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# Increasing Milking Intervals Decreases the Mammary Blood Flow and Mammary Uptake of Nutrients in Dairy Cows

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## ABSTRACT

Increasing the milking intervals reduces milk yield. The aims of this study were to determine whether the reduction in milk yield could be explained by a decrease in mammary uptake of the nutrients or a decrease in the efficiency of the mammary gland in using the milk precursors to synthesize milk components, or both. In a Latin square design with 5 periods, 4 multiparous lactating dairy cows in midlactation were milked at 8-, 12-, 16-, or 24-h intervals over a period of 7 d. The cows were surgically prepared to estimate the net mammary balance of nutrient precursors of milk components (glucose, $\alpha$-amino nitrogen, acetate, $\beta$-hydroxybutyrate, and total glycerol). The efficiency of the mammary gland in synthesizing milk components was estimated by the mammary uptake:milk output ratio. After 7 d of treatment, the decrease in milk yield of 6.1 kg/d between 8- and 24-h milking intervals was associated with a reduction in the uptake of nutrients by the mammary gland, whereas the efficiency of the mammary gland in synthesizing milk components remained relatively unchanged. The mammary uptake decreased by 26% for glucose, 32% for $\alpha$-amino nitrogen, 18% for acetate, 24% for total glycerol, and 24% for $\beta$-hydroxybutyrate, respectively. These reductions in nutrient uptake were due to a decrease in the mammary blood flow (1.23 ± 0.24 L/min). For milk fat precursors (acetate, $\beta$-hydroxybutyrate, and total glycerol), the decrease in mammary blood flow explained the entire reduction in the mammary uptake. For glucose and the milk protein precursors, the reduction in the mammary blood flow explained 60% of the decrease in the mammary uptake, with the other 40% being accounted for by a reduction in the mammary extraction of nutrients. The nutrient uptake was altered as milk yield decreased. These decreases began with the 16-h milking interval and were higher at the 24-h milking interval.

**Key words:** dairy cow, milking frequency, mammary blood flow, mammary nutrient uptake

## INTRODUCTION

Decreasing the milking frequency reduces milk yield (Davis et al., 1999; Rémond and Pomiès, 2004), which could be linked to a decline in the mammary uptake of nutrients associated with a change in the efficiency of the mammary gland in converting these nutrients into milk components. Indeed, Fleet and Peaker (1978) reported a reduction in the mammary uptake of glucose, acetate, and oxygen 2 d after cessation of milking in the goat. A decrease in the mammary nutrient uptake could be due to a decrease in the mammary blood flow (**MBF**) or a decrease in ability of the gland to extract nutrients from the blood compartment, or both. Previously, we showed that the extraction ability of the mammary gland could be affected in the dairy cow following an increase in the milking interval from 8 to 24 h (Delamaire and Guinard-Flament, 2006). Milk volume fell by 25% and the extraction rates of milk component precursors (glucose, $\alpha$-amino nitrogen, BHBA, and total glycerol) declined from 32 to 27% between 8- and 24-h milking intervals. Other studies have suggested that a reduction in MBF may also occur. In cows milked once daily for 7 d, Guinard-Flament and Rulquin (2001) observed a 28% decline in milk yield and a 10% reduction in the MBF. In the goat, a lengthening of the interval between milkings from 26 to 36 h caused a 50% reduction in the MBF (Stelwagen et al., 1994; Farr et al., 2000). On the other hand, the effects of changing to a milking frequency of more than twice daily are less clear. In goats milked 5 times in 12 h, milk secretion increased by 24% and the MBF increased by 44% (Prosser and Davis, 1992). However, in goats milked hourly for 8 h, milk secretion increased by 15%, but there was no change in the MBF (Maltz et al., 1984).

The aim of the present study was to determine whether the reduction in milk yield observed in response to a reduced milking frequency was associated with a reduction in the mammary uptake of nutrients, partly due to a reduction in the MBF or in the efficiency of the mammary gland to convert the plasma nutrients into milk components, or both. This study established dose–response curves for the mammary utilization of nutrients as a function of increasing the milking interval from 8 to 24 h with a constant nutrient intake.

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## MATERIALS AND METHODS

### Treatments, Cows, and Experimental Design

Treatments, cows, and sample analyses were as described previously by Delamaire and Guinard-Flament (2006). Treatments consisted of 4 milking frequencies under a constant level of feeding: milking 3 times daily, milking twice daily, milking 3 times in 2 d, and milking once daily, which corresponded to 8-, 12-, 16-, and 24-h milking intervals, respectively.

