Regulation of rat foetal lipogenesis in brown adipose tissue *in vivo* and in isolated brown adipocytes during the last day of, and after prolonged, gestation

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Rates of lipogenesis in foetal isolated brown adipocytes from 22-day-pregnant rats were significantly increased by lactate plus pyruvate as major substrates in the incubation medium, in comparison with the endogenous rates. Insulin stimulated foetal brown-adipocyte lipogenesis, and adrenaline or noradrenaline and isoprenaline decreased lipogenesis. Glucagon had no effect on the lipogenic rate in brown adipocytes. Progesterone administration to the mother significantly increased the rates of lipogenesis in brown adipose tissue and in isolated brown adipocytes from 22-day foetuses. Prolongation of gestation by progesterone to day 23 decreased the rates of brown-adipose-tissue lipogenesis *in vivo* and in isolated cells in the post-mature foetuses.

INTRODUCTION

Brown-adipose-tissue lipogenesis increases during late foetal life in the rat (Pillay & Bailey, 1982). At birth, the rate of lipogenesis in brown fat becomes maximally stimulated (Benito *et al.*, 1984). Thus the administration of glucose fails to increase the lipogenic rate 1 h after birth, even though plasma insulin concentration is increased (Benito *et al.*, 1984).

The foetal–neonatal transition in the rat is concurrent with several metabolic changes in brown adipose tissue; a decrease in lipogenesis (Benito *et al.*, 1984) and an initiation of thermogenesis (Nicholls & Locke, 1984). In addition, significant hormonal changes occur at that moment. Thus plasma insulin concentration decreases from day 21 of foetal life (Lorenzo *et al.*, 1983), and plasma glucagon and adrenaline increase immediately after birth (Cuezva *et al.*, 1982; Martin *et al.*, 1981).

Accordingly, the aim of the present work was to study the rates of lipogenesis in isolated brown adipocytes from foetal rats on day 22 of gestation, and its short-term regulation by substrates and hormones such as insulin, glucagon and adrenaline or noradrenaline. Concurrently, we have investigated long-term regulation of lipogenesis by progesterone, on day 22 and after prolonged gestation to day 23, in brown adipose tissue *in vivo* and in isolated brown adipocytes.

EXPERIMENTAL

Animal treatment

Albino Wistar pregnant rats (300–350 g) fed on a stock laboratory diet were injected with 0.2 ml of progesterone [1.25 mg in olive oil/ethanol (1:1, v/v)/animal per day; Caswell *et al.*, 1983] on day 21 of gestation. Controls were injected with 0.2 ml of 0.9% NaCl and the carrier solution used. Other group of animals received a second injection of progesterone on day 22, and gestation was prolonged by 1 day (Klepac, 1981). Conception was

assumed by the presence of spermatozoa in the vagina, and gestational age was verified by the foetal weight.

Determination of lipid synthesis *in vivo*

Rats on days 22 and 23 of gestation were injected intraperitoneally with 5 mCi of $^3\text{H}_2\text{O}$; 55 min later they were anaesthetized with Nembutal (50 mg/kg body wt.), and 5 min later the abdomen was opened and maternal blood was collected from the aorta for determination of specific radioactivity of plasma water. An external-standard curve for quenching corrections was used for the calculations of radiolabelling. Foetuses were delivered by rapid hysterectomy, decapitated and exsanguinated. Samples of pooled brown adipose tissue (0.3 g) were added to 3 ml of 30% (w/v) KOH in 95% (v/v) ethanol, and the lipids were saponified and extracted by the method of Stansbie *et al.* (1976). Lipogenesis was expressed in terms of μmol of $^3\text{H}_2\text{O}$ incorporated into fatty acids/h per g wet wt.

Determination of lipid synthesis in isolated brown adipocytes

Adipocytes from foetal-rat brown adipose tissue were prepared essentially by a collagenase-dispersion method containing Krebs (1933) phosphate Ringer buffer (Elliott *et al.*, 1984). Cell viability was determined by dye exclusion and was routinely higher than $95 \pm 3\%$. Incubation of adipocytes $[(5-7) \times 10^5 \text{ cells}]$ were carried out in closed plastic tubes (0.9 cm $\times$ 7 cm) in a total volume of 0.3 ml containing Krebs–Henseleit (1932) bicarbonate buffer plus 4% (w/v) bovine serum albumin, in the presence of 0.75 mCi of $^3\text{H}_2\text{O}$. In other incubations, several substrates and hormones were added. Cells were incubated for 60 min at 37 °C in a shaking water bath (100 cycles/min). The reaction was stopped by addition of 30 μl of 60% (w/v) HClO_4 . Lipogenesis was measured as described by Harris (1975). An external-standard curve for quenching corrections was used for radiolabelling. Lipid synthesis was

