Adaptations in lipid metabolism of bovine adipose tissue in lactogenesis and lactation

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Abstract The timing and magnitude of metabolic adaptations in adipose tissue during lactogenesis and lactation were determined in first lactation bovines. In vitro rates of lipogenesis and palmitate esterification were measured to estimate in vivo synthesis. Lipolysis was measured in the basal state and as maximally stimulated by norepinephrine or epinephrine to estimate physiological adaptations as well as the changes in catecholamine responsiveness. Subcutaneous adipose tissue was biopsied at −1, −0.5, +0.5, 1, 2, and 6 months from parturition. From 1 to 0.5 months prepartum there was a 54% reduction in lipogenesis, a 16% reduction in esterification, a 54 and 77% increase in norepinephrine- and epinephrine-stimulated free fatty acid (FFA) release, respectively, and a 28% increase in epinephrine-stimulated glycerol release. The immediate postpartum period (0.5 and 1 month) was marked by a decrease in lipogenesis to 5% and esterification to 50% of −1 month rates. During this period, norepinephrine-stimulated FFA release increased 50% above −1 month rates, epinephrine-stimulated FFA release increased 128%, and norepinephrine- and epinephrine-stimulated glycerol release increased 30 and 87%, respectively. Midlactation (2 and 6 months) was marked by a dramatic rebound in lipogenesis and esterification to 14-fold and 2.5-fold prepartum rates, respectively. Basal glycerol release doubled during this period, while basal FFA release declined to near prepartum levels. Catecholamine-stimulated FFA and glycerol release decreased from the peak during midlactation, but remained elevated compared to prepartum levels. Bovine adipose tissue adapts prepartum for increased release of energy, meets peak lactation demand by ceasing synthesis and increasing lipolysis, and recovers synthesis dramatically to replenish body energy stores while also maintaining elevated levels of lipolysis in support of lactation.—**McNamara, J. P., and J. K. Hillers.** Adaptations in lipid metabolism of bovine adipose tissue in lactogenesis and lactation. *J. Lipid Res.* 1986. **27:** 150–157.

Supplementary key words gestation • lipogenesis • lipolysis • catecholamines • adaptation

Adipose tissue metabolism adapts biphasically during pregnancy to first store (midpregnancy) and then release (late pregnancy) energy for fetal growth and subsequent lactation. Pregnancy and lactogenic hormones initiate these adaptations prior to increased energy demand on the animal; in fact, adipose tissue lipid synthesis decreases and lipolysis increases while serum insulin and triglyceride levels are elevated (1–4). This coordination of metabolism in support of a dominant physiological process is called homeorhesis (3). This type of adaptation has been discovered in rats (1), sheep (5), swine (6), and man (2). Lipogenesis is lower in adipose tissue of 'lactating' (ca. 2–4 months) versus 'pregnant' (midpregnancy) cows (7), and lipolysis increases during the peripartum period in cows (8). The presence of a similar adaptation in all placental mammals yet tested illustrates a basic biological principle of energy metabolism regulation. Although both synthetic and lipolytic pathways are affected, the timing and magnitude of the changes in these pathways during gestation and lactation are undefined.

The dairy cow, genetically selected for increased rates of milk synthesis and length of lactation, provides a scientifically stimulating model for study of genetic, endocrine, and metabolic components of homeorhetic regulation (3). The objectives of this study were *1*) to define the adaptations occurring in the lipogenesis, esterification, and lipolysis pathways in subcutaneous adipose tissue during lactogenesis and lactation in the bovine; and *2*) to determine the involvement of the adrenergic system in the adaptations of adipose tissue lipolysis during lactogenesis and lactation.

METHODS AND MATERIALS

Animal handling and tissue sampling

Fifteen Holstein heifers were maintained under normal lighting conditions outdoors and fed ad libitum. The animals were artificially inseminated at approximately 15 months of age and gave birth to healthy young between November 1, 1983 and June 1, 1984. Prior to parturition, animals were fed a hay diet ad libitum. During lactation, a mixed forage/grain ration was offered ad libitum in relation to lactation requirements. Immediately prior to preparation for sampling, a single blood sample was removed from the tail vein. Samples were left refrigerated overnight and serum was collected for free fatty acid (FFA)

Abbreviations: FFA, free fatty acids; TG, triglyceride; NE, (−)norepinephrine; E, (−)epinephrine bitartrate.