Four multiparous Holstein cows (635 ± 30 kg of BW) in their second or third lactation at 72 ± 3 d postpartum at the start of the experiment were used. The cows were surgically prepared to estimate the net mammary balance of nutrients in the left-half udder, according to the method described by Guinard et al. (1994). One month before the beginning of the experiment, 2 permanent catheters were inserted into the left carotid and subcutaneous vein. An ultrasonic flow probe (Probe 20 S, i.d. 20 mm, 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, to measure the MBF. The flow probe cable was protected with Silastic tubing (Silclear medical grade silicone tubing, i.d. 3 mm, o.d. 6 mm; VWR International SAS, Briare, France). Two rings of Dacron (Mersutures, TS53; Ethicon, Issy-Les-Moulineaux, France) were placed along the cable and at the level of exteriorization to prevent any spread of infection.

The experiment was conducted using a Latin square design with 4 cows and 5 periods. The duration of each period was 2 wk. The first week provided a transition when cows were milked twice daily (0630 and 1830 h). The second week was the experimental week, with cows milked according to the treatments allocated. A fifth period was subsequently added because one ultrasonic flow probe stopped emitting a signal during the first period; this cow was eliminated. During the third period, one cow experienced digestive problems. Hence, during the fifth period, this cow received the treatment planned for the third period. Two other cows were subjected to the 2 extreme treatments, i.e., milking once and 3 times daily. Consequently, the results are for 3 cows.

### Measurements, Sampling, and Analyses

***MBF.*** The MBF was continuously measured throughout the experimental period. The sampling rate of the 2 flow meters (T208D; Transonic Systems Inc.) was fixed at 200 Hz. The MBF and heart rate were averaged every minute and recorded using IOX software (EMKA Technologies, Paris, France). The cows were fitted with a sensor to record their position (standing, lying). Because MBF varies according to the position of the animal, the MBF and animal position were recorded simultaneously to study the variations in the MBF as a function of the position.

***Blood.*** Analyses were performed as described previously (Delamaire and Guinard-Flament, 2006). Briefly, concurrently with MBF recording, 12 blood samples were collected simultaneously, during the last 24 h of the period, from the artery and vein using heparinized syringes (S-Monovette, 7.5 mL; Sarstedt, Nümbrecht, Germany). Samples were pooled by cow and period. The concentrations of glucose (precursor of lactose), $\alpha$-amino nitrogen, and AA (precursors of milk proteins), acetate, BHBA, NEFA, and total glycerol (precursors of milk fat) were determined from the arterial and venous plasma to analyze the mammary use of blood nutrients. Heparinized plasma was used to determine the levels of glucose, $\alpha$-amino N, BHBA, total glycerol, and NEFA, and deproteinized plasma was used to determine the levels of acetate. Plasma was acidified with 50% sulfosalicylic acid (vol/vol), centrifuged at 3,000 $\times g$ for 5 min at 4°C, and then diluted in a buffer solution (vol/vol) to analyze AA concentrations. Samples were pooled by cow and period and analyzed according to the methods described by Moore and Stein (1954) using chromatography on a cation-exchange resin column with a Biotronick LC3000 autoanalyser (Biotronick, Maintal, Germany), and were quantified by reaction with ninhydrin. Oxygen and carbon dioxide concentrations were determined by a gas analyzer (ABL 625, Radiometer Copenhagen, Brønshøj, Denmark) from blood samples collected using special "blood gas" heparinized syringes (S-Monovette, 2 mL, Sarstedt; Nümbrecht, Germany.

***Milk.*** During the 7 d of treatment, the cows were milked by each half gland. The milk yield was recorded, and the fat and protein contents were determined by infrared analysis (MilkoScan; Foss Electric, Hillerød, Denmark). After 7 d of treatment, the milk lactose and milk fatty acid levels were analyzed as previously described (Delamaire and Guinard-Flament, 2006).

### Calculations and Statistical Analyses

The results are given for 3 cows (n = 14). Data concerning milk performance, the MBF, and the net mammary balance of nutrients are given after 7 d of treatment.

The arterial flow is equal to the arterial concentration × mammary plasma flow. The mammary plasma flow is calculated from the MBF (mean of the whole day) corrected for arterial hematocrit values. The mammary uptake is equal to the mammary plasma flow × arteriovenous difference except for oxygen and carbon dioxide

analyzed in the blood. Mammary efficiency for use of nutrients for milk synthesis was estimated using the uptake:milk output ratio. De novo synthesis of fatty acid yield was estimated based on the hypothesis that all fatty acids from $C_4$ to $C_{14}$ were synthesized by the mammary gland, and that only 50% of $C_{16}$ was synthesized by the mammary gland (Palmquist et al., 1969). For essential AA (**EAA**), nonessential AA (**NEAA**), and total AA (**TAA**), milk protein yield was corrected by 3.5% to take into account proteins issuing from the plasma.