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Table 1. Rates of lipogenesis in isolated brown adipocytes from foetuses on day 22 of gestation: effect of several substrates

For details see the Experimental section. Results are means $\pm$ S.E.M. ($n = 6-14$). Rates of lipogenesis are expressed as μmol of $^3\text{H}_2\text{O}$ incorporated into fatty acids/h per 10^7 cells. Values that are significantly different by Student's t test from those for endogenous substrates are shown by *** $P < 0.001$.

Substrates	Rate of lipogenesis
Endogenous	0.083 ± 0.003
Lactate (10 mM) + pyruvate (1 mM)	$0.225 \pm 0.015^{***}$
Glucose (5 mM)	$0.136 \pm 0.004^{***}$
Lactate + pyruvate + glucose	$0.248 \pm 0.013^{***}$
Acetate (8 mM)	$0.106 \pm 0.004^{***}$
β -Hydroxybutyrate (4 mM)	$0.114 \pm 0.007^{***}$
Acetoacetate (4 mM)	$0.128 \pm 0.007^{***}$
Dihydroxyacetone (5 mM)	0.085 ± 0.002

expressed in terms of μmol of $^3\text{H}_2\text{O}$ incorporated into fatty acids/h per 10^7 cells.

The conversion factors used in Table 3 to convert cell number/wt. of tissue were determined as follows. A suspension of brown-fat cells was counted under a microscope. Several samples in duplicate of cell suspension containing 10^6-10^7 cells/ml were placed in pre-weighed plastic tubes. One series of those was centrifuged (10000 g , 2 min) and the cell pellets were weighed. The other was desiccated (100 $^\circ\text{C}$ for 24 h) and the residues were weighed. Conversion factors of 1 mg dry wt. = 3 mg wet wt. or 5×10^6 cells were established.

Determination of hormones and metabolites

Foetal plasma insulin was measured by radioimmunoassay by the method of Hales & Randle (1963). Neutralized HClO_4 extracts were used to determine foetal plasma glucose concentration (Krebs *et al.*, 1963, 1964).

RESULTS AND DISCUSSION

Short-term regulation of foetal lipogenesis in brown adipocytes on the last day of gestation

Isolated adipocytes from foetal-rat brown adipose tissue have been demonstrated as a tool for metabolic studies (Elliott *et al.*, 1984; Roncero *et al.*, 1986). Lipogenesis *in vivo* in brown fat increases during the last day of foetal life in the rat (Pillay & Bailey, 1982), reaching its maximal rate at birth (Benito *et al.*, 1984). At this stage, we have studied the lipogenic capacity of isolated brown adipocytes from foetuses on day 22 of gestation, and tested its response to several substrates and hormones for 1 h (Table 1).

The presence of 8 mM-acetate as exogenous substrate (a well-known lipogenic precursor for lipogenesis in monogastric mammals; Snoswell *et al.*, 1982) increases the rates of endogenous lipogenesis by 28% (Table 1). Ketone bodies have been described as good lipogenic substrates for foetal brown adipose tissue *in vivo* (Secombe *et al.*, 1977) and for brown adipose tissue

Table 2. Rates of lipogenesis in isolated brown adipocytes from foetuses on day 22 of gestation: effects of hormones

For details see the Experimental section. Results are means $\pm$ S.E.M. ($n = 6-14$). Rates of lipogenesis are expressed as μmol of $^3\text{H}_2\text{O}$ incorporated into fatty acids/h per 10^7 cells. Values that are significantly different by Student's t test from those for endogenous substrates (*) or for lactate plus pyruvate (†) in the absence of hormones are shown by: *, † $P < 0.05$; †† $P < 0.01$.

