# High NEFA concentration in postpartum dairy cows is not strongly correlated with hyperketonemia; are there associated genomic regions?

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## Introduction

Early postpartum dairy cows are subject to physiological changes, including adaptations to support rapidly increasing energy output in milk in excess of energy intake. During this period of negative energy balance (NEB), cows respond by mobilizing lipid and protein reserves in order to support lactation and vital functions. Fat reserves are mobilized as non-esterified fatty acids (NEFA) that can be extracted by the liver and either oxidized or re-esterified, then exported as very low density lipoproteins or accumulated in the liver tissue. Cows vary in their success in supporting these physiological functions during NEB. Some cows adapt very well to NEB; however, other cows do not, resulting in excessive ketone body synthesis.

Our hypothesis is that, within the same environment and management conditions, there is a genetic difference that triggers the capability of some cows to utilize NEFA efficiently whereas other cows develop hyperketonemia. The objective of this study was to identify cows with elevated postpartum NEFA concentrations, with or without concurrent hyperketonemia, and identify genomic regions associated with development of hyperketonemia in early postpartum Holstein cows.

## Materials and Methods

The study population consisted of 123 Holstein dairy cows from 2 different studies: In the first study, 754 cows (parity $\geq$ 2) were enrolled after calving and blood was collected from coccygeal vessels from 3 to 16 DIM 3 times per week. Blood was tested for NEFA concentration (Wako HR series NEFA-HR colorimetric) and $\beta$-hydroxybutyrate (BHBA; Precision Xtra meter). An extra blood sample was harvested once to perform DNA extraction and subsequent sequencing. Hyperketonemia was defined as a BHBA concentration $\geq$1.2 mmol/L. In the second study, 84 cows (parity $\geq$ 2) were enrolled and blood was sampled 3 times per week from -21d to +21d relative to calving. DNA was extracted and sequenced from muscle biopsies. Blood samples were tested for concentrations of BHBA and NEFA as above.

All BHBA and NEFA measurements were grouped using incremental area under the curve (AUC) in order to identify individuals with the most variation. The trapezoidal rule was used to measure AUC by summing the area of all the trapezoids formed between 2 time points (Chiou, 1978). They were divided into 4 different phenotype groups: 1) high NEFA and high BHBA; 2) low NEFA and high BHBA; 3) low NEFA and low BHBA; and 4) high NEFA and low BHBA.

High AUC was considered at values above 7.2 and 4.2 for BHBA and NEFA, respectively. Multivariable ANOVA Wilcoxon/Kruskal Walis Test was performed to compare difference between NEFA AUC and BHBA AUC among the 4 phenotype groups. Linear regression was performed between NEFA AUC and BHBA AUC by PROC REG procedure (SAS 9.3).

Whole-genome genotypes of 777K single-nucleotide polymorphisms (SNPs) were generated using the Illumina Bovine High-density beadchip. A GWAS is being performed to establish correlation between low frequency variants allele (0.5 to 5%) and development of hyperketonemia in early postpartum Holstein dairy cows.

## Results

The mean NEFA AUC and BHBA AUC were 3.62 and 3.84, respectively. The frequency of the 4 different phenotype groups was: 1) 4 cows (3.25%); 2) 4 cows (3.25%); 3) 67 cows (54.47 %); and 4) 48 cows (39.02%).

The mean NEFA AUC for group 3 was 2.34. It differed from the group 1 mean 5.1 ($P = 0.0009$), group 2 mean 3.47 ($P = 0.0351$), and group 4 mean 5.29 ($P < 0.0001$), but the mean NEFA AUC for groups 1 and 4 were not different ($P = 0.7184$).

The mean BHBA AUC for group 3 was 2.94. It differed from group 1 mean 8.23 ($P = 0.0009$), group 2 mean 8.97 ($P = 0.0009$), and group 4 mean 4.29 ($P < 0.0001$). However, the mean BHBA AUC were not different for group 1 and 2 ($P = 0.885$).

Linear regression between NEFA AUC, and BHBA AUC gave an R-square of 0.21.

## Significance

For this study population, the linear regression R-square suggested a low strength of relationship between BHBA synthesis and NEFA concentration in blood. Other factors likely influence ketone production in these cows. Given that these cows were managed under similar conditions, there may be a quantitative trait locus associated with the differences among the 4 phenotype groups.

Identification of quantitative trait loci may allow us to recognize high risk animals and develop preventative measures that decrease the development of hyperketonemia. Identification of this trait may additionally allow improved genetic selection criteria.