Protein and fat mobilization and associations with serum β -hydroxybutyrate concentrations in dairy cows

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ABSTRACT

The objective of this study was to obtain information on variation between dairy cows in muscle and fat tissue mobilization around parturition and to study the association between protein and fat mobilization and serum β -hydroxybutyrate (BHBA) concentrations (hyperketonemia) in this period. Thirty-four cows kept under similar conditions at a university dairy farm (no experimental treatments) were monitored from 4 wk before until 8 wk after calving. Mobilization of muscle protein was investigated by analysis of plasma 3-methylhistidine concentrations (3-MH, analyzed by a recently developed HPLC tandem mass spectrometry method) and ultrasound measurements of longissimus muscle thickness. Mobilization of fat tissue was monitored by serum nonesterified fatty acid (NEFA) concentrations and ultrasound measurements of backfat thickness. Large variation was observed between cows in onset and duration of periparturient protein and fat mobilization. Plasma 3-MH concentrations and muscle thickness profiles indicated that protein mobilization started, on average, before parturition and continued until approximately wk 4 of lactation. Serum NEFA concentrations and backfat thickness profiles showed that fat mobilization occurred from parturition until the end of the study. Thus, muscle protein mobilization occurred in advance of fat mobilization in most cows from this study. We hypothesized that this might be due to a prepartum amino acid deficiency in the absence of negative energy balance. The incidence of hyperketonemia in this study was $16/34 = 47\%$. With the exception of 3 cows defined as having severe hyperketonemia, cows with lower 3-MH concentrations had higher serum BHBA concentrations. A possible explanation for this observation might be that higher mobilization of protein around calving might restrict ketone body production due to the higher availability

of glucogenic precursors in the period of most severe negative energy balance and highest fat mobilization. The validity of this hypothesis needs to be confirmed, but data from this study indicate that further research on the role of protein mobilization in the etiology of hyperketonemia in dairy cows is needed.

Key words: protein mobilization, fat mobilization, β -hydroxybutyrate, dairy cow

INTRODUCTION

Energy requirements of dairy cows are generally not matched by feed intake in early lactation, and over the years, this gap has grown larger due to continuous genetic selection for high milk production (Veerkamp et al., 2003). Cows experience negative energy balance (NEB) for several weeks after parturition, and fat reserves and muscle protein are mobilized to compensate for the energy deficit. The amount of energy that cows mobilize from muscle protein during NEB is limited compared with that mobilized from fat reserves (Tamminga et al., 1997; Komaragiri et al., 1998; Van Knegsel et al., 2007). Calculations on protein balances (indicated by mass or energy retained as body protein) show that protein mobilization in cows ends approximately 4 wk after parturition, whereas fat mobilization continues until at least 8 wk postpartum (Tamminga et al., 1997; Komaragiri et al., 1998; Van Knegsel et al., 2007). Profiles of plasma 3-methylhistidine (**3-MH**), used as an indicator of muscle protein breakdown in cattle, also show that protein mobilization from muscle is restricted to the first 3 to 5 wk of lactation (Blum et al., 1985; Zurek et al., 1995; Doepel et al., 2002). The few studies on prepartum 3-MH profiles indicate that protein breakdown from muscle may occur before parturition in cows (Blum et al., 1985; Doepel et al., 2002). Most research on protein mobilization during NEB has been done in experimental feeding trials (Vandehaar et al., 1999; Doepel et al., 2002; Phillips et al., 2003; Kokkonen et al., 2005; Van Knegsel et al., 2007), and information on biological variation in dairy cows is therefore limited.

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Hyperketonemia (ketosis) is considered an important metabolic disorder in dairy cows due to its association with reduced reproductive performance (Walsh et al., 2007) and the occurrence of other periparturient disorders such as displaced abomasum (Geishauser et al., 1997; Duffield et al., 2009). Higher prepartum BCS and larger fat mobilization around parturition have been associated with an increased risk for ketosis in dairy cows in early lactation (Rukkwamsuk et al., 1999; Busato et al., 2002). In contrast, the association between muscle protein mobilization and blood BHBA profiles has rarely been studied in dairy cows. It can be hypothesized that muscle breakdown is of significant importance during NEB, as it provides the cow with glucogenic amino acids to be allocated for liver gluconeogenesis. Consequently, greater muscle breakdown around parturition would result in greater availability of glucose precursors in the period of most severe fat mobilization, which may restrict ketone body production in early lactation. More knowledge on protein mobilization during NEB may therefore aid in understanding the etiology of hyperketonemia in dairy cows.

