The Effects of Glucose, Free Fatty Acids and Lipid Depletion on the Metabolism *in vitro* of Brown Fat from Newborn Rabbits

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Brown adipose tissue supplies the major part of the extra heat produced when newborn rabbits are exposed to cold (Hull & Segall, 1965*a*). O₂ consumption and therefore heat production, in brown fat is stimulated by the release of noradrenaline from sympathetic nerve endings within the tissue (Hull & Segall, 1965*b*; Knight & Myant, 1970). During prolonged exposure of newborn rabbits to cold the lipid droplets within brown adipose tissue gradually decrease in size and finally disappear (Hull & Segall, 1966). When the lipid content of the tissue is low the rabbits are unable to raise their O₂ consumption in response to the cold (Hull & Segall, 1965*c*). Since the animals are probably hypoglycaemic by this time, it is not possible to discover from these results whether the inability of lipid-depleted brown fat to liberate heat is merely a reflection of a lack of oxidizable substrate or whether there is an impairment of some part of the mechanism which stimulates the O₂ consumption of the tissue.

The present paper reports experiments that were performed to evaluate the relative importance of glucose, plasma free (non-esterified) fatty acids and intracellular lipid as substrates for oxidation in brown fat, and to investigate the requirements of the tissue for the maintenance of heat production during exposure of newborn rabbits to cold.

Materials and methods

The rabbits were of the Dutch strain. Lipid-replete brown fat was obtained from 3-day-old rabbits that had been left with their mother until the morning of the experiment and kept at 37°C until they were killed. Lipid-depleted brown fat was obtained from 3-day-old rabbits that had been kept, unfed, at 22°C for 48 h before the experiment.

The methods used for the preparation, incubation and analysis of brown fat have been described previously (Knight & Myant, 1970). DNA was assayed by the method described by Burton (1956). For the determination of the specific radioactivity of ¹⁴C-labelled fatty acids the incubation medium was neutralized and extracted as described by Dole (1956). The heptane phase was assayed for fatty acids by the method of Mosinger (1965), and for radioactivity. Bovine serum albumin (fraction V) was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. It was

defatted by the procedure described by Chen (1967). Palmitic acid or [¹⁴C]palmitic acid was added to the defatted albumin when required. The desired amount of fatty acid was neutralized in 0.1 M-NaOH at 60°C and added dropwise to a stirred solution of albumin (5–10%, w/v) in water at 35°C. The solution was filtered and either freeze-dried or used immediately. [U-¹⁴C]Glucose and [U-¹⁴C]palmitic acid were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Student's *t* test was used to check the significance of differences between mean values.

Results and discussion

The DNA content of lipid-replete brown fat was 1.44 ± 0.15 mg/g fresh wt. and that of lipid-depleted tissue was 2.51 ± 0.28 mg/g fresh wt. This increase is slightly, but not significantly, greater than would be expected from the loss of glyceride from the tissue. Thus there is probably little loss of water or protein from brown fat during starvation of newborn rabbits in the cold.

Table 1 shows that the O₂ consumption of lipid-depleted brown fat incubated with glucose was less than that of lipid-replete tissue, both in the presence and in the absence of noradrenaline. Moreover the increase in O₂ consumption on the addition of noradrenaline was much smaller in lipid-depleted tissue than in lipid-replete tissue. This is consistent with results obtained *in vivo*, which show that the ability of newborn rabbits to raise their metabolic rate when exposed to cold is impaired if the fat content of brown adipose tissue is allowed to fall to a low value (Hull & Segall, 1965*c*; Hardman *et al.*, 1969). Although the oxidation of glucose by brown fat in the presence of noradrenaline was depressed by lipid depletion (Tables 1 and 2), the proportion of the total CO₂ that was derived from glucose was increased. The respiratory quotient of lipid-depleted brown fat incubated with 10 mM-glucose and noradrenaline was 1.02 ± 0.04 , which is significantly higher ($P < 0.001$) than the value of 0.80 ± 0.02 found for lipid-replete tissue.

Experiments were performed with different concentrations of glucose in incubation media containing noradrenaline. As the concentration of glucose in the medium was lowered the amount of glucose oxidized was significantly decreased. In lipid-replete brown fat

Table 1. *Effect of noradrenaline on O₂ consumption and CO₂ production in lipid-replete and lipid-depleted brown fats*

The incubation medium was Krebs–Ringer bicarbonate buffer, pH 7.4 (with half the recommended Ca²⁺) (Umbreit *et al.*, 1964), containing bovine serum albumin (fraction V) (5%, w/v), glucose (10 mM), free fatty acids (0.5 μ mol/ml), insulin (0.1 unit/ml) and either [U-¹⁴C]glucose (0.1 μ Ci/ μ mol of glucose) or [U-¹⁴C]palmitate (0.4 μ Ci/ μ mol of free fatty acid). Noradrenaline (1 μ g/ml) was present where indicated. Results are given as μ mol/h per mg of DNA for O₂ consumption or μ g-atoms of C/h per mg of DNA for CO₂ production, and as means $\pm$ S.E.M. with the numbers of rabbits used for each pair in parentheses.

