Fatty Acid Synthesis from Lactate in Growing Cattle[1]

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ABSTRACT Rates of fatty acid synthesis from lactate and acetate and activities of selected lipogenic and NADPH-generating enzymes were determined in subcutaneous, intermuscular and intramuscular adipose tissues of cattle that were 11–19 months of age. Fatty acid synthesis from lactate and acetate increased from 11 to 13 months of age in subcutaneous and intermuscular adipose tissues; synthesis from lactate increased until 17 months of age. In intramuscular adipose tissue, synthesis from lactate also increased until 17 months of age while that from acetate continually increased. Activities of NADPH-generating enzymes increased in all three fat depots from 11 to 13 months in age, and little change occurred thereafter. Acetyl-CoA carboxylase activity was constant over entire growth period in all depots. Activity of ATP-citrate lyase increased from 11 to 13 months of age in subcutaneous and intermuscular adipose tissues, but did not increase until 19 months of age in intramuscular adipose tissue. In all cases, activities of ATP-citrate lyase were sufficient to support synthesis from lactate; therefore, lactate conversion to fatty acids in bovine adipose tissues seems to use the citrate cleavage pathway for generation of cytosolic acetyl-CoA. J. Nutr. *111:* 1454–1461, 1981.

INDEXING KEY WORDS lactate · fatty acid synthesis · cattle · citrate cleavage pathway

In nonlactating ruminants, acetate, not glucose, is the principal precursor for fatty acids (1–3), and adipose tissue is the principal site of lipogenesis (1, 2). The inability of ruminants to utilize glucose for fatty acid synthesis has been attributed to the low activity of the citrate cleavage pathway (1). Recent investigations have demonstrated that lactate is a fatty acid precursor in sheep and in bovine adipose tissue (4–6) and have challenged the explanation that low citrate cleavage pathway activity results in poor utilization of glucose for lipogenesis in ruminant tissues (1). No studies of lactate utilization for lipogenesis in cattle at several stages of growth and development have been performed.

Our objective was to quantify the use of lactate and acetate for lipogenesis by subcutaneous, intermuscular and intramuscular adipose tissues from cattle at several different ages. To test the hypothesis that lactate conversion to fatty acid requires the citrate cleavage pathway, activity of adenosine triphosphate (ATP)-citrate lyase was determined in the same depots. Furthermore, activities of acetyl-coenzyme A (-CoA) carboxylase and the principal nicotinamide-adenine dinucleotide phosphate (reduced)

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(NADPH)-generating enzymes in ruminants (7, 8) were measured to identify possible rate-limiting steps in lactate conversion to fatty acids.

MATERIALS AND METHODS

Experimental design. Thirty-two crossbred steers, progeny of Limousin, Maine-Anjou, Angus and Simmental sires and crossbred cows, of similar birthdates were used. Cows were either two-way or three-way crosses among Angus, Hereford, Holstein and Brown Swiss breeds.

Steers were assigned at random to four slaughter groups. Steers were housed outdoors in the same pen and fed ad libitum a ration consisting of, on a dry-matter basis, 72% ground shelled corn, 22% alfalfa-brome haylage or oatlage and 6% pelleted 32%-protein supplement. At 11, 13, 17 and 19 months of age, eight randomly selected steers were slaughtered at Iowa State University's Meat Science Laboratory. Steers were transported from their feedlot at the McNay Research Center at Chariton, IA, to Ames, a distance of approximately 80 miles, about 20 hours before slaughter and housed in the Meat Laboratory overnight where feed and water were provided.

Tissue sampling. Immediately after stunning and exsanguination, we removed a portion of the right M. longissimus and associated fat corresponding to the 10th to 12th rib. Subcutaneous adipose tissue over the 12th rib and intermuscular and intramuscular adipose tissues of the longissimus muscle were removed and kept in 0.9% sodium chloride solution at 37° until assayed for fatty acid synthesis or until homogenization for enzymatic assays. Subcutaneous and intermuscular adipose tissues were obtained within 10 minutes after longissimus muscle removal, and an additional 20 minutes generally were needed to dissect intramuscular adipose tissue from the longissimus muscle.

