Glucose metabolism and its regulation in pieces of perirenal adipose tissue from foetal lambs

James P. ROBERTSON,* Anne FAULKNER and Richard G. VERNON
*Department of Lactational Physiology and Biochemistry, Hannah Research Institute, Ayr,
Scotland KA6 5HL, U.K.*

(Received 8 October 1982/Accepted 2 November 1982)

1. The following were measured in pieces of perirenal adipose tissue obtained from foetal lambs at about 120 days of gestation or within 3 days of term, and 9-month-old sheep: the rates of synthesis from glucose of fatty acids, acylglycerol glycerol, pyruvate and lactate; the rate of glucose oxidation to CO_2 and the proportions contributed by the pentose phosphate cycle, pyruvate dehydrogenase and the tricarboxylic acid cycle; the activities of hexokinase, glucose 6-phosphate dehydrogenase, phosphofructokinase, pyruvate kinase and pyruvate dehydrogenase. 2. The total rate of glucose utilization was lower in pieces of adipose tissue from near-term lambs than 120-day foetal lambs and the pattern of glucose metabolism differed, with, for example, a much smaller proportion of glucose carbon being used for fatty acid synthesis, whereas a greater proportion of glucose oxidation occurred via the tricarboxylic acid cycle in the near-term lambs. In general, these differences in glucose metabolism were not associated with differences in the activities of the various enzymes listed above. 3. The rates of glucose utilization per fat-cell by 120-day foetal lambs and 9-month-old sheep were very similar but, again, the proportions metabolized to the various products differed. In particular, there was a smaller proportion of glucose oxidized via the pentose phosphate cycle and a greater proportion oxidized via pyruvate dehydrogenase and the tricarboxylic acid cycle in adipose tissue from foetal lambs. These differences were matched by a lower activity of glucose 6-phosphate dehydrogenase and a higher pyruvate dehydrogenase activity in fat-cells from the foetal lambs.

As little glucose is obtained from the diet, the adult ruminant synthesizes over 90% of its glucose requirements by gluconeogenesis (see Ballard *et al.*, 1969; Leng, 1970; Lindsay, 1978). The rate of glucose turnover (per kg body wt.) is much higher in the foetal than in the adult ruminant (cf. Leng, 1970; Prior, 1982) and, in contrast with the adult, glucose oxidation accounts for about 50% of the CO_2 produced by the foetus (see Battaglia & Meschia, 1978; Girard *et al.*, 1979). Most, if not all, of the glucose used by the foetus is obtained from the mother, although some may be produced by foetal gluconeogenesis (see Battaglia & Meschia, 1978; Girard *et al.*, 1979; Prior, 1982).

There is very little information available on the metabolism of glucose by individual tissues of foetal ruminants. Some aspects of glucose metabolism by

liver (see Ballard *et al.*, 1969) and perirenal adipose tissue (Robertson *et al.*, 1981; Vernon *et al.*, 1981b) have been described. The purpose of the present study was to explore in more detail the pathways of glucose metabolism in perirenal adipose tissue from foetal lambs, and, by comparison with glucose metabolism in adipose tissue from 9-month-old sheep, provide further insight into the regulation of glucose metabolism in adipose tissue of ruminant animals.

Methods

Animals

Foetal lambs were obtained from Cheviot ewes (about 6 years old). The mothers were fed on hay (*ad libitum*) plus a cereal mix (200 g/day until day 100 of pregnancy, then increasing gradually to 1000 g/day at 140 days of pregnancy). The ewes were kept in an open field and allowed to graze. The

* Present address: Department of Biology, University of York, Heslington, York YO1 5DD, U.K.

gestation period was about 148 days in these ewes. Ewes were killed at 10:00h with a captive-bolt humane killer. Foetal lambs were removed from the mother and killed in the same way. Samples of perirenal adipose tissue were removed from the foetal lambs and either frozen in liquid N₂ or placed in 0.9% NaCl at about 37°C.