The data were analyzed using the GLM procedure of SAS (SAS Institute, 1990) according to the following statistical model:

$$Y_{ijk} = \mu + \text{cow}_i + \text{period}_j + \text{treatment}_k + \varepsilon_{ijk}$$

with $Y_{ijk}$ being the variable dependent on cow $i$ receiving treatment $k$ for period $j$, $\mu$ being the mean, and $\varepsilon_{ijk}$ being the residual error associated with each $ijk$ observation. The linear, quadratic, and cubic effects of treatments were analyzed by orthogonal contrasts. Results were expressed as least squares means with the root mean square error. The threshold of significance was set at $P \leq 0.05$ and trends were noted at $P \leq 0.10$.

## RESULTS

### Milk Yield

The milk yield, milk protein, and lactose yields of the left-half udder were more markedly reduced than the milk fat yield with increased milking intervals after 7 d of treatment. Milk yield and milk protein yield tended to decrease linearly by 3.7 kg/d and 116 g/d between 8- and 24-h milking intervals, respectively (Table 1). The milk protein content remained constant with increasing milking intervals. The milk lactose yield decreased in a curvilinear manner by 213 g/d between 8- and 24-h milking intervals; the content in milk remained unchanged by the treatments. The milk fat yield did not vary significantly in response to treatments; it fell by only 84 g/d between the 16- and 24-h milking intervals. The milk fat content rose linearly by 7.2 g/kg to peak at the 24-h milking interval.

### MBF

After 7 d of treatment, daily MBF and mammary plasma flow values decreased linearly by 1.23 and 0.89 L/min between the 8- and 24-h milking intervals, respectively. The heart rate and MBF:milk yield ratio remained unchanged with the treatments (Table 2).

The MBF decreased linearly by 0.99 L/min with increasing milking intervals when cows were in an upright position. In a supine position, it decreased by 1.53 L/min in response to treatment. The MBF decreased by 0.81 L/min between the 12- and 16-h milking intervals and by 0.61 L/min between the 16- and 24-h milking intervals. The time spent in an upright position remained unaffected by the different treatments.

### Plasma Metabolites

The arterial flow of nutrients decreased with increasing milking intervals (Table 3). These reductions were linear for glucose and $\alpha$-amino nitrogen (–2.6 and –3.3 mmol/min, respectively, from 8- to 24-h milking intervals) but resulted in a trend with total glycerol (–40 mmol/min). The arterial flows of acetate and NEFA decreased curvilinearly; they did not vary between 8 and 16 h and were reduced by 1.5 mmol/min and 104 $\mu$mol/min between 16 and 24 h, respectively. The arterial flow of BHBA did not change between the 8- and 24-h milking intervals.

With the exception of NEFA and total glycerol, the mammary uptake of nutrients decreased from the 8- to 24-h milking intervals (Table 3). For glucose, $\alpha$-amino nitrogen, and acetate, these decreases were linear (–1.24, –1.21, and –1.47 mmol/min, respectively, between the 8- and 24-h milking intervals). The mammary uptake of BHBA decreased curvilinearly; it did not vary between 8 and 12 h but decreased by 0.79 mmol/min between the 12- and 24-h milking intervals.

The mammary uptake of AA was not modified when milking intervals were increased (Table 4). The mammary uptakes of the sum of EAA, NEAA, and TAA were not affected by the different treatments.

### Mammary Uptake:Milk Output Ratio

The ratio of glucose uptake:lactose output remained constant with all treatments (Table 4). The (acetate + BHBA) uptake:($C_4$ to $C_{16}$/2) output ratio decreased; it did not vary between 8- and 12-h milking intervals but decreased by 0.45 points between the 12- and 16-h milking intervals and remained unchanged between the 16- and 24-h milking intervals. For AA, the uptake:output ratio of EAA, NEAA, and TAA remained unchanged when the milking intervals increased.

### Blood Gases

The arterial flows of oxygen and carbon dioxide decreased linearly by 6.7 and 36 mmol/min, respectively (Table 5). The mammary uptake of oxygen did not change between the 8- and 24-h milking intervals ($P$ = 0.160). The mammary output of carbon dioxide decreased curvilinearly, falling by 2.9 mmol/min between

**Table 1.** Effect of milking frequency on milk yield of the left-half udder in dairy cows after 7 d of treatment

| | Milking interval, h | | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | 8 | 12 | 16 | 24 | RMSE[1] | L | Q | C |
| Milk yield, kg/d | 18.4 | 18.2 | 17.3 | 14.7 | 1.61 | † | NS | NS |
| Milk protein, g/d | 540 | 534 | 511 | 424 | 53.3 | † | NS | NS |
| Milk protein, g/kg | 29.2 | 29.2 | 29.8 | 29.2 | 0.66 | NS | NS | NS |
| Milk fat, g/d | 728 | 721 | 765 | 681 | 66.7 | NS | NS | NS |
| Milk fat, g/kg | 39.5 | 39.3 | 44.1 | 46.7 | 3.15 | * | NS | NS |
| Milk lactose, g/d | 925 | 914 | 885 | 712 | 82.6 | * | † | NS |
| Milk lactose, g/kg | 50.2 | 50.5 | 51.0 | 48.3 | 1.35 | NS | NS | NS |

[1]Root mean square error.