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analysis. Adipose tissue from the dorsal subcutaneous depot immediately cranial and lateral to the first lumbar vertebra was sequentially sampled according to the following procedure. Tissue samples were taken at 1 and 0.5 months prior to expected parturition date (1 and 0.5 months prepartum); at 0.5, 1, 2, and 6 months postpartum and at 0.5 month after lactation ceased (0.5 month postlactation; approximately 10 months postpartum). This final sample is also 1.5 month prior to the cow's second parturition, allowing a 60-day nonlactating period between lactations. Sampling sites alternated with period on either side of the spine. Animals were prepared for aseptic surgery and a subdermal block using lidocaine was administered in a 'U' pattern, the base of the 'U' parallel to the spine. An incision approximately 5 to 10 cm long was made and adipose tissue was removed with a scalpel. Incisions were closed with #2 gut and treated with a topical antibiotic, antifungal agent. No further antibiotic treatment was administered. During the lactation periods (0.5, 1, 2, 6 months), some animals had no or a very reduced amount of dissectible adipose tissue. Thus, on some animals, one or more variables were not measured in that period. Actual numbers of animals providing tissue for that variable in that period are given in the figure legends. All fifteen animals started and completed the study. Daily milk production for the first 6 months averaged 26.5 $\pm$ 0.9 kg/day.

Tissue slice incubations

Lipid synthesis rates were estimated using in vitro tissue slice incubations. Tissue samples were held in Krebs buffer containing 118 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.16 mM $MgSO_4$, 1.16 mM KH_2PO_4, 25 mM $NaHCO_3$, and 10 mM HEPES buffer (KB/HEPES) at pH 7.4 and 37°C. Tissues were sliced on a Stadie-Riggs hand microtome to approximately 100 mg and 500 μm thickness. Tissue incubations were conducted in triplicate for each variable and begun within an hour after sampling. All incubations were run under conditions of linear incorporation of substrates or release of fatty acid and glycerol. All media used for tissue incubations utilized the KB/HEPES buffer containing 3% bovine serum albumin (BSA). Lipogenesis from sodium acetate was measured in KB/HEPES/BSA media containing 10 mM sodium acetate, 5 mM glucose, and approximately 1 μCi/ml of [2-^{14}C]acetate.

Esterification of palmitate into tissue triglycerides (TG) was measured in KB/HEPES/BSA media containing 250 μM sodium palmitate complexed to BSA (9) and approximately 0.5 μCi/ml of [1-^{14}C]palmitic acid. Incubations were carried out for 2 hr in 2 ml of media at pH 7.4 and 37°C in stoppered 25-ml Erlenmeyer flasks in a shaking water bath. Reactions were stopped by removing tissue from media and placing it into 5 ml of 10% KOH in MeOH for lipogenesis sample extraction or into 5 ml of isopropanol–heptane–1 N H_2SO_4 4:1:0.1 solvent for esterification sample extraction. Free fatty acids or TG were extracted and quantitated as previously reported (10, 11).

Duplicate incubations of tissue from one cow were conducted at six time points from zero to 2 hr to test linearity of activity and uptake of radioactive substrate into the tissue precursor pool. For lipogenesis incubations, the equation relating nanomoles of acetate incorporated per gram of tissue (y) with time in hours (x) was $y = -35.4 + 177.1x$; $n = 12$, $r^2 = 0.93$. The good linear fit indicates that adequate mixing of extra- and intracellular precursor pools was achieved. Also, no curvilinearity existed to indicate a slowing of reaction rate. For esterification incubations, nanomoles of palmitate taken up into TG (y) versus time in hours (x), the equation was $y = -1.64 + 50.0x$, $n = 12$, $r^2 = 0.93$. Further, radioactive palmitate taken up into tissue FFA was 30.0 $\pm$ 3.4 (n = 12) nmol/g of tissue, averaged from 0.25 to 2 hr. By 45 min of incubation, the tissue uptake of radioactive palmitate into the FFA pool had stabilized, indicating a complete mixing of extra- and intracellular precursor pools of radiolabeled FFA. All results are presented as nmol of substrate incorporated per gram tissue per 2 hr.