The objective of this study was to obtain information on variation between dairy cows in muscle protein and fat mobilization during negative energy balance. Plasma 3-MH concentrations were one of the indicators used for protein mobilization in this study. We used a recently developed, highly specific analytical method based HPLC tandem mass spectrometry to differentiate between 3-MH and 1-methylhistidine in plasma samples (Houweling et al., 2012). Implementation of this analysis may be valuable in research in peripartum cows, provided that substantial variation in blood profiles of 3-MH around parturition can be observed. Specifically, measuring blood 3-MH concentration may be of clinical relevance when studying the etiology of hyperketonemia, as cows that can generate more glucose precursors from muscle protein around parturition might have a lower risk of developing ketosis. To explore whether further research on the role of protein mobilization in the development of hyperketonemia in cows would be needed, we investigated whether plasma 3-MH concentrations and serum NEFA concentrations were associated with serum BHBA concentrations in cows in this study.

MATERIALS AND METHODS

Animals, Feeding, and Management

Sampling procedures of this study were evaluated and approved by the Ethical Committee on Animal Experiments from Utrecht University. From April 2008 until March 2009, 34 Holstein-Friesian and Holstein-Friesian

crossbred dairy cows of different parities were enrolled in the study. Cows were housed at the organic dairy farm "De Tolakker" of the Faculty of Veterinary Medicine, Utrecht University. The herd consisted of 62 dairy cows, and all cows with expected calving dates between May 2008 and January 2009 were included in the study. Because the objective was to study the variability in protein and fat mobilization within and between cows on a commercial farm, no experimental treatments were applied and cows were kept according to normal husbandry practices. Lactating cows were kept in a loose-housing system with sawdust-bedded cubicles. Lactating cows were grazed on pasture from 1600 h until 0700 h the next morning (except for milking time in the evening) from May until August 2008 and from 2200 h until 0700 h the next morning from September to October 2008. During the day, all cows were kept indoors and fed corn silage (2 to 6 kg of DM per cow per day) combined with either fresh grass or grass silage ad libitum. From November 2008 until March 2009, all cows were kept indoors and fed ad libitum with a basal diet consisting of, on a DM basis, approximately 50% grass silage, 45% corn silage, and 5% rapeseed expeller. Concentrate pellets were provided in 3 equal portions per day with an automatic concentrate feeder (minimal feed refusals). One kilogram of concentrates was fed on the first day postpartum and this amount was increased by 200 g/d up to 7 kg for heifers and 8 kg for multiparous cows. Total diets (including concentrates) during pasture season were formulated to contain, on average, 6.72 MJ/kg DM of NE_L, 158 g/kg DM CP and 77 g/kg DM of intestinal digestible protein (**DVE**; Tamminga et al., 1994). Total diets during winter were formulated to contain, on average, 6.78 MJ/kg DM of NE_L, 164 g/kg DM of CP, and 83 g/kg DM of DVE. Diets were intended to meet Dutch requirements (Dutch feeding tables; CVB, 2007). Cows were milked 3 times a day at 0700, 1500, and 2200 h. Milk production data of each milking were automatically stored in the farm's management program. Average daily milk production (kg/d) of cows was calculated based on wk 2 to wk 8 of lactation (milk production data from wk 1 postpartum were incomplete or absent for most cows). Dry cows were kept indoors in a large, straw-bedded pen. The dry cow diet was fed ad libitum and consisted of, on a DM basis, a mixture of approximately two-thirds grass silage (average content 5.75 MJ/kg DM of NE_L, 150 g/kg DM CP and 59 g/kg DM DVE), one-third corn silage (average content 6.62 MJ/kg DM of NE_L, 77 g/kg DM of CP, and 50 g/kg DM of DVE), and dry cow minerals; no concentrates were supplied and dry cows were not grazed on pasture. The BCS of all cows was assessed weekly by the same, trained farm technician according to the method of Edmonson et al. (1989).

Monitoring Protocol and Sample Analysis

Cows were monitored from 4 wk before the expected calving date (wk -4 to -1) until 8 wk after parturition (wk 1 to 8). Blood sampling and ultrasound measurements of muscle and backfat thickness were performed weekly throughout this period; parturition occurred in the 1-wk interval between sampling moments at wk -1 and wk 1. Blood samples were drawn from the coccygeal vein into serum and heparinized tubes between 1000 and 1100 h, centrifuged at $2,800 \times g$ for 10 min, and serum and heparin plasma were frozen at -20°C until analysis. Serum samples were analyzed for NEFA and BHBA using Randox test kits (NEFA: FA 115 kit; BHBA: Ranbut kit; Randox Laboratories Ltd., Crumlin, UK). Heparin plasma samples were analyzed for 3-MH according to the method developed by Houweling et al. (2012), in which 3-MH concentrations were analyzed in plasma samples using HPLC tandem mass spectrometry. This analytical method was developed to distinguish between 3-MH, which is specifically released upon myofibrillar degradation in muscle, and 1-methylhistidine, another histidine derivative that can be present in mammalian blood. Thus, by separating the plasma profile of 3-MH from that of 1-MH, a more specific indication of the actual breakdown of myofibrillar muscle protein could be obtained in this study.

