Materials and Methods

Three weeks before the due date, 198 Holstein cows were affixed with neck collars containing rumination loggers (Hr-Tag rumination monitoring system, Israel) providing rumination time (RT) at 2-h intervals. Blood was collected 12 to 72 h after calving for NEFA, BHBA, and serum total calcium determination from a subset of cows (n=136). Cows were considered in negative energy balance (NEB) if serum NEFA concentrations were > 0.5 mmol/L, positive for subclinical ketosis (SCK) if serum BHBA concentrations were ≥ 1.4 mmol/L, and hypocalcemic (HYC) if serum CA concentrations were < 8.0 mg/dL. Mixed models for repeated measures were developed for the analysis of 2-h RT at the pre-calving (PRECLV; -7 to -1 d before calving) and post-calving (POSTCLV; 1 to 7 d after calving) periods. Regression coefficients and correlation between time relative to calving and 2-h rumination values was estimated for the PRECLV and POSTCLV periods for affected and healthy cows. In addition, the correlation between blood parameters and total daily RT was measured for the day of blood sample collection.

Results

Overall, 57.7%, 18.4%, and 47.7% of cows were diagnosed with NEB, SCK, and HYC, respectively. The repeated measures analysis indicated that NEB ($P<0.001$) and time ($P<0.001$) were associated with RT in the PRECLV period. During the POSTCLV period time ($P<0.001$) and NEB*time interaction ($P<0.001$) were significant. In both periods, cows without NEB had higher rumination activity across time. For BHBA, SCK ($P<0.001$) and time ($P<0.001$) were associated with RT in the PRECLV period. Similarly, in the POSTCLV period SCK ($P<0.001$) and time ($P<0.001$) were also associated with RT. In both periods, cows without SCK had higher rumination activity across time. For CA, HYC ($P<0.001$), time ($P<0.001$), and HYC*time interaction ($P<0.001$) were associated with RT in the PRECLV period. During the POSTCLV period only, time ($P<0.001$) and HYC*time interaction ($P=0.025$) were significant. In both periods, cows without HYC had higher rumination activity across time. During the PRECLV period, the correlation coefficient (r) for time to calving and RT were -0.23 ($P<0.001$) and -0.19 ($P<0.001$) for cows with and without NEB, respectively. During the POSTCLV period, the correlation coefficients for time after calving and RT were 0.18 ($P<0.001$) and 0.16 ($P<0.001$) for cows with and without NEB, respectively. During the PRECLV period, the correlation coefficients for time to calving and RT were the same for cows with or without SCK (r = -0.21; $P<0.001$). However, during the POSTCLV period, the correlation coefficients were 0.16 ($P<0.001$) and 0.17 ($P<0.001$) for cows with and without SCK, respectively. During the PRECLV period, the correlation coefficient for time to calving and RT were -0.25 ($P<0.001$) and -0.22 ($P<0.001$) for HYC and healthy cows, respectively. During the POSTCLV period, the correlation coefficients for time to calving and RT were 0.20 ($P<0.001$) and 0.15 ($P<0.001$) for HYC and healthy cows, respectively. Coefficients for the correlation between NEFA, BHBA, and CA levels and total daily RT on the day of blood sampling were -0.39 ($P<0.001$), -0.14 ($P=0.17$), and 0.51 ($P<0.001$), respectively.

Significance

Rumination time was reduced during the pre- and post-calving periods in cows with postpartum elevations in serum NEFA, BHBA, or low serum total CA concentrations compared with healthy cows. In particular, the association of reduced rumination time in the pre-calving period with worse metabolic status after calving indicates that this monitoring tool may have diagnostic potential in transition cows. Further research into the utility of rumination activity for decision-making at the individual and group level in the management of transition cow health is warranted.