[2]Linear (L), quadratic (Q), and cubic (C) effects. NS = not significant

NS = $P > 0.10$; †$P \leq 0.10$; *$P \leq 0.05$.

the 16- and 24-h milking intervals. The respiratory quotient remained unaffected by treatments.

## DISCUSSION

### *Reduction in Milk Yield Associated with Reduction in Mammary Gland Uptake of Nutrients with Increasing Milking Interval*

Very few data are available in the literature for the mammary uptake of nutrients in response to variations in milking frequency. To our knowledge, no trial has been conducted with dairy cows. Mammary uptake of nutrients was studied in goats but at 2 d after stopping milking and on only 3 metabolites (glucose, acetate, and oxygen; Fleet and Peaker, 1978).

We observed a decrease in milk yield of 3.7 kg/d when the milking interval increase was accompanied by a reduction in the mammary gland uptake of nutrients and oxidative metabolism on the left-half udder. Our results support those of Fleet and Peaker (1978), but their data were obtained after 48 h of milk accumulation in the udder of the goat and with larger reductions in the mammary uptake of oxygen (–60%), glucose (–75%), and acetate (–65%). In the present study, we found that the mammary uptake of nutrients began to decrease between the 12- and 16-h milking intervals and reduced more between the 16- and 24-h milking intervals. When milking intervals of 8 and 24 h were compared, the uptake of glucose, a precursor of milk lactose, fell by 26%. The mammary uptake of milk protein precursors ($\alpha$-amino nitrogen) was reduced by 32%. With the exception of NEFA, uptake of milk fat precursors by the mammary gland was also reduced. The decrease in the mammary uptake of de novo synthesized fatty acid precursors reached nearly 20% for acetate and 30% for BHBA. The uptake of total glycerol fell by nearly 25% ($P = 0.119$). The mammary uptake of oxygen and mammary output of carbon dioxide decreased (oxygen: $P = 0.160$).

In parallel, the efficiency of the mammary gland in converting these nutrients into milk components, estimated by the mammary uptake:milk output ratio, did

**Table 2.** Effects of milking frequency on the mammary blood flow (MBF) and mammary plasma flow (MPF) of the left-half udder, time spent in an upright position, heart rate, and MBF:milk yield ratio in dairy cows after 7 d of treatment

| | Milking interval, h | | | | | Effects[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | 8 | 12 | 16 | 24 | RMSE[1] | L | Q | C |
| Daily MBF[3], L/min | 7.02 | 6.94 | 6.12 | 5.79 | 0.340 | ** | NS | NS |
| Daily MPF[3], L/min | 4.93 | 4.91 | 4.23 | 4.04 | 0.240 | ** | NS | NS |
| MBF in an upright position,[3] L/min | 6.49 | 6.48 | 5.58 | 5.50 | 0.338 | * | NS | NS |
| MBF in a supine position,[3] L/min | 7.72 | 7.61 | 6.80 | 6.19 | 0.375 | ** | † | NS |
| Time in a standing position, % | 58.6 | 59.0 | 56.3 | 60.1 | 4.39 | NS | NS | NS |
| Heart rate,[3] beats per minute | 73 | 75 | 74 | 76 | 1.8 | NS | NS | NS |
| MBF/milk yield,[3,4] L/kg | 550 | 552 | 502 | 566 | 54.8 | NS | NS | NS |

[1]Root mean square error.

[2]Linear (L), quadratic (Q), and cubic (C) effects. NS = not significant.

[3]Mean of the whole day.

[4]Calculated from MBF and milk yield for the left mammary gland after 7 d of treatment.

NS = $P > 0.10$; †$P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$.

