

# Identifying Biomarkers for Pre-Onset Insulin Resistance Using Mass Spectrometry-Based Metabolomics: A Pilot Study of Lean and Overconditioned Prepartum Holstein Dairy Cows.

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Metabolomics is a systems biology analytical approach used to study disease phenotypes, an established field in biomedicine that is emerging in the dairy sciences. Parallel with ‘transcriptome’ and ‘proteome’, the comprehensive set of small molecules in biological systems constitutes its ‘metabolome’. Because no single analytical methodology is suited to identify all metabolites, a combination of untargeted and targeted methods (measurement of any molecule that ionizes within a specific mass range, and measurement of specific metabolites, respectively) are employed using gas chromatography (GC) or liquid chromatography (LC), coupled with mass spectrometry (MS) systems. Metabolome screening provides the opportunity to discover molecules (biomarkers) that are associated with disease progression. High-throughput metabolite profiling has been used to identify fatty acylcarnitines, ceramides, glycerophospholipids, fatty acylglycerols, as well as branched chain amino acids, and TCA cycle intermediates as potential predictors for insulin resistance risk in overweight humans and rodents. Similar to overweight monogastrics, overconditioned transition dairy cows experience greater insulin resistance compared with lean cows. Since insulin resistance accelerates NEFA mobilization, overconditioned dairy cattle are at greater risk of developing postpartum disease. Therefore, the objective was to screen the bovine metabolome using GC/MS and LC/MS technologies in search for metabolic phenotypes associated with decreased insulin sensitivity. Our data set included multiparous Holstein cows grouped according to BCS at d-30 prepartum: lean (BCS <3.25; n=21) or overconditioned (BCS >3.75, n=26), with blood samples collected at d-45, -30, -15 and -7, relative to expected calving, and at d1 and 4 postpartum. Relative to lean, overconditioned cows had reduced insulin sensitivity and greater NEFA mobilization at d-30, -15 and -7 prepartum ( $P < 0.05$ ). For untargeted detection, derivatized plasma methanol extracts were analyzed using GC/MS operated in electron ionization mode. For targeted analysis, plasma chloroform-methanol extracts were analyzed by LC coupled to an electrospray ionization source of a triple quadrupole tandem MS system operated in positive ionization mode. Following normalization, log transformation, and median-scaling, data were analyzed using ANOVA and cluster analysis. GC/MS and LC/MS analysis detected lactate, urea, glycerol, branched chain amino acids, ceramides, fatty acylcarnitines, citrate, mono- and disaccharides, saturated and unsaturated free FA, uric acid, fatty acylglycerols, vitamin E, nonesterified cholesterol, and others. Currently used markers (e.g. NEFA and BHBA) have limited predictive power for pre-onset insulin resistance, as they are delayed indications of metabolic stress. Metabolomics may improve our ability to predict prepartum cows at risk of developing greater insulin resistance.

## KEYWORDS

Insulin resistance  
Metabolomics  
Transition cow