Alteration of the nutrient uptake by the udder over an extended milking interval in dairy cows

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ABSTRACT

Little is known about modifications of the mammary utilization of nutrients circulating in blood plasma when milk yield is strongly decreased by once-daily milking. A trial was carried out to describe the mammary nutritional adjustments linked to the downregulation of milk synthesis as milk accumulated over an extended milking interval in the bovine udder. Three Holstein dairy cows yielding 34.0 kg/d of milk were fitted with an ultrasound flow probe around the left external pudic artery and with catheters inserted into the left carotid and milk vein to estimate mammary blood flow (MBF) and mammary uptake of acetate, β-hydroxybutyrate, nonesterified fatty acids, glycerol, glucose, O_2, and CO_2 release. The trial was carried out over 2 consecutive weeks, with wk 2 repeating wk 1. Cows were milked twice daily at 12-h milking intervals. On d 3, cows were milked at 0630 h and were not milked for 36 h until d 4 at 1830 h. Over the following days, twice-daily milking was resumed using 12-h milking intervals. Each half-udder was milked separately. Secretion rates of milk and milk proteins decreased 67% during the 12-to-36-h interval of milk accumulation, whereas that of milk fat fell 30%. Timing of changes in MBF and lactose levels in blood plasma was concomitant and significant after 19.5 and 21.5 h of milk accumulation, respectively. The MBF decreased, most likely because the usual increases in MBF no longer occurred when the udder was full of milk. After 24 h of milk accumulation, MBF did not increase further when cows lay down, and did not increase as usual 3 h after a meal, suggesting a possible physical effect of milk accumulated in the udder on MBF, complementing metabolic regulation. Mammary uptake or release of nutrients was lowered before 24 h for glucose, acetate, and β-hydroxybutyrate and after 24 h for total glycerol, O_2, and CO_2, mostly associated with the impaired MBF. However, these decreases ranged from 12 to 17%, and cannot entirely explain the −45 and −20% decreases in milk secretion rates observed during the entire 36 h of milk accumulation, thus confirming the primary role of intramammary metabolic regulation in the downregulation of milk secretion. The larger amount of nutrients taken up by the udder could explain the enhanced milk fat levels, involving a strongly modified metabolic fate of nutrients.

Key words: dairy cow, milk accumulation, mammary gland, nutrient uptake

INTRODUCTION

Among management practices that modify milk production in dairy cows, once-daily milking (**ODM**) is of interest to provide a better understanding of how the mammary gland uses nutrients circulating in blood plasma for milk synthesis. Once-daily milking strongly decreases milk yield and should modify nutrient needs of the mammary gland for milk synthesis. The amount of milk produced by dairy cows fell by an average of 20 to 30% from the first day of ODM application with slight modifications in milk composition (Davis et al., 1999; Rémond and Pomiès, 2005). Some of the changes in milk composition are related to the induced disruption of the mammary epithelium, leading to increased exchange between alveolar and blood plasma compartments. Lactose content decreased in milk and increased in blood plasma (Stelwagen et al., 1994a; Stelwagen et al., 1998). Similarly, protein content could increase in milk due to increases in whey proteins such as serum albumin (Delamaire and Guinard-Flament, 2006b; Guinard-Flament et al., 2007a). In contrast, some milk composition changes, such as for milk fat, could be related to different regulatory mechanisms governing the aqueous phase of milk and ultimately increasing milk fat content (Davis et al., 1999; Rémond and Pomiès, 2005).

Several experiments have aimed to explain milk yield and composition changes in response to ODM by studying nutrient flow to the udder and nutrient extraction by the udder (Delamaire and Guinard-Flament, 2006a,b; Guinard-Flament et al., 2007a). However, the measurements have all been carried out after 6 d of

ODM when the mammary gland has adapted to its new milking frequency, and have not made it possible to determine the mammary adjustments linked to the downregulation of milk synthesis. Studies examining the short-term effects of intramammary milk accumulation have mostly only described blood supply to the udder (Stelwagen et al., 1994b; Farr et al., 2000), rarely tackling nutrients and mammary blood flow (**MBF**; Kuhn and Linzell, 1970; Fleet and Peaker, 1978), and only a few nutrients have been assessed to understand the nutritional mechanisms that lead to decreased milk production (glucose, Kuhn and Linzell, 1970; glucose, acetate, lactate, and oxygen, Fleet and Peaker, 1978). In addition, these studies were performed on goats, which showed only moderate changes in milk yield compared with dairy cows (Komara et al., 2009). In the first 24 h after cessation of milking in goats, no significant changes in milk secretion and MBF were observed (Fleet and Peaker, 1978). In contrast, and also in goats, Farr et al. (2000) reported lower MBF after 26 to 28 h of intramammary milk accumulation, whereas Stelwagen et al. (1994b) demonstrated that MBF began to decrease after 21 h of milk accumulation (i.e., roughly the same time that mammary epithelium became disrupted and milk secretion began to decrease). After d 1 of cessation of milking, the glucose and acetate uptakes fell in goats due to decreases in both MBF and arteriovenous differences (**AVD**) in concentrations, whereas the fall in oxygen consumption was related to the decrease in MBF alone (Fleet and Peaker, 1978).

Thus, little information is available on changes in mammary uptakes of nutrients circulating in the blood plasma as milk accumulates in the udder of dairy cows. In addition to the availability of nutrients in the arterial pool, mammary net uptakes of nutrients depend on metabolic activity within the udder and on MBF, both modified during ODM. The objective of the present study was to characterize the acute changes in mammary uptakes of the major precursors of milk fat and lactose synthesis (acetate, BHBA, NEFA, glycerol, and glucose) and mammary oxidative metabolism (O_2 and CO_2) during 36-h intramammary milk accumulation. To better understand MBF variations, the timing of changes in MBF and mammary epithelium permeability was determined, and variations in MBF were examined according to the cow's position (supine or upright) as milk accumulates in the udder.