Lipolysis was measured in tissue incubated in KB/HEPES/BSA media without substrates at pH 7.4 and 37°C. Tissues were first placed in the 2 ml of media in the flask and preincubated for 20 min to remove the effects of slicing and handling on lipid release. Media were then aspirated and replaced with 2 ml of fresh media for a subsequent 2-hr incubation. Reactions were stopped by removing the tissue from the media. Media were frozen until assayed for FFA and glycerol. At the end of the incubations, tissue slices from the triplicate incubations were blotted, pooled together, and frozen. The tissue FFA content of this pool was extracted and quantitated colorimetrically as described in (6, 12). Thus, the same incubations were used to measure FFA and glycerol release as well as FFA pool size of the tissue in basal and catecholamine-stimulated states. Lipolysis was also measured in the presence of (−)norepinephrine (NE) and (−)epinephrine bitartrate (E), separately, at 10^{-4} M to estimate the maximal stimulation of lipolysis by these two adrenergic agonists.

FFA and glycerol assays

An enzymatic assay for FFA, synthesizing the acyl CoA form and then linking the ester to a dye absorbing at 550 nm was utilized (Wako Chemicals, Dallas, TX) with the following modifications. Liquid reagents were diluted with distilled water in the ratio of 1:1. After dilution, reagent A was used at 350 μl and reagent B was used at 750 μl; sample size was 100 or 200 μl of incubation media. Sample size for serum was 50 μl. Reactions were carried out for 20 min. Interassay coefficient of variation using a repeated media sample was 14%.

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Glycerol was assayed enzymatically using a kit from Boehringer-Mannheim Biochemicals (Indianapolis, IN) with the following modifications. Reagent 1 was diluted in the ratio of 1:2 with distilled water. Reagents 2 and 3 were diluted 1:5. Assay times for both endogenous substrate removal and for glycerol assay were 45 min. Interassay coefficient of variation was 10% using a repeated media sample. All basal and stimulated lipolysis incubations from each animal at each sampling period were determined in one assay. All data are expressed as nmol released per g of tissue per 2 hr.

Experimental design and analysis

The experiment was designed with sample period as a main effect. Analysis of variance (13) using SAS (14) was performed and significant effects of sampling period were determined using the sample-by-animal interaction term. Because of the large effect of period, standard errors of each period mean (SEM) are shown for lipogenesis. For the other variables, SEM for the sample-by-animal interaction term for each variable are shown in the figure. For all variables, the effect of period was tested against the error mean square for the animal-by-period interaction. All differences due to sample period reported are significant at $P < 0.05$ unless otherwise noted.

Materials

All substrates were of reagent grade. Radiolabeled compounds were from New England Nuclear (Boston, MA). Bovine serum albumin (fatty acid-free) was from Sigma Chemical Co. (St. Louis, MO) or from Boehringer-Mannheim (Indianapolis, IN); catecholamines were from Sigma Chemical Co.

RESULTS

Adaptations in synthetic pathways

De novo lipogenesis from acetate was the pathway most affected by changing lactation state (**Fig. 1**). From 1 to 0.5 month prepartum, lipogenesis decreased ($P < 0.05$) 50%. From 0.5 month prepartum to 0.5 month postpartum, lipogenesis decreased to 5% of 1-month prepartum rates. At 1 month postpartum, rates were still only 7% of 1-month prepartum rates; however, the range of values among animals was much greater than at 0.5 month postpartum. By 2 months postpartum, lipogenesis rates were increased 5-fold compared to those at 1 month prepartum, and nearly 100 times greater than at 1 month postpartum. This recovery continued to at least 6 months postpartum, when rates were 14-fold greater than 1-month prepartum rates, approximately 210-fold greater than 1 month postpartum, and 2.5-fold greater than at 2 months postpartum. In the animals that were sampled 0.5 month after lacta-