Fatty acid synthesis assay. A 100 to 200-mg section of adipose tissue was incubated under an atmosphere of 95% O_2 and 5% CO_2 for 2 hours at 37° in 3 ml of Krebs-Ringer bicarbonate buffer [Ca^{2+}-free] (9) containing either 150 μmoles of L(+)-sodium lactate plus 15 μmoles of glucose and 1μCi of L(+)-sodium-[U-^{14}C]-lactate (New England Nuclear, Boston, MA) or 75 μmoles of sodium acetate plus 15 μmoles of glucose and 1 μCi of sodium-[1-^{14}C]-acetate (Amersham Searle, Arlington Heights, IL). Fifty millimolar lactate was necessary to attain maximal rates of lactate conversion to fatty acids. Triplicate determinations were conducted for each adipose tissue sample and substrate combination. Incubations were terminated by addition of 0.5 ml of 1.5 *N* H_2SO_4. Tissue sections incubated in the presence of H_2SO_4 served as controls.

After termination of incubations, tissue sections were separated from the incubation media by filtering through glass wool and then washed with a 0.9% sodium chloride solution. Lipids were extracted from tissue overnight on a wrist-action shaker with 20 ml of chloroform:methanol (2:1, v/v; 10). Resulting lipid extracts then were washed as described by Folch et al. (10) and evaporated before saponification for 2 hours at 85° in 3% methanolic KOH. Fatty acids were separated from saponified lipid extracts with hexanes (mixture of isomers of hexane) as described by Pothoven and Beitz (11) and, after extracts were evaporated, the fatty acids were assayed for radioactivity by liquid scintillation counting (Model LS-8000 liquid scintillation spectrophotometer, Beckman Instruments, Irvine, CA). Quenching was corrected by the external standard method.

Tissue homogenization. A weighed amount of adipose tissue was homogenized (Model 45, Virtis Co., Gardiners, NY) at 37° for 30 seconds in a buffer solution containing 0.25 M sucrose, 1mM ethylenediaminotetraacetate (EDTA), 1 mM glutathione and 10 mM Tris-HCl, pH 7.4. The ratio of homogenization buffer to tissue was 2:1 (v:w). Tissue homogenates were centrifuged at 15,000 *g* for 20 minutes at 23°. The solution above the pellet and below the fatcake was removed, and the volume was measured. Aliquots were utilized for enzymatic as-

says and protein determination. Protein concentration was determined as described by Lowry et al. (12) using bovine serum albumin as a standard.

Enzyme assays. Acetyl-CoA carboxylase (EC 6.4.1.2) was assayed at 37° as described by Dakshinamurti and Desjardens (13) as modified by Anderson et al. (14). Bovine serum albumin in incubation media was defatted before use as described by Fain (15). The total assay volume was 1.0 ml and contained 0.6 ml of tissue homogenate. Assays were started by addition of $NaH^{14}CO_3$ (Amersham Searle, Arlington Heights, IL), ATP and acetyl-CoA after a 30-minute preincubation with citrate at 37°. Aliquots of 0.2 ml were removed 1 minute and 2 minutes after addition of substrates and then added to 0.3 ml of 1 *N* HCl to liberate the unfixed $NaH^{14}CO_3$ (13, 14). Acetyl-CoA and ATP were omitted in controls. Triplicate determinations were performed with each adipose tissue depot.

Activity of ATP-citrate lyase (EC 4.1.3.8) was determined at 23° as described by Srere (16). The total reaction volume was 1.0 ml and contained 0.2 ml of tissue homogenate from subcutaneous or intermuscular adipose tissue and 0.1 ml of tissue homogenate from intramuscular adipose tissue. Reaction was initiated by addition of ATP, and change in absorbance at 340 nm was followed with a recording spectrophotometer (Model 2000, Gilford Instruments, Oberlin, OH). All assays were conducted against a control with ATP and coenzyme A omitted.