	Without noradrenaline		With noradrenaline	
	Lipid-replete	Lipid-depleted	Lipid-replete	Lipid-depleted
O ₂ consumption	54.9 $\pm$ 4.6	31.6 $\pm$ 3.3*	179.5 $\pm$ 10.4 (7)	70.5 $\pm$ 4.9* (4)
Total CO ₂ production	47.0 $\pm$ 12.3	34.7 $\pm$ 6.1	148.6 $\pm$ 8.0 (7)	66.4 $\pm$ 8.1* (4)
CO ₂ from [¹⁴ C]glucose	9.7 $\pm$ 1.9	10.2 $\pm$ 0.1	30.9 $\pm$ 2.8 (7)	21.4 $\pm$ 2.8 (4)
CO ₂ from [¹⁴ C]palmitate	—	—	11.0 $\pm$ 0.9 (3)	5.3 (2)

* Statistically significant ($P < 0.05$) effect of lipid depletion; all effects of noradrenaline are statistically significant ($P < 0.05$).

the loss of glucose oxidation was balanced by an increase in the oxidation of fatty acids, since the O₂ consumption remained constant and the respiratory quotient significantly fell. This implies that the rate of respiration in lipid-replete brown fat *in vitro* after stimulation by noradrenaline is limited, not by the availability of substrate, but by the supply of O₂ or, more probably, the activity of the citric acid cycle. In lipid-depleted brown fat the oxidation of fatty acids did not increase as the oxidation of glucose was lowered. Between 5 mM- and 1 mM-glucose the production of CO₂ from [U-¹⁴C]glucose fell from 21.4 $\pm$ 2.8 to 6.6 $\pm$ 0.2 μ g-atoms of C/h per mg of DNA and the O₂ consumption fell by almost the same amount, from 66.4 $\pm$ 3.6 to 52.8 $\pm$ 1.4 μ mol/h per mg of DNA. Despite the obvious lack of fatty acids for oxidation in lipid-depleted brown fat, the oxidation of glucose was not greater than in lipid-replete tissue, even when the concentration of glucose in the incubation medium was raised to 20 mM. Thus, although fatty acids can replace glucose as a fuel for thermogenesis in brown fat, the oxidation of glucose is maximally stimulated by noradrenaline and cannot increase further to compensate for a lack of fatty acids. This is again consistent with results obtained *in vivo*. Hardman *et al.* (1969) have reported that the inability of cold-exposed newborn rabbits to maintain a high metabolic rate cannot be overcome by the administration of glucose. They found that a significant recovery could be demonstrated if the rabbits were given a milk feed and kept at 36°C for 4 h. The use of extracellular free fatty acids for respiration in brown fat was studied by incubating lipid-replete and lipid-depleted tissues in medium containing high or low concentrations of free fatty acids or [U-¹⁴C]palmitate. Increasing the concentration of free fatty acid in the

medium increased the net uptake of free fatty acid into both tissues (Table 2), although this does not necessarily mean that they were incorporated into the fat-cells themselves. Indeed, it is clear from the results shown in Table 2 that extracellular free fatty acids did not affect the oxidative metabolism *in vitro*, at least during the first 2 h of incubation. In particular the O₂ consumption of lipid-depleted brown fat was not raised by increasing the availability of free fatty acid. Further, extracellular free fatty acids provided only a small proportion of the total CO₂ produced by lipid-replete tissue *in vitro*, and the amount of extracellular fatty acid oxidized was not increased by lipid depletion (Table 1). These experiments with brown fat *in vitro* indicate that the recovery after a milk feed observed by Hardman *et al.* (1969) cannot be merely attributed to an increased availability and oxidation of plasma free fatty acids.

The behaviour of brown fat *in vitro* suggests that intracellular lipid, which supplies about 65% of the CO₂ produced by lipid-replete tissue, is an essential substrate for the maintenance of heat production in brown fat. The depression of respiration in lipid-depleted tissue can be accounted for almost entirely by a decrease in the oxidation of endogenous lipid. It is not clear from these results, however, whether the inability of lipid-depleted brown fat to respond to noradrenaline is entirely due to the lack of substrate, or whether the period of starvation in the cold causes an impairment, through the depletion of lipid or some other factor, of the mechanism of stimulation of respiration of the tissue. The restoration of the response of cold-exposed newborn rabbits observed by Hardman *et al.* (1969) was not accompanied by a detectable increase in the amount of lipid in brown fat, so it is possible that the period of exposure to cold

Table 2. *Metabolism of lipid-replete and lipid-depleted brown fats in media containing low or high concentrations of free fatty acids*

The incubation medium (see Table 1) contained glucose (10 mM), [U-¹⁴C]glucose (1 μCi/ml), insulin (0.1 unit/ml), noradrenaline (1 μg/ml) and bovine serum albumin (fraction V) (5 %, w/v) fortified with or depleted of fatty acids (see under ‘Materials and methods’). Incubations were continued for 2 h. Values for CO₂ from [¹⁴C]glucose are given as μg-atoms of C/h per mg of DNA and all others as μmol/h per mg of DNA. The results shown are means ± S.E.M. of values with tissue from three rabbits in each case.

	High concn. of free fatty acids (2.2 μmol/ml)		Low concn. of free fatty acids (0.2 μmol/ml)	
	Lipid-replete	Lipid-depleted	Lipid-replete	Lipid-depleted
O ₂ consumption	180.2 ± 3.5	59.4 ± 1.6*	178.8 ± 1.1	55.4 ± 2.4*
CO ₂ production	142.8 ± 6.2	55.0 ± 6.0*	142.1 ± 10.4	61.0 ± 2.4*
CO ₂ from [¹⁴ C]glucose	36.2 ± 0.4	19.6 ± 1.2*	36.2 ± 2.1	19.6 ± 1.0*
Net output of free fatty acids	2.29 ± 1.66	-3.83 ± 2.91	12.47 ± 1.94†	2.83 ± 0.24*†

* Statistically significant (*P* < 0.05) effect of lipid depletion.
† Statistically significant (*P* < 0.05) difference between results obtained with high and low concentrations of free fatty acids.

causes changes in the tissue, apart from a depletion of lipid, that can be reversed by keeping the rabbit at 36°C.

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