Ultrasound measurements were performed on the right side of the animal with a scanner 100 (Pie Medical, Maastricht, the Netherlands) and linear transducer (5.0 MHz). Prior to measurement, skin spots were brushed and greased with rapeseed oil, but not clipped. Muscle thickness (longissimus muscle) was assessed at the transversal process of the fourth lumbar vertebra (perpendicular to vertebral column) as the largest diameter between the muscular fascias at that site. Backfat thickness (subcutaneous fat) was measured in the pelvic region at a hand's width before the tuber ischiadicum, as described by Schröder and Staufienbiel (2006). A triangle-shaped structure of the fascia of the underlying gluteus muscle was used as a landmark to determine backfat thickness at the same site in consecutive weeks.

Data Analysis

The final data set contained 11 ($n = 8$) or 12 ($n = 26$) repeated measurements of blood and ultrasound variables for each cow, depending on the cows' actual calving date. All data analysis was done with Statistical Package R (version 2.9.1, R Foundation for Statistical Computing, Vienna, Austria). Box plots were drawn for plasma 3-MH concentrations, serum NEFA concentrations, and ultrasound measurements of muscle and fat

thickness per week to show time trends and variation between cows.

Plasma 3-MH concentrations, longissimus muscle thickness (ultrasound), serum NEFA concentrations, and backfat thickness (ultrasound) were analyzed as dependent variables in linear mixed-effects models to analyze observed time patterns during the study. Plasma 3-MH concentrations and serum NEFA concentrations were logarithmically transformed before analysis to achieve normally distributed model residuals. For each dependent variable, cow was included as random effect (random intercept per cow) in the statistical model, because repeated measurements per cow were performed over time. A random time effect was included in all models (random coefficient for each cow) to model time-dependent correlations between observations within cows. Time effects (week relative to parturition), parity effects (parity 1, $n = 11$; parity 2, $n = 10$; and parity 3 and higher, $n = 13$), and time $\times$ parity interaction effects were investigated as fixed effects in the models. Model parameters were estimated with the maximum likelihood method. Models were checked (visual inspection) by Q-Q plots of residuals (normality) and plots of predicted values versus residuals (linearity and constant variance). The mean and standard deviation of model residuals were calculated for each week.

Total mobilization of muscle or backfat tissue in cows during the trial was defined as muscle or fat thickness at the start of the trial (wk -4 or wk -3) minus muscle or fat thickness at wk 8, respectively. Linear regression analysis was performed to study associations between muscle thickness at the start of the trial and total muscle tissue mobilization, and between backfat thickness at the start and total fat mobilization.

The second objective of the study was to explore whether blood profiles of BHBA (i.e., degree of hyperketonemia) were associated with concentrations of 3-MH and NEFA. A box plot of serum BHBA concentrations was drawn to show variation in cows during the study. Hyperketonemia was defined as serum BHBA concentration $\geq 1,200 \mu\text{mol/L}$, which was shown to be the lowest threshold level at which a negative effect on cow health was observed (Duffield et al., 2009). The incidence of hyperketonemia was calculated as the proportion of cows experiencing hyperketonemia in at least 1 wk during the observational study. To study associations between degree of hyperketonemia and, respectively, total protein mobilization and total fat mobilization, areas under the curve (AUC) were calculated for serum BHBA concentrations (AUC BHBA), plasma 3-MH concentrations (AUC 3-MH), and serum NEFA concentrations (AUC NEFA). Multivariate linear regression analysis was performed with AUC BHBA

Figure 1. Plasma 3-methylhistidine (3-MH) concentrations of cows in the last 4 wk of the dry period and the first 8 wk of lactation ($n = 34$, except wk -4 , which included 26 observations). The dashed line indicates parturition; boxes represent median and interquartile range; whiskers include all cases.

as dependent variable and AUC 3-MH and AUC NEFA as predictive variables. No collinearity was present between AUC 3-MH and AUC NEFA. By inspecting individual cow profiles, we identified 3 cows with severe hyperketonemia in the data set. These cows had a BHBA concentration $>1,200$ $\mu\text{mol/L}$ in 5 or 6 wk of the study and an average BHBA concentration after parturition above this threshold ($1,670 \pm 210$ $\mu\text{mol/L}$, compared with 840 ± 190 $\mu\text{mol/L}$ for other cows). We investigated whether the association between the AUC of blood variables differed for the cows with severe hyperketonemia and cows without this metabolic condition. The same linear regression procedure was repeated after exclusion of these 3 cows with severe hyperketonemia from the data set. Results from both regression analyses are reported.