Because of the low activity of ATP-citrate lyase reported in bovine adipose tissue (1), reagents for assay of ATP-citrate lyase activity were monitored by measuring the same activity in a 100,000 *g* supernatant preparation from rat liver prepared as described earlier for homogenization of adipose tissue except that a 15,000 *g* supernatant was centrifuged at 100,000 *g* for 90 minutes at 0–4° with an ultracentrifuge (Model L-2, Beckman Instruments, Irvine, CA) fitted with a 40.3 rotor. Homogenization of rat liver and centrifugation at 15,000 *g* were conducted at 0–4°. The 100,000 *g* supernatant fraction of rat liver was stored at −20° and remained active for at least 18 months.

Activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were assayed by the coupled substrate procedure of Glock and McLean (17). Increases in absorbance at 340 nm were followed as described for assay of ATP-citrate lyase. Final assay volume of 2.4 ml contained 0.05 ml of tissue homogenate from either subcutaneous or intermuscular adipose tissue or 0.1 ml of tissue homogenate from intramuscular adipose tissue. Reactions were started by addition of 0.1 ml of 50 mM glucose-6-phosphate or 0.1 ml of 50 mM glucose-6-phosphate plus 50 mM of 6-phosphogluconate.

Activity of NADP-isocitrate dehydrogenase (EC 1.1.1.42) was assayed as described by Ochoa (18). Volumes of tissue homogenate from specific adipose tissues used for assay were identical with those used for assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Substrate (DL-isocitrate) was omitted from controls, and increases in absorbance at 340 nm were determined as indicated previously.

Adipose tissue cellularity. A weighed (approximately 200 mg) section of adipose tissue was fixed with 2% osmium tetroxide in 50 mM collidine chloride buffer solution at pH 7.6 for 72 hours (19). Fixed adipose tissues then were treated with a 8 M urea solution to solubilize connective tissue as described by Etherton et al. (20). The fixed, urea-treated adipocytes were freed by washing with a 0.9% sodium chloride solution containing 0.01% Triton X-100 through a 250-μm nylon mesh filter and collected on a 20-μm nylon mesh filter. The freed adipocytes then were washed with 0.9% sodium chloride solution into a pre-weighed 400-ml plastic beaker; volume was increased to approximate 350 ml with the sodium chloride solution, and the beaker was reweighed.

Number of adipocytes was determined by counting ten 0.5-ml aliquots (Model ZB, Coulter Electronics, Hialeah, FL) in the linear mode. A 400-μm aperture was

TABLE 1

Fatty acid synthesis from lactate and acetate in subcutaneous, intermuscular and intramuscular adipose tissues of steers during growth[1]

	Subcutaneous		Intermuscular		Intramuscular	
Age	Lactate	Acetate	Lactate	Acetate	Lactate	Acetate
months	*nmoles substrate converted to fatty acid(2 hours × 10^6 cells)*					
11	1,051 ± 331[a]	2,824 ± 1,111[a]	417 ± 113[ac]	1,271 ± 386[a]	43 ± 11[a]	185 ± 56[a]
13	3,685 ± 713[ac]	9,017 ± 2,827[b]	1,696 ± 378[ac]	3,192 ± 933[b]	244 ± 41[a]	297 ± 48[a]
17	6,771 ± 2,174[b]	7,604 ± 1,268[b]	3,587 ± 1,077[b]	2,387 ± 537[b]	1,419 ± 271[abc]	370 ± 133[a]
19	9,011 ± 1,322[b]	6,694 ± 894[b]	3,268 ± 373[ab]	2,939 ± 271[ab]	1,448 ± 272[bc]	722 ± 95[b]

[1] Means ± SEM for eight steers unless noted otherwise. [2] Means ± SEM for seven steers. [a,b] Means in same column with different superscripts are significantly different ($P < 0.05$). [c] Means within a given adipose tissue are significantly different ($P < 0.05$) from that for acetate of same age group.

used. Base channel threshold was set to exclude material less than 20 μm in diameter. The total number of adipocytes per gram of tissue then was calculated by multiplying the average of the ten 0.5-ml counts by difference in weight of full and empty beaker and dividing by density of sodium chloride solution (1.0053 g/ml) and by tissue weight. The result was doubled to correct for the counting of the 0.5-ml aliquot.