MATERIALS AND METHODS

Cow Management and Experimental Design

Three Holstein dairy cows averaging 632 ± 39 kg of BW and 141 ± 2 DIM and yielding 34.0 ± 2.2 kg/d of milk at the start of the experiment were used according to National legislation on animal care (A35802). The cows were in second or third lactation, and were surgically prepared 43 ± 3 d after calving to study the mammary uptake of nutrients in the left half-udder (Guinard et al., 1994; Delamaire and Guinard-Flament, 2006a,b). Venal blood was sampled from the subcutaneous abdominal vein with no occlusion of the pudic and perineal veins, as it is technically very difficult to occlude these two veins. Two permanent catheters were inserted into the left carotid and subcutaneous abdominal vein. An ultrasound flow probe (Probe 20 S, 20-mm i.d., cable length: 2.5 m; Transonic Systems Inc., Ithaca, NY) was implanted around the left external pudic artery before the S-shaped bend in the artery. The flow probe cable was protected with Silastic tubing (Silclear medical-grade silicone tubing, 3-mm i.d., 6-mm o.d.; VWR International SAS, Briare, France). Two Dacron rings (Mersutures, TS53; Ethicon SAS, Issy-Les-Moulineaux, France) were placed along the cable and catheters and at the level of exteriorization to prevent any spread of infection.

The cows were housed indoors in individual stalls and fed a diet of 60% corn silage, 20% energy concentrate (containing 22% barley, 21.5% wheat, 20% beet pulp, 17% fine wheat bran, 15% soybean meal, 2% molasses, and 2.5% minerals, all on a DM basis), 10% soybean meal, and 10% dehydrated alfalfa on a DM basis. The diet was calculated to meet the energy and protein requirements of cows milked twice daily (Institut National de la Recherche Agronomique, 1989). The cows were fed twice daily throughout the experiment and had free access to food for 8 h after each allocation (from 0630 to 1430 h and from 1830 to 0230 h the next day).

The experiment was carried out during 2 consecutive weeks, with wk 2 repeating wk 1. Cows were milked twice daily at 12-h milking intervals (0630 and 1830 h). On d 3, cows were milked at 0630 h and were not milked for 36 h until d 4 at 1830 h. Over the following days, the cows were milked twice daily at 0630 and 1830 h. Each half-udder was milked separately during the experiment. No mastitis was observed. The SCC were <400 × 10³/mL on the 2 d before the 36-h milking intervals.

Sampling and Analyses

Milk. Milk yield was recorded per half-udder at each milking. Every day, except the seventh day of each experiment week, the fat and protein contents and SCC of each half-udder were determined for each milking by infrared analysis (Milkoscan; Foss Electric A/S, Hillerød, Denmark). On d 3 and 4, 2 L of milk, representative

of the milking of the left half-udder, was collected and stored at either 4°C for nitrogen analysis or −20°C for subsequent milk composition analysis. The milk was analyzed for lactose using a colorimetric enzymatic reaction (lactose/D-galactose kit; Roche, Meylan, France) on a multiparameter analyzer (Kone Instruments Corp., Espoo, Finland), total nitrogen using the Kjeldahl procedure (Kjeldahl, 1883), and CN (precipitation at pH 4.6 with 10% acetic acid and 1 M sodium acetate). Milk fatty acids were extracted to determine the percentages of short-, medium-, and long-chain fatty acids, according to the method described by Bauchard and Duboisset (1983), using 0.5 mL of an ethanol/HCl (100:5 vol/vol) solution, followed by 5 mL of hexane. Briefly, milk fatty acids were transesterified with 1 mL of a methanol-NaOH solution (100/2, vol/wt), followed by 0.5 mL methanol/boron trifluoride (100/20, vol/vol) and 2 mL of hexane. Fatty acid methyl esters were then injected into a gas chromatograph (Varian 3400; Varian Inc., Les Ulis, France) equipped with an electron ionization detector. The carrier gas was helium. The oven temperature was increased from 70 to 220°C at 100°C/min and held for 32 min. The injector and detector temperatures were 220 and 250°C, respectively.

To estimate mammary epithelial permeability, milk Na^+ and K^+ concentrations were assayed using flame spectrometry (SpectraAA-20; Varian Inc.). The milk BSA concentration was measured using a radial immunodiffusion assay (rabbit anti-BSA antiserum; IDBiotech, St-Genès-Champanelle, France; Levieux, 1991).

Mammary Blood Flow. The MBF monitoring was continuous throughout the experiment. The sampling rate of the flowmeters (T208D; Transonic Systems Inc., Ithaca, NY) was fixed at 200 Hz. The MBF and heart rate were averaged every minute and recorded using IOX software (emka Technologies SA, Paris, France). The cows were also fitted with a position-monitoring sensor to evaluate MBF in supine or upright position, as MBF varies according to the animal position.