RESULTS

Cows produced (mean $\pm$ SD) 38.0 ± 6.4 kg of milk per day during the trial (wk 2 to 8 of lactation). The BCS of cows (mean $\pm$ SD) was 3.2 ± 0.4 at the start, and BCS was reduced by 0.4 ± 0.3 points to 2.8 ± 0.5 at the end of the study. Some cows experienced clinical mastitis ($n = 4$), subclinical mastitis ($n = 2$), milk fever ($n = 2$), lameness ($n = 3$), or displaced abomasum ($n = 1$) during the study. All cases recovered quickly

after treatment and their data were therefore included in the analyses. Later analysis showed that the mean concentrations of plasma 3-MH, serum NEFA, and serum BHBA of these cows were not different from those of cows that remained healthy throughout the study ($P > 0.05$, tested with 2-sample t -test; results not shown).

Figure 1 shows a box plot of plasma 3-MH concentrations of cows during the study. Neither parity nor time $\times$ parity interaction was associated with any of the blood or ultrasound variables in the study. Results from the final linear mixed model on plasma 3-MH concentrations are shown in Table 1. Highest plasma 3-MH values were observed in the first week after parturition (wk 1) compared with other weeks ($P < 0.01$). Figure 2 shows a box plot of longissimus muscle thickness of cows during the study. Results from the final linear mixed model on muscle thickness are shown in Table 1. On average, muscle thickness decreased before parturition (wk -1 compared with wk -4 ; $P < 0.001$), which corresponds with observations on plasma 3-MH concentrations. The largest decrease in longissimus muscle was observed in the week of parturition (between wk -1 and wk 1).

Figure 3 shows a box plot of serum NEFA concentrations of cows during the study. Results from the final linear mixed model on serum NEFA concentrations are shown in Table 2. Highest serum NEFA concentrations

Figure 2. Longissimus muscle thickness of cows in the last 4 wk of the dry period and the first 8 wk of lactation ($n = 34$, except wk -4 , which included 26 observations). The dashed line indicates parturition; boxes represent median and interquartile range; whiskers include all cases.

Figure 3. Serum NEFA concentrations of cows in the last 4 wk of the dry period and the first 8 wk of lactation ($n = 34$, except wk -4 , which included 26 observations). The dashed line indicates parturition; boxes represent median and interquartile range; whiskers include all cases.

Figure 4. Backfat thickness of cows in the last 4 wk of the dry period and the first 8 wk of lactation; the dashed line indicates parturition ($n = 34$, except wk -4 , which included 26 observations). Boxes represent median and interquartile range; whiskers include all cases.

were observed in the first 3 wk of lactation compared with precalving weeks and from wk 4 of lactation onward. Figure 4 shows a box plot of backfat thickness of cows during the study. Results from the final linear mixed model on backfat thickness are shown in Table 2. Backfat tissue mobilization started, on average, after

parturition ($P < 0.001$) and lasted until the end of the study.

Ultrasound measurements of muscle and fat thickness and total amounts and proportions of mobilized muscle and fat tissue are shown in Table 3. We found a tendency toward a larger total decrease in muscle thickness for cows with larger muscle thickness at the

Table 1. Results of final linear mixed effects models to analyze time patterns in weekly observed plasma 3-methylhistidine (3-MH) concentrations ($\mu\text{mol/L}$, log-transformed) and longissimus muscle thickness (cm) in 34 cows around parturition¹