Substrates	Hormone added...	None	Insulin (1 nM)	Glucagon (1 μM)	Adrenaline (0.1 μM)	Noradrenaline (0.1 μM)	Isoprenaline (0.1 μM)	Phenylephrine (0.1 μM)	Propranolol (100 μM) + phenylephrine (10 μM)
Endogenous		0.083 ± 0.003	$0.096 \pm 0.005^*$	0.081 ± 0.003	0.078 ± 0.003	$0.155 \pm 0.004^{††}$	$0.165 \pm 0.004^{††}$	0.205 ± 0.007	—
Lactate (10 mM) + pyruvate (1 mM)		0.225 ± 0.015	$0.284 \pm 0.029^{††}$	0.220 ± 0.010	$0.178 \pm 0.006^{††}$	$0.155 \pm 0.004^{††}$	$0.165 \pm 0.004^{††}$	0.205 ± 0.007	0.210 ± 0.020

Table 3. Effect of progesterone treatment on the rates of lipogenesis in foetal brown adipose tissue and in isolated brown adipocytes, and on the concentrations of glucose and insulin in foetal plasma, on day 22 of gestation and after prolonged gestation

For details see the Experimental section. Rates of lipogenesis *in vivo* are expressed as μmol of $^3\text{H}_2\text{O}$ incorporated into fatty acids/h per g wet wt. Rates of lipogenesis in isolated brown adipocytes are expressed as μmol of $^3\text{H}_2\text{O}$ incorporated into fatty acid/h per 10^7 cells. Conversion factors are: 1 mg dry wt. = 3 mg wet wt. or 5×10^6 cells. Results are means $\pm$ S.E.M. ($n = 6-12$). Values that are significantly different by Student's *t* test from those for pregnant control rats on day 22 (*) or for pregnant rats treated with progesterone on day 22 (†) are shown by: * $P < 0.005$; ***, ††† $P < 0.001$.

Treatment	Foetal age (days)	Rates of lipogenesis			[Glucose] ($\mu\text{mol/ml}$)	[Insulin] ($\mu\text{units/ml}$)
		<i>In vivo</i> ($\mu\text{mol/h per g wet wt.}$)	Endogenous substrates ($\mu\text{mol/h per } 10^7$ cells)	Lactate (10 mM) + pyruvate (1 mM) ($\mu\text{mol/h per } 10^7$ cells)		
0.9% NaCl plus carrier solution	22	41 ± 2	0.083 ± 0.002	0.206 ± 0.012	2.1 ± 0.2	90 ± 7
Progesterone (24 h)	22	$49 \pm 3^*$	$0.134 \pm 0.004^{***}$	$0.279 \pm 0.016^{***}$	$3.4 \pm 0.7^{***}$	89 ± 8
Progesterone (48 h)	23	$24 \pm 2^{\dagger\dagger\dagger}$	$0.052 \pm 0.006^{\dagger\dagger\dagger}$	$0.110 \pm 0.026^{\dagger\dagger\dagger}$	3.4 ± 0.7	$35 \pm 5^{\dagger\dagger\dagger}$

in vivo in cold-adapted rats (Wright & Agius, 1983). The addition of 4 mM-acetoacetate or 4 mM- β -hydroxybutyrate to the incubation medium increases the endogenous rates by 54% and 35% respectively (Table 1).

Dihydroxyacetone (5 mM), which was found by Harris (1975) to be the best lipogenic precursor in hepatocytes from fed rats, is not a lipogenic substrate in foetal brown adipocytes (Table 1). The rate of lipogenesis in the presence of 5 mM-glucose (an important lipogenic precursor for brown adipose tissue *in vivo* and *in vitro*; Agius *et al.*, 1983) increases by 63% as compared with the endogenous rate (Table 1).

The presence of 10 mM-lactate plus 1 mM-pyruvate as exogenous substrates increases by 171% the rate of lipogenesis observed in isolated brown adipocytes in its absence (Table 1). Thus lactate plus pyruvate are the best substrates for lipogenesis in brown adipocytes from 22-day foetuses, as previously observed in foetal hepatocytes (Lorenzo *et al.*, 1986). Finally, the presence of 10 mM-lactate plus 1 mM-pyruvate plus 5 mM-glucose produces the maximal stimulation of the lipogenic flux, increasing by 198% the endogenous rate (Table 1).

The lipogenic response to hormones in isolated brown adipocytes from foetuses on day 22 of gestation is shown in Table 2. In the presence of 1 nM-insulin, the rate of lipogenesis increased by 16%. When lactate plus pyruvate were present as exogenous substrates, insulin produced a higher effect, increasing the lipogenic flux by 26% (Table 2). A stimulation by insulin of the rate of lipogenesis in brown adipose tissue *in vivo* has been described (McCormack *et al.*, 1986). These results seem to indicate that insulin works on the rate of lipogenesis in isolated brown adipocytes as it does *in vivo*, presumably by mechanisms involving activation of pyruvate dehydrogenase and acetyl-CoA carboxylase (McCormack & Denton, 1977).