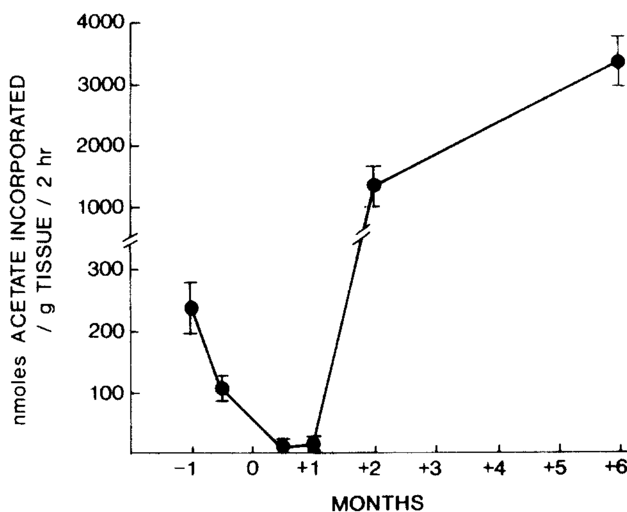

Fig. 1. De novo lipogenesis from acetate in bovine adipose tissue in vitro. Subcutaneous adipose tissue was biopsied at the noted times and lipogenesis was estimated as in Methods. Standard error of the mean at each period is shown. Effect of period was significant at $P < 0.0001$. Data represent means of triplicate incubations from 15, 15, 14, 12, 12, and 14 animals in each period, respectively.

tion was stopped (ca. 10 months postpartum), rates had declined from the peak levels at 6 months to 862 ± 207 nmol per g of tissue per 2 hr, (n = 12); or 364% of 1-month prepartum rates. Thus, at 1.5 months prior to second parturition, animals were recovering adipose fatty acids at rates more than 3-fold faster compared to the same approximate time prior to first parturition (1-month prepartum sample).

The pathway following de novo lipogenesis for the storage of lipid in adipose tissue is esterification. Esterification rates were also sensitive to changing physiological state as shown in **Fig. 2**. The pattern of adaptation in esterification paralleled that of de novo lipogenesis, but the magnitude of the change was not as great. Esterification at 0.5 month prepartum was 86% of 1-month prepartum rates; and subsequently declined to 62 and 46% of 1-month prepartum rates at 0.5 and 1 month postpartum, respectively. This pathway recovered to 25% higher than 1-month prepartum rates at 2 months postpartum and to 150% higher at 6 months postpartum. Rates at 0.5 month postlactation were 135.9 ± 20.3 (n = 12) nmol of palmitate incorporated into TG per g of tissue per 2 hr; or 46% higher than 0.5-month prepartum rates.

Adaptations in lipolytic pathways

Adipose tissue lipid storage is also regulated by the rate of FFA release from triglycerides. Adipose tissue reutilizes some of the FFA and the rest is released for use in other tissues. Adipose tissue does not reutilize glycerol; therefore, glycerol release measures hormone-sensitive lipase

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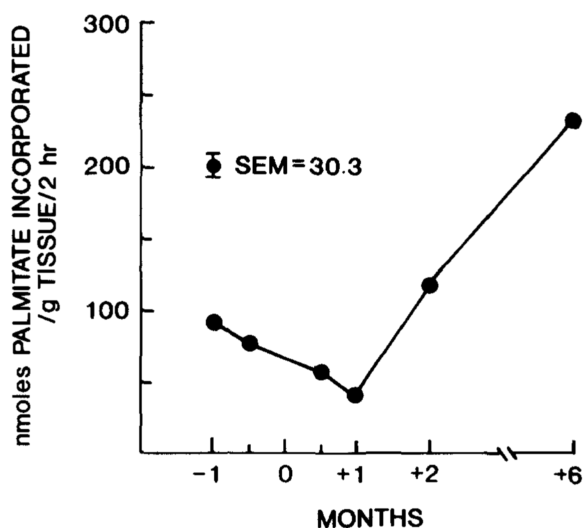

Fig. 2. Esterification of palmitate into bovine adipose tissue triglycerides in vitro. Tissues were obtained and esterification of palmitic acid into tissue triglycerides was estimated as in Methods. Standard error of the mean for the sample-by-animal error term is reported in upper left. Effect of period was significant at $P < 0.0001$. Data represent means of triplicate incubations from 15, 15, 14, 11, 11, and 13 animals in each period, respectively.