Terms ²	Plasma 3-MH				Longissimus muscle thickness			
	Estimate	SE	P-value	Residuals ³ (mean $\pm$ SD)	Estimate	SE	P-value	Residuals ³ (mean $\pm$ SD)
Week -4^4 (Ref)	1.628	0.083	<0.001	0.246 $\pm$ 0.184	4.126	0.106	<0.001	0.129 $\pm$ 0.130
Week -3	+0.158	0.082	0.055	0.210 $\pm$ 0.158	-0.032	0.042	0.439	0.099 $\pm$ 0.064
Week -2	+0.169	0.083	0.041	0.221 $\pm$ 0.184	-0.077	0.043	0.076	0.121 $\pm$ 0.085
Week -1	+0.305	0.084	<0.001	0.261 $\pm$ 0.202	-0.179	0.046	<0.001	0.133 $\pm$ 0.107
Week 1	+0.548	0.085	<0.001	0.207 $\pm$ 0.181	-0.400	0.049	<0.001	0.129 $\pm$ 0.090
Week 2	-0.001	0.087	0.987	0.204 $\pm$ 0.155	-0.528	0.053	<0.001	0.144 $\pm$ 0.139
Week 3	-0.393	0.090	<0.001	0.311 $\pm$ 0.256	-0.584	0.057	<0.001	0.111 $\pm$ 0.099
Week 4	-0.556	0.093	<0.001	0.210 $\pm$ 0.181	-0.697	0.062	<0.001	0.081 $\pm$ 0.065
Week 5	-0.549	0.096	<0.001	0.197 $\pm$ 0.159	-0.718	0.067	<0.001	0.076 $\pm$ 0.063
Week 6	-0.681	0.099	<0.001	0.180 $\pm$ 0.137	-0.762	0.072	<0.001	0.086 $\pm$ 0.063
Week 7	-0.691	0.103	<0.001	0.198 $\pm$ 0.180	-0.774	0.078	<0.001	0.082 $\pm$ 0.059
Week 8	-0.773	0.107	<0.001	0.187 $\pm$ 0.126	-0.735	0.084	<0.001	0.091 $\pm$ 0.069

¹Random intercepts and random coefficients were included for individual cows in both models.

²Time was investigated as week relative to parturition (fixed factor).

³Absolute values of model residuals.

⁴Week -4 was used as reference (intercept) in the model for plasma 3-MH concentrations and longissimus muscle thickness; estimates for each subsequent week represent the change relative to wk -4 .

Table 2. Results of final linear mixed effects models to analyze time patterns in weekly observed serum NEFA concentrations ($\mu\text{mol/L}$, log-transformed) and backfat thickness (cm) in 34 cows around parturition¹

Terms ²	Serum NEFA				Backfat thickness			
	Estimate	SE	P-value	Residuals ³ (mean $\pm$ SD)	Estimate	SE	P-value	Residuals ³ (mean $\pm$ SD)
Week -4 ⁴ (Ref)	4.818	0.088	<0.001	0.129 $\pm$ 0.112	0.761	0.075	<0.001	0.101 $\pm$ 0.098
Week -3	+0.024	0.092	0.790	0.168 $\pm$ 0.167	+0.062	0.024	0.011	0.058 $\pm$ 0.057
Week -2	+0.111	0.093	0.235	0.215 $\pm$ 0.176	+0.066	0.025	0.009	0.072 $\pm$ 0.088
Week -1	+0.339	0.094	<0.001	0.277 $\pm$ 0.256	+0.059	0.026	0.027	0.074 $\pm$ 0.056
Week 1	+1.289	0.097	<0.001	0.306 $\pm$ 0.249	+0.022	0.028	0.437	0.067 $\pm$ 0.060
Week 2	+1.158	0.099	<0.001	0.274 $\pm$ 0.180	-0.060	0.030	0.048	0.055 $\pm$ 0.049
Week 3	+1.012	0.102	<0.001	0.355 $\pm$ 0.207	-0.090	0.032	0.006	0.042 $\pm$ 0.043
Week 4	+0.692	0.106	<0.001	0.294 $\pm$ 0.224	-0.143	0.035	<0.001	0.058 $\pm$ 0.050
Week 5	+0.539	0.110	<0.001	0.263 $\pm$ 0.218	-0.188	0.038	<0.001	0.039 $\pm$ 0.038
Week 6	+0.481	0.114	<0.001	0.236 $\pm$ 0.147	-0.211	0.041	<0.001	0.042 $\pm$ 0.037
Week 7	+0.466	0.119	<0.001	0.190 $\pm$ 0.148	-0.258	0.044	<0.001	0.042 $\pm$ 0.034
Week 8	+0.507	0.124	<0.001	0.230 $\pm$ 0.168	-0.282	0.047	<0.001	0.046 $\pm$ 0.043

¹Random intercepts and random coefficients were included for individual cows in both models.

²Time was investigated as week relative to parturition (fixed factor).

³Absolute values of model residuals.

⁴Week -4 was used as reference (intercept) in the models for serum NEFA concentrations and backfat thickness; estimates for each subsequent week represent the change relative to wk -4.

start of observations (wk -4 or -3; $P < 0.10$; Figure 5A). Cows with higher backfat thickness at the start of observations (wk -4 or -3) mobilized a greater amount of backfat thickness during the study ($P < 0.001$; Figure 5B).