Blood. Blood samplings were carried out only during the 36-h milking intervals. To obtain representative mammary venous blood, cows were sampled while they were standing to ensure as much venal blood as possible was leaving the udder via the subcutaneous abdominal vein (Delamaire and Guinard-Flament, 2006b). Blood samplings were carried out at 0.5, 1.5, 3.0, 5.5, 8.5, 11.5, 12.5, 13.5, 15.0, 17.5, 19.5, 21.5, 23.5, 24.5, 25.5, 27, 29.5, 31.5, 33.5, and 35.5 h after the d-3 milking. Blood samples were simultaneously collected from the carotid and subcutaneous abdominal vein using heparinized syringes (7.5 mL) and special blood gas heparinized syringes (2 mL, S-Monovette; Sarstedt AG & Co., Nümbrecht, Germany). Concentrations of O_2 and CO_2 in blood were determined within 30 min after collection on a gas analyzer (ABL 625; Radiometer Copenhagen A/S, Brønshøj, Denmark). Other 7.5-mL blood samples were kept on ice until centrifugation at 2,500 × g for 10 min at 4°C. Plasma was stored at −20°C for subsequent determination of mammary epithelial permeability (lactose in venous plasma) and mammary nutrient uptake (glucose, acetate, BHBA, NEFA, and total glycerol in arterial and venous plasma) based on colorimetric reactions with a multiparameter analyzer (Kone Instruments Corp.). Enzymatic kits were used for lactose (β-galactosidase, β-galactose dehydrogenase; lactose/D-galactose kit; Roche), glucose (hexokinase, glucose-6-phosphate dehydrogenase; glucose hexokinase kit; Roche), NEFA [acetyl-coenzyme A (**CoA**) synthetase, acyl-CoA oxidase, peroxidase method; Wako kit; Oxoid S.A., Dardilly, France], total glycerol (lipase, glycerol kinase, glycerol-3-phosphate oxidase, peroxidase method; Biotrol Diagnostic SA, Chennevières-lès-Louvres, France), and BHBA (3-HB-dehydrogenase method; 3-D-hydroxybutyrate kit; Randox Laboratories Ltd., Crumlin, UK). Deproteinized plasma (with 50%, vol/vol perchloric acid by filtration) was used to measure acetate concentrations (acetyl-CoA synthetase, citrate synthetase, malate dehydrogenase method; Sigma, Saint-Quentin-Fallavier, France).

Calculations and Statistical Analyses

The 36-h milking interval was repeated twice on the 3 cows. However, data from one cow was discarded in wk 2 due to a failure of the blood flow cable (n = 5). Nutrients, O_2 and CO_2 data were plotted using AVD or venoarterial differences (**VAD**) of concentrations, extraction rates (AVD/arterial concentration × 100), arterial flow [arterial concentration × MBF or mammary plasma flow (**MPF**)] and mammary uptake or output (AVD or VAD × MBF or MPF). To describe mammary kinetics, MBF was averaged over 1 h (30 min before and 30 min after blood sampling), and MPF was calculated by correcting the MBF for hematocrit value. The MPF was only used for nutrients, not for O_2 or CO_2. Blood data were also divided into three 12-h periods to analyze variations between periods. The area under the curve was determined for the 3 periods using the trapezoidal rule because of non-equidistant sampling, except for MBF, which was recorded every minute. The timing of changes in MBF was determined using the 1-h averaged MBF recorded during the 36-h intramammary milk accumulation compared with the MBF recorded over 36 h on d 1 and 2 of the week when cows were milked twice daily at a 12-h milking interval.

Milk and 12-h period blood data were analyzed using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC). The statistical model included milk

accumulation period for milk or 12-h period for blood as fixed effects with repetition as a random effect to account for repeated measurements for each cow. The timing of changes in MBF and plasma lactose levels was analyzed on averaged data using the MIXED procedure considering kinetic time as a repeated measure. Results were expressed as least squares means with standard error of the mean. The threshold of significance was set at $P \leq 0.05$ and trends were noted at $P \leq 0.10$.

RESULTS

Milk

Milk accumulation in the udder reduced secretion rates for milk, milk fat and milk proteins. Comparing the 12 to 36-h interval of milk accumulation to the 0 to 12-h interval, secretion rates of milk, fat, and proteins were decreased by 0.96 kg/h, 18.3 g/h, and 30.2 g/h (i.e., −67%, −30%, and −68%, respectively; Table 1). Milk fat content and SCS increased strongly during the 12-to-36-h accumulation interval (+6.22 percentage points and +3.3 units, i.e., +152% and +118%, respectively), whereas milk protein content remained unchanged.

Similar variations were found for milk of the left-half udder (Table 2). The decrease in milk fat secretion rate was half the decrease in secretion rates of total milk and milk proteins (−32 versus −67 and −68%, respectively). Milk fat content increased by 7.75 percentage points with slight modifications in fatty acid composition. The C16 and C4-to-C10 fatty acid contents decreased or tended to decrease to the benefit of C18 fatty acids (+3.9 percentage points). The saturated fatty acid content decreased 3 percentage points. Milk lactose content did not vary significantly, but was numerically lower (−1.45 percentage point; $P = 0.132$). The ratio and contents of milk protein, CN, and whey protein did not change.

Indicators of Mammary Epithelium Permeability

In response to 36-h intramammary milk accumulation, the milk BSA content increased 48% (Table 3). The milk Na^+ content tended to increase, whereas the milk K^+ content remained unchanged. The milk Na^+-to-K^+ ratio did not vary significantly, although being numerically higher in milk collected after 36-h milk accumulation ($P = 0.103$). Plasma lactose concentrations rose significantly after 21 h of intramammary milk accumulation (Figure 1).

Mammary Blood Flow

The MBF started to decrease from the second 12-h period of milk accumulation and decreased further over the third 12-h period (−0.44 and −1.38 L/min compared with the first 12-h period, respectively; Table 4). To determine more precisely the time of change in MBF in response to milk accumulation, the MBF measured over the 36-h milk accumulation was compared with the MBF measured over 36 h on d 1 and 2 as cows were milked at 12-h milking intervals (Figure 2). The MBF decreased after 19 h of milk accumulation in the udder. This decrease tended to be greater when cows were lying instead of standing (−1.27 vs. −0.53 L/min, respectively; SEM = 0.206; $P = 0.066$). The time spent in the upright position ranged between 60 and 73%, and did not vary significantly. Heart rates only tended to be higher during the night.

