

LIVER GENE EXPRESSION DURING THE TRANSITION PERIOD IS ALTERED BY LEVEL OF ENERGY INTAKE DURING THE DRY PERIOD

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TAKE HOME MESSAGES

- Microarray technology is a new molecular tool that allows simultaneous analysis of thousands of genes in a tissue sample. We are the first research group in the world to apply this advanced technology to problems of dairy cattle physiology and nutrition.
- Restricted energy intake before calving resulted in earlier (i.e. day -14 relative to parturition) up-regulation of genes with key functions in liver fatty acid oxidation, gluconeogenesis, and cholesterol synthesis, among others.
- Ad libitum feeding up-regulated a number of genes associated with liver triglyceride synthesis and pro-inflammatory cytokines; these responses corresponded closely with increased liver triglyceride concentrations early postpartum.
- Moderate overfeeding of energy in the dry period results in transcriptional changes predisposing cows to fatty liver, which may compromise overall liver health during the periparturient period.
- Restricted energy intake may confer an advantage to the cow by triggering adaptations of molecular mechanisms in the liver well ahead of parturition.

INTRODUCTION

The DNA microarray “chip” is a new tool used to monitor the level of expression of thousands of genes simultaneously by measuring the abundance of RNA in a tissue sample. Information necessary for the synthesis of enzymes and proteins needed in biological processes by an organism is contained in RNA. The messenger RNA (mRNA) thus serves as the “go-between” that connects the information contained as DNA in the cell nucleus to production of enzymes or proteins to carry out that information in the cell. By measuring the degree of up- or down-regulation of a gene from an RNA sample of a cow’s tissue, we can begin the formidable task of defining roles for each gene and understanding the interactions between sets of genes in a particular tissue during different physiological states.

Suboptimal nutritional management during the transition period can impact the incidence of clinical or subclinical disease and thus animal well-being. Many of the common periparturient

health disorders (e.g. fatty liver, ketosis) are strongly linked to energy balance. Increasing evidence implicates subclinical ketosis and fatty liver as predisposing factors for energy-associated disease as well as other metabolic and infectious problems. Function of the adult liver as well as the performance of essential functions in the body is controlled through the coordinated expression of a large number of genes. Environmental signals, such as nutrition, exert powerful control of liver gene expression.

Current practice is to increase energy density of pre-calving diets and to maximize feed intake before parturition. Our recent data suggest that cows that are moderately overfed during the dry period, even without becoming visually fatter, may be placed at *greater* risk for periparturient health problems. A consistent finding in our studies and others is that cows allowed ad libitum access to higher energy diets during the dry period have larger decreases in feed intake before parturition, and lower feed intake post-partum. Therefore, lower energy diets may be beneficial in maintaining more consistent feed intake around and after parturition.

Genomic technologies may help identify regulatory mechanisms in liver that are sensitive to nutrient balance during the dry period. Here we report temporal expression profiling of more than 6,300 unique genes in liver of dairy cows moderately overfed or restricted-fed a moderate energy diet throughout the dry period using a cattle-specific, high-density cDNA. Evidence indicates that moderate overfeeding of energy during the dry period results in transcriptional changes predisposing cows to fatty liver and compromising overall liver health.

METHODS

Cows were selected from two groups of 12 cows enrolled in a large experiment to assess the effects of ad libitum or restricted intake of moderate-energy diets during the entire dry period on pre-partum metabolism and post-partum metabolism and performance. A corn silage-based diet (26% of diet dry matter) providing 0.72 Mcal/lb during the far-off dry period (first 5 wk of an 8-wk dry period) or providing 0.73 Mcal/lb during the close-up period (last 3 wk of the dry period) was fed for ad libitum (160% of calculated energy requirements) or restricted (80% of requirement) intake. After calving, all cows were fed a common lactation diet, balanced according to NRC (2001) recommendations. Four multiparous Holstein cows were randomly selected from each group. All cows underwent normal calving and were free from disorders.

Total RNA was extracted from liver tissue biopsies obtained on day -65 (dry-off), -30, -14, +1, +14, +28, and +49 relative to parturition. The relative abundance of 7,872 mRNAs in each of the samples was measured by DNA microarray developed at the University of Illinois. By using a 'reference' pool of mRNA collected from tissues not including liver, we were able to calculate the abundance of those genes present in liver relative to their amount in the 'reference' sample.

RESULTS AND DISCUSSION

A total of 85 genes were 'affected' by the interaction of time and pre-partum diet, and 122 genes were affected exclusively by pre-partum diet. Many of these genes are involved in key aspects of lipid metabolism (e.g. fatty acid oxidation, triglyceride formation), carbohydrate metabolism (e.g. synthesis of glucose or 'gluconeogenesis'), cholesterol synthesis, and inflammatory responses (e.g. proteins that activate the immune system). Some of the changes in liver gene expression explain nutrition-induced differences in the capacity of liver to esterify or oxidize

long-chain fatty acids. For example, in cows fed ad libitum prepartum (**AA**; Fig. 1A), we observed a linear increase in expression from day -30 to +14 relative to parturition of genes associated with fat synthesis in liver such as 1-acylglycerol-3-phosphate-acyltransferase (*AGPAT1*). In contrast, expression of genes with key functions in liver fatty acid oxidation such as acyl-CoA dehydrogenase-very long-chain (*ACADVL*), carnitine palmitoyl transferase-1 (*CPT1A*), and adiponectin receptor-2 (*ADIPOR2*) was nearly 2-fold higher around parturition in restricted-fed (**RR**, Fig. 1B) cows. These data show that restricted energy feeding prepartum confers an advantage to the cow by triggering molecular adaptations in liver well ahead of parturition, which are necessary to deal with greater influx and uptake of nonesterified fatty acids (NEFA) by liver after calving. Taken together, these changes are consistent with greater likelihood for fat accumulation in the liver after parturition in cows that had free access to moderate-energy diets prepartum.

