J. Dairy Sci. 100:1–13
https://doi.org/10.3168/jds.2016-12454

Liver transcriptome analysis reveals important factors involved in the metabolic adaptation of the transition cow

N.-T. Ha,*†[1] C. Drögemüller,‡ C. Reimer,† F. Schmitz-Hsu,§ R. M. Bruckmaier,* H. Simianer,† and J. J. Gross*
*Veterinary Physiology, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland
†Animal Breeding and Genetics Group, Department of Animal Sciences, University of Goettingen, 37075 Goettingen, Germany
‡Institute for Genetics, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland
§Swissgenetics, 3052 Zollikofen, Switzerland

ABSTRACT

During early lactation, dairy cows experience a severe metabolic load often resulting in the development of various diseases. The inevitable deficiency in nutrients and energy at the onset of lactation requires an optimal adaptation of the hepatic metabolism to overcome metabolic stress. We conducted a whole-liver transcriptome analysis for the transition cow to identify novel factors crucial for metabolic adaptation. Liver samples were obtained from 6 Red Holstein dairy cows (parity 2 to 7, mean ± standard deviation: 3.7 ± 2.3) at 3 time points: T1 = 22 ± 4 d antepartum, T2 = 10 ± 2 d postpartum, and T3 = 17 ± 2 d postpartum. Using RNA sequencing (RNA-seq), we studied the transcriptomic profile of the transition cow before and after parturition. We performed a differential gene expression analysis (DGEA) and gene-set enrichment analysis (GSEA) for biological processes (gene ontology, GO) and pathways (Kyoto Encyclopedia of Genes and Genomes, KEGG). Among the 10,186 expressed genes, we discovered 1,063 differentially expressed genes (false discovery rate = 5%). The GSEA revealed 16 biological processes and 7 pathways significantly (false discovery rate = 5%) associated with the hepatic changes of the transition cow. Our results confirm that major hepatic changes are related to energy mobilization after parturition; in particular, they are related to fatty acid oxidation/metabolism, cholesterol metabolism, and gluconeogenesis. Using the STRING database (https://string-db.org/), we investigated interactions between significant genes and identified 9 key genes (*CYP7A1*, *APOA1*, *CREM*, *LOC522146*, *CYP2C87*, *HMGCR*, *FDFT1*, *SGLE*, and *CYP26A1*) through which the different processes involved in the metabolic adaptation interact. Comparing our main results with the literature, we could identify further genes that have not yet been associated with the transition period (e.g., *CPT1B*, *ADIPOR2*, *LEPR*, *CREB3L3*, and *CCND1*) and that are mainly involved in processes controlled by AMP-activated protein kinase, an important regulator of energy homeostasis.
Key words: RNA sequencing (RNA-seq), transition cow, metabolic adaptation, hepatic transcriptome

INTRODUCTION

The transition period of a dairy cow, mostly defined as the period from wk 3 antepartum until 3 wk postpartum, is characterized by abrupt changes in physiology, metabolism, and nutrition of the animal (Gross et al., 2011a,b). Intensive fetal growth before parturition, morphological and endocrine changes related to mammary gland development, and the tremendous increase in energy and nutrient demand with the onset of lactation are the most substantial challenges the dairy cow has to face during this period. Hence, optimal metabolic adaptation is required to avoid the development of metabolic and infectious diseases.

The liver is the key organ controlling and regulating metabolic adaptation. Therefore, several studies have been carried out to quantify the molecular adaptations during the transition period (e.g., Greenfield et al., 2000; Reynolds et al., 2003; Drackley et al., 2005; Loor et al., 2005; Graber et al., 2010; Schlegel et al., 2012; Ostrowska et al., 2013) to improve the understanding of the complexity of the transition cow's biology. The main purpose of these studies was to assess the transcriptomic profile of candidate genes known to be involved in liver metabolism during the transition period using mRNA abundances determined by real-time PCR (Greenfield et al., 2000; Graber et al., 2010; Schlegel et al., 2012; Ostrowska et al., 2013). Whereas most of these studies focused mainly on specific metabolic processes that are already known, a more extensive study conducted by Loor et al. (2005) that included the gene expression profiles of more than 6,300 genes using microarray technology.

Received December 14, 2016.
Accepted July 20, 2017.
[1]Corresponding author: nha@gwdg.de

Table 1. Milk yield on test day, energy balance in the test week, and metabolic status of the 6 studied cows

Period	Day of sampling (relative to parturition)	Milk yield (kg)	Energy balance (MJ of NE_L/d)	Plasma concentration (mmol/L)		
				Glucose	Fatty acids	BHB
T1	−22 ± 4	—	26.0 ± 12.7	4.25 ± 0.28	0.17 ± 0.05	0.29 ± 0.04
T2	10 ± 2	31.6 ± 2.2	−42.8 ± 7.9	3.31 ± 0.18	0.80 ± 0.21	0.86 ± 0.31
T3	17 ± 2	35.5 ± 3.0	−34.5 ± 14.7	3.48 ± 0.30	0.76 ± 0.34	0.74 ± 0.32

According to our knowledge, the present study is one of the first using RNA-sequencing (**RNA-seq**) to investigate changes in the whole-liver transcriptome of dairy cows from late pregnancy to early lactation. This study may, first, validate the results established by earlier studies and, second, reveal new possible candidate genes crucial for the hepatic adaptation. A similar approach was taken by McCabe et al. (2012), who analyzed the whole-liver transcriptome using RNA-seq to compare cows divergent in negative energy balance (**NEB**). However, in practice, whether a cow can successfully adapt to the new physiological state of lactation may not depend only on the extent of NEB. Indeed, cows with an optimal adaptive performance could overcome even an extremely severe NEB without any occurrence of health disorders, whereas more vulnerable cows are more prone to fail even under less