**Table 3.** Effects of milking frequency on the arterial flow and mammary uptake of glucose, lactate, α-amino nitrogen, acetate, BHBA, total glycerol, and NEFA of the left-half udder in dairy cows after 7 d of treatment

| | Milking interval, h | | | | | Effects[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | 8 | 12 | 16 | 24 | RMSE[1] | L | Q | C |
| Glucose, mmol/min | | | | | | | | |
| Arterial flow | 17.6 | 16.8 | 14.7 | 15.0 | 1.38 | * | NS | NS |
| Mammary uptake | 4.79 | 4.58 | 3.95 | 3.55 | 0.466 | * | NS | NS |
| α-Amino nitrogen, mmol/min | | | | | | | | |
| Arterial flow | 16.4 | 15.6 | 12.8 | 13.1 | 1.15 | * | NS | NS |
| Mammary uptake | 3.78 | 3.62 | 2.48 | 2.57 | 0.583 | * | NS | NS |
| Acetate, mmol/min | | | | | | | | |
| Arterial flow | 10.9 | 11.5 | 10.6 | 9.1 | 0.24 | ** | ** | NS |
| Mammary uptake | 7.97 | 8.34 | 6.78 | 6.50 | 0.602 | * | NS | NS |
| BHBA, mmol/min | | | | | | | | |
| Arterial flow | 5.45 | 6.53 | 5.87 | 5.16 | 0.989 | NS | NS | NS |
| Mammary uptake | 2.20 | 2.34 | 1.87 | 1.55 | 0.269 | * | † | NS |
| Total glycerol, μmol/min | | | | | | | | |
| Arterial flow | 331 | 329 | 309 | 291 | 20.3 | † | NS | NS |
| Mammary uptake | 117 | 115 | 111 | 89 | 16.1 | NS | NS | NS |
| NEFA, μmol/min | | | | | | | | |
| Arterial flow | 383 | 380 | 353 | 249 | 21.5 | ** | ** | NS |
| Mammary uptake | –27.9 | –42.1 | 15.9 | –94.1 | 76.96 | NS | NS | NS |

[1]Root mean square error.

[2]Linear (L), quadratic (Q), and cubic (C) effects. NS = not significant.

NS = $P > 0.10$; †$P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$.

not appear to be markedly affected after 7 d of treatment. Indeed, the MBF:milk yield ratio remained unchanged. Whatever the milking interval, the quantity of blood necessary to synthesize 1 kg of milk remained the same, 543 ± 54.8 L, which is consistent with the results of Linzell (1974). Although the efficiency of the mammary gland in converting nutrients into milk components was generally preserved, there were some slight deviations, depending on the type of nutrient. Thus, the efficiency of the mammary gland in converting glucose into lactose did not appear to be markedly affected. The ratio between glucose taken up and lactose secreted remained stable no matter what the milking interval. The glucose uptake of the left-half udder decreased by 1.79 mol/d between the 8- and 24-h milking intervals, whereas that of lactose decreased

**Table 4.** Effects of milking frequency on mammary uptake of AA in the left half-udder and mammary uptake:milk output ratios in dairy cows after 7 d of treatment

| | Milking interval, h | | | | | Effects[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | 8 | 12 | 16 | 24 | RMSE[1] | L | Q | C |
| Uptake, mmol/min | | | | | | | | |
| EAA[3] | 1.28 | 1.28 | 1.25 | 1.13 | 0.124 | NS | NS | NS |
| NEAA[4] | 0.56 | 0.62 | 0.58 | 0.42 | 0.233 | NS | NS | NS |
| TAA[5] | 1.84 | 1.91 | 1.82 | 1.55 | 0.357 | NS | NS | NS |
| Uptake:output, mol of C/mol of C | | | | | | | | |
| EAA | 1.06 | 1.10 | 1.09 | 1.18 | 0.066 | NS | NS | NS |
| NEAA | 0.34 | 0.42 | 0.40 | 0.34 | 0.141 | NS | NS | NS |
| TAA | 0.66 | 0.72 | 0.70 | 0.71 | 0.093 | NS | NS | NS |
| Glucose:lactose | 1.29 | 1.25 | 1.08 | 1.21 | 0.115 | NS | NS | NS |
| (Acetate + BHBA):($C_4$ to $C_{16}$/2)[6] | 1.74 | 1.84 | 1.39 | 1.39 | 0.132 | * | NS | † |

[1]Root mean square error.

[2]Linear (L), quadratic (Q), and cubic (C) effects. NS = not significant.

[3]Essential AA (Lys, His, Arg, Thr, Val, Met, Ile, Leu, Phe).

[4]Nonessential AA (Asn, Asp, Ser, Glu, Gln, Pro, Gly, Ala, Tyr, Trp).

[5]Total AA.

[6]De novo synthesis of fatty acid yield was estimated based on the hypothesis that all fatty acids from $C_4$ to $C_{14}$ were synthesized by the mammary gland and that only 50% of $C_{16}$ was synthesized (Palmquist et al., 1969).

NS = $P > 0.10$; †$P \leq 0.10$; *$P \leq 0.05$.