During the transition period, there was increased expression of a key gene (pyruvate carboxylase, *PC*) involved in liver glucose synthesis in **RR** cows. Increased *PC* mRNA was likely associated with the need to maintain blood glucose levels in **RR** cows; whereas in **AA** cows greater influx of propionate (from greater grain intake), greater liver glucose output, and the resulting high blood insulin levels could have decreased *PC* expression through a feedback mechanism. Lower post-partum *PC* expression for **AA** cows implies that liver use of lactate and glucogenic amino acids for glucose synthesis (i.e. gluconeogenesis) was reduced, which may have hindered the ability of cows to cope with increasing glucose needs for milk production.

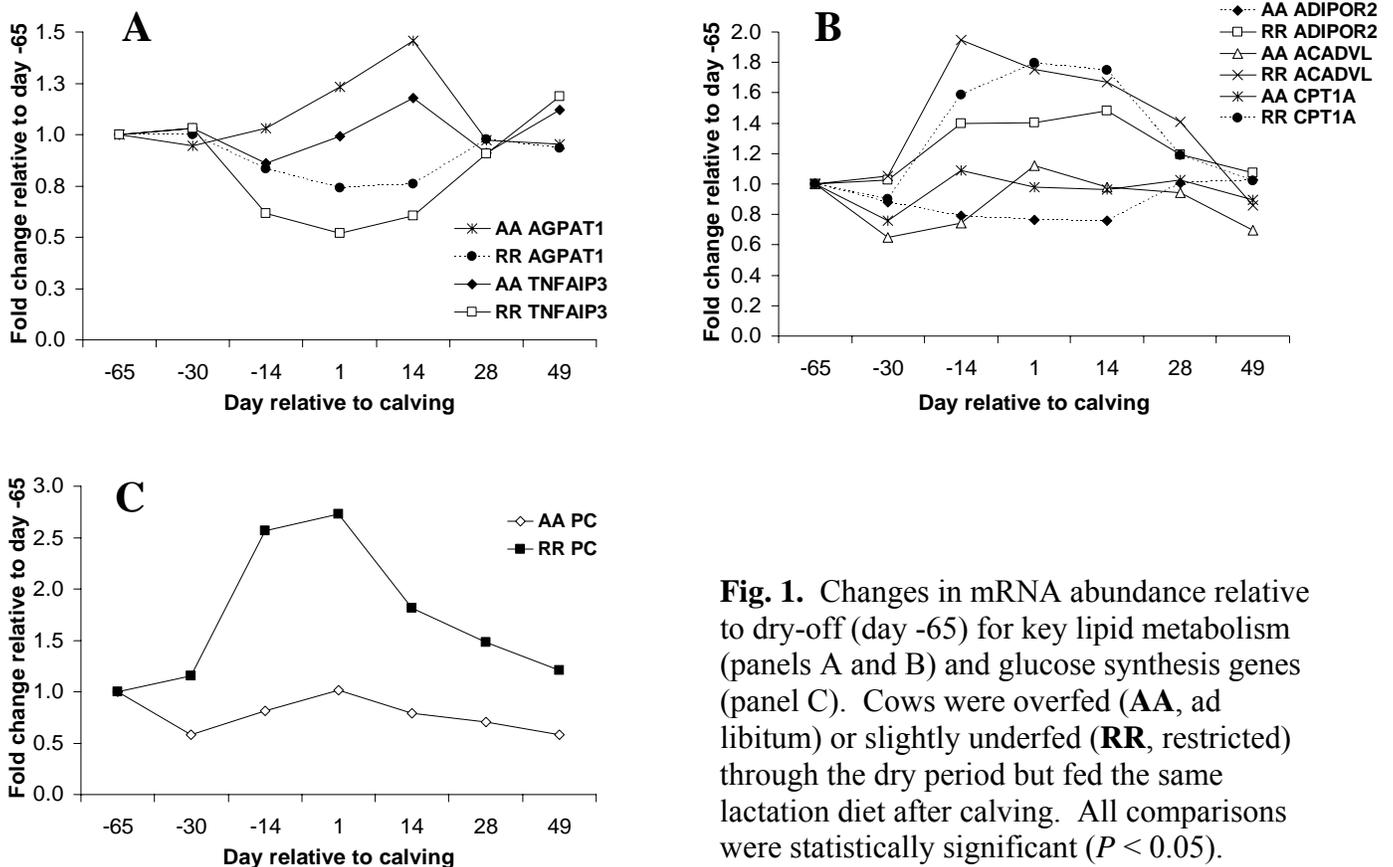


Fig. 1. Changes in mRNA abundance relative to dry-off (day -65) for key lipid metabolism (panels A and B) and glucose synthesis genes (panel C). Cows were overfed (**AA**, ad libitum) or slightly underfed (**RR**, restricted) through the dry period but fed the same lactation diet after calving. All comparisons were statistically significant ($P < 0.05$).