis in foetal rat liver decreases during the last 2 days of gestation (Lorenzo *et al.*, 1981). These changes may be consistent with the accumulation of glycogen in foetal liver during this period (Devos & Hers, 1974). We have previously reported the existence of an inverse relationship between lipogenesis and glycogen synthesis in foetal rat liver during late gestation. This relationship was also observed in foetal liver when glycogen synthesis was enhanced by treatment with dexamethasone through the maternal circulation (Benito *et al.*, 1982). On the other hand, the rates of lipogenesis in foetal-rat liver observed during the last 2 days of gestation do not seem to correlate with plasma insulin concentration in the foetus at the same period (Benito *et al.*, 1982), as described for maternal liver (Lorenzo *et al.*, 1981; Benito *et al.*, 1982). These results seem to suggest that glucose availability may be a main factor in the control of foetal lipogenesis in the rat.

Accordingly, the aim of the present work was to study in the rat the effect of different experimental conditions, such as maternal glucose intubation, streptozotocin-induced diabetes and starvation, on the regulation of lipogenesis *in vivo* in maternal and foetal tissues during late gestation.

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Experimental

Pregnant albino Wistar rats (300–350g) fed on a stock laboratory diet were given an single oral load of 5ml of 0.9% NaCl or 1M-glucose for 1h before ^3H_2O injection (Robinson & Williamson, 1977). Diabetes was induced by injecting streptozotocin (45mg/kg body wt.) in 50mM-sodium citrate buffer, pH4.5, into the tail vein of pregnant rats on day 5 of gestation (Cuezva *et al.*, 1982). Diabetes was confirmed 48h after streptozotocin administration by measuring tail-vein blood glucose. An animal was considered diabetic when its mean blood glucose concentration, measured at intervals of 3–4 days, was 15mM or higher from days 7 to 20, 21 or 22 of gestation. Pregnant rats were starved for 48h but allowed water *ad libitum*, and were used for the experiments on days 20, 21 or 22 of gestation. Rats were injected intraperitoneally with 5mCi of ^3H_2O (The Radiochemical Centre, Amersham, Bucks., U.K.). For further experimental details see Benito *et al.* (1982). Concentrations of metabolites and insulin in maternal and foetal plasma were measured by methods previously described (Benito *et al.*, 1982). Neutralized $HClO_4$ extracts were used to determine glucose (Krebs *et al.*, 1963, 1964) and acetoacetate

and D-3-hydroxybutyrate (Williamson *et al.*, 1962).

Experiments were performed concurrently with those reported in Benito *et al.* (1982), and thus control values for blood glucose, non-esterified fatty acids, triacylglycerols and insulin are as reported in that paper.

Results and discussion

Rates of lipogenesis in maternal tissues

The oral load of glucose between 09:00 and 10:00h did not affect the rates of lipogenesis observed in the liver and adipose tissue during the last 2 days of gestation (Table 1). However, glucose administration increased plasma glucose and decreased plasma non-esterified fatty acid concentrations. These results are consistent with the operation of the glucose/fatty acid cycle *in vivo* in the pregnant rat, as described for non-pregnant ones (Randle *et al.*, 1963). In addition, plasma insulin concentration slightly increased at days 20–22 of gestation, which may account for the absence of a direct effect of glucose on lipogenesis in maternal tissues (Tables 1 and 2).

Table 1. *Rates of lipogenesis in vivo in maternal and foetal tissues during late gestation in fed control, glucose-intubated and streptozotocin-diabetic and starved rats*

For details see the Experimental section. The results are means ± S.E.M. ($n = 6$–20). Rates of lipogenesis are expressed as μmol of ^3H_2O incorporated into lipid/h per g wet wt. Values that are significantly different by Student's t test from those for fed control rats on the corresponding day of gestation are shown by: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