Statistical analysis. Data were analyzed as a completely randomized design (21). Sums of squares for each variable were partitioned according to age effects and pairwise comparisons of means within an adipose tissue depot were performed with Student's *t*-test by using the pooled error mean square for each measurement within an adipose tissue depot from the analysis of variance.

RESULTS

Fatty acid synthesis during growth. To study the importance of lactate as a precursor for fatty acids during growth of cattle, fatty acid synthesis from lactate and acetate was compared in adipose tissue taken from three major fat depots of growing cattle. The cattle available at each age group weighed an average of 352, 427, 542 and 640 kg at 11, 13, 17 and 19 months of age, respectively, resulting in an average daily gain of 1.20 kg of body weight. Rates of lipogenesis in subcutaneous, intermuscular and intramuscular adipose tissues, expressed on a cellular basis, are shown in table 1. Fatty acid synthetic rates from lactate and acetate increased from 11 to 13 months of age in subcutaneous and intermuscular adipose tissues. Synthetic rates from lactate in subcutaneous and intermuscular adipose tissues increased significantly from 11 to 17 months of age; rates did not change significantly from 17 to 19 months of age. Synthetic rates from acetate in these two tissues did not change during growth from 13 to 19 months of age.

Rates of fatty acid synthesis from both lactate and acetate increased with increasing age in intramuscular adipose tissue. Synthetic rates from lactate increased about 35-fold over the post-weaning growth period while synthetic rates from acetate increased only about 4-fold. Fatty acid synthetic rates were significantly greater from lactate than from acetate after 13 months of age.

Activity of lipogenic enzymes. Specific activities, hereinafter simply called activities, of acetyl-CoA carboxylase, ATP-citrate lyase and selected NADPH-generating enzymes during growth were determined to relate lipogenic capacity to possible rate-limiting enzyme activities. Another major objective was to determine if sufficient ATP-citrate lyase activity existed to support fatty acid synthesis from lactate via the citrate cleavage pathway. Activity of the lipogenic enzymes for the three adipose tissue depots is shown in table 2.

Activity of acetyl-CoA carboxylase did not change significantly during the growing period in any of the adipose tissue

TABLE 2

Activity of acetyl-CoA carboxylase and ATP-citrate lyase in subcutaneous, intermuscular and intramuscular adipose tissues of steers during growth[1]

	Subcutaneous		Intermuscular		Intramuscular	
Age	Acetyl-CoA carboxylase	ATP-citrate lyase	Acetyl-CoA carboxylase	ATP-citrate lyase	Acetyl-CoA carboxylase	ATP-citrate lyase
months	*nmoles substrate converted to product/(minute × 10^6 cells)*					
11	17 ± 6[a]	58 ± 13[a]	16 ± 8[a]	52 ± 11[a]	60 ± 26[a]	424 ± 50[a]
13	35 ± 6[a]	310 ± 43[b]	35 ± 11[b]	230 ± 55[b]	53 ± 13[a]	449 ± 77[a]
17	30 ± 6[a]	230 ± 74[b]	7 ± 3[a]	109 ± 33[a]	35 ± 9[2a]	440 ± 54[2a]
19	29 ± 6[a]	216 ± 37[b]	34 ± 17[2b]	96 ± 21[2a]	48 ± 26[a]	835 ± 146[b]

[1] Mean ± SEM for eight steers unless noted otherwise. [2] Mean ± SEM for seven steers. [a,b] Means in same column with different superscripts are significantly different ($P < 0.05$).

depots studied. In subcutaneous adipose tissue, activity of ATP-citrate lyase increased from 11 to 13 months of age and did not change significantly thereafter. In intermuscular adipose tissue, ATP-citrate lyase increased during growth from 11 to 13 months of age and decreased from 13 to 19 months of age. Activity of ATP-citrate lyase did not increase until 19 months of age in intramuscular adipose tissues. Activities of ATP-citrate lyase in intramuscular adipose tissue was greater than the same activities in subcutaneous and intermuscular adipose tissues taken from cattle at all ages.