The other Cheviot sheep used in the study were about 9 months old. They were fed on hay *ad libitum* plus a cereal mix (400g/day) for at least 1 month before sampling. These sheep were anaesthetized before sampling as described previously (Vernon *et al.*, 1981a). Samples of perirenal adipose tissue were again removed and placed immediately in liquid N₂ or 0.9% NaCl at about 37°C. Sheep were killed with a captive-bolt humane killer before they recovered from anaesthesia.

Measurement of metabolic activities

Pieces of adipose tissue weighing about 5 mg were cut with scissors. Samples of these pieces (total wt. about 250 mg) were transferred to sterile 250 ml Erlenmeyer flasks containing 25 ml of M199 medium (Medium 199 containing Earle's salts, L-glutamine and 25 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.3, from Gibco Biocult, Paisley, Scotland, plus 60 µg of penicillin/ml, 100 µg of streptomycin sulphate/ml, 10 µg of neomycin/ml, 5 µg of amphotericin B/ml and 100 ng of insulin/ml). Insulin was obtained from Sigma (London) Chemical Co. These flasks were maintained at 37°C for 2 h without shaking, after which samples of adipose tissue weighing about 50 mg in all were transferred to Erlenmeyer flasks containing 2.5 ml of M199 medium and the amount of incorporation of ¹⁴C from [U-¹⁴C]glucose into CO₂, fatty acids and acylglycerol glycerol, and the rate of production of pyruvate, L-lactate and glycerol were determined as described previously [Robertson *et al.* (1982a) and Vernon *et al.* (1981a) respectively]. Medium 199 contains 5.5 mM-glucose and 0.6 mM-acetate.

Measurement of enzyme activities

Enzyme activities were measured in adipose tissue without any prior incubation, unless otherwise stated in the text. Pyruvate dehydrogenase activity was measured in the samples of adipose tissue, which had been frozen in liquid N₂ immediately after removal from the animals. Details of homogenization and assay procedures have been described previously (Robertson *et al.*, 1982a).

Determination of adipocyte volume and number of adipocytes per g of tissue

Preparation of perirenal adipocytes from foetal lambs and 9-month-old sheep have been described previously [Vernon *et al.* (1981b) and Robertson *et*

al. (1982a) respectively]. The lipid content of pieces of adipose tissue was determined after incubation (as described above) and the number of fat-cells per g of tissue pieces was calculated as described previously (Vernon, 1977).

Calculation of metabolic fluxes

The net total rate of glucose utilization was calculated by summation of the rate of glucose carbon conversion into fatty acid, acylglycerol glycerol, CO₂, lactate and pyruvate: it was assumed that very little glucose was converted into, or formed from, glycogen and that all lactate and pyruvate produced was formed from glucose (Robertson *et al.*, 1982a). The rate of glucose oxidation by the pentose phosphate cycle and other metabolic fluxes was calculated as described previously (Robertson *et al.*, 1982a).

Statistical analysis

Results are expressed as means ± S.E.M. Statistical analysis was performed by using the Mann-Whitney U-test (Siegel, 1956).

Results

Fat-cells from foetal lambs were very much smaller than those from adult sheep (Table 1). In view of the huge difference in the numbers of adipocytes per g of tissue between foetal and 9-month-old sheep, results in subsequent Tables are expressed per fat-cell and, in the case of enzyme activities, per mg of protein.

Changes in the number of fat-cells per g of tissue are not paralleled exactly by changes in the amount of protein per g of tissue (Table 1). The larger fat-cells from the 9-month-old sheep appear to contain about twice as much protein as those from foetal lambs, but this comparison is complicated by the presence of extracellular protein in the tissue, which may make a different contribution to the total protein content at the various ages. The amount of protein/g of tissue fell on incubation for 2 h of pieces of adipose tissue from near-term lambs and 9-month-old sheep ($P < 0.001$ and < 0.05 respectively, using Student's *t* test for paired observations); incubation for a further 2 h had no effect on the amount of protein/g of tissue pieces and values reported in Table 1 are the means of those obtained after 2 h and 4 h of incubation. The reason for the fall in protein concentration was not determined, but it would appear that oedema was at least partly responsible as, at all three ages studied, pyruvate kinase activity/mg of protein (measured by using 3 mM-phosphoenolpyruvate and 3 mM-fructose 1,6-bisphosphate) was the same before and after incubation of adipose tissue for 2 h or 4 h.