State of rats	Gestation period (days)	Maternal tissues: Liver	Maternal tissues: Adipose	Foetal tissues: Liver	Foetal tissues: Lung
Fed control	20	26.1 ± 1.5	4.9 ± 0.8	16.7 ± 1.2	6.5 ± 0.5
	21	18.5 ± 0.8	6.0 ± 0.4	12.5 ± 0.8	8.0 ± 0.4
	22	13.4 ± 1.0	8.3 ± 1.0	9.5 ± 0.4	9.0 ± 0.4
Fed glucose-intubated	20	25.2 ± 2.4	4.0 ± 0.5	16.9 ± 0.5	6.5 ± 0.5
	21	18.2 ± 1.4	5.7 ± 0.5	15.9 ± 0.5***	8.2 ± 0.4
	22	13.9 ± 0.8	8.2 ± 0.4	13.3 ± 0.5***	8.5 ± 0.4
Fed streptozotocin-diabetic	20	12.0 ± 1.2***	5.3 ± 0.7	16.2 ± 1.2	6.3 ± 0.5
	21	13.4 ± 1.0***	6.3 ± 0.7	12.4 ± 1.0	7.0 ± 0.8
	22	10.0 ± 1.0**	6.6 ± 1.0	9.4 ± 0.9	8.7 ± 0.8
48h-starved	20	12.1 ± 0.7***	3.4 ± 0.2**	11.9 ± 0.7***	4.8 ± 0.3*
	21	12.3 ± 0.4***	4.8 ± 0.3***	9.5 ± 0.5**	5.7 ± 0.4***
	22	10.5 ± 0.7**	4.1 ± 0.4***	6.8 ± 0.3***	5.0 ± 0.6***

Table 2. *Concentrations of metabolites and insulin in maternal plasma during late gestation in fed control, glucose-intubated and streptozotocin-diabetic and starved rats*

Blood was collected from the aorta, and the metabolites and insulin were determined as described in the Experimental section. The results are means ± S.E.M. ($n = 6$–10). Values that are significantly different from those for fed control rats on the corresponding day of gestation are shown by: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Control values for glucose, non-esterified fatty acids, triacylglycerols and insulin are taken from Benito *et al.* (1982).

State of rats	Gestation period (days)	Glucose (μmol/ml)	Non-esterified fatty acids (μmol/ml)	Ketone bodies (μmol/ml)	Triacylglycerols (μmol/ml)	Insulin (μunits/ml)
Fed control	20	4.2 ± 0.3	1.60 ± 0.10	0.20 ± 0.07	5.1 ± 0.3	110 ± 11
	21	4.4 ± 0.3	1.90 ± 0.30	0.20 ± 0.02	5.6 ± 0.5	73 ± 11
	22	3.5 ± 0.4	0.90 ± 0.10	0.30 ± 0.02	1.7 ± 0.1	27 ± 8
Fed glucose-intubated	20	6.4 ± 0.7**	0.46 ± 0.02***	—	4.7 ± 0.4	154 ± 25*
	21	5.9 ± 0.4**	0.42 ± 0.04***	—	4.3 ± 0.5	108 ± 20*
	22	6.1 ± 0.4***	0.36 ± 0.02***	—	1.5 ± 0.4	39 ± 9
Fed streptozotocin-diabetic	20	22.3 ± 0.3***	0.76 ± 0.10***	0.60 ± 0.06*	4.1 ± 0.2**	24 ± 4***
	21	21.6 ± 1.7***	0.60 ± 0.06***	0.45 ± 0.06*	4.4 ± 0.3**	32 ± 3***
	22	23.1 ± 0.6***	0.60 ± 0.08***	0.48 ± 0.05*	4.2 ± 0.6***	30 ± 3
48h-starved	20	3.7 ± 0.1	1.30 ± 0.10	4.20 ± 0.60***	1.3 ± 0.3	40 ± 2***
	21	3.8 ± 0.3	1.40 ± 0.10	4.30 ± 0.40***	1.8 ± 0.3	31 ± 2**
	22	3.9 ± 0.1	1.30 ± 0.07	4.20 ± 0.50***	2.1 ± 0.5	34 ± 5