Mammary Nutrient Utilization

No variation in arterial concentrations of nutrients occurred, except an increase for glucose and total glycerol (Table 5), which were greater in the third 12-h period of milk accumulation. Arteriovenous differences of concentrations did not vary significantly between periods for any nutrient. Extraction rates decreased for glucose, total glycerol, and acetate but not for BHBA. Extrac-

Table 1. Effect of milk accumulation in the udder on milk secretion rate and milk composition in dairy cows

Item	Accumulation period		SEM	Effect (*P*-value)
	0–12 h[1]	12–36 h[2]		
Milk (kg/h)	1.44	0.48	0.062	0.009
Milk fat (g/h)	61.5	43.2	3.37	0.038
Milk protein (g/h)	44.3	14.1	2.16	0.009
Fat content (%)	4.09	10.31	1.104	0.055
Protein content (%)	3.16	3.02	0.169	NS
SCS	2.8	6.1	0.37	0.035

[1]Milk collected after a 12-h milking interval.

[2]Milk produced from 12 to 36 h post-milking (determined by calculating the difference between the 36-h milk and the 12-h milk).

Table 2. Effect of milk accumulation in the udder on milk composition from the left-half udder in dairy cows

Item	Accumulation period		SEM	Accumulation effect (P-value)
	0–12 h[1]	12–36 h[2]		
Milk secretion rate (kg/h)	0.69	0.23	0.037	0.018
Milk fat				
g/h	29.1	19.7	2.80	0.046
%	3.87	11.62	2.025	0.106
Milk fatty acids				
C4 to C10 (%)	18.0	16.9	0.93	0.065
C14 (%)	13.6	13.1	0.31	NS
C16 (%)	37.2	34.9	1.53	0.034
C18 (%)	29.7	33.6	0.66	0.029
Saturated fatty acids (%)	75.4	72.4	0.64	0.014
Milk lactose				
g/h	34.3	8.3	2.14	0.015
%	4.90	3.45	0.306	NS
Milk protein				
g/h	21.6	6.9	1.42	0.022
%	3.08	2.97	0.094	NS
CN				
g/h	17.5	5.3	1.33	0.023
%	2.54	2.32	0.112	NS
Whey protein				
g/h	4.0	1.5	0.25	0.025
%	0.62	0.72	0.104	NS
Ratio				
Milk CN/protein	0.80	0.77	0.031	NS
Whey protein/CN	0.26	0.31	0.052	NS

[1]Milk collected after a 12-h milking interval.

[2]Milk produced from 12 to 36 h post-milking (determined by calculating the difference between the 36-h milk and the 12-h milk).

tion rates were altered for glucose and total glycerol in the third 12-h period (−7.5 and −9.6 percentage points between the first and third periods, respectively) due to higher arterial concentrations and numerically lower AVD. It was decreased for acetate from the second 12-h period (−7 percentage points) due to numerically lesser AVD. Arterial flow did not vary between periods for glucose and total glycerol, and decreased or tended to decrease over the third 12-h period for acetate, NEFA, and BHBA ($P = 0.1051$) in a similar range of variation to MBF (by about −27 vs. −25%, respectively). Mammary nutrient uptake (except NEFA) decreased as milk accumulated in the udder. It decreased or tended to decrease from the second 12-h period for glucose, acetate, and BHBA (by −15, −12, and −18%, respec-

tively), whereas the only decrease noted at the third 12-h period concerned total glycerol.

Oxidative Mammary Metabolism

The arterial concentration, AVD, and extraction rates of O_2 did not change between periods in response to milk accumulation (Table 6). Mammary arterial flow of O_2 decreased from the second 12-h period and decreased strongly during the third 12-h period, whereas mammary O_2 uptake decreased only from the third 12-h period. The arterial concentration and VAD of CO_2 did not vary between periods. However, CO_2 output by the udder tended to decrease in the third 12-h period. The respiratory quotient remained unchanged,

Table 3. Effect of milk accumulation in the udder on indicators of mammary epithelium integrity in dairy cows (from the left-half udder milk)

Item	Accumulation period		SEM	Accumulation effect (P-value)
	0–12 h	0–36 h		
BSA (mg/L)	200	295	19.2	0.031
Na^+ (mM)	13.7	23.3	3.44	0.089
K^+ (mM)	51.7	47.5	4.92	NS
Ratio Na^+/K^+	0.279	0.524	0.1306	NS

Table 4. Effect of milk accumulation in the udder over 36 h on mammary blood flow (MBF) according to position (upright versus supine) of dairy cows

Item	Accumulation period			SEM	Period effect (*P*-value)
	0–12 h	12–24 h	24–36 h		
MBF (L/min)	5.50[a]	5.06[b]	4.12[c]	1.052	0.001
MBF in upright position (L/min)	5.20[a]	4.98[a]	4.20[b]	1.032	0.005
MBF in supine position (L/min)	5.93[a]	5.20[b]	3.95[c]	1.129	0.003
Relative time in upright position (%)	60	72	73	4.7	NS
Heart rate (beats/min)	67.6[b]	73.0[a]	67.3[b]	2.82	0.059

[a–c]Within each row, values with different superscript letters differ at $P < 0.05$.

despite being numerically lower in the third 12-h period (−0.14 units).