Table 1. *Adipocyte mean volume, lipid and protein concentration and number of adipocytes per g of tissue of pieces of perirenal adipose tissue and protein concentration of adipose tissue from foetal and adult sheep*

Foetal lambs were at either about 120 days of gestation or near (less than 3 days) term. Lipid concentration and hence adipocytes per g of tissue were determined in pieces of adipose tissue after incubation for a total of 4 h (2 h pre-incubation plus 2 h incubation). Protein concentrations were determined in supernatants and homogenates of adipose tissue without prior pre-incubation and in pieces of adipose tissue after incubation for 2 h and 4 h; as the values obtained after 2 h and 4 h of incubation were the same, mean values are reported. Results are means $\pm$ S.E.M. of five or six observations. *, ** and *** indicate values significantly different from that obtained for adult sheep at $P < 0.05$, $P < 0.01$ and $P < 0.002$ respectively; † and †† indicate values significantly different from that obtained for near-term foetal lambs at $P < 0.05$ and $P < 0.01$ respectively.

	Age ...	Foetal		Adult
		120 days	Near term	
Adipocyte volume (pl)		6.0 $\pm$ 1.3***	4.5 $\pm$ 1.1***	188 $\pm$ 23
Lipid concentration (mg/g of tissue pieces)		295 $\pm$ 26**	306 $\pm$ 26***	646 $\pm$ 18
10 ⁻⁶ $\times$ no. of adipocytes/g of tissue pieces		70.0 $\pm$ 3.4**	86.1 $\pm$ 12.1***	4.0 $\pm$ 0.6
15 000 g-supernatant protein (mg/g of tissue)		32.3 $\pm$ 1.3***, ††	41.0 $\pm$ 1.9**	4.9 $\pm$ 1.1
(mg/g of tissue pieces)		29.1 $\pm$ 1.1***	27.3 $\pm$ 1.9**	3.7 $\pm$ 0.6
Total protein (mg/g of tissue)		60.8 $\pm$ 4.6**	80.1 $\pm$ 6.2***	11.1 $\pm$ 2.4

Table 2. *Metabolic fate of glucose carbon in pieces of perirenal adipose tissue from foetal and adult sheep*

Foetal lambs were at either about 120 days of gestation or near (less than 3 days) term. Glucose uptake was determined by measuring the amount of glucose removed from the medium during the course of the incubation. Results are expressed as means $\pm$ S.E.M. of six (120-day-foetal lambs) or five (others) observations. Other details are given in the text. *, ** and *** indicate values significantly different from that obtained for adult sheep at $P < 0.05$, $P < 0.01$ and $P < 0.002$ respectively; †, †† and ††† indicate values significantly different from that obtained for near-term foetal lambs at $P < 0.05$, $P < 0.01$ and $P < 0.002$ respectively.

		Glucose carbon converted into product					
		(ng-atoms/2 h per 10 ⁶ cells)			(% of total glucose carbon used)		
State ...		Foetal		Adult	Foetal		Adult
Product	Age ...	120 days	Near term	9 months	120 days	Near term	9 months
CO ₂		671 $\pm$ 212††	100 $\pm$ 20	738 $\pm$ 330	16.5 $\pm$ 1.8†††	6.7 $\pm$ 0.7**	20.9 $\pm$ 6.6
Fatty acid		252 $\pm$ 90††	4 $\pm$ 3*	102 $\pm$ 72	6.9 $\pm$ 1.9*, †††	0.3 $\pm$ 0.2*	2.5 $\pm$ 1.6
Acylglycerol glycerol		111 $\pm$ 33**	58 $\pm$ 11**	294 $\pm$ 48	2.9 $\pm$ 0.3***, †	3.9 $\pm$ 0.9**	11.2 $\pm$ 1.3
Pyruvate		172 $\pm$ 54*	75 $\pm$ 11**	444 $\pm$ 120	4.4 $\pm$ 0.3***	5.3 $\pm$ 0.7**	16.3 $\pm$ 2.5
Lactate		2598 $\pm$ 807*, †	1244 $\pm$ 125	1272 $\pm$ 180	69.3 $\pm$ 3.7*, †††	83.8 $\pm$ 1.6**	49.7 $\pm$ 5.8
All products		3803 $\pm$ 1124†	1483 $\pm$ 188	2838 $\pm$ 600	100	100	100
Glucose uptake		4500 $\pm$ 2280	1380 $\pm$ 540	—			