The incubation with 1 μM -glucagon does not produce any effect on the rate of lipogenesis in all the conditions studied (Table 2). Thus glucagon does not seem to regulate the rate of lipogenesis in brown adipocytes, as previously indicated by Elliott *et al.* (1984), where glucagon did not produce any effect on cyclic AMP accumulation in the cells or on the rate of lipolysis studied.

The presence of 0.1 μM -adrenaline in the incubation medium did not change the endogenous lipogenesis, but produced a decrease in lipogenesis of 21% of that when lactate plus pyruvate were present (Table 2). The addition of 0.1 μM -noradrenaline in the presence of lactate plus pyruvate as substrates produced a significant inhibition of the rates of lipogenesis, by 31% (Table 2), in agreement with the results found *in vivo* by McCormack *et al.* (1986). These results are consistent with the adrenergic stimulation of thermogenesis in brown adipose tissue at birth (Nicholls & Locke, 1984). In addition we have tested a β -adrenergic agonist (0.1 μM -isoprenaline), an α_1 -adrenergic agonist (0.1 μM -phenylephrine) and an α_1 -adrenergic agonist in the presence of a β -blocker (10 μM -phenylephrine + 100 μM -propranolol) on the rates of lipogenesis observed in the presence of lactate plus pyruvate. The rates of lipogenesis decreased by 27% with isoprenaline, but did not change with 0.1 μM -phenylephrine or 10 μM -phenylephrine in the presence of propranolol (Table 2). These results show that the inhibitory effect of adrenaline or noradrenaline on brown-adipose-tissue lipogenesis is mainly exerted through a β -adrenergic mechanism.

Long-term regulation of foetal lipogenesis in brown adipose tissue *in vivo* and in isolated brown adipocytes on the last day and after prolonged gestation

The rates of lipogenesis in foetal brown adipose tissue increase during the last 2 days of gestation, reaching a maximum around parturition (Pillay & Bailey, 1982). The administration of progesterone to the mother on day 21 of gestation increased the rates of lipogenesis *in vivo* in foetal brown adipose tissue on day 22 (Table 3) in comparison with the non-treated animals. Plasma insulin in the foetus was not affected by progesterone administration, but plasma glucose concentration was significantly higher in the progesterone-treated animals (Table 3). Owing to the ability of maternal progesterone to cross the placenta into the foetal circulation, and to the similarity of maternal and foetal plasma progesterone concentrations (Martin *et al.*, 1977), it is reasonable to suggest a role for maternal progesterone in the control of brown-adipose-tissue lipogenesis in the foetuses.

Moreover, the administration of progesterone through

the mother on day 21 of gestation increased significantly the rates of endogenous lipogenesis in foetal isolated brown adipocytes from pregnant rats on day 22, as compared with values for non-treated rats. In the presence of lactate plus pyruvate as exogenous substrates, the rates of endogenous lipogenesis increased significantly in the 24 h-progesterone-treated animals (Table 3). A similar effect of progesterone on foetal lipogenesis has been observed in the liver and in isolated hepatocytes (Lorenzo *et al.*, 1986). Our results indicate that the administration of progesterone for 24 h during the last day of gestation increased the rates of foetal brown-adipose-tissue lipogenesis as a consequence of enhanced lipogenic capacity as well as an increased substrate availability to the foetuses.

The administration of progesterone for 48 h on day 21 of gestation produced a prolonged gestation, to day 23. Foetuses from prolonged-gestation rats showed a higher body weight (6.7 ± 0.3 g) in comparison with untreated or progesterone-treated 22-day foetuses (5.3 ± 0.2 g). Prolonged gestation by progesterone administration, however, decreased significantly the rates of foetal lipogenesis *in vivo* in the brown adipose tissue as compared with those values from progesterone-treated rats on day 22 (Table 3). In addition, the decrease in lipogenesis was parallel to the fall in plasma insulin concentration found in foetuses from 48 h-progesterone-treated rats (Table 3).

