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Glucose, Acetate, and Lactate Metabolism in Perirenal Adipose Tissue of Fetal and Newborn Calves

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ABSTRACT. Rates of utilization of glucose, acetate, and lactate and activities of selected enzymes were determined *in vitro* to characterize the nature of lipogenesis and metabolite utilization in perirenal adipose tissue from 6- to 7-month old fetal and 3- to 4-h-old unsuckled newborn calves. Contribution of the pentose phosphate cycle to glucose metabolism was estimated using specifically labeled glucose. Rates of fatty acid synthesis from all three substrates and oxidation of glucose were much greater in fetal than in newborn adipose tissue. In fetal adipose tissue, acetate and lactate were major sources of carbon for fatty acid synthesis; glucose functioned mainly by metabolism via the pentose phosphate cycle to provide reducing equivalents for fatty acid synthesis and by incorporation into glyceride glycerol for fatty acid esterification. Pentose phosphate cycle contributed 58 and 12% to glucose metabolism in adipose tissue of fetal and newborn calves, respectively. Adipose tissue metabolism of newborn calves was characterized by greatly depressed rates of fatty acid synthesis despite high enzyme activities and elevated rates of glyceride glycerol synthesis. (*Pediatr Res* 20: 542–544, 1986)

Most studies of fetal metabolism have been carried out in sheep with substrate utilization usually being determined on the basis of rates of oxygen consumption and uptake of different substrates. Various acute and chronic preparations and techniques such as the isolated fetus, the exteriorized fetus, and the chronically catheterized fetal lamb preparation, have enabled researchers to gain an insight into fetal metabolism and physiology (1). Although these studies have provided valuable data, detailed information on the metabolism of substrates by individual fetal tissues and organs is still lacking, particularly in the bovine.

Substrates available to the fetus are used for three purposes: synthesis of new tissue, oxidative metabolism, and building of energy reserves. The primary energy reserves that accumulate within the fetus in the latter part of gestation are glycogen and lipids. Together, these represent the major stores that provide for energy needs of the newborn ruminant in the immediate postnatal period (2). In the sheep fetus accumulation of lipids appears to occur primarily via *de novo* synthesis from various substrates (2), and the adipose tissue appears to be an important site of fatty acid synthesis (3). With the abrupt introduction of the fetus to an extrauterine existence, immediate changes may be expected to occur in lipid metabolism. Answers to many questions concerning such changes in lipid metabolism have yet to be obtained. The present investigation involved studies of adipose tissue from fetal and unsuckled newborn calves. Rates of utilization of glucose, acetate, and lactate, as substrates for oxidation and for synthesis of fatty acids and glyceride glycerol, were determined *in vitro* to characterize the nature of lipogenesis and patterns of metabolite utilization. Specifically labeled glucose was employed to evaluate the contribution of the pentose phosphate cycle to glucose metabolism and to the reducing equivalents needed for fatty acid synthesis. In addition, activities of selected enzymes related to lipogenesis in adipose tissue were also assayed.

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MATERIALS AND METHODS

Perirenal adipose tissue samples were obtained from five fetal calves (180–210 days gestation or 64–75% of full term) and eight newborn unsuckled calves (3–4 h after birth). Newborn calves were sacrificed by exsanguination prior to tissue removal. Adipose tissue samples were placed in warm saline (37° C) for transport to the laboratory, cleaned of connective tissue and blood vessels, and cut into small (30–50 mg) pieces. Adipose tissue pieces weighing 50–100 mg were placed in siliconized 25-ml Erlenmeyer flasks containing 2 ml of incubation medium (Table 1). Concentrations of glucose, acetate, lactate/pyruvate, and insulin in the incubation medium were determined in the preliminary experiment to achieve maximum rates of substrate utilization. The flasks were gassed with a mixture of 95% O_2 and 5% CO_2, capped with self-sealing rubber serum caps fitted wth plastic center wells, and incubated for 2 h at 37° C in a shaking (100 cycles/min) water bath. At the end of incubation, 0.5 ml of 1 N H_2SO_4 was injected into the flasks and 0.2 ml of hyamine hydroxide (Sigma, St. Louis, MO) was injected into the center wells. Flasks were shaken for another 1 h to allow CO_2 absorption by hyamine. Incorporation of labeled substrates into triacyl glycerol and fatty acids were determined by the method of Dole (5), as modified by Rodbell (6). The air dried triacyl glycerol and fatty acid extracts and CO_2 absorbed in hyamine were dissolved in 0.4% Omniflour (New England Nuclear Co., Boston, MA) toluene counting fluid. Radioactivity was determined in a Packard liquid scintillation spectrometer. Substrate conversions were calculated from initial substrate specific radioactivity and radioactivities of the products.