For adipose tissue pieces from foetal lambs, the estimated rate of glucose utilization, determined by summation, did not differ significantly from the measured rate of glucose uptake by the tissue pieces (Table 2). The rate of glucose uptake per g of tissue by tissue pieces from 9-month-old sheep is very low and could not be measured directly in the system with sufficient accuracy. However, preliminary experiments with adipose tissue from 9-month-old Finn-Dorset sheep, showed that the average amount of glucose taken up from the medium by adipose tissue pieces during a 24 h incubation

(2.2 $\pm$ 0.2 μ mol/2 h per g of tissue) did not differ significantly from the mean of the estimated rates of glucose utilization, obtained by summation, measured at the beginning and at the end of the 24 h incubation (2.7 $\pm$ 0.5 μ mol/2 h per g of tissue).

The net total rate of glucose utilization by fat-cells from foetal lambs at about 120 days of gestation did not differ significantly from that of 9-month-old sheep (Table 2) but the pattern of glucose metabolism differed with smaller proportions being converted into acylglycerol glycerol and pyruvate and larger proportions into lactate and fatty acids (Table 2).

Table 3. *Contribution of the pentose phosphate cycle, pyruvate dehydrogenase and the tricarboxylic acid cycle to CO₂ production from glucose carbon in pieces of perirenal adipose tissue from foetal and adult sheep*

Foetal lambs were at either about 120 days of gestation or near (less than 3 days) to term. Results are means $\pm$ s.e.m. of six (120-day-foetal lambs) or five (others) observations. *, ** and *** represent values significantly different from that obtained for adult sheep at $P < 0.05$, $P < 0.01$, $P < 0.002$ respectively; †, ††, ††† represent values significantly different from that for near-term foetal lambs at $P < 0.05$, $P < 0.01$, $P < 0.002$ respectively.

Conversion of glucose carbon into CO ₂ via different reactions (% of total glucose carbon converted into CO ₂)				
Reaction	State ... Age ...	Foetal		Adult
		120 days	Near term	9 months
Pentose phosphate pathway		28.2 $\pm$ 8.6***, ††	5.0 $\pm$ 1.4**	80.3 $\pm$ 3.9
Pyruvate dehydrogenase		36.7 $\pm$ 1.7***	33.4 $\pm$ 1.3**	9.3 $\pm$ 2.6
Tricarboxylic acid cycle		35.3 $\pm$ 7.2*, †††	61.5 $\pm$ 1.5**	10.1 $\pm$ 3.4

The proportion of glucose carbon oxidized to CO₂ was similar at both ages, but, as shown in Table 3, glucose oxidation to CO₂ via the pentose phosphate cycle made a much smaller contribution, whereas CO₂ production from glucose carbon by pyruvate dehydrogenase and the tricarboxylic acid cycle made much greater contributions in adipose tissue from 120-day foetal lambs as compared with 9-month-old sheep.

The rate of glucose utilization by adipose tissue pieces from near-term lambs was generally lower than that of adipose tissue pieces from both 120-day-foetal lambs and 9-month-old sheep (Table 2). The patterns of glucose metabolism of adipose tissue from near-term lambs also differed from that of 9-month-old sheep and generally resembled that of 120-day-foetal lambs, except that the proportion converted into CO₂ and fatty acids was lower, whereas that converted into lactate was higher (Table 2). The contribution of the various pathways to CO₂ production from glucose carbon also differed, with the contribution of the pentose phosphate pathway being particularly low and that of the tricarboxylic acid cycle relatively higher in adipose tissue pieces from the near-term foetus.