DISCUSSION

To gain deeper insights into the mammary utilization of nutrients circulating in blood plasma for the synthesis and secretion of milk compounds in response to ODM, the present study was designed to describe the mammary nutritional adjustments linked to the downregulation of milk synthesis as milk accumulated in the udder. We elected to study mammary nutrient uptake over a single time interval rather than several successive intervals to describe the activation of mammary adaptive mechanisms and pinpoint the key points of mammary use of nutrients. The trial was repeated a second time, but after a week-long interval to eliminate any residual effects of the first repetition (Wheelock et al., 1966). The time interval studied was 36 h, which runs over the 24-h interval for ODM to amplify the peak of the phenomena measured and partly resolve the limited statistical power of the experimental set-up due to the small number of animals used (n = 3) and the data losses caused by the breakage of a flowmeter probe in one animal.

Timing of Changes in Mammary Epithelium Permeability and MBF

The effects of 36-h intramammary milk accumulation on milk yield and composition were larger but in agreement with previous reports on cows switched to ODM
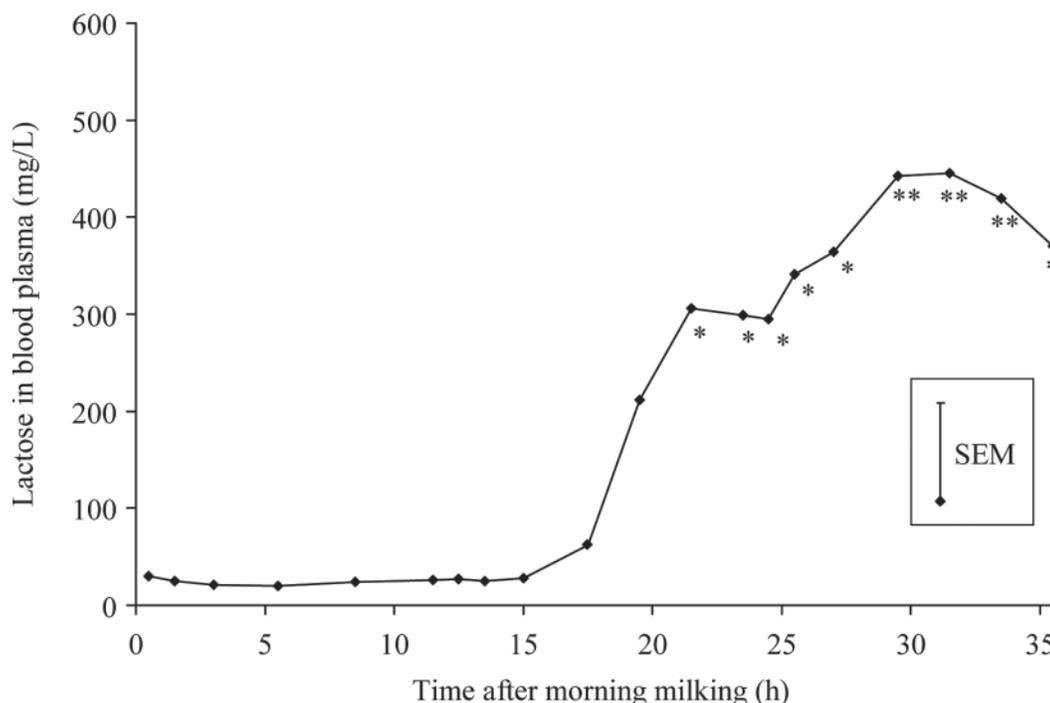
Figure 1. Lactose concentrations in blood plasma during 36-h milk accumulation in the udder in dairy cows. ** and * = significantly different from lactose at time 0.5, 1.5, 3, 5.5, 8.5, 11.5, 12.5, 13.5, 15, and 17.5 h after morning milking, at $P < 0.01$ and 0.05, respectively.
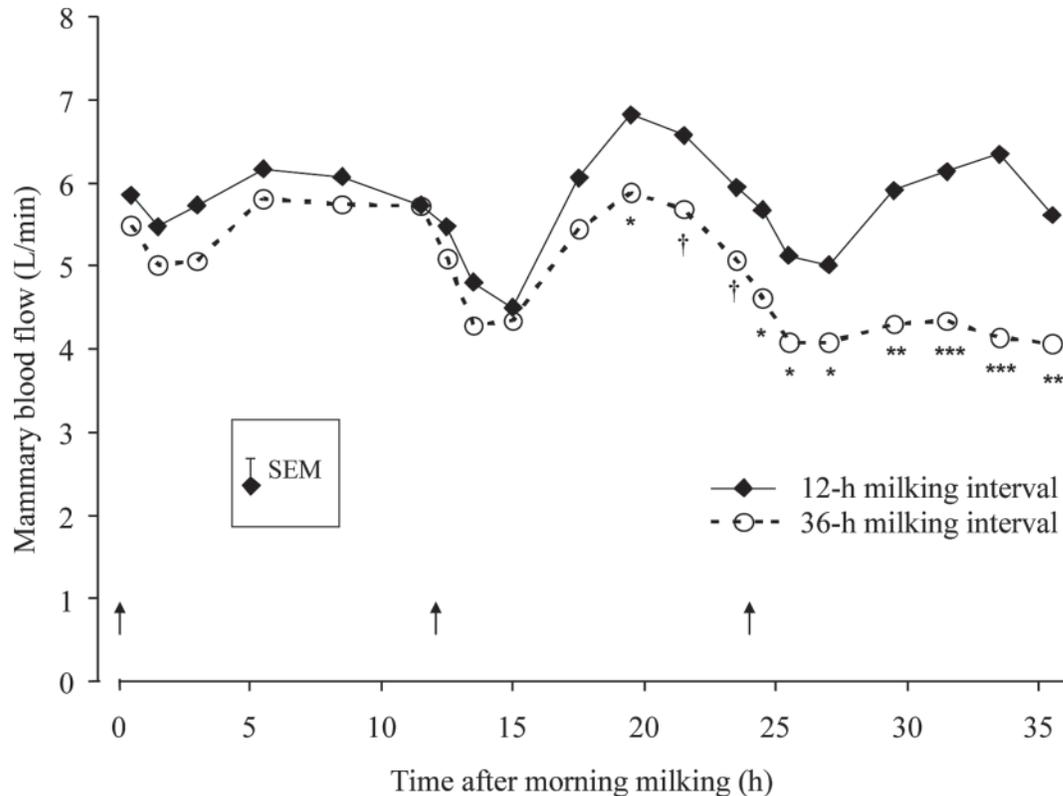
Figure 2. Mammary blood flow over 36 h in dairy cows milked every 12 h or after a 36-h milking interval. ***, **, *, and † = significantly different at $P < 0.001$, 0.01, 0.05, and 0.10, respectively. Vertical arrows (↑) indicate time of feed distribution.