activity, while measuring FFA release also incorporates FFA reesterification. The enzyme activity is important to compare biochemical changes; however, taking FFA reesterification into account is more correct for determining tissue metabolism of fatty acids. Both products were measured to estimate the biochemically (glycerol) and metabolically (FFA) important adaptations. A change in enzyme activity may be affected by other alterations in tissue metabolism of FFA. Glycerol and fatty acid release rates (**Fig. 3** and **Fig. 4**, respectively), in the basal state remained at similar levels from 1 month prepartum to 1 month postpartum. Basal fatty acid release was only slightly (11%) increased at 2 and 6 months postpartum as compared to 1 month prepartum. Glycerol release, however, increased to 43% and 210% greater than 1-month prepartum rates at 2 and 6 months postpartum, respectively. The maintenance of basal FFA release while glycerol release increased is consistent with the increased rates of esterification observed during this period (Fig. 2). At 0.5 month postlactation, basal FFA release had declined to 2632 ± 446 nmol of FFA released per g of tissue per 2 hr or 123% of 1 month prepartum and basal glycerol release was 691 ± 291 or 87% of 1 month prepartum. Comparing rates of lipogenesis, esterification, and lipolysis at 1 month prior to first lactation with those measured approximately 1.5 months prior to the second lactation, adipose tissue was in a relatively more synthetic state at the latter time. After the first lactation ceased, rates of lipogenesis and esterification remained elevated while lipolysis was similar to or only slightly increased compared to before the first lactation. These data are consistent with these cows being in a body fat recovery phase at this time.

Bovine adipose tissue responded well to either NE or E in vitro. These catecholamines were used to test the adaptations in the adrenergic responsiveness of adipose tissue during changing physiological states. Rates of glycerol release at 1 month prepartum increased 3.3-fold in response to NE and 3.5-fold in response to E. Fatty acid release rates increased only 32% and 79% in response to NE and E, respectively. Differences in reesterification rates and in the tissue/media partitioning of FFA can account for the lower catecholamine response of FFA release as compared to glycerol release. The response of FFA release to NE was less than that to E throughout the study period. Glycerol release response to NE was also less than to E, but the difference in glycerol response to the two catecholamines was not as great as it was for FFA release. These results are consistent with either *1*) a different potency of NE and E in bovine adrenergic adipose tissue systems, or *2*) the presence of both alpha (lipolysis-inhibiting) and beta (lipolysis-promoting) adrenergic receptors in bovine adipose tissue.

The response of FFA release to NE and E increased 54 and 77%, respectively, from 1 to 0.5 month prepartum. The NE-stimulated rates remained at approximately the −1 month value at 0.5 and 1 month postpartum, declined somewhat at 2 months but remained above prepartum rates at 6 months. The E-stimulated FFA release was more affected by stage of lactation than that of NE-stimulated rates. At 0.5 and 1 months postpartum, E-stimulated rates were 60 and 128% higher than at 1 month pre-

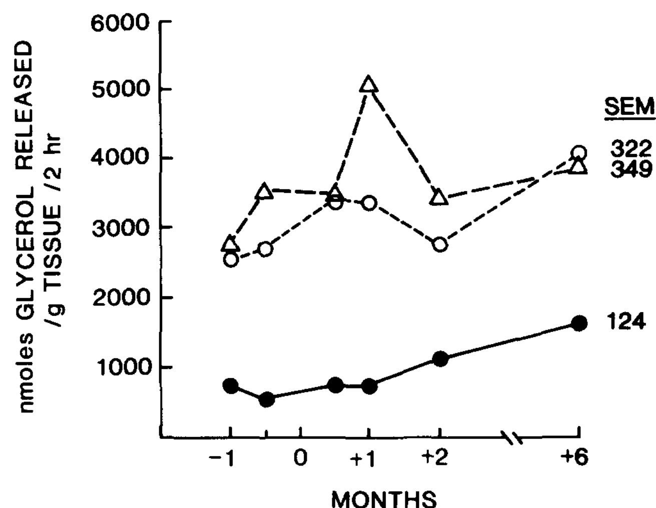

Fig. 3. Glycerol release from bovine subcutaneous adipose tissue in vitro. Glycerol release from adipose tissue was determined as in Methods on the media from incubations. Standard errors of the mean (from the sample-by-animal error mean square) are reported at right for each variable. Lipolysis was estimated under basal conditions (●) or in the presence of 10^{-4} M norepinephrine (○) or epinephrine (△). Values are means of triplicate incubations from 15, 14, 14, 12, 12, and 11 animals per period for basal and NE; and 8, 10, 6, 5, 8, and 8 animals for E. Basal, NE, and E glycerol release increased significantly ($P < 0.001$).