The molar ratios of L-lactate/pyruvate released into the incubation medium by the tissue pieces were 16.3 ± 1.8 , 17.5 ± 3.0 and 3.3 ± 0.5 for the 120-day-foetal lambs, near-term lambs and 9-month-old sheep respectively (results are means $\pm$ s.e.m.): the ratio for the 9-month-old sheep was significantly less than those of the foetal lambs ($P < 0.002$ and < 0.01 respectively).

The activities of most of the enzymes, per mg of protein, measured were the same in adipose tissue from foetal lambs near term and at about 120 days of gestation: the exceptions were glucose 6-phosphate dehydrogenase and phosphofructokinase (measured using 0.1 mM-fructose 6-phosphate),

which were lower in adipose tissue from near-term lambs (Table 4). Comparisons with enzyme activities per mg of protein of adipose tissue from 9-month-old sheep showed that pyruvate dehydrogenase activity (both the active form and total) was much higher in foetal lambs (Table 4). In addition pyruvate kinase activity was higher and glucose 6-phosphate dehydrogenase activity lower in adipose tissue from near-term lambs than in that from 9-month-old sheep.

As the amount of protein per fat-cell appears to be greater in 9-month-old than in foetal sheep, enzyme activities were also expressed on a per cell basis. This calculation in the present study is complicated by the suspected oedema during incubation of the tissue pieces. Results presented in Tables 4 and 5 are not corrected for any oedema. Statistical analysis was also performed with activities and fluxes corrected for possible oedema using the ratio of protein/g of tissue found in tissue before and after incubation, and using enzyme activities measured in pieces of adipose tissue from 9-month-old sheep incubated for 2 h. Identical conclusions were drawn from all of these statistical analyses of results for 120-day-foetal lambs and 9-month-old sheep; hence only one series of results is presented in Tables 4 and 5. As shown in Table 4, glucose 6-phosphate dehydrogenase activity per fat-cell was lower and pyruvate dehydrogenase activity per cell (both active form and total) was higher in fat-cells from 120-day-foetal lambs than from 9-month-old sheep, whereas other activities per cell did not differ at the two ages.

The activity of phosphofructokinase measured with 0.1 mM-fructose 6-phosphate as a fraction of that measured with 3 mM-fructose 6-phosphate was 0.46 ± 0.07 , 0.43 ± 0.03 and 0.65 ± 0.10 for 120-day-foetal lambs, near-term lambs and 9-month-old sheep respectively; the values do not differ significantly. Similarly, the activity of pyruvate kinase

Table 4. *Enzyme activities of perirenal adipose tissue from foetal and adult sheep*

Foetal lambs were at either about 120 days of gestation or near (less than 3 days) to term. Phosphofructokinase activity was measured using both 0.1 mM- and 3.0 mM-fructose 6-phosphate (F6P), whereas pyruvate kinase activity was measured using 0.05 mM-phosphoenolpyruvate (PEP) and 3 mM-phosphoenolpyruvate + 3 mM-fructose 1,6-bisphosphate (FDP). Results are expressed as means $\pm$ S.E.M. of six (120 days gestation) or five (others) observations. *, ** and *** indicate values significantly different from that obtained for adult sheep at $P < 0.05$, $P < 0.01$ and $P < 0.002$ respectively; † and †† indicate values significantly different from that obtained for near-term foetus at $P < 0.05$ and $P < 0.01$ respectively.