**Table 5.** Effects of milking frequency on the arterial flows of oxygen and carbon dioxide and their mammary uptake (output) in the left-half udder in dairy cows after 7 d of treatment

| | Milking interval, h | | | | | Effects[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | 8 | 12 | 16 | 24 | RMSE[1] | L | Q | C |
| Oxygen, mmol/min | | | | | | | | |
| Arterial flow | 42.0 | 40.7 | 37.8 | 35.3 | 2.13 | * | NS | NS |
| Mammary uptake | 11.8 | 12.0 | 10.7 | 9.8 | 1.50 | NS | NS | NS |
| Carbon dioxide, mmol/min | | | | | | | | |
| Arterial flow | 186 | 177 | 164 | 150 | 14.3 | * | NS | NS |
| Mammary output | 17.0 | 17.4 | 16.5 | 13.6 | 1.57 | † | † | NS |
| Respiratory quotient[3] | 1.45 | 1.43 | 1.52 | 1.39 | 0.115 | NS | NS | NS |

[1]Root mean square error.

[2]Linear (L), quadratic (Q), and cubic (C) effects. NS = not significant.

[3]Blood carbon dioxide output:blood oxygen mammary uptake.

NS = $P > 0.10$; †$P \leq 0.10$; *$P \leq 0.05$.

by 0.59 mol/d. Assuming that 2 molecules of glucose are used to synthesize 1 molecule of lactose, the fall in the amount of glucose taken up by the udder and converted into lactose averaged 1.18 mol/d, 66% of the decrease in the glucose taken up.

Amino acid metabolism did not seem to be markedly disturbed by increasing the milking interval: The uptake:output ratio of TAA and NEAA remained constant. Only the uptake:output ratio of EAA appeared to indicate a reduction in the efficiency of the mammary gland to convert EAA into milk protein components for the 24-h treatment (linear effect; $P = 0.110$). In contrast, the mammary gland seemed more efficient in converting acetate and BHBA into short- and medium-length milk fatty acids when the milking interval was at least 16 h long. Indeed, the (acetate + BHBA):($C_4$ to $C_{16}$/2) ratio fell as the milking interval increased. This increase in mammary efficiency to convert the acetate and BHBA into short- and medium-length milk fatty acids may contribute to maintaining the synthesis of milk fatty acids and at least partially explain the small reduction in the production of milk fatty acids when compared with that of protein components and milk volume.

### *Reduction in Nutrient Uptake by the Mammary Gland Is Associated with Reductions in MBF and Nutrient Extraction*

Variations in the MBF were consistent with results reported in the literature (Prosser and Davis, 1992; Guinard-Flament and Rulquin, 2001). Thus, neither the MBF (+0.08 L/min) nor the milk yield (+0.6 kg/d) differed with the 8- or 12-h milking intervals. With regard to a difference between the 12- and 16-h intervals, when a reduction in milk yield had been triggered, the MBF fell by 12%. This reduction was amplified between the 12- and 24-h milking intervals (–17%). These results confirm those obtained by Guinard-Flament and Rulquin (2001) in dairy cows milked once daily, in which the MBF fell by –10% and the milk yield by –28%.

The reduction in MBF allowed a decrease in the mammary uptake of nutrients (Table 3). Previously, a reduction in the arteriovenous difference from 10 to 15% for glucose and $\alpha$-amino nitrogen occurred when the milking interval increased from 8 to 24 h (Delamaire and Guinard-Flament, 2006). This reduction in the arteriovenous difference did not account for even half the reductions in the mammary uptake of glucose and $\alpha$-amino nitrogen (which decreased from 25 to 30%; Table 3). Thus, in this study, the drop in MBF explained 60% of the reduction in glucose and $\alpha$-amino nitrogen uptake, with the remaining 40% being due to a reduction in the extraction of these nutrients by the mammary gland. In the case of milk fatty acid precursors, MBF was the only factor responsible for a reduction in the uptake, because the arteriovenous difference was either kept constant for BHBA and total glycerol (Delamaire and Guinard-Flament, 2006) or it depended on the arterial concentration with respect to acetate (Delamaire and Guinard-Flament, 2006).

