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 bovine 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.

Materials and Methods

A convenience sample of 269 multiparous Holstein cows with repeated measurements of blood NEFA and BHBA (3 wks before to 3 wks after calving) was used in this study. Data originated from 4 separate studies carried out by our research groups and were combined into a single dataset. To represent data over time, area under the curve (AUC) was calculated for all prepartum, postpartum, and combined prepartum and postpartum time-points (total) using the midpoint rule. Pearson correlation coefficients for NEFA and BHBA AUC were analyzed using Proc CORR in SAS (v. 9.3, Cary, NC). Day of maximum concentration for both analytes and the incidence of hyperketonemia (blood BHBA ≥ 1.2 mmol/L) were determined during the postpartum period for each animal. In addition, cows were dichotomized based on their average milk production during weeks 1 through 3 and body condition score (BCS) in the first week postpartum.

Results

Prepartum AUC of NEFA and BHBA were not correlated (r=-0.07, *P*=0.31). Postpartum and total AUC for NEFA and BHBA showed only a weak correlation (r=0.26 and 0.21, *P*<0.001). The mean (± SD) of day of maximum NEFA and BHBA concentration was 6.8 (± 5.3) and 9.6 (± 6.1). The peak incidence of hyperketonemia was on day 4 postpartum. In 16.5 % of cows maximum NEFA and BHBA occurred on the same day. Although the correlation of postpartum NEFA and BHBA was smaller in cows with a postpartum BCS ≤ 3.25 (n=201, r=0.10, *P*=0.14) compared with those that had a BCS > 3.25 (n=67, r=0.38, *P*=0.002), and smaller in cows with an average milk production >84 lb (>38 kg)(n=140, r=0.16, *P*=0.06) than in cows that produced less milk (n=127, r=0.39, *P*<0.001), correlations remained weak.

Significance

Using longitudinal data of NEFA and BHBA by computing AUC did not improve correlations compared to values previously reported in a cross-sectional sample. Although there is evidence that production and body condition characteristics have an effect on the relationship between both markers, there was no meaningful improvement in correlations when taking these into account. Overall, the correlation between the commonly used markers of negative energy balance in peripartum dairy cattle is poor. In conclusion, caution should be exerted when extrapolating the relationship between concentrations of NEFA and BHBA in transition dairy cows.

A comparative evaluation of 2 cow-side meters and a milk test for the diagnosis of subclinical ketosis

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Introduction

Subclinical ketosis (SCK) is a metabolic disease of dairy cows that has significant economic effects on dairy farms due to its effect on reproductive performance, milk production, and future risk of disease. Subclinical ketosis has been defined as being present when serum β-hydroxybutyrate (βHB) concentrations are ≥1.2 mmol/L. Currently there are 2 electronic hand- held meters marketed for cow-side determination of βHB levels. One meter, the Nova Vet Blood Ketone and Glucose Monitoring System, is a new veterinary specific entrant to the market, and no performance data on it exists. The other, Precision Xtra, is a human meter validated for use in cows. The objective of this study was to evaluate the diagnostic test performance of 2 different cow-side handheld meters and a milk βHB test to a reference laboratory testing method.

Materials and Methods

This study was conducted on 13 herds in southeastern Minnesota. Blood samples on cows 3 to 14 days in milk (DIM) were collected immediately after milking on the same day as milk samples were taken. Milk samples were collected by Minnesota Dairy Herds Improvement Association and analyzed for βHB concentrations using fourier transform infrared technology. Whole blood samples were tested immediately cow-side using both the meters. A second blood sample was taken and the separated serum was sent to a reference laboratory for analysis of serum βHB concentrations using colorimetric methods. Performance was evaluated by calculating sensitivity (SE), specificity (SP), and concordance correlation coefficient. The optimum cut-points for each βHB method were determined using receiver operator curve analysis.