		Activity (nmol of substrate converted/min)				
		(per 10 ⁶ cells)		(per mg of protein)		
State	...	Foetal	Adult	Foetal		Adult
Age	...	120 days	9 months	120 days	Near term	9 months
Hexokinase		8.2 $\pm$ 2.7	11.1 $\pm$ 2.4	12.4 $\pm$ 1.1	12.3 $\pm$ 3.2	12.0 $\pm$ 3.8
Glucose 6-phosphate dehydrogenase		20.6 $\pm$ 7.6*	56.7 $\pm$ 16.5	30.3 $\pm$ 3.8††	14.0 $\pm$ 1.9**	65.8 $\pm$ 23.8
Phosphofructokinase						
0.1 mM-F6P		34.6 $\pm$ 14.4	37.3 $\pm$ 9.3	48.6 $\pm$ 6.1†	28.4 $\pm$ 3.8	32.6 $\pm$ 10.7
3.0 mM-F6P		88.6 $\pm$ 45.9	65.0 $\pm$ 13.2	112.3 $\pm$ 23.8	69.0 $\pm$ 12.4	53.3 $\pm$ 11.2
Pyruvate kinase						
0.05 mM-PEP		67.7 $\pm$ 25.3	54.9 $\pm$ 12.2	90.3 $\pm$ 19.3	141.4 $\pm$ 41.7**	48.1 $\pm$ 7.4
3 mM-PEP + 3 mM-FDP		356.1 $\pm$ 99.4	230.4 $\pm$ 36.9	585.3 $\pm$ 163.4*	792.5 $\pm$ 159.7**	220.8 $\pm$ 49.8
Pyruvate dehydrogenase						
Active form		22.3 $\pm$ 5.8**	4.0 $\pm$ 1.2	21.8 $\pm$ 5.6***	19.1 $\pm$ 2.5**	2.0 $\pm$ 0.7
Total		28.3 $\pm$ 6.6**	7.3 $\pm$ 2.0	29.1 $\pm$ 8.1***	25.3 $\pm$ 2.5**	3.8 $\pm$ 1.4

Table 5. *Net substrate flux through enzymes in pieces of perirenal adipose tissue from foetal and adult sheep*

Foetal lambs were at either about 120 days of gestation or near (less than 3 days) to term. Maximum net fluxes were assumed to be equal to the enzyme activities reported in Table 4, using saturating concentration of substrates. ATP citrate lyase for the adult sheep was 4.7 $\pm$ 1.2 nmol/min per 10⁶ cells. Results are means $\pm$ S.E.M. of five or six observations. * and ** indicate values significantly different from that obtained for adult sheep at $P < 0.05$ and $P < 0.01$ respectively; †, †† and ††† indicate values significantly different from that obtained for near-term lambs at $P < 0.05$, $P < 0.01$ and $P < 0.002$ respectively.

		Net flux			
		(nmol of substrate converted/min per 10 ⁶ cells)		(% of maximum flux)	
State	...	Foetal		Adult	
Age	...	120 days	Near term	9 months	
Hexokinase		5.3 $\pm$ 1.6†	2.1 $\pm$ 0.3	4.0 $\pm$ 0.8	79.5 $\pm$ 14.5**
Glucose 6-phosphate dehydrogenase		2.0 $\pm$ 1.1†††	0.04 $\pm$ 0.01**	4.8 $\pm$ 2.0	10.2 $\pm$ 4.5
Phosphofructokinase		4.7 $\pm$ 1.4†	2.1 $\pm$ 0.3	2.4 $\pm$ 0.3	7.6 $\pm$ 1.3*
Pyruvate kinase		9.6 $\pm$ 2.9†	4.0 $\pm$ 0.5	5.5 $\pm$ 1.0	3.0 $\pm$ 0.1
Pyruvate dehydrogenase (active)		1.9 $\pm$ 0.5†††,*	0.3 $\pm$ 0.1	0.8 $\pm$ 0.5	10.7 $\pm$ 3.1
ATP-citrate lyase		1.0 $\pm$ 0.4†††	0.02 $\pm$ 0.01*	0.4 $\pm$ 0.3	13.2 $\pm$ 6.3

measured with 0.05 mM-phosphoenolpyruvate as a fraction of that measured with 3 mM-phosphoenolpyruvate plus 3 mM-fructose 1,6-bisphosphate was 0.17 $\pm$ 0.03, 0.20 $\pm$ 0.06 and 0.24 $\pm$ 0.04 respectively for 120-day foetal lambs, near-term lambs and 9-month-old sheep; again, the values do not differ significantly. Incubation of perirenal adipose tissue from 9-month-old sheep for 4 h led to an increase in the ratio of pyruvate kinase activity measured with

0.05 mM-phosphoenolpyruvate to that with 3 mM-phosphoenolpyruvate and 3 mM-fructose 1,6-bisphosphate (Robertson *et al.*, 1982a) but this was not observed with tissue pieces from foetal lambs.

