Nonesterified Fatty Acids and Glucose in Lactating Dairy Cows: Diurnal Variations and Changes in Responsiveness During Fasting to Epinephrine and Effects of Beta-Adrenergic Blockade

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ABSTRACT
To examine whether the typical nightly rise of nonesterified fatty acids in high yielding dairy cows is due to enhanced sympathoadrenal activity, the β-adrenergic blocker, propranolol, was infused from 1800 to 0805 h. Concentrations of nonesterified fatty acids continuously increased, whereas those of glucose and insulin decreased. Nonesterified fatty acid concentration decreased within minutes in response to concentrate feeding, starting at 0700 h, in association with an increase of insulin and glucose. In a second experiment, adrenaline (.82 μmol/kg/min) was infused from 1800 to 1810 h, 0600 to 0610 h, and 0600 to 0610 h on the 1st, 2nd and 3rd d. After the second infusion, food was withdrawn for 23 h. Concentrations of adrenaline increased similarly. Nonesterified fatty acids and glucose responses were higher during the second than the first infusion. During fasting, nonesterified fatty acid concentrations increased, whereas glucose and insulin concentrations decreased. During the third infusion nonesterified fatty acids responses were unchanged, whereas glucose responses were decreased. Thus, the nightly rise of nonesterified fatty acids was not the consequence of enhanced β-adrenergic activity.

INTRODUCTION
During early lactation, in dairy cows, fat oxidation is enhanced, whereas glucose oxidation is reduced, thus sparing glucose for lactose synthesis (3). Nevertheless, glucose concentrations are usually low as a consequence of the enhanced demand and insufficient gluconeogenesis (5, 10, 20). Concentrations of nonesterified fatty acids (NEFA) are elevated (9, 10). The magnitude of the rise depends on the degree of energy deficiency (9, 20). Concentrations of NEFA increase during the night in association with decreasing concentrations of immunoreactive insulin (IRI) as a consequence of decreased feed, i.e., energy intake (9). Glucose concentrations increase during the night, especially in the energy-deficient animals, likely as a result of low circulating insulin, which favors gluconeogenesis and glycolysis (9).

Although somatotropin is the most potent hormone stimulating milk production (4), several other hormones regulate metabolism in a direction that favors milk secretion (12, 18). The possible role(s) of catecholamines (CA) for nutrient repartitioning in lactating cows has not been well-studied. This is somehow surprising, because adrenaline (epinephrine, E) and noradrenaline (norepinephrine, NE) cause NEFA and glucose to rise (8, 11). In addition, they cause insulin resistance (Blum, unpublished observations), as does somatotropin, at least during short-lasting administration (15). There is some evidence that CA can change metabolism in a direction typical for lactation. Thus, the number of β-adrenergic receptors on...
fat cells increase during lactation compared with numbers during the dry period (17). In addition, NEFA and glycerol responses are greater after parturition than before in cows as a consequence of reduced reesterification of NEFA and an increase in the active form of lipase in adipose tissue (23). Variable provision of food energy may be in part responsible for enhanced sensitivity to E, because responses of NEFA and glucose to E and NE are modified by fasting and low circulating IRI (1, 8, 13, 24, 26, 27). Also, E decreases acetate conversion to fatty acids (2). Furthermore, during the administration of somatotropin to lactating cows, effects of E on NEFA became enhanced (21). Endogenously produced somatotropin is usually elevated during lactation, especially when compared with levels during the dry period (14, 16, 20), which could contribute to enhanced fat mobilization.

Experiments were conducted to study a possible contribution of endogenous CA on the nightly rise of NEFA and glucose, by inhibiting β-adrenergic effects with propranolol. Possible diurnal modifications and changes caused by fasting of NEFA, glucose, and lactate responses to the administration of E were also investigated.

**MATERIALS AND METHODS**

**Experimental Procedures**

**Effects of Propranolol on Plasma Non-esterified Fatty Acids, Glucose, and Immunoreactive Insulin.** Six cows (two experiments with three animals) in their second to fourth lactations were studied 4 to 6 wk after parturition. Their milk production during 305 d of the preceding lactation was 5949 ± 291 kg, and milk yield on the day before the start of the experiments was 32.8 ± 2.0 kg/d. Animals were milked at 0515 and 1600 h.