Activity of the principal NADPH-generating enzymes increased in subcutaneous and intermuscular adipose tissues from 11 to 13 months of age and did not change significantly during the rest of the growing period (table 3). In intramuscular adipose tissue, activity of glucose-6-phosphate dehydrogenase increased significantly from 11 to 13 and from 17 to 19 months of age. Activity of 6-phosphogluconate dehydrogenase increased from 11 to 13 months of age in intramuscular adipose tissue and did not change thereafter. Activity of NADP-isocitrate dehydrogenase in intramuscular adipose tissue did not change from 11 to 13 months of age but increased between 13 and 17 months of age and remained unchanged thereafter. In each adipose tissue depot, activity of glucose-6-phosphate dehydrogenase was an average of 3-fold greater than

TABLE 3

Activity of glucose-6-phosphate, 6-phosphogluconate and isocitrate dehydrogenases (GPDH, PGDH and ICDH) in subcutaneous, intermuscular and intramuscular adipose tissues of steers during growth[1]

	Subcutaneous			Intermuscular			Intramuscular		
Age	GPDH	PGDH	ICDH	GPDH	PGDH	ICDH	GPDH	PGDH	ICDH
months	*nmoles substrate converted to product/(minute × 10^6cells)*								
11	5,415 ± 1,072[a]	2,277 ± 180[a]	5,337 ± 957[a]	5,556 ± 1,146[a]	2,371 ± 183[a]	6,625 ± 537[a]	3,597 ± 811[a]	1,632 ± 273[a]	3,625 ± 546[a]
13	33,503 ± 6,120[b]	11,848 ± 3,408[b]	16,300 ± 2,097[b]	17,075 ± 2,823[b]	6,629 ± 1,444[b]	11,806 ± 1,050[b]	6,730 ±1,507[b]	± 3,691 ± 785[b]	3,514 ± 371[a]
17	27,340 ± 5,909[b]	10,231 ± 2,194[b]	19,440 ± 3,447[b]	15,032 ± 3,590[b]	4,502 ± 705[b]	14,270 ± 1,770[b]	7,705 ± 1,594[2b]	2,629 ± 458[2b]	6,730 ± 1,077[2b]
19	22,502 ± 3,564[b]	9,321 ± 1,334[b]	18,010 ± 1,626[b]	13,942 ± 2,274[2b]	5,597 ± 797[2b]	16,866 ± 2,956[2b]	12,439 ± 2,664[c]	3,556 ± 400[b]	7,940 ± 881[b]

[1] Mean ± SEM for eight steers unless noted otherwise. [2] Mean ± SEM for seven steers. [a,b,c] Means in same column with different superscripts are significantly different ($P < 0.05$).

that for 6-phosphogluconate dehydrogenase. Activities of the three NADPH generating enzymes in intramuscular adipose tissue tended to be less than those observed in subcutaneous and intermuscular adipose tissues at each respective age.

DISCUSSION

Pothoven and Beitz (10) observed decreases in rates of fatty acid synthesis in bovine adipose tissue with increasing steer weight when rates were expressed on a tissue-weight basis. Similar observations have been made for growing lambs (3) and rats (22). When rates were expressed on a cellular basis, however, no decrease in fatty acid synthesis was observed in rat adipose tissue with advancing age (23). In our study, fatty acid synthetic rates in bovine adipose tissues were expressed on a cellular basis. Whether lactate or acetate was utilized as the substrate, fatty acid synthetic rates were observed to increase with advancing age in the three major adipose tissue depots of growing and fattening cattle.

When acetate was the substrate, previous studies have shown that rates of fatty acid synthesis are greater in subcutaneous adipose tissue than in internal or intramuscular adipose tissues (24–26). In our study, fatty acid synthetic rates from lactate and acetate were greater at all ages in subcutaneous than in intermuscular or in intramuscular adipose tissues. This result further confirms the important role of subcutaneous adipose tissue in lipid accretion in cattle.