The proportion of pyruvate dehydrogenase in the active state was 0.77 $\pm$ 0.03, 0.75 $\pm$ 0.04 and 0.56 $\pm$ 0.06 respectively for 120-day-foetal lambs, near-term lambs and 9-month-old sheep; the values for the foetal lambs were significantly greater than

those for 9-month-old sheep ($P < 0.002$ and < 0.01 respectively).

Net metabolic fluxes through the reactions catalysed by the enzymes listed in Table 5 were lower in fat-cells from near-term lambs than in those from 120-day-foetal lambs. No statistically significant differences in the fluxes were found when comparing those of fat-cells from 120-day-old foetal lambs and 9-month-old sheep, except for a higher flux through pyruvate dehydrogenase in the former (Table 5). When fluxes were expressed as a percentage of the enzyme activity/cell (measured by using saturating substrate concentrations), the net flux, as a percentage of the measured enzyme activity, through hexokinase and phosphofructokinase was significantly greater in fat-cells from 120-day-old foetal lambs than from 9-month-old sheep.

Discussion

The results of the present study confirm and extend our previous study (Vernon *et al.*, 1981b). Adipocyte mean volumes of the foetal lambs were rather smaller in the present study and appeared to decrease rather than increase towards term, but this is probably a reflection of the much colder winter. Rates of fatty acid, acylglycerol glycerol and CO_2 production from glucose in perirenal adipose tissue pieces from foetal lambs were very similar in both studies. Results of the previous study suggested that the rate of glucose oxidation/fat-cell and the activity of glucose 6-phosphate dehydrogenase/mg of protein fell during the last month of gestation; these trends were confirmed in the present study.

During the last month of gestation there is both a fall in the rate of glucose utilization and change in the pattern of glucose utilization by fat-cells from foetal lambs. In general, the lower fluxes found in pieces of adipose tissue from near-term lambs cannot be attributed to diminished activities of the various enzymes measured. Some of these changes can be related to the fall in the rate of fatty acid synthesis from acetate (the major precursor) during this period (Vernon *et al.*, 1981b; Robertson *et al.*, 1981). The total rate of fatty acid synthesis, measured using $^3\text{H}_2\text{O}$, was 610 ± 196 and 23 ± 7 ng-atoms of carbon/2h per 10^6 cells respectively in pieces of adipose tissue from the 120-day- and near-term foetal lambs. This would account for the diminished flux through the pentose phosphate cycle and to some extent the lower rate of acylglycerol glycerol synthesis. The latter will also be influenced by the rate of lipolysis and hence the availability of fatty acids for re-esterification. The rate of glycerol release was 26.7 ± 9.0 and 8.5 ± 1.8 nmol/2h per 10^6 cells respectively for tissue pieces from 120 days and

near-term foetal lambs. To re-esterify the fatty acids released by lipolysis plus those produced by fatty acid synthesis would require a rate of acylglycerol glycerol synthesis of 118 and 28 ng-atoms of carbon/2h per 10^6 cells respectively, which compared reasonably well with the measured values (Table 2).

The 9-month-old sheep used in this study were true ruminants and at the beginning of the fattening phase of development (see Vernon, 1980). The similar rates of total glucose utilization per cell and the similar hexokinase activity of perirenal adipose tissue from 120-day-foetal lambs and 9-month-old sheep are rather surprising as glucose utilization is thought to be restricted in the adult ruminant, whereas glucose is thought to be more freely available in the foetus. However, differences in the patterns of glucose metabolism at the two ages were found. The greater proportion of CO_2 production occurring via the pentose phosphate cycle and the greater glucose 6-phosphate dehydrogenase activity/cell in the 9-month-old sheep is in accordance with the higher total rate of fatty acid synthesis, 2292 ± 352 ng-atoms of carbon/2h per 10^6 cells, measured by summing the rates of fatty acid synthesis from glucose and acetate, the major lipogenic precursor in ruminants (see Vernon, 1980). The greater proportion of glucose carbon used for acylglycerol glycerol synthesis in 9-month-old sheep is probably due to the greater total rate of fatty acid synthesis plus a higher rate of basal lipolysis, 42.3 ± 9.6 nmol of glycerol released/2h per 10^6 cells, than in fat-cells from foetal lambs. The mean rate of acylglycerol glycerol synthesis required to esterify the fatty acids produced by synthesis *de novo* and lipolysis is 270 ng-atoms of carbon/2h per 10^6 cells, which is in good agreement with the observed rate (Table 2). The greater proportion of glucose carbon converted into CO_2 via pyruvate dehydrogenase and the tricarboxylic acid cycle in the foetal lambs is in agreement with the finding that glucose is a more important oxidative fuel in the foetal lamb than in the adult sheep (see the introduction).