Prior to the experiment, animals received hay and corn silage ad libitum and concentrates twice daily (at 0700 and 1700 h). From 0700 to 1900 h the animals received only hay ad libitum, which corresponded to an intake of 135 ± 3 MJ NEI and 2.84 ± 1.64 kg crude protein. Then feed was completely removed until 0700 h of the next morning.

Catheters were implanted in both jugular veins at 1800 h of the experiment. Epinephrine (.82 nmol/kg/min for 10 min) was infused starting on the 1st d at 1800 h (infusion A), on the 2nd d at 0600 h (infusion B), and on the 3rd d again at 0600 h (infusion C). Blood samples were obtained from the contralateral veins at 10.5 and .5 min before and 4, 6, 8, 10, 15, 20, 30, 45, and 60 min after the start of the 10-min infusions.

**Laboratory Procedures**

Blood samples were immediately transferred to tubes containing heparin (50 U USP/ml blood) or in ice-cold perchloric acid (1 ml/1 ml blood) and centrifuged at 4°C within 2 h after collection. Plasma aliquots of heparinized blood samples were transferred to polystyrol cups and

Infusions of the β-adrenergic agonist isoproterenol (286 pmol/kg/min from 0800 to 0805 h) were performed in addition to propranolol infusions through the same catheter.

Blood samples were obtained from the contralateral vein at 1750, 1755, and 1759 (before the start of the propranolol infusions), 1900, 2000, 2100, 2200, 2300, 2400, 0100, 0200, 0300, 0400, 0500, 0515, 0530, 0545, 0600, 0615, 0630, 0645, 0700, 0730, 0755, 0758, 0800 (before the isoproterenol infusions), 0804, 0805, 0806, 0810, and 0820 h.

**Effects of Epinephrine on Plasma Non-esterified Fatty Acids, Glucose, and Lactate in Normally Fed and Fasted Cows.** Nine cows (three experiments with three animals) in their second to fourth lactations were studied 4 to 6 wk after parturition. Their milk production during 305 d of the preceding lactation was 7424 ± 466 kg and milk yield on the day before the start of the experiments was 36.1 ± 1.2 kg/d. The animals were milked twice daily (at 0515 and 1600 h).

Prior to 0700 h of the 2nd d of the experiment animals received hay and corn silage ad libitum and concentrates twice daily (at 0700 and 1700 h). This corresponded to an intake of 135 ± 3 MJ NEI and 2.84 ± 1.64 kg crude protein. From 0700 to 1900 h the animals received only hay ad libitum, which corresponded to an intake of 39.9 ± 1.3 MJ NEI and 39 ± .13 kg crude protein. Then feed was completely removed until 0700 h of the next morning.

Catheters were implanted in both jugular veins at 1800 h of the experiment. Epinephrine (.82 nmol/kg/min for 10 min) was infused starting on the 1st d at 1800 h (infusion A), on the 2nd d at 0600 h (infusion B), and on the 3rd d again at 0600 h (infusion C). Blood samples were obtained from the contralateral veins at 10.5 and .5 min before and 4, 6, 8, 10, 15, 20, 30, 45, and 60 min after the start of the 10-min infusions.
frozen at -20°C until analyzed for E, NE, IRI, triiodothyronine (T₃), NEFA, and glucose. Deproteinized supernatants were filtered and frozen at -20°C until analyzed for the determination of acetoacetate (AAC) and l-lactate.

Concentrations of E and NE were determined by a radioenzymatic method, IRI and T₃ by radioimmunassay, and metabolites colorimetrically (9). All samples from one experiment were determined within the same assay. Control sera were measured to evaluate interassay variations.

Drugs

DL-Propranolol was obtained from Imperial Chemical Industries, Macclesfield/England; epinephrine-bitartrate from Fluka AG, Buchs/Switzerland; and isoproterenol-HCl from Winthrop Products Co., Surbiton on Thames/England. Epinephrine and isoproterenol were dissolved in .9% NaCl. Solutions of propranolol also contained 31.5 mg citric acid in 100 ml. During the experiments the solutions were kept on ice in light-protected bottles.

Statistical Analysis

Means (± SE) at various times during the infusions were compared to mean basal concentrations by paired t-test and total incremental or decremental changes after calculation of the area under the concentration curve (concentration × volume⁻¹ × min) for each individual.