### *Reduction in MBF Is Not Explained by an Increase in Time Cows Spend Standing*

The reduction in MBF observed following a change from twice daily to once daily milking (Guinard-Flament and Rulquin, 2001), or when the milking interval was extended in the goat (Stelwagen et al., 1994; Farr et al., 2000), may have resulted partly from the increased time the animals spent standing. Indeed, the behavior of animals can be modified when the milking frequency decreases. Thus, Österman and Redbo (2001) observed that animals milked 3 times daily spent less time standing than those milked twice a day (+64 vs. 128 min, 4 h before morning milking). Brulé et al. (2003) suggested that a change to milking once daily caused dairy cows

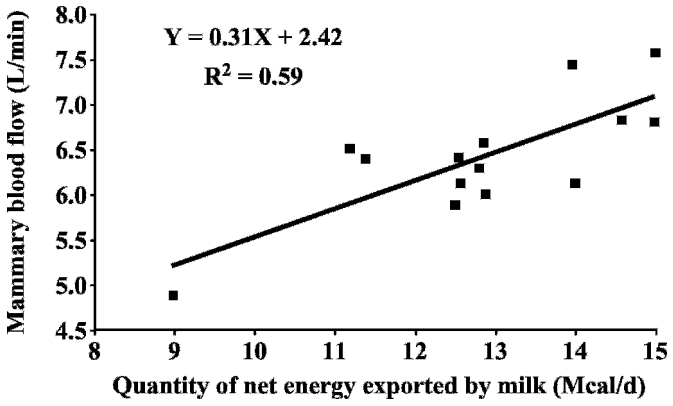


**Figure 1.** Mammary blood flow (corrected for cow and period effects) according to the quantity of net energy exported in milk after 7 d of treatment (left-half udder in dairy cows). The quantity of net energy exported by milk was calculated, assuming that the energy value of 1 kg of milk (containing 40 and 31 g/kg of milk fat and protein, respectively) is 740 kcal (INRA, 1989) and that the energy values of incremental grams of fat or protein in milk are 9.15 and 5.78 kcal/g, respectively (Sjaunja et al., 1991).

some discomfort, resulting in an increase in the time they spent standing at the beginning of lactation before the morning milking. In the current study, the MBF was higher by 25% when the animal was lying down than when it was in the upright position, according to the results of Rulquin and Caudal (1992), and the time the animal spent standing did not vary significantly with an increase in the milking interval (Table 2). Thus, the decrease in the daily MBF when the milking interval was increased from 8 to 24 h was not due to an increase in the time the animals spent standing.

The MBF fell whether the animal was standing or supine when the milking interval was extended (Table 2), although the drop was more marked when the animal was supine than when it was standing (–1.53 vs. –0.99 L/min). This decline could be of local origin, as suggested by Prosser et al. (1996), and by the lack of disturbance of the heart rate (Table 2). This result is also consistent with the hypothesis of a dual regulatory mechanism, as previously suggested by Guinard-Flament and Rulquin (2001).

The reduction in the MBF could result from lowered metabolic activity of the mammary gland following the drop in milk yield. According to Cant and McBride (1995), MBF would be controlled as a function of mammary requirements, producing sufficient ATP to cover needs. Results obtained in the present study could support this hypothesis. Assuming that energy exported in milk reflects the ATP needs of the mammary gland, the relationship between the MBF and the quantity of net energy exported in milk after 7 d of treatment was linear and positive (Figure 1); the MBF fell when the net quantity of energy exported by milk diminished.

The second regulatory mechanism causing a reduction in the MBF may be attributed to a physical effect exerted by milk accumulating in the mammary alveoli. The hypothesis of a physical downregulation of MBF has been discussed in the literature. Studies attempting to mimic increased intramammary pressure by air insufflations or infusions of isosmotic sucrose solution or milk into the lumen of the gland (Pearl et al., 1973; Peaker, 1980) have failed to show consistent results and demonstrate such a mechanism. According to Farr et al. (2000), low flow during extended milking intervals results from capillary closure. Assuming that the physical control of MBF occurs directly by compressing the blood capillaries or indirectly by inhibiting the metabolic activity producing vasoactive agents, the blood would be unable to circulate in the closed capillaries, especially when the animal is supine because of an increased intramammary pressure. As a result, this negative retrocontrol may slow down the increase in MBF when the animal lies down. Indeed, the difference in MBF values when the animal was standing or supine (Table 2) decreased as the milking interval increased, falling from 1.23 L/min with an 8-h milking interval to 0.69 L/min with a 24-h milking interval.

## CONCLUSIONS

After 7 d of treatment, the reduction in milk yield observed following an increase in the milking interval was more closely associated with a drop in mammary gland nutrient uptake than with a loss of efficiency of the mammary gland in converting these nutrients into milk components. This reduction in mammary gland nutrient uptake occurs, partially or wholly depending on the type of nutrient, as a result of a drop in the MBF. The latter probably results from a dual regulation of local origin—a metabolic regulation (the mammary gland could control the MBF as a function of its requirements) associated with a physical regulation (linked to the quantity of milk accumulated in the mammary gland). However, it could be interesting to determine the relative importance of these 2 regulatory mechanisms in reducing the MBF.