in short-term trials (Delamaire and Guinard-Flament, 2006b; Guinard-Flament et al., 2007a). Milk accumulation in the udder led to major and proportional decrease in milk and milk protein secretion rates that ranged 67 to 68% during the 12-to-36-h interval (i.e., 45% when calculated over the entire 36-h milk accumulation). Such variations in milk and milk protein secretion rates have already been described in dairy cows (roughly −35%; Wheelock et al., 1966) and goats (−48 and −46%, respectively; Ben Chedly et al., 2009). Milk fat secretion rate was affected less (−30% during the 12-to-36-h interval), leading to an increase in milk fat content associated with slight increases in the proportion of C18 and unsaturated fatty acids. In the present study, no measurement of residual milk was performed after milking, which impairs the quality of the estimation of milk fat yield. However, similar variation in milk fat content in dairy cows after a 36-h period of milk accumulation was observed when removing residual milk at each milking by giving an injection of 20 IU of oxytocin (Wheelock et al., 1966). The increase in milk fat content observed in the present study (+2.1 percentage points when calculated over the entire 36-h milk accumulation) was greater but remained consistent with

the +1.3 percentage points reported by Wheelock et al. (1966).

When extended milking intervals were applied just once, the literature reports constant milk secretion rates up to 12 h or even 16 h of milk accumulation in the udder (Wheelock et al., 1966; Davis et al., 1998; Stelwagen et al., 2008) that subsequently decrease (−16 and −21% at 18 and 24 h of milk accumulation, respectively; Stelwagen et al., 2008). After 16 h, continued milk accumulation distends the udder, causing many effects. The cause-effect relationships have not been clearly established, but the rate of milk secretion begins to decrease as mammary epithelium permeability increases with lactose leaking into the blood compartment (Stelwagen et al., 1997; Stelwagen, 2001). Our findings on plasma lactose, which indicate changes in mammary epithelium leakiness, are consistent with the above studies. In the present study, plasma lactose increased significantly from 21.5 h of milk accumulation in the udder versus 21 h in goats and 18 h in dairy cows (Stelwagen et al., 1994b and 1997, respectively).

As previously observed in goats (Stelwagen et al., 1994b), here, in cows, the effects of milk accumulation in the udder on plasma lactose and MBF proved con-

Table 5. Time-course evolution in arterial concentrations, arteriovenous differences (AVD) of concentrations, extraction rates, arterial flows, and mammary uptake of glucose, acetate, BHBA, total glycerol, and NEFA during 36-h milk accumulation in the udder in dairy cows

Item	Accumulation period			SEM	Period effect (P-value)
	0–12 h	12–24 h	24–36 h		
Plasma flow (L/min)	3.81[a]	3.54[b]	2.86[c]	0.753	0.001
Glucose					
Arterial concentration (mM)	3.37[b]	3.41[b]	4.23[a]	0.089	0.002
AVD (mM)	0.95	0.88	0.86	0.038	NS
Extraction rate (%)	28.1[a]	26.2[a]	20.6[b]	0.74	0.007
Arterial flow (mmol/h)	759	718	736	162.3	NS
Uptake (mmol/h)	211[a]	180[b]	148[c]	37.2	0.004
Total glycerol					
Arterial concentration (μM)	75.1[b]	81.5[b]	96.6[a]	4.92	0.027
AVD (μM)	27.5	26.6	25.3	1.65	NS
Extraction rate (%)	35.9[a]	32.6[a]	26.3[b]	2.05	0.019
Arterial flow (mmol/h)	17.0	17.2	16.4	3.49	NS
Uptake (mmol/h)	6.18[a]	5.65[a]	4.38[b]	0.853	0.034
Acetate					
Arterial concentration (mM)	2.06	2.22	1.98	0.106	NS
AVD (mM)	1.51	1.46	1.25	0.062	NS
Extraction rate (%)	74.6[a]	67.6[b]	65.4[b]	2.29	0.049
Arterial flow (mmol/h)	475[a]	464[a]	335[b]	77.8	0.030
Uptake (mmol/h)	353[a,a]	309[a,b]	218[b,c]	55.9	0.007
BHBA					
Arterial concentration (mM)	1.19	1.34	1.21	0.118	NS
AVD (mM)	0.54	0.48	0.49	0.002	NS
Extraction rate (%)	46.3	39.2	42.2	3.17	NS
Arterial flow (mmol/h)	278	292	203	79.8	NS
Uptake (mmol/h)	122[a,a]	100[ab,b]	82[b,b]	22.3	0.046
NEFA					
Arterial concentration (μM)	84.8	85.4	81.4	4.71	NS
AVD (μM)	−10.7	−13.5	−14.8	2.74	NS
Arterial flow (mmol/h)	19.7[a]	18.4[a]	14.5[b]	4.52	0.021
Uptake (mmol/h)	−2.5	−2.9	−2.4	0.85	NS