RESULTS

Effects of Propranolol on Plasma Nonesterified Fatty Acids, Glucose, and Immunoreactive Insulin

Concentrations of NEFA, after a slight initial decrease (after 1 h; P<.05) continuously increased (higher than preinfusion concentrations at 2400 and later; P<.05), then rapidly decreased when animals were fed at 0700 h to values lower than those before the start of the infusions (P<.05), as shown in Figure 1. Concentrations of glucose started to decrease at 2200 h and were lower at 0100 h than preinfusion concentrations (P<.05) and rapidly increased towards preinfusion concentrations after the animals were again fed at 0700 h. Concentrations of IRI decreased during the first hours of the propranolol infusions (P<.05) and then remained low until 0700 h, after which time they rapidly increased to concentrations slightly higher than preinfusion concentrations. From the start of the experiment to 0700 h and from 0700 to 0800 h, IRI was negatively related to NEFA (r = -.88 and -.97, respectively). During infusions of isoproterenol (from 0800 to 0805 h) concentrations of IRI, NEFA, and glucose did not change.

Effects of Epinephrine on Plasma Nonesterified Fatty Acids, Glucose, and Lactic Acid in Normally Fed and Fasted Cows

Concentrations of NEFA before the three infusions increased during the experiment (P<.01) and transiently increased in response to the E infusions (Figure 2). The total incremental change (mmol · L⁻¹ · 60 min) was significantly smaller in response to the first infusion (A) than in response to the following infusions, but changes in response to infusions B and C were similar.

Concentrations of glucose were highest before the second infusion (B), lowest before the third infusion (C), and different before infusions A and B as well as B and C (P<.001). The total incremental changes (mmol · L⁻¹ · 60 min) were different from each other (P<.05) and greatest in response to infusion B and smallest in response to infusion C.

Concentrations of lactate before the infusions were similar. The total incremental changes (mmol · L⁻¹ · 60 min) were highest in response to infusion B and lowest in response to infusion C, but there were no significant differences between the three infusions.

Concentrations of E (not shown) were higher before infusion C (.60 ± .06 µmol/L) than before infusions A and B (.36 ± .04 and .43 ± .04 µmol/L, respectively) (P<.01), reached maximal concentrations between 8 and 10 min of the infusions (8.47 ± .42, 8.72 ± .48, and 8.76 ± .51 µmol/L above basal concentrations during infusions A, B, and C, respectively; P<.001), and then rapidly decreased. The total incremental changes were similar during the infusions (150 ± 8, 152 ± 9, and 155 ± 9 µmol · L⁻¹ · 60 min). Basal concentrations of NE were similar (2.1 ± .3, 1.9 ± .3, and 2.1 ± .3 µmol/L before infusions A, B, and C, re-
Figure 1. Changes of nonesterfied fatty acids, glucose and insulin in blood of lactating cows during the night in the presence of propranolol, combined with fasting, realimentation, and 5-min isoproterenol infusions.
Figure 2. Changes of nonesterified fatty acids, glucose and lactate in blood of lactating cows in response to 10-min infusions of epinephrine, starting on the 1st d at 1800 h and on the 2nd and 3rd d at 0600 h. The animals were fasted, starting after the second until the end of the third infusion.
spectively). Norepinephrine concentrations did not change significantly during the E infusion (not shown). Concentrations before infusions A, B, and C of IRI (0.67 ± .16, .26 ± .05, and .09 ± .04 μg/L, respectively) and T3 (2.76 ± .11, 2.25 ± .15, and 1.94 ± .11 nmol/L) continuously decreased and were different from each other (P<.05) (not shown).

Concentrations of (AAC) before infusion A (108 ± 28 μmol/L) were higher than before infusion B (61 ± 31 μmol/L) and highest before infusion C (188 ± 33 μmol/L) but basal AAC concentrations were different only between infusion B and C (P<.05) (not shown).

Milk yield was 36.1 ± 1.2 kg/d on the day before the start of the experiment, 36.0 ± 1.0 kg on the 1st d, 36.8 ± 1.0 kg on the 2nd d, 31.7 ± 1.4 kg on the 3rd d of the experiment, and 35.1 ± 1.1 kg on the day after the experiment (not shown).

DISCUSSION

Feed restriction during the night in experiments with propranolol simulated the normal situation, in which feed intake is markedly reduced during the night in dairy cows (9). In the present study cows were unaffected by the short time without feed, and metabolic and endocrine changes were relatively small. Feed removal starting at 0600 h for 23 h was characterized by lowered glucose, IRI, and T3 concentrations and elevated NEFA and ketone bodies, which are typical for cattle during energy restriction (10, 20). The increase of E concentrations during fasting was possibly due to hypoglycemia. Thus, insulin-induced reduction of glucose concentrations was associated with an immediate increase of E and NE concentrations in calves (7, 22), as found in other species.