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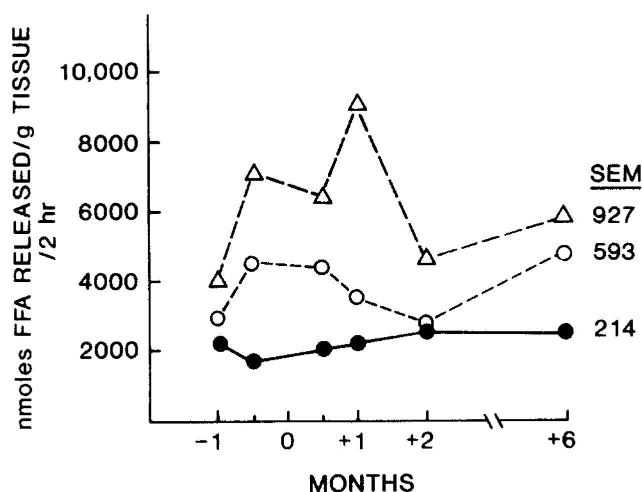

Fig. 4. Fatty acid release from bovine subcutaneous adipose tissue in vitro. Tissues were obtained and lipolysis was determined in the same incubations reported in Fig. 3 as in Methods. Standard errors of the means for each variable (from the sample-by-animal error mean square) are shown at right. Values are means of triplicate incubations from 15, 15, 14, 12, 12, and 10 animals at each period for basal and NE; 8, 10, 7, 5, 8, and 8 animals for E incubations. The effect of period was significant for basal FFA release at $P < 0.05$; for NE at $P < 0.04$ and E at $P < 0.09$.

partum. The response to E also declined at 2 and 6 months, but remained higher than prepartum rates. At 0.5 month after lactation ceased, NE-stimulated FFA release was 5647 ± 934 nmol per g of tissue per 2 hr (n = 6); or 191% of 1 month prepartum, while E-stimulated rates were 8070 ± 973, or 201% of 1 month prepartum.

NE-stimulated glycerol release was unchanged prior to parturition. Stimulated release increased to 29 and 30% above 1-month prepartum levels at 0.5 and 1 month postpartum, respectively. Rates then returned to near prepartum rates at 2 months postpartum, but at 6 months were 27% above 1-month prepartum rates. At 0.5 month after lactation ceased, NE-stimulated glycerol release was 4842 ± 103 nmol per g per 2 hr (n = 5) or 190% of 1-month prepartum rates. Glycerol release in response to E increased 28% from 1 month to 0.5 month prepartum and was 21 and 87% higher than 1-month prepartum rates at 0.5 and 1 month postpartum, respectively. Rates were still 23% higher than prepartum rates at 2 months postpartum and remained elevated through 180 days. At 0.5 month after lactation ceased, rates were 4530 ± 978 nmol per g per 2 hr (n = 5) or 164% of rates at 1 month prepartum.

Serum FFA levels at time of tissue sampling and in vitro adipose tissue FFA content at the end of the 2-hr incubation period were also measured (**Table 1**). Serum FFA did not change during the peripartum period; however, there was wide among-animal variation. Levels fell in midlactation, after the period of peak lipolysis and energy demand. After 0.5 month post-lactation, serum FFA had returned to peripartum levels. These data are consistent with the relative energy balance during these lactation periods. Basal tissue FFA content fluctuated from sampling period to sampling period, with no consistent pattern. The tissue FFA pool size in the basal state shows that measures of fatty acid esterification (Fig. 2) and release (Fig. 4) were not dependent on tissue FFA pool size and were significant physiological adaptations. Tissue FFA responded to E and NE as did FFA release, with response being highest in the first 2 months of lactation and returning to peripartum levels by the dry period. Thus the effect of these catecholamines is to increase total FFA release from adipose tissue TG. This hormone receptor-mediated system is altered with changing lactational state.

DISCUSSION

Anabolism predominates in adipose tissue lipid metabolism during midpregnancy. The tissue shifts to catabolism prior to parturition during the period of increased fetal growth, and follows with tremendous catabolism as lactation begins. The presence of these adaptations in every species tested thus far indicates an involvement of evolutionarily protected regulatory mechanisms. Midpregnancy is a time of increased adipose tissue lipid synthesis and decreased lipolysis in rats (1, 4), pigs (6), and women (2) resulting in increased body fat at this time (15-17). At some species-dependent time-point in the last third of pregnancy, de novo lipogenesis and esterification decrease, lipolysis increases, and lipoprotein lipase activity decreases (4). In all species investigated, this catabolic shift precedes the energy demand for lactation. The quantitative effect of energy needs for fetal growth on maternal adipose tissue metabolic activity during this period has not yet been determined, especially in the larger animals.