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## REFERENCES


Brulé, A., V. Brocard, B. Portier, and V. Racine. 2003. Effets de la réduction de la fréquence de traite sur le bien-être de la vache laitière. Renc. Rech. Ruminants 10:77–80.

Cant, J. P., and B. W. McBride. 1995. Mathematical analysis of the relationship between blood flow and uptake of nutrients in the mammary glands of a lactating cow. J. Dairy Res. 62:405–422.

Davis, S. R., V. C. Farr, and K. Stelwagen. 1999. Regulation of yield loss and milk composition during once-daily milking: A review. Livest. Prod. Sci. 59:77–94.

Delamaire, E., and J. Guinard-Flament. 2006. Longer milking intervals alter mammary epithelial permeability and the udder's ability to extract nutrients. J. Dairy Sci. 89:2007–2016.

Farr, V. C., C. G. Prosser, and S. R. Davis. 2000. Effects of mammary engorgement and feed withdrawal on microvascular function in lactating goat mammary glands. Am. J. Physiol. Heart Circ. Physiol. 279:H1813–H1818.

Fleet, I. R., and M. Peaker. 1978. Mammary function and its control at the cessation of lactation in the goat. J. Physiol. 279:491–507.

Guinard, J., H. Rulquin, and R. Vérite. 1994. Effect of graded levels of duodenal infusions of casein on mammary uptake in lactating cows. 1. Major nutrients. J. Dairy Sci. 77:2221–2231.

Guinard-Flament, J., and H. Rulquin. 2001. Effect of once vs. twice daily milking on mammary blood flow (MBF) in dairy cows. Livest. Prod. Sci. 51:180.

INRA (Institut National de la Recherche Agronomique). 1989. Dairy cows. Pages 73–91 in Ruminant Nutrition: Recommended Allowances and Feed Table. R. Jarrige, ed. John Libbey, Eurotext, London.

Linzell, J. L. 1974. Mammary blood flow and methods of identify and measuring precursors of milk. Pages 143–225 in Lactation. Vol. I. B. L. Larson and V. R. Smith, ed. Academic Press, New York.

Maltz, E., D. R. Blatchford, and M. Peaker. 1984. Effects of frequent milking on milk secretion and mammary blood flow in the goat. Q. J. Exp. Physiol. 69:127–132.

Moore, S., and W. H. Stein. 1954. Procedures for the chromatographic determination of amino acids on four per cent cross-linked sulfonated polystyrene resins. J. Biol. Chem. 211:893–906.

Österman, S., and I. Redbo. 2001. Effects of milking frequency on lying down and getting up behaviour in dairy cows. Appl. Anim. Behav. Sci. 70:167–176.

Palmquist, D. L., C. L. Davis, R. E. Brown, and D. S. Sachan. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of D(−)$\beta$-hydroxybutyrate. J. Dairy Sci. 52:633–638.

Peaker, M. 1980. The effect of raised intramammary pressure on mammary function in the goat in relation to the cessation of lactation. J. Physiol. 301:415–428.

Pearl, S. L., H. F. Downey, and T. L. Lepper. 1973. Intramammary pressure and mammary blood flow in lactating goats. J. Dairy Sci. 56:1319–1323.

Prosser, C. G., and S. R. Davis. 1992. Milking frequency alters the milk yield and mammary blood flow response to intra-mammary infusion of insulin-like growth factor-I in the goat. J. Endocrinol. 135:311–316.

Prosser, C. G., S. R. Davis, V. C. Farr, and P. Lacasse. 1996. Regulation of blood flow in the mammary microvasculature. J. Dairy Sci. 79:1184–1197.

Rémond, B., and D. Pomiès. 2004. Effect of once daily milking in dairy cows: A review of recent French experiments. 55th EAAP Annual Meeting, Wageningen Acad. Press, Bled, Slovenia.

Rulquin, H., and J. P. Caudal. 1992. Effects of lying or standing on mammary blood flow and heart rate of dairy cows. Ann. Zootechnol. 41:101.

SAS Institute. 1990. SAS User's Guide: Statistics. Version 6, Edition 4. SAS Inst., Inc., Cary, NC.

Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setala. 1991. Measurement of the total energy content of cow's milk and the energy value of milk fat and milk protein. Pages 152–155 in Performance Recording of Animals: State of the Art 1990. EAAP Publication no. 50. PUDOC, Wageningen, The Netherlands.

Stelwagen, K., S. R. Davis, V. C. Farr, C. G. Prosser, and R. A. Sherlock. 1994. Mammary epithelial cell tight junction integrity and mammary blood flow during an extended milking interval in goats. J. Dairy Sci. 77:426–432.