Figure 6 shows a box plot of serum BHBA concentrations of cows during the trial. The incidence of hyperketonemia in the present study was 47% ($n = 16$). No cases of clinical ketosis were observed during the study. Results from linear regression analysis on associations between degree of hyperketonemia and muscle and fat mobilization, measured as the AUC of serum BHBA, plasma 3-MH, and serum NEFA concentrations, respectively, are shown in Table 4. When data of all cows were analyzed, muscle mobilization was not associated with degree of hyperketonemia, whereas fat mobilization was associated with degree of hyperketonemia ($P < 0.05$). Three cows developed severe hyperketonemia during the study and had different mean profiles of serum BHBA and serum NEFA compared with other cows. The AUC for BHBA, 3-MH, and NEFA were (mean $\pm$ SD) 13.63 ± 1.13 , 60.99 ± 12.78 , and 4.81 ± 1.27 , respectively, for cows with severe hyperketonemia compared with 7.90 ± 1.51 , 48.29 ± 13.27 , and 2.66 ± 0.79 , respectively, for the other 31 cows. The analysis was repeated after exclusion of the 3 cows with severe hyperketonemia from the data set. Analysis of this restricted data set ($n = 31$) differed from the previous analysis and showed that muscle mobilization was associated with degree of hyperketonemia in cows ($P < 0.05$), whereas fat mobilization was not.

DISCUSSION

The present study was performed to collect information on variation in muscle and fat mobilization from 4 wk before until 8 wk after parturition in dairy cows kept under similar conditions at the dairy farm of the Dutch Faculty of Veterinary Medicine, a farm managed as a commercial dairy farm. Herd size and yearly milk production per cow were representative for the average Dutch dairy farm, whereas some management and feeding conditions may have been different, as cows in this study were kept at an organic university farm. Mobilization of muscle protein was investigated by plasma 3-MH (assessed by using a newly developed mass spectrometry method) and ultrasound measurements of longissimus muscle thickness. Mobilization of fat tissue was monitored by serum NEFA concentrations and ultrasound measurements of backfat thickness. We observed large variation between cows in onset and duration of periparturient protein and fat mobilization. Protein mobilization occurred before parturition, whereas fat mobilization started after parturition in most cows. Differences in protein and fat mobilization around parturition may influence the susceptibility of cows to hyperketonemia in early lactation. In this study, higher plasma 3-MH concentrations were associated with lower serum BHBA concentrations, with the exception of 3 cows that experienced severe hyperketonemia.

Large variation was observed in plasma 3-MH concentrations of cows in this study, as the average co-

Table 3. Thickness and mobilization of muscle and fat tissue in dairy cows ($n = 34$) in the period from 3 to 4 wk before the expected calving date (start trial) to wk 8 of lactation (end trial), as assessed by ultrasound measurements

Item	Muscle (cm)		Fat (cm)	
	Mean	SD	Mean	SD
Thickness				
Start trial (wk -4 or -3)	4.13	0.57	0.77	0.39
At parturition (wk -1)	3.95	0.60	0.82	0.41
End trial (wk 8)	3.39	0.60	0.48	0.32
Mobilization				
Before parturition (wk -4 or -3 to wk -1)	0.18	0.30	-0.05	0.20
Week of parturition (wk -1 to 1)	0.22	0.18	0.04	0.10
After parturition (wk 1 to 8)	0.34	0.39	0.30	0.22
Total	0.74	0.41	0.29	0.23
Mobilized tissue (%)	18	9	35	26

efficient of variation in individual weeks was 42.2% (35.2 to 51.8%). Total amount of muscle and backfat thickness mobilized during the study varied from 0.03 to 1.84 cm for longissimus muscle and from -0.14 cm (i.e., net fat deposition) to 0.84 cm for backfat in individual cows. Tamminga et al. (1997) also reported large differences between cows in calculated protein and fat mobilization in early lactation. Earlier studies showed that the composition of prepartum or early lactation diets could influence protein mobilization (Komaragiri et al., 1998; Doepel et al., 2002) and fat mobilization (Komaragiri et al., 1998; Van Knegsel et al., 2007). Dietary variation throughout the year may therefore have contributed to the observed variation between cows in the present study, but the moment of enrollment in the study was not associated with mean concentrations of 3-MH and NEFA or total muscle and backfat tissue mobilization in cows (results not shown). Part of the observed variation in mobilization of body reserves may be explained by differences in milk production, because genetic differences in milk yield were associated with differences in feed intake, energy balance, and metabolic adaptation during NEB in cows (Veerkamp et al., 2003). Fat mobilization in cows is increased by a fat body condition before calving (Komaragiri et al., 1998; Kokkonen et al., 2005), which was confirmed by the association between fat thickness at the start and the total change in backfat thickness in our study. Additionally, we found a tendency that higher muscle thickness at wk -4 was associated with a larger loss in muscle thickness in cows. It is not well known whether larger prepartum protein stores allow for more protein mobilization around parturition (Bell et al., 2000), but in earlier studies, an effect of prepartum dietary protein supply on postpartum protein mobilization was not observed (Doepel et al., 2002; Phillips et al., 2003).