Treatment with streptozotocin decreased the rate of maternal liver lipogenesis on days 20, 21 and 22 of gestation (Table 1). Thus this treatment prevents the inhibition of the rate of hepatic lipogenesis observed during late gestation. These results are in agreement with previous evidence obtained in the pancreatectomized pregnant rat *in vivo* (Fain & Scow, 1966), and in maternal liver and adipose-tissue fatty acid synthesis *in vitro* in pregnant diabetic pigs (Kasser *et al.*, 1981). On the other hand, treatment with streptozotocin increased plasma glucose and decreased plasma non-esterified fatty acid concentrations at 20–22 days of gestation (Table 2). These results are consistent with a modification of the glucose/fatty acid cycle in diabetes by accelerating the release and oxidation of fatty acids as a consequence of insulin deficiency (Randle, 1966). In fact, a plasma insulin concentration fell considerably in streptozotocin-diabetic pregnant rats as compared with those values from control rats, which may account for the decreased rates of lipogenesis in the liver described in the treated rats (Tables 1 and 2). Conversely, treatment with streptozotocin prevents the increase in the rate of lipogenesis in adipose tissue observed between 21 and 22 days in the untreated rats (Table 1). In addition, there are high plasma triacylglycerol and low ketone-body concentrations at 22 days of gestation in the treated rats (Table 2). The explanation for this might be an accelerated hepatic triacylglycerol biosynthesis because of elevated fatty acid substrate concentrations in the liver (Bierman *et al.*, 1975) and increased enzymic capacity for hepatic triacylglycerol biosynthesis (Woods *et al.*, 1981) in the diabetic rats, which is consistent with the low rates of lipogenesis in adipose tissue observed at 22 days of gestation (Table 1). After 48h of starvation, the rates of lipogenesis in the liver and adipose tissue were significantly lower than those in fed control rats on days 20, 21 and 22 of gestation (Table 1). In addition, maternal starvation was associated with low blood glucose and high blood ketone bodies (Table 2). The decrease in the rate of hepatic lipogenesis *in vivo* caused by maternal starvation may account for the significantly lower concentration of plasma triacylglycerol found at 20–22 days of gestation, and is consistent with the low plasma insulin concentration observed during late gestation (Table 2).

Rates of lipogenesis in foetal tissues

The oral load of glucose increased rates of lipogenesis in foetal liver observed at 21 and 22 days of gestation (Table 1). No effect was seen at 20 days, when the rates of lipogenesis in foetal liver reached their maximal value. These results are consistent with the suggestion that glucose is passing from the maternal into the foetal circulation to produce hepatic lipogenesis in the rat (Table 1). In addition, glucose administration, through the mother, to the foetus prevents the inhibition in the rate of lipogenesis observed between days 20–21 and 21–22 respectively (Table 1). These results seem to suggest that glucose availability becomes a rate-limiting factor in controlling foetal hepatic lipogenesis during late gestation. In addition, the foetal hepatic lipogenic responses to glucose are not mediated by insulin at 21 days of gestation. Conversely, the oral load of glucose doubled plasma insulin concentration at day 22 (Table 3), which is consistent with the idea that the mechanism for insulin

Table 3. *Concentrations of metabolites and insulin in foetal plasma during late gestation in fed control, glucose-intubated and streptozotocin-diabetic and starved rats*

Whole foetal blood was collected, and the metabolites and insulin were determined as described in the Experimental section. The results are means ± S.E.M. ($n = 6$–10). Values that are significantly different from those for foetuses from fed control rats on the corresponding day of gestation are shown by: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Control values for glucose and insulin are taken from Benito *et al.* (1982).

State of rats	Gestation period (days)	Glucose (μmol/ml)	Ketone bodies (μmol/ml)	Insulin (μunits/ml)
Fed control	20	1.0 ± 0.1	0.19 ± 0.05	72 ± 9
	21	1.4 ± 0.1	0.20 ± 0.04	128 ± 9
	22	2.1 ± 0.2	0.22 ± 0.04	90 ± 7
Fed glucose-intubated	20	1.0 ± 0.1	—	66 ± 10
	21	1.3 ± 0.2	—	135 ± 9
	22	2.9 ± 0.2**	—	185 ± 21***
Fed streptozotocin-diabetic	20	14.5 ± 1.2***	0.40 ± 0.07	58 ± 7
	21	15.0 ± 1.7***	0.50 ± 0.08	103 ± 9
	22	15.7 ± 2.3***	0.40 ± 0.08	102 ± 6
48h-starved	20	0.9 ± 0.1	5.50 ± 0.30***	54 ± 5
	21	1.4 ± 0.2	4.30 ± 0.40***	71 ± 8***
	22	2.1 ± 0.1	4.40 ± 0.50***	63 ± 4**

secretion mediated by glucose is developed in foetal pancreas near term.