The greater flux through the pyruvate dehydrogenase reaction in adipose tissue from 120-day-foetal lambs is associated with a higher pyruvate dehydrogenase/cell than in 9-month-old sheep. This observation is consistent with our view that pyruvate dehydrogenase has an important role in regulating the fate of glucose carbon in ruminant adipose tissue (Robertson *et al.*, 1980, 1982a). However, changes in flux through pyruvate dehydrogenase can occur in sheep adipose tissue without any change in measurable enzyme activity as shown previously (Robertson *et al.*, 1982a) and as found during the last month of gestation in the present study. In adipose tissue from both foetal and 9-month-old sheep, only 15–20% of the pyruvate produced by glycolysis is metabolized via pyruvate

dehydrogenase, the rest being released from the cell, primarily as lactate.

As found in a previous study with tissue from 9-month-old sheep (Robertson *et al.*, 1982a), the flux through pyruvate kinase as a percentage of the measured enzymic activity does not change, whereas that through both hexokinase and phosphofructokinase may vary. In addition, the ATP-citrate lyase activity per fat-cell of 120-day-foetal lambs and 9-month-old sheep were very similar, despite differences in the net flux through the reaction (Robertson *et al.*, 1982b). We proposed (Robertson *et al.*, 1982a) that pyruvate kinase has a critical role in regulating the rate of glycolysis in ruminant adipose tissue; the results of the present study do not contradict this view.

We thank Mr. D. Fullarton and Mr. C. E. Park for care of the animals. J. P. R. was supported by an Agricultural Research Council Scholarship.

References

- Ballard, F. J., Hanson, R. W. & Kronfeld, D. S. (1969) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **28**, 218–231
- Battaglia, F. C. & Meschia, G. (1978) *Physiol. Rev.* **58**, 499–527
- Girard, J., Pintado, E. & Ferre, P. (1979) *Ann. Biol. Anim. Biochim. Biophys.* **19**, 181–197
- Leng, R. A. (1970) *Adv. Vet. Sci.* **14**, 209–260
- Lindsay, D. B. (1978) *Biochem. Soc. Trans.* **6**, 1152–1156
- Prior, R. L. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 117–122
- Robertson, J. P., Faulkner, A. & Vernon, R. G. (1980) *FEBS Lett.* **120**, 192–194
- Robertson, J. P., Faulkner, A. & Vernon, R. G. (1981) *Biochim. Biophys. Acta* **665**, 511–518
- Robertson, J. P., Faulkner, A. & Vernon, R. G. (1982a) *Biochem. J.* **206**, 577–586
- Robertson, J. P., Faulkner, A. & Vernon, R. G. (1982b) *Proc. Nutr. Soc.* **41**, 58A
- Siegel, S. (1956) *Non-Parametric Statistics for the Behavioural Sciences*, McGraw-Hill, London
- Vernon, R. G. (1977) *Biol. Neonate* **32**, 15–23
- Vernon, R. G. (1980) *Prog. Lipid Res.* **19**, 23–106
- Vernon, R. G., Clegg, R. A. & Flint, D. J. (1981a) *Biochem. J.* **200**, 307–314
- Vernon, R. G., Robertson, J. P., Clegg, R. A. & Flint, D. J. (1981b) *Biochem. J.* **196**, 819–824