[a–c]Within each row, values with different superscript letters differ at $P < 0.05$ (in italics when period effect ≤0.10).

comitant. We found that MBF was significantly lower after 19.5 h of milk accumulation in the udder. The nadir of this effect was not as low as described previously: after 36 h of milk accumulation, MBF fell to 74% of initial MBF versus 50% in goats (Stelwagen et al., 1994b). However, 3 hypotheses explain the fall in MBF. It could result from a decrease in the metabolic activity of mammary epithelial cells via a local self-regulatory mechanism or from a compression of the blood vessels by milk accumulated in the udder, or a

Table 6. Time-course evolution in arterial concentrations, arteriovenous differences (AVD) of concentrations, extraction rates, arterial flows, and mammary uptake or output of oxygen and carbon dioxide during 36-h milk accumulation in the udder in dairy cows

Item	Accumulation period			SEM	Period effect (P-value)
	0–12 h	12–24 h	24–36 h		
Oxygen					
Arterial concentration (mM)	6.03	6.01	6.15	0.093	NS
AVD (mM)	1.90	1.87	1.80	0.103	NS
Extraction rate (%)	31.7	31.2	29.3	1.93	NS
Arterial flow (mol/h)	1.98[a]	1.83[b]	1.53[c]	0.398	0.004
Uptake (mol/h)	0.60[a]	0.55[a]	0.44[b]	0.098	0.022
Carbon dioxide					
Arterial concentration (mM)	25.3	24.8	25.8	0.42	NS
VAD[1] (mM)	2.73	2.81	2.45	0.098	NS
Output (mol/h)	0.90[a]	0.83[a]	0.60[b]	0.178	0.069
Respiratory quotient	1.50	1.48	1.36	0.083	NS

[a–c]Within each row, values with different superscript letters differ at $P < 0.05$ (in italics: $P < 0.10$).

[1]Venoarterial differences of concentrations.

combination of both. To date, studies have tended to describe the decrease in MBF as resulting from altered mammary metabolic activity (Peaker, 1980; Stelwagen et al., 1994b; Farr et al., 2000), prompted by the fact that MBF does not return to initial levels immediately after the udder is emptied (Stelwagen et al., 1994b; Farr et al., 2000) and because no decrease in MBF occurred when infusing an isosmotic sucrose solution into the lumen to increase intramammary pressure within a physiological range in goats (Peaker, 1980). However, our results argue for the possibility of a physical effect of milk accumulated in the udder on MBF, which would not lead to a decrease in MBF but would prevent its increase.

The arguments for a physical effect are based on several observations. First, the decrease in MBF occurs concomitantly with the impairment of the mammary epithelial permeability, which itself could result from the increase in intramammary pressure caused by milk accumulated in the udder (Stelwagen, 2001). Furthermore, Farr et al. (2000) showed that 26- to 28-h milk stasis in the udder triggered a decrease in the number of capillaries perfused, suggesting blood capillary de-recruitment. This capillary de-recruitment could be due to their compression induced by milk accumulated. Second, the decrease in MBF observed in this trial is partly explained by the fact that usual increases in MBF no longer occurred when the milk had heavily accumulated in the udder. This fact can be illustrated in 2 different ways: (1) as a rule, MBF decreases in the 3 h following a meal, maybe as a response to blood flow being rechanneled toward splanchnic tissues to support digestive and absorption functions, after which it increases again. This increase, which would have been expected with metabolic regulation, although being lower, no longer occurred between 24 and 36 h of milk accumulation; and (2) in animals milked twice daily, the MBF is higher when the animals are lying down than when they are standing. Here, we found that supine MBF turned out to be significantly higher than upright MBF, averaging 0.72 L/min higher over the first 12 h of milk accumulation. As the milk progressively accumulates, this pattern becomes less clear, or is even reversed. After 12 h of milk accumulation, MBF measurements were no longer significantly different between supine and upright positions, and by the 24-to-36-h period, supine MBF had become lower in numerical terms than upright MBF. However, even if these observations suggest a physical effect of milk accumulated in the udder on MBF, this might not be a regulatory driver of milk secretion in response to milk accumulation in the udder. This physical effect could coexist alongside metabolic regulation of MBF, as a response to the decreased metabolic activity of the udder.

Decreased Intramammary Metabolic Activity

In this study, downregulation of mammary metabolic activity was much stronger than the downregulation on mammary nutrient uptake, thus demonstrating the key role of decreased mammary metabolic activity in the decrease of milk secretion. Assuming that measurements performed over the first 12 h of milk accumulation provide a sound basis for estimating quantitative nutrient uptake in animals milked every 12 h over 36 h (data multiplied by 3), then the total quantity of nutrient uptake over 36 h of milk accumulation decreased 12 to 17%, depending on the target nutrient, whereas the total milk and milk protein secretion rates decreased 45% over the entire 36-h milk accumulation. The experiment reported here did not study the metabolic activity of mammary epithelial cells per se, but this activity can still be gauged from indicators of nutrient uptake. A decrease in AVD or extraction rate without changes in arterial nutrient concentrations indicates changes in mammary metabolic activity, the udder adjusting its extraction efficiency according to its nutrient requirements (Miller et al., 1991; Guinard and Rulquin, 1994). Arteriovenous differences, measured in this study, did not vary significantly but extraction rates showed that mammary metabolic activity decreased before 24 h of milk accumulation in the udder. Indeed, the acetate extraction rate decreased between 12 and 24 h of milk accumulation in the udder. This decreased metabolic activity was associated with lower mammary uptake or release of nutrients mostly due to the impaired MBF. The quantitative decrease in nutrient uptake was observed for glucose, acetate, and BHBA between 12 and 24 h of milk accumulation and for all nutrients except NEFA between 24 and 36 h. Similarly, the same variations were found, but on the second day after milking was stopped in goats (Kuhn and Linzell, 1970; Fleet and Peaker, 1978).