The present data provide a detailed analysis of the timing and magnitude of the major metabolic adaptations in bovine adipose tissue during lactogenesis and lactation. The pattern of decreased lipid synthesis and storage prior to parturition along with increased rates of lipolysis, especially catecholamine-stimulated lipolysis, is consistent with the pattern in other mammals. The details provided in this report allow some initial conclusions and hypotheses on the mechanisms involved in this homeorhetic adaptation. The sharp prepartum drop in lipogenesis, the functionally nonexistent rates during the first month of lactation, and the rebound to the highest rates during midlactation all point to a specific regulatory role of this pathway in adipose metabolism during lactation. Lipogenic activity decreases in rat adipose tissue during lactogenesis (18). The magnitude of the decrease in lipogenesis in this study without a similarly dramatic decrease in esterification is consistent with a specific hormonal inhibition of de novo lipogenesis. Although the lipogenic

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TABLE 1. Subcutaneous adipose tissue free fatty acid pool content in vitro and serum free fatty acids of dairy cows in late gestation and lactation

	Adipose Tissue FFA Pool Size[a]							
	Period (Months about Parturition)							
	Gestation		Lactation					
	−1	−0.5	0.5	1	2	6	Dry	SEM
	nmol/g							
Basal	5504	3889	4897	5173	6017	5604	2438	656
10^{-4} M Norepinephrine	6188	5235	7035	8278	7896	7483	6974	907
10^{-4} M Epinephrine	4317	5133	6679	8269	10055	6009	5562	688[b]
Serum FFA (nmol/l)[c]	484.3	465.4	441.1	442.9	204.2	118.1	470.6	50.7[b]

[a]Tissue fatty acid pool size was measured at the end of 2 hr lipolysis incubation as described in Methods. Tissues from triplicate incubations from each animal were pooled before extraction and fatty acid determination. Basal, tissue incubated in Krebs-HEPES/BSA buffer; 10^{-4} M norepinephrine, tissue incubated in KH/BSA with 10^{-4} M NE present; 10^{-4} M epinephrine, tissue incubated in KH/BSA with 10^{-4} M E present. These incubations are the same ones used for FFA and glycerol release (Figs. 3 and 4).
[b]Main effect of period was significant at $P < 0.05$, SEM calculated from the animal within period error mean square.
[c]FFA assayed enzymatically on tail-vein sample taken immediately prior to biopsy.

enzymes were not measured in this study, acetyl CoA carboxylase is the key rate-limiting enzyme in this pathway. The decrease in lipogenic activity prior to the energy demand for lactation is further evidence for endocrine regulation. This same time period (30 days to 7 days prepartum) in the bovine is also the time of mammary differentiation (19) and rapid fetal growth. This differentiation is initiated by prolactin and is inhibited by progesterone (3). In the rat, the decrease in adipose tissue lipogenesis can be prevented by inhibition of prolactin release (18) and by maintenance of high progesterone levels (20, 21). The metabolic alterations discovered here in the bovine are consistent with the timing of mammary differentiation; and a similar endocrine regulation to that described for the mammary gland is highly likely for adipose tissue metabolism.

Adipose tissue lipolytic pathways are also coordinated as shown by an increased lipolysis during late pregnancy in rats (18), swine (16), and cows (8 and in this study). The present study determined that adipose tissue responsiveness to both norepinephrine and epinephrine, the major sympathetic and adrenal neurotransmitters in vivo, increases in the period between 1 and 0.5 month prepartum in the 270-day bovine gestation. The catecholamine-stimulated lipolysis alterations during this period are consistent with the adrenergic system being a key to this adaptation in adipose tissue metabolism. This adrenergic adaptation has now been shown in cattle and swine (6). The in vivo role of the adrenal catecholamine, epinephrine, in this type of metabolic adaptation is not proposed; however, it is a specific research tool to determine adrenergic receptor changes. Jaster and Wegner (22) have reported an increase in beta adrenergic binding capacity in bovine adipocytes between the period 30 days prepartum and 39 days postpartum. The observations of the present study are consistent with this and extend the result to include an increased biological response (increased lipolysis). The time period of this adaptation is before 15 days prepartum (Figs. 3 and 4). The work of Metz and van den Bergh (8) also indicates an adaptation prior to 15 days prepartum without much further increase in catecholamine-stimulated lipolysis in early lactation.