Plasma 3-MH concentrations and prepartum longissimus muscle profiles indicated that muscle protein

mobilization started before parturition in most cows in the present study. Longissimus muscle thickness decreased significantly between the start of study and the last measurement prepartum. Other studies show that protein mobilization can start at 1 or 2 wk before calving (Doepel et al., 2002; Kokkonen et al., 2005). To our knowledge, increased 3-MH concentrations before 2 wk before parturition, such as observed in this study, have not yet been reported in dairy cows. The largest decrease in longissimus muscle thickness and highest 3-MH concentration occurred around parturition, indicating the period of most severe protein mobilization. Plasma concentrations of 3-MH suggested that average net muscle protein mobilization largely decreased upon wk 4 after calving in this study, which corresponds with earlier reports on 3-MH profiles (Zurek et al., 1995; Doepel et al., 2002; Phillips et al., 2003) and studies that have calculated protein balances in cows (Tamminga et al., 1997; Komaragiri et al., 1998; Van Knegsel et al., 2007). Similarly, longissimus muscle thickness stabilized in most cows from wk 4 of lactation onward. Plasma 3-MH concentrations and longissimus muscle change may not result in identical profiles of protein mobilization, because 3-MH reflects muscle breakdown only, whereas longissimus muscle thickness is the result of net protein turnover. Uterine involution might also contribute to postpartum plasma 3-MH concentrations, but Tian and Noakes (1991) observed no association between plasma 3-MH and change in uterine diameter after calving in a small number of cows. In contrast to protein mobilization, we observed that backfat thickness did not decrease and serum NEFA concentrations did not increase before calving. Backfat thickness in cows decreased from parturition until the end of the study, which suggests that fat mobilization continued until at least wk 8 of lactation, as was observed in earlier studies (Tamminga et al., 1997; Komaragiri et al., 1998; Van Knegsel et al., 2007). Serum NEFA profiles

Figure 5. Association between thickness at the start of the trial (3 to 4 wk before parturition) and amount of tissue mobilized for muscle (A) and fat tissue (B) between start of the trial and wk 8 of lactation (including linear regression line; dots represent individual cows, $n = 34$).

of cows in this study only partly correspond to this finding, but this discrepancy is probably because serum NEFA concentrations do not represent lipolysis itself but the net result of NEFA release by adipose tissue and NEFA uptake by peripheral organs.

Results from the present study indicate that significant muscle protein mobilization may occur before par-

Figure 6. Serum BHBA concentrations of cows in the last 4 wk of the dry period and the first 8 wk of lactation ($n = 34$, except wk -4, which included 26 observations). The dashed line indicates parturition; boxes represent median and interquartile range; whiskers include all cases.

turition in advance of fat mobilization. This observation suggests differences in the regulation of protein and fat mobilization in dairy cows. Although it is known that fat mobilization during NEB is under control of hormones such as insulin, growth hormone, and catecholamines, and further modulated by autocrine and paracrine factors produced by adipose tissue [reviewed by McNamara (1991) and Vernon (2005), among others], regulatory mechanisms of protein mobilization, especially skeletal muscle proteolysis, are still relatively poorly understood in ruminants (Bell et al., 2000; Tesseraud et al., 2007) and humans (Combaret et al., 2001; Prod'homme et al., 2004). Ubiquitin-mediated protein breakdown (during which 3-MH is formed) is the major proteolytic pathway in the body (Lecker et al., 1999; Melstrom et al., 2007), and was shown to be upregulated during early lactation in dairy cows (Chibisa et al., 2008). Insulin plays a key role in muscle protein metabolism by stimulating protein synthesis and inhibition of muscle proteolysis by this ubiquitin-proteasome pathway (Prod'homme et al., 2004; Tesseraud et al., 2007). In the present study, prepartum negative energy balance and low insulin levels were not likely, because a simultaneous mobilization of fat tissue was lacking. Therefore, this cannot explain the proteolysis before calving. On the other hand, amino acids also play a role in the regulation of protein metabolism, as a low availability of amino acids is thought to reduce the inhibi-