Very few studies have investigated the decreased metabolic activity of mammary epithelial cells during the acute-phase regulatory response to milk accumulation in the udder of dairy ruminants. To the best of our knowledge, this metabolic activity has essentially only been described in time-course trials designed to highlight cell remodeling and apoptosis effects in mammary tissue during milk accumulation in the udder (Singh et al., 2005, 2008; Ben Chedly et al., 2009). In these trials, the indicator used to describe metabolic activity was the expression levels of specific milk protein genes, and more rarely protein levels, whereas enzyme activity has never yet, at least to our knowledge, been used. Gene expression analysis shows that mRNA expression of milk protein genes is quickly regulated, reportedly decreasing within at least 24 h of milk accumulation in

dairy cows for CN αs1 and α-LA and 36 h for β- and κ-CN (Singh et al., 2008). Similar results have been reported in goats for α-LA and κ-CN on mammary tissue biopsies sampled after 36 h of milk accumulation (Ben Chedly et al., 2009). However, to date, no results have been reported for the proteins involved in milk fat synthesis.

Less Downregulated Milk Fat Yield

The strong increase in milk fat content found in this study is probably due to milk fat synthesis being affected less compared with lactose and proteins. Indeed, it seems unlikely that fat synthesis was decreased to the same extent, and accumulated in the udder because of the large size of fat globules such that they could not pass through the mammary epithelium. No increase in milk CN content or tendency was observed here. This would have been expected because of their micellar structure.

The strong increase in fat content could be due to a lower decrease in the number or activity, or both, of enzymes responsible for fat synthesis compared with enzymes involved in synthesis of other milk components. Our data do not provide information about milk secretion throughout the extended interval and the timing of inhibition of secretion of fat and aqueous phases. However, with regard to uptake of nutrients by the udder, which is fairly related to milk component synthesis and secretion, glucose, acetate, and BHBA began to decrease in the same 12-to-24-h period of milk accumulation. Because acetate and BHBA are both precursors for milk short- and medium-chain fatty acids, this suggests that the decrease in milk fat secretion could coincide with the decrease in the aqueous phase. Therefore, milk fat synthesis could be simultaneously decreased but could be relatively less affected than the aqueous phase in the course of milk accumulation, leading to a progressive exacerbated fat concentration in milk.

As a result, this was associated with a modified partitioning of glucose between mammary metabolic pathways. The ratio number of moles of lactose C exported in milk-to-number of moles of glucose C taken up by the udder fell from 90% over the 12-h interval to 53% over the 36-h interval ($P = 0.0321$). However, using lactose content measured on 36-h milk to estimate this ratio leads to an underestimation of mammary lactose secretion because of lactose leakage into the blood compartment. Correcting this 36-h lactose content by assigning the 12-h-interval lactose content still highlights a decrease, falling from 90 to 58% ($P = 0.0499$). This decrease in the proportion of glucose taken up and converted to lactose is large and this is the first study

to report such variations in the potential contribution of glucose to lactose supply. This large variation could illustrate a transient response corresponding to the acute-phase adaptation of metabolic activity in mammary epithelial cells in response to a highly extended milking interval. Because the glucose taken up cannot participate in lactose synthesis, it could potentially be oxidized or converted to glycerol and subsequently incorporated in the milk triglycerides. Given the important increase in milk fat yield found in this trial, the hypothesis of a strongly enhanced conversion toward glycerol synthesis appears highly likely. Guinard-Flament et al. (2007b) previously reported improved glucose-to-glycerol conversion in response to extending milking interval. This improvement, estimated after 6 d of treatment, remained mild, only increasing from 6.5 to 8.8% between cows milked every 8 and 24 h, respectively. In the same way, a greater conversion of acetate and BHBA uptake toward short- and medium-chain fatty acid synthesis was reported (Delamaire and Guinard-Flament, 2006a; Guinard-Flament et al., 2007b).

The biological significance of the strong increase in milk fat content warrants further investigation to better understand the driving force of milk fat modifications. Indeed, it could be interpreted as an udder adaptation designed to curtail the decrease in milk energy value for newborns while milk production is significantly reduced (Davis et al., 1999). If true, this suggests that this metabolic pathway has priority in the udder. In another way, from a nutritional point of view, lower inhibition of fat synthesis enabled utilization of the energy made freshly available by the decrease in milk secretion and milk proteins, suggesting that milk fat synthesis is a buffer metabolic pathway able to absorb lack or excess of energy supplied to the udder.

CONCLUSIONS

In the present study, the timing of changes in the permeability of the mammary epithelium and in MBF were concomitant, and occurred at the time known to mark the point when milk secretion rate began to fall in response to milk accumulation in the udder. These changes could result from the increased intramammary pressure, making MBF unable to increase as it used to, because of blood vessel compression by the milk accumulated in the udder. However, the physical effect of milk accumulated in the udder on MBF has to be demonstrated, given that its role in the downregulation of milk synthesis could remain limited. Here, the mammary uptakes of nutrients decreased less than milk yield and milk protein yield, underlining the involvement of intracellular regulation in the decrease in milk secre-

tion. The sharp drop in mammary conversion of glucose toward lactose synthesis associated with slight decrease in milk fat suggested a highly modified partitioning of nutrients between metabolic pathways.

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