Moreover, the rates of lipogenesis from endogenous sources and in the presence of lactate plus pyruvate as substrates in isolated foetal adipocytes from prolonged-gestation rats were significantly decreased as compared with those rates for progesterone-treated rats on day 22 (Table 3). These results are in agreement with those seen above *in vivo*, and indicate that the regulation of lipogenesis by insulin in brown adipose tissue is operating in the post-mature foetal rat, as described in the newborn rat at term immediately after birth (Benito *et al.*, 1984).

In conclusion, the rate of lipogenesis in isolated brown adipocytes from 22-day foetuses is significantly stimulated by lactate plus pyruvate as major substrates at physiological concentrations. Insulin and adrenaline or noradrenaline exert a short-term regulatory effect on lipogenesis in brown adipocytes in the foetal-neonatal transition in the rat, as they are *in vivo* under physiological conditions. Glucagon, however, does not seem to regulate the lipogenic rate in brown adipocytes, as it does not have any effect on cyclic AMP accumulation in the isolated cells. Progesterone has a long-term regulatory role on lipogenesis *in vivo* and in isolated brown adipocytes in foetuses at the end of gestation. Prolongation of gestation by progesterone

indicated that post-mature foetal rats had decreased rates of brown-adipose-tissue lipogenesis *in vivo* and in isolated cells, owing to the substantial decrease in foetal plasma insulin concentration, as occurred in the newborn rat at term immediately after birth.

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REFERENCES

- Agius, L., Watts, D. J. & Sugden, M. C. (1983) *Horm. Metab. Res.* **15**, 482–485
- Benito, M., Lorenzo, M. & Caldés, T. (1984) *Biochem. J.* **224**, 823–828
- Caswell, A. M., Higham, F. C. & Bailey, E. (1983) *J. Dev. Physiol.* **5**, 229–305
- Cuezva, J. M., Burkett, E. S., Kerr, D. S., Rodman, H. M. & Patel, M. S. (1982) *Pediatr. Res.* **16**, 632–637
- Elliott, K. R. F., Roncero, C., Fernández, M. & Benito, M. (1984) *Biosci. Rep.* **4**, 397–401
- Hales, C. N. & Randle, P. J. (1963) *Biochem. J.* **88**, 137–146
- Harris, R. A. (1975) *Arch. Biochem. Biophys.* **169**, 168–180
- Klepac, R. (1981) *Biol. Neonate* **39**, 165–170
- Krebs, H. A. (1933) *Hoppe-Seyler's Z. Physiol. Chem.* **217**, 191–228
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–36
- Krebs, H. A., Bennet, D. A. H., De Gasquet, P., Gascoyne, T. & Yoshida, T. (1963) *Biochem. J.* **86**, 22–27
- Krebs, H. A., Dierks, C. & Gascoyne, T. (1964) *Biochem. J.* **93**, 112–121
- Lorenzo, M., Benito, M., Caldés, T. & Medina, J. M. (1983) *Biochem. J.* **216**, 695–699
- Lorenzo, M., Roncero, C. & Benito, M. (1986) *Biochem. J.* **239**, 135–139
- Martin, A., Caldés, T., Benito, M. & Medina, J. M. (1981) *Biochim. Biophys. Acta* **672**, 262–267
- Martin, C. E., Cake, M. H., Hartmann, P. E. & Cook, I. F. (1977) *Acta Endocrinol. (Copenhagen)* **84**, 167–176
- McCormack, J. G. & Denton, R. M. (1977) *Biochem. J.* **166**, 627–630
- McCormack, J. G., Gibbins J. M. & Denton, R. M. (1986) *Biochem. Soc. Trans.* **14**, 227–230
- Nicholls, D. & Locke, R. M. (1984) *Physiol. Rev.* **64**, 1–64
- Pillay, D. & Bailey, E. (1982) *Int. J. Biochem.* **14**, 511–517
- Roncero, C., Peñas, M., Domínguez, M. J., Fernández, M. & Benito, M. (1986) *Biochem. Soc. Trans.* **14**, 284
- Secombe, D. W., Harding, P. G. R. & Possmayer, F. (1977) *Biochim. Biophys. Acta* **488**, 402–416
- Snoswell, A. M., Trimble, R. P., Fishlock, R. C., Storer, F. B. & Topping, D. L. (1982) *Biochim. Biophys. Acta* **716**, 290–297
- Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Wright, J. & Agius, L. (1983) *Biochim. Biophys. Acta* **753**, 244–248