Table 4. Mean, standard deviation, and results from linear regression analysis of the area under the curve (AUC) of plasma 3-methylhistidine (3-MH) and AUC of serum NEFA concentrations as predictive variables for the AUC of serum BHBA concentrations for the data set of all cows ($n = 34$) and the data set excluding cows with severe hyperketonemia ($n = 31$)

Variable	Mean $\pm$ SD	Estimate	SE	P-value
All cows				
AUC 3-MH	49.42 $\pm$ 13.54	-0.0100	0.0268	0.713
AUC NEFA	2.85 $\pm$ 1.02	0.9171	0.3550	0.015
Excluding severe hyperketonemia				
AUC 3-MH	48.29 $\pm$ 13.27	-0.0424	0.0200	0.043
AUC NEFA	2.66 $\pm$ 0.79	0.0365	0.3362	0.914

tive effect of insulin on proteolysis, thereby facilitating muscle protein degradation (Prod'homme et al., 2004; Tesseraud et al., 2007). A negative protein balance (i.e., amino acid deficiency) in absence of negative energy balance can occur when protein requirements are not met in the last stage of gestation and may thus explain why cows mobilized muscle protein in advance of fat tissue in this study. Negative protein balance may occur because uterine uptake of amino acids appears to be high in cows, implying that amino acids are not only used for fetal growth but are also catabolized in uterine tissues in this period (Bell, 1995). In addition, the CP content of the dry cow diet at the organic dairy farm from this study could have been inadequate, because the average CP content of the grass silages fed (150 g/kg of DM) was relatively low compared with the targeted CP content for grass silages (160 to 190 g/kg of DM). Moreover, one-third of the dry cow diet consisted of corn silage, which has a high energy but low protein content, which could have further contributed to an inadequate prepartum protein supply.

Protein balances in cows have been calculated to become positive around wk 4 of lactation, whereas NEB continues (Tamminga et al., 1997; Komaragiri et al., 1998; Van Knegsel et al., 2007). The availability of amino acids might therefore similarly influence the postpartum reduction of protein mobilization in cows. In addition, body protein mobilization was shown to be restricted by an increased inhibitory effect of insulin on proteolysis in early lactation goats (Tesseraud et al., 2007). Postpartum restriction of muscle degradation in cows might therefore result from an increasing availability of amino acids when feed intake increases in early lactation and an increased sensitivity of muscle tissue for the antiproteolytic effect of insulin during NEB.

To our knowledge, associations between protein mobilization and serum BHBA concentrations in dairy cows have rarely been studied. Proteolysis in muscle predominantly releases alanine and glutamine, the main amino acids to be taken up by the liver for gluconeogenesis in humans (Frayn, 2003). We hypoth-

esized that, under similar dietary conditions, greater protein mobilization around parturition might restrict ketone body production in cows because of increased gluconeogenesis in the period of most severe NEB and highest fat mobilization. Controlled feeding trials would be necessary to prove such a hypothesis. We explored if any association between protein mobilization and hyperketonemia could be detected in this study to indicate whether further research on the role of protein mobilization in the development of hyperketonemia in cows is needed. We observed that lower plasma 3-MH concentrations were associated with a greater degree of hyperketonemia in cows, whereas serum NEFA concentrations were not associated with degree of hyperketonemia, with the exception of 3 cows that experienced severe hyperketonemia. These 3 cows had higher serum NEFA concentrations compared with other cows. We speculate that release of NEFA from fat tissue in these cows was very large relative to the amount of glucogenic amino acids that could be mobilized from muscle protein. In the other cows, protein and fat mobilization might have been more in balance, thereby preventing the occurrence of severe hyperketonemia.

CONCLUSIONS

This study shows large variation in periparturient protein and fat mobilization in cows kept under similar conditions on a university dairy farm, which could be due to differences between animals in milk production, feed intake, metabolic adaptation to NEB, or prepartum fat and muscle thickness. The observation that protein mobilization occurred before parturition and in advance of fat mobilization indicates that protein mobilization is not just the result of NEB. Furthermore, cows with lower plasma 3-MH concentrations had higher serum BHBA concentrations in this study. We hypothesized that greater muscle breakdown might, to a certain extent, restrict ketone body production in early lactation dairy cows. Whether this is valid needs to be confirmed in further research on the interaction of protein and fat mobilization in the etiology of hyperketonemia.

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