Stimulation of lipolysis in cattle by CA is mediated entirely by β-adrenergic receptors on fat cells because the rise of NEFA concentrations in response to the administration of E, NE, and β-adrenergic agonists can be suppressed by β-adrenergic blockade with propranolol (8). The continuous increase of NEFA during the night in the presence of propranolol was comparable to results of a previous study performed in the absence of propranolol (9). Because propranolol could not prevent the increase of NEFA during the night indicates that enhanced lipolysis was not the consequence of an increased sympathoadrenal activity. Propranolol was infused in amounts sufficient to inhibit the typical rise of NEFA in response to the administration of the β-adrenergic agonist isoproterenol seen otherwise in the absence of β-adrenergic blockade (9). The high negative relationship between concentrations of NEFA and IRI and in particular the rapid fall of NEFA concentrations in response to the increase of IRI concentrations during refeeding suggest that insulin is important for the regulation of NEFA concentrations.

In this study, glucose concentrations decreased during the night in the presence of propranolol, suggesting that reduction of sympathoadrenal activity was responsible for this effect. However, amounts of plasma E and NE do not change significantly during the night in lactating dairy cows (9). The increase of glucose concentrations observed after the intake of concentrates in the morning was also surprising because glucose concentrations decreased in the absence of propranolol in previous studies (9). Thus, β-adrenergic blockade obviously reversed the normal pattern of glucose during the night and in response to food intake.

The greater responses of both NEFA and glucose in response to the administration of E in the morning vs. night suggested enhanced sensitivity or responsiveness of fat and liver cells to the catecholamine. Because lactate response was not changed, glycogenolysis in liver and muscle is probably modified differently by time of day. The marked diurnal variations of NEFA and glucose responses to E has to our knowledge not been previously reported. In our study, diurnal variations in sensitivity or responsiveness of target organs to E were likely modified by changes in blood concentrations of insulin, which were lower in the morning than night, thus favoring lipolysis and glycogenolysis in the morning. In lactating cows, IRI concentrations and sensitivity to insulin were lower in the morning than night and were associated with elevated glucose concentrations and reduced clearance rates of glucose in the morning (25). It is possible that other hormones, whose blood concentrations also exhibit diurnal variations as well as inherent diurnal patterns in the activity of
enzymes involved in lipo- and glycogenolysis, also contributed to changes in sensitivity to E.

Greater NEFA and glycerol responses and reduced glucose and lactate responses to E after 3 or 5 d of fasting has been reported previously in cattle (8, 13). In starvation ketosis, the increase of NEFA to the administration of E was enhanced (19). The present study shows that during fasting the time required for fat tissue to become more responsive and for liver and muscle to become less responsive to E is only about 1 d, much shorter than noted previously. The marked decrease of circulating insulin presumably favors enhanced E-stimulated lipolysis. Elevated blood somatotropin concentrations were associated with enhanced responsiveness of NEFA to E (21).

The reduced increase of glucose in response to E after 24 h of feed withdrawal was presumably in part the consequence of decreased glycogen stores in the liver. The decrease of plasma T3 concentrations during fasting probably modified the sensitivity of fat, liver, and muscle cells to E. Low thyroid hormone concentrations in hypothyroidism are usually associated with reduced lipolysis (6), which was not the case in our experiment. Because identical amounts of E were administered and since E concentrations in plasma increased in a comparable manner, amounts of E expected to interact with target organs were similar and do not explain diurnal variations or altered responses to E during fasting of glucose and NEFA.

The interesting new information from this investigation is the marked diurnal variations of NEFA and glucose responses to E. Changes in metabolic responses to E during fasting could be observed within 24 h. Thus, high yielding dairy cows rapidly adapt in response to changes in feed intake. We speculate that metabolic alterations under basal conditions are regulated by CA largely at target organs and possibly only to a minor extent by changes in sympathoadrenal activity and circulating CA concentrations. This holds in part also for other hormones (12). Changes in partitioning of nutrients in favor of the mammary gland are considered to be largely the consequence of altered responsiveness of key tissues to homeostatic signals (3).

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