Samples of adipose tissue from each animal were immediately homogenized in 0.14 M KCl (37° C) with a Potter-Elvehjem homogenizer. Tissue homogenates were centrifuged in a Sorvall refrigerated centrifuge for 20 min at 35,000 × *g*. The aqueous fractions were assayed for enzyme activities. Citrate cleavage enzyme (EC 4.1.3.7), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), NADP-isocitrate dehydrogenase (EC 1.1.1.42), and NAD-malate dehydrogenase (EC 1.1.1.37) were assayed as described by Baldwin and Milligan (7). NADP-malate dehydrogenase (malic en-

zyme, EC 1.1.1.40), and lactate dehydrogenase (EC 1.1.1.27) were assayed according to Ochoa (8) and Bergmeyer *et al.* (9), respectively. Enzyme assays were conducted in the linear range of activity at 25° C using a Gilford spectrophotometer fitted with a multiple absorbance recording system. Protein was determined in the aqueous fractions by the method of Lowry *et al.* (10), using bovine serum albumin as the standard.

Bovine insulin and other biochemicals were purchased from Sigma. Sources of labeled substrates were: U-^{14}C glucose and 2-^{14}C lactate (Amersham Searle, Arlington Heights, IL); 1-^{14}C glucose (ICN Pharmaceutical Inc, Irvine, CA), and 6-^{14}C glucose (Research Products International, Elkgrove Village, IL).

RESULTS

Metabolic rates for adipose tissue (Tables 2 and 3) are expressed per mg soluble protein for reasons given in the discussion. Soluble protein content (Table 2) in adipose tissue from fetal calves was significantly greater ($p < 0.001$) than that from newborn calves. All enzyme activities appeared very much depressed in newborn calves when expressed on a tissue wet weight basis (results not shown), but activities expressed on a soluble protein basis (Table 2) exceeded or equalled those of fetal adipose tissue except for citrate cleavage enzyme. Even the reduced activity of citrate cleavage enzyme was at least 80- and 40-fold higher than the observed rates of fatty acid synthesis from glucose and lactate and, therefore, does not appear to limit fatty acid synthesis in the newborn.

Table 1. *Composition of incubation medium*

Krebs Ringer bicarbonate buffer, pH 7.4*	90 ml
Bovine serum albumin†	2 g
Amino acid mixture‡	10 ml
Glucose§	20 mM
Acetate‖	5 mM
L-Lactate/pyruvate (9:1)¶	5 mM
Insulin	100 mU/ml
Final volume, made up with buffer	100 ml

* Krebs buffer containing 1/2 recommended [Ca], gassed for 1 h with 95% O_2 + 5% CO_2 mixture.

† Defatted (4) and dialysed for 48 h against double deionized water.

‡ 100 ml amino acid mixture contained 300 mg casein hydrolysate, 10 mg methionine, and 10 mg tryptophan.

§ Combined with U-^{14}C, 1-^{14}C, or 6-^{14}C glucose.

‖ Combined with 1-^{14}C acetate.

¶ Combined with 2-^{14}C lactate.

Table 2. *Enzyme activities in the perirenal adipose tissue of fetal and newborn calves*

Enzyme*, †	Fetal	Newborn
CCE	0.098 ± 0.025	0.019 ± 0.011‡
ME	0.034 ± 0.006	0.172 ± 0.043§
MDH	0.632 ± 0.097	5.580 ± 0.933‡
G6-PD	0.185 ± 0.033	0.199 ± 0.035
6-PGD	0.087 ± 0.010	0.541 ± 0.121§
ICD	0.593 ± 0.084	4.060 ± 0.841‡
LDH	3.590 ± 0.403	11.550 ± 2.190§
Soluble protein (mg/g)	16.91 ± 1.79	2.49 ± 0.87‖

* Units in μmol substrate converted per min per mg tissue soluble protein; mean ± SEM; $n = 5$ fetal and 8 newborn calves.