As was found for fatty acid synthetic capacity, lipogenic enzyme activities also tended to increase with increasing age in all three adipose tissue depots. The notable exception, however, was that the acetyl-CoA carboxylase was not significantly changed over the entire period from 11 to 19 months of age. Similarly, little change in acetyl-CoA carboxylase was observed in adipose tissue of growing swine by Anderson and Kauffman (27). No previous information is available on activities of acetyl-CoA carboxylase of adipose tissue of growing cattle. Increases in activity of ATP-citrate lyase and NADPH-generating enzymes have been observed in growing swine (28, 29). Pothoven and Beitz (10) observed slight, but not statistically significant, increases in activity of glucose-6-phosphate dehydrogenase in bovine adipose tissues with increasing animal weight; however, their data were not expressed on a cellular basis. Hanson and Ballard (30) demonstrated that livers of mature ruminants had lower specific activity of ATP-citrate lyase than did livers of fetal ruminants, but little information is available concerning changes in activity of ATP-citrate lyase during postweaning growth in cattle.

One objective of our study was to determine if sufficient ATP-citrate lyase activity existed to support lactate conversion to fatty acids and to identify possible rate-limiting enzymes for lactate conversion to fatty acids. At all ages and in all adipose tissue depots, observed activities of ATP-citrate lyase were greater than those required to support observed rates of lactate conversion to fatty acids. This result suggests that fatty acid synthesis from lactate could occur by way of the citrate cleavage pathway in bovine adipose tissues.

Activities of major NADPH-generating enzymes in ruminants (7) were considerably greater than those needed to supply the NADPH required for lipogenesis from lactate in all three adipose tissue depots. In studies of lactate use for lipogenesis in the presence of glucose by rat adipose tissue, Katz and Wals (31) observed that generation of cytosolic reducing equivalents closely balanced the requirement for reducing equivalents. Our estimates of NADPH-generating potential based on enzymatic activities suggest that lactate use for lipogenesis would not be limited by NADPH supply; the balance of NADPH formation and use during fatty acid biosynthesis from lactate has not been determined for bovine adipose tissues.

In subcutaneous and intermuscular adipose tissues, average rates of lactate conversion to fatty acids for all age groups were 5,130 and 2,209 nmoles/(2 hours ×

10^6 cells), respectively, and were not significantly different ($P > 0.05$) from mean activities for acetyl-CoA carboxylase of 3,324 and 2,750 nmoles/(2 hours × 10^6 cells) for the same two tissues. These comparisons of rates suggest that acetyl-CoA carboxylase activity limits the rate of fatty acid synthesis from lactate in these two tissues. In a recent report, Smith and Prior[5] also have suggested that fatty acid synthesis from lactate in subcutaneous adipose tissue of cattle was limited by activity of acetyl-CoA carboxylase. This conclusion is consistent with the generally accepted rate-limiting role of acetyl-CoA carboxylase in fatty acid biosynthesis (32).

In our study, activity of acetyl-CoA carboxylase in intramuscular adipose tissue was always observed to be greater than that required to support rates of fatty acid synthesis from lactate. The limiting factor for conversion of lactate to fatty acids in intramuscular adipose tissue is not clear; under in vivo conditions, the concentration of lactate in situ logically would be a possible limiting factor. It is also not clear why acetyl-CoA carboxylase activity did not vary with age as did rates of fatty acid synthesis from lactate.

Current concepts of lipogenesis in bovine adipose tissue state that glucose use is limited for fatty acid synthesis because of low citrate cleavage pathway activity (1, 8). On the basis of known metabolic pathways, lactate as well as glucose is catabolized to pyruvate as a common metabolic intermediate. Lactate as well as pyruvate is converted to fatty acids at rates several-fold greater than glucose in bovine adipose tissue (5). Concentrations of specific isoenzymes of lactate dehydrogenase (EC 1.1.1.27) suggest that lactate would be converted to pyruvate (33). Furthermore, both pyruvate and lactate are decarboxylated before being converted to fatty acids (6).

Data in our study clearly indicate that ATP-citrate lyase activity was more than sufficient to support fatty acid synthesis from lactate in subcutaneous, intermuscular and intramuscular adipose tissues of cattle. Smith and Prior[5] also have suggested that lactate conversion to fatty acids is not limited by ATP-citrate lyase activity in subcutaneous adipose tissue of cattle. Therefore, the citrate cleavage pathway, as described by Ballard and Hanson (1), seems operative in bovine adipose tissue and would explain the generation of cytosolic acetyl-CoA from lactate for fatty acid biosynthesis.

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