These results confirm and extend the initial findings of Metz and van den Bergh (8) on changes in NE responsiveness of bovine adipose tissue during lactogenesis. They observed a similar percentage increase in NE responsiveness between 20 and 12 days prepartum and a further increase to 4 days prepartum. The present study discovered the same change between 1 and 0.5 months prepartum, narrowing the initial adaptation period to between 20 and 15 days prepartum in the bovine. A temporary plateau of increased responsiveness was noted in both studies from approximately 12 to 20 days postpartum. Both studies observed a further increase in NE responsiveness to approximately 30 days; in the present study, no further increase in responsiveness was noted and a slight decline actually occurred during maintenance of lactation. Information on the timing and magnitude of adaptation in the adrenergic control of lipolysis during late gestation and lactogenesis is unavailable for other species. The adaptation of the adipose tissue adrenergic system begins prior to the start of lactation in the bovine. After the lactation begins, the energy demand then adds additional stimulation to the adrenergic pathways. Catecholamine-stimulated lipolysis remains elevated throughout lactation and into the dry period, indicating a long-lasting adaptation of the adrenergic system to support lactation and, possibly, fetal growth.

These results on adipose tissue slices may be discussed in relation to adipocyte metabolism. Measures of adipocyte cell size and number were not determined in this study. A recent study in the dairy cow (23) has shown a

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decrease in adipocyte size between 1 and 8 weeks postcalving, followed by an increase of approximately the same magnitude (as the initial decrease in size) from weeks 9 to 18. That study represents the sum knowledge of changes in adipocyte size during bovine lactation. Given the decrease in synthetic activity, the increase in lipolysis, and the resultant net mobilization of body fat, it can be deduced that adipocyte size would decrease in early lactation (causing an increase in cells per g tissue) and increase in mid to late lactation (decreasing cells/g of tissue). A thorough quantitation of these processes awaits further experimental investigation. Relating the tissue metabolism reported here with the direction of adipocyte size changes (23) and the resultant change in adipocytes/g of tissue, the following may be deduced.

Activity of synthetic pathways per adipocyte would decrease in early lactation as cells/g of tissue increase and synthetic activity/g of tissue decreases. During mid and late lactation, synthesis/cell would rebound at greater rates than for tissue, as activity/g of tissue increases and cells/g decrease. Lipolytic activity would be reciprocal to synthesis but a large increase in lipolysis/cell in early lactation would not be necessary to explain the present data. During mid to late lactation, lipolysis/g of tissue maintains or decreases slightly, while cells/g of tissue would decrease (as cell size recovers); thus, lipolysis per cell would maintain or increase slightly during this period in support of the continuing lactation. Thus, the known and deduced changes in adipocyte size and number strengthen the physiological differences reported here for adipose metabolism. This discussion points out *1*) the need for quantitation of adipocyte cellularity changes during bovine lactation, and *2*) the concerted coordination of adipose synthetic and lipolytic systems in support of lactation and body fat recovery.

Subcutaneous adipose tissue metabolism does not exactly parallel that of other depots (eg., abdominal, perigonadal) during growth, obese states, or nutritional manipulation. However, the dairy cow, and rat as well, loses a large percentage of mobilizable body fat during early lactation (3, 5, 24). The increase in energy demand in early lactation results in a need for rapid mobilization of stored lipid. Therefore, although minor differences may exist in the net rates of mobilization among depots, subcutaneous adipose tissue metabolism is probably representative of total body adipose tissue metabolism during this physiological state.

Bovine adipose tissue adapts prepartum via a dramatic decrease in de novo lipogenesis, a smaller decrease in esterification, and an increase in rates of lipolysis. After lactation begins, further decreases in synthesis and increases in lipolysis support peak lactation, after which time synthetic rates rebound dramatically to replenish body lipid stores during concomitant lactation. Rates of basal lipolysis are maintained at elevated levels at least through 180 days of lactation, while catecholamine-stimulated rates remain elevated into the dry period. Differentiation in both lipogenic and lipolytic pathways prior to lactational energy demand is evidence for homeorhetic regulations, likely through prolactin and progesterone. Separation of metabolic pathway control in adipose tissue (i.e., increasing synthesis concomitant with elevated lipolysis) during mid and late lactation suggests that body energy storage recovery (de novo lipogenesis) is driven by homeorhetic hormonal signals, while lactation energy demand maintains high activity of adrenergically regulated lipolytic pathways through homeostatic mechanisms.

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