† CCE, citrate cleavage enzyme; ME, malic enzyme; MDH, malate dehydrogenase; G6-PD, glucose-6-phosphate dehydrogenase; 6-PGD, 6-phosphogluconate dehydrogenase; ICD, isocitrate dehydrogenase; LDH, lactate dehydrogenase.

‡ $p < 0.01$, § $p < 0.05$, ‖ $p < 0.001$ significant differences, (Student's t test).

The sum of the rates of oxidation of glucose, acetate, and lactate in adipose tissue from newborn calves was nearly 6-fold higher than that from fetal calves (Table 3). However, the pattern of substrate oxidation differed in the two groups; the rate of glucose oxidation in adipose tissue from fetal calves exceeded ($p < 0.01$) that of newborn calves, while the opposite was true for rates of oxidation of acetate and lactate ($p < 0.01$).

Rates of conversion of glucose, acetate, and lactate to fatty acids were depressed by 11-, 2.4-, and 20-fold, respectively, in adipose tissue of newborn calves when compared with those of fetal calves. On the basis of acetyl-CoA units incorporated into fatty acids in fetal adipose tissue, acetate exceeded glucose and lactate by 5– and 1.5-fold, respectively. The rate of incorporation of glucose carbons into glyceride glycerol in newborn adipose tissue was significantly greater ($p < 0.05$) than that of fetal adipose tissue. The ratio of glucose-1-^{14}C/glucose-6-^{14}C conversion to CO_2, an index of the activity of pentose phosphate cycle (11), was 4.4 and 1.8 for adipose tissue from fetal and newborn calves, respectively. The contribution of the pentose phosphate cycle to glucose metabolism was calculated using specific $^{14}CO_2$ yields and fatty acid ratios from glucose 1-^{14}C and 6-^{14}C, as described by Katz *et al.* (12). The reference value used to calculate specific yields was computed by summing glucose incorporation into CO_2 and triacyl glycerol. Calculated percentage contribution of the pentose phosphate cycle to glucose metabolism in adipose tissue was signficantly greater ($p < 0.001$) in fetal (58 ± 2) than in newborn calves (12 ± 5).

DISCUSSION

Difficulties encountered in establishing a satisfactory and meaningful reference base for expression of metabolic and enzyme data from adipose tissue have been noted by several workers (3, 6, 13–15). Expression of results on a cellular or a protein basis appear to be preferred methods for evaluating adipose tissue metabolism (3, 13). However, in neonatal animals cellular hypertrophy and marked variations in adipocyte sizes in animals of the same chronological age can complicate the evaluation of age-related changes in adipose tissue metabolism (3, 15). Fat cells from fetal calves can be expected to be smaller than those of newborn calves (3). Thus, changes in metabolic activity per unit weight of tissue may merely reflect changes in adipocyte number rather than true age-related differences. In the present study, the observation of a 6-fold reduction in soluble protein content of adipose tissue between the fetal and newborn state (Table 2) provides indirect evidence for the occurrence of marked cellular hypertrophy and lipid accumulation in perirenal adipose tissue during the last trimester of gestation. In order to compensate for these changes in cellularity, and with the above noted limitations in mind, all results of the present study were expressed on the basis of tissue soluble protein content.

Acetate, lactate, and glucose were preferred in that order as substrates for fatty acid synthesis in both fetal and newborn calf adipose tissue (Table 3). A similar order of preference also exists in the adipose tissue of adult ruminants (3). That acetate traverses the placenta and is used in oxidative and synthetic reactions by the fetal lamb has been established (16). The ratios of acetate carbons:glucose carbons incorporated into fatty acids in adipose tissue of adult ruminants vary between 10–100 (3). The same ratio, when computed for fetal calves in the present study, was 5, indicating a greater capacity of fetal adipose tissue to extract and utilize glucose for fatty acid synthesis. This capability may also be reflected in the relatively high activities of both citrate cleavage and malic enzymes in adipose tissue of fetal and newborn calves in comparison to activities reported (3) for adult ruminants. This study provides the first *in vitro* evidence for the role of lactate as an important precursor for fatty acid synthesis in adipose tissue of the bovine fetus (Table 3). Activities of lactate

Table 3. *Patterns of glucose, acetate, and lactate metabolism in perirenal adipose tissue of fetal and newborn calves**