However, the oral load of glucose did not increase the rate of lipogenesis in the foetal lung at 20–22 days of gestation (Table 1), a fact consistent with the suggestion that the glycogen depletion that occurs in foetal rat lung before birth provides glucose available for lipid synthesis in the lung (Maniscalco *et al.*, 1978).

On the other hand, treatment with streptozotocin did not affect the rate of lipogenesis in the liver and lung observed between days 20 and 22 of gestation. These results do not coincide with previous work on diabetic mothers by pancreatectomy, where a marked inhibition in fatty acid synthesis in the foetal liver occurred at 20 days (Fain & Scow, 1966), and with mothers made diabetic before pregnancy, where a slight inhibition in foetal liver lipogenesis has been described at 22 days (Pillay & Bailey, 1983). However, a possible explanation why the hyperglycaemia in the foetuses from diabetic mothers stimulated insulin release in the foetal pancreas, to such an extent that there was no insulin present at the time fatty acid synthesis was measured, is not appropriate in our case. Actually the chronic streptozotocin-diabetic state of pregnant rats in our experiments brought about a high plasma glucose concentration in the foetus without insulin variation as compared with those values found in the control rats (Table 3). In addition, the increased adipose-tissue fatty acid release and accelerated hepatic triacylglycerol synthesis discussed above in the diabetic mother might result in a possible increased placental transfer of lipid to the foetus, as suggested by Pillay & Bailey (1983). If so, lipid deposition in the liver does not decrease foetal liver lipogenesis at 20–22 days in our experimental conditions. However, we cannot exclude the possibility that the persistent state of hyperglycaemia and low insulin in the mother may have affected the foetal metabolism. Maternal starvation decreased the rates of lipogenesis in foetal liver and lung at 20–22 days of gestation (Table 1). These results do not agree with previous work, where the rate of lipogenesis in foetal liver slices was not decreased by maternal starvation (Miguel & Abraham, 1976). In addition, it has been suggested that maternally derived ketone bodies are used for lipogenesis in the rat (Seccombe *et al.*, 1977). In fact, as a consequence of maternal starvation, we found a high concentration of foetal plasma ketone bodies on days 20–22 of getation (Table 3). These results seem to suggest that the increase in ketone bodies caused by maternal starvation does not contribute to maintain the rates of lipogenesis observed in foetal tissues in the fed control rats. Therefore glucose rather than ketone bodies is likely to be the main and the limiting precursor for foetal lipogenesis in the liver. In conclusion, maternal liver lipogenesis seems to be regulated by insulin rather than by blood glucose available for hepatic lipid synthesis. Although there is a slight, statistically significant ($0.05 > P > 0.01$), increase in maternal insulin on glucose intubation on days 20 and 21, we do not consider these changes to be physiologically important when compared with those seen during normal pregnancy or dexamethasone treatment (Benito *et al.*, 1982). Our results suggest that the glucose potentially made available by the maternal blood supply (which may not be seen as a significant increase in foetal glycaemia, owing to rapid glucose utilization in late foetal life), rather than plasma insulin is the main factor involved in controlling foetal liver lipogenesis. In addition, because glucose administration increases foetal liver lipogenesis and plasma insulin concentration at 22 days, it may be suggested that the regulation of liver lipogenesis by insulin observed in adult rats works on foetal lipogenesis before birth. Under non-physiological conditions, however, chronic hyperglycaemia developed by streptozotocin administration to the mother does not result in more glucose being available for lipid synthesis in the foetal liver at 20–22 days of gestation. At 22 days, foetal hyperglycaemia is not related to an increased plasma insulin concentration. These results seem to suggest that the mechanism for insulin secretion mediated by glucose has not been developed in foetal pancreas from diabetic rats.

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