Fetuin-A dynamics in transition cows' plasma

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Introduction

Around parturition and during early lactation, cows enter a state of negative energy balance (NEB) driven by the onset of lactation and a reduced dry matter intake. The major metabolic adaptation to NEB is adipose tissue lipolysis that releases nonesterified fatty acids (NEFA) into systemic circulation. High plasma NEFA predisposes cows to metabolic and inflammatory based diseases that negatively impact animal welfare and increase economic losses to dairy producers. However, not all cows with increased plasma NEFA concentrations develop a disease, thus underscoring the need for novel lipid mobilization-related biomarkers that could improve accuracy of disease prediction. In humans,

high fetuin-A concentrations have been identified as an independent marker of insulin resistance and a predictor of type 2 diabetes and non-alcoholic fatty liver. Furthermore, elevated human fetuin-A concentrations are strongly associated with dyslipidemias. Fetuin-A is an abundant carrier of NEFA in plasma and is mainly expressed in hepatocytes, monocytes/macrophages, and adipocytes. Fetuin-A inhibits insulin receptor signaling and therefore increases lipolysis and NEFA efflux from adipose tissues. Despite its strong link with lipolytic processes, fetuin-A expression in plasma and its relationship with metabolic markers in transition cows remains poorly understood. The objective of this study was to evaluate the dynamics of serum fetuin-A and its association with NEFA during the transition period.

Materials and Methods

Holstein cows (n=14) in their second or third lactation were enrolled in a longitudinal cohort study conducted in a large Michigan herd. At the time of selection, cows were non-lactating, had more than 230 days of gestation, and had a body condition score of 3.5 to 3.75. Blood samples were collected at 4 different time points relative to calving: dry-off (DO, -28.4 ± 5 d), close-up (CU, -7.2 ± 4.6 d), fresh (FR, 8.0 ± 3.0 d), and early lactation (EL, 26.9 ± 5.6 d). Blood was collected using EDTA and non-anticoagulant tubes. Plasma NEFA was measured using a colorimetric assay. Plasma fetuin-A concentrations were assessed using a semi-quantitative western blot analysis. Diluted serum samples (1:100 in PBS) were electrophoresed onto tris-glycine gels, transferred to PVDF membranes, and then incubated with a goat anti bo-

vine fetuin-A antibody (1:200 dilution). Protein bands were visualized by chemiluminescence after incubation with donkey anti-goat secondary antibody labeled with horseradish peroxidase (1:10,000). Band densities were quantified using the Quantity One software and these values were normalized to that of the DO sample. Continuous variables were analyzed as repeated measures using a mixed model procedure, and the relation between fetuin-A and NEFA was examined by simple Pearson's correlation.

Results

Lipolysis increased plasma NEFA during the transition period (P=0.002) reaching a peak concentration at FR (0.77 mEq/L), then reducing to values similar to DO (0.18 mEq/L) and CU (0.43 mEq/L) by 4 weeks into lactation (EL=0.43 mEq/L). Plasma fetuin-A relative expression increased as lactation progressed: DO=1.00, CU=1.21, FR=1.91, and EL=2.06 (P=0.08). Remarkably, fetuin-A concentrations were positively correlated with plasma NEFA concentrations (r=0.39; P=0.02) in cows that had plasma NEFA above 0.4 mEq/L at prepartum or 0.7 mEq/L at postpartum.

Significance

Plasma concentrations of fetuin-A tended to increase during the transition period and are positively associated with high lipolysis rates in dairy cows. Future work will determine the value of fetuin-A as a disease risk predictor that could be used together with plasma NEFA to increase sensitivity and specificity of disease prediction.

The relationship between concentrations of non-esterified fatty acids and β-hydroxybutyric acid in transition dairy cows

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Introduction

Both non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHBA) concentrations have been used as cow and herd-level markers of negative energy balance during the transition period of dairy cows to monitor disease risk and reproductive and milking performance.

Prior evidence based on cross-sectional data suggests that the NEFA and BHBA are not well correlated. The objective of this study was to describe the correlation between NEFA and BHBA concentrations of dairy cows in the peripartum period. We hypothesized that accounting for possible lag times between elevation of NEFA and BHBA by using longitudinal data could improve their relationship.