Substrate and label	Fetal	Newborn	Fetal	Newborn	Fetal	Newborn
Products	CO_2		Fatty acids		Glyceride glycerol	
Glucose	575.5	288.5†	154.3	13.6‡	73.7	176.3§
U-^{14}C	±73.3	±23.5	±36.5	±3.1	±5.8	±32.0
Glucose	740.0	358.6‡	91.3	21.5‡	15.9	117.8§
1-^{14}C	±92.3	±26.4	±17.8	±6.9	±4.0	±32.4
Glucose	167.2	203.6	237.9	20.9†	154.1	273.7
6-^{14}C	±21.4	±22.7	±68.3	±3.7	±32.2	±51.5
Acetate	249.4	5194.8†	1554.1	642.2†	72.2	54.4
1-^{14}C	±38.8	±968.0	±152.1	±191.8	±50.8	±26.7
Lactate	443.6	1557.6†	1036.0	52.0‡	48.2	17.1†
2-^{14}C	±69.5	±260.1	±191.7	±14.1	±11.3	±4.0

* Values given in nanomoles substrate converted to products per 2 h per mg tissue soluble protein; mean ± SEM; $n = 5$ fetal and 8 newborn calves.

† $p < 0.01$, ‡ $p < 0.001$, § $p < 0.05$ significant differences, (Student's *t* test).

dehydrogenase in both fetal and newborn calf adipose tissue appear nonlimiting and are far in excess of the sum of the rates of lactate oxidation and incorporation into triacyl glycerol (Tables 2 and 3). The importance of lactate as a major exogenous nutrient for the developing fetal lamb has been recognized (17, 18).

The ratios of glucose 1-^{14}C/glucose 6-^{14}C conversion to CO_2 and the calculated (see "Results") contribution of the pentose phosphate cycle to glucose metabolism indicate that metabolism of glucose via pentose phosphate cycle is increased in adipose tissue of fetal and depressed in adipose tissue of newborn calves. However, activities of glucose-6-phosphate dehydrogenase were similar in both groups of calves, while those of 6-phosphogluconate dehydrogenase were increased in the newborn (Table 2). The equations used in this study to calculate the contribution of pentose phosphate cycle to glucose metabolism were developed by Katz *et al.* (12) for white adipose tissue of the rat. Baldwin and Smith (11) have noted that the assumptions involved in these equations may not be applicable to adipose tissue of the adult ruminant. Whether these assumptions are applicable to adipose tissue of the bovine neonate is not known. Thus, calculations done in the present study may only provide a general idea of the magnitude of the pentose phosphate cycle. Assuming that the calculated values of 58 and 12% are reasonable estimates, further calculations based on the methods of Rognstad and Katz (19) show that pentose phosphate cycle contributes 100% of the NADPH requirement for fatty acid synthesis from acetate in fetal calves and 42% of the same in newborn calves. Pentose phosphate cycle is thought to make a major contribution to NADPH production in adipose tissue of adult ruminants (3). Two other potential sources of reducing equivalents in the adipose tissue are NADP-malate dehydrogenase and the NADP-linked isocitrate dehydrogenase enzyme. It appears, from the activities of these two enzymes (Table 2), that they may also play a role in NADPH production in adipose tissue of the bovine neonate.

The most striking difference observed between fetal and newborn calves (Tables 2 and 3) is the markedly depressed rate of fatty acid synthesis in adipose tissue of the newborn despite high enzyme activities and increased rates of glyceride glycerol synthesis. Calculations show that *de novo* fatty acid synthesis in newborn adipose tissue could account for only 7% of the total fatty acids esterified and indicate apparently high rates of lipolysis in this tissue. The potential ability of perirenal adipose tissue to mobilize lipids to meet energy needs of the neonatal ruminant has been recognized previously (2).

This study provides additional insight into the metabolism of glucose, acetate, and lactate in perirenal adipose tissue of the bovine neonate. It appears that in fetal adipose tissue both acetate and lactate are major sources of carbon for fatty acid synthesis and that glucose functions mainly to provide reducing equivalents for fatty acid synthesis and glyceride glycerol for fatty acid esterification. In newborn adipose tissue fatty acid synthesis is depressed and lipolysis appears to be elevated. The latter observation may relate to mobilization of free fatty acids to meet energy demands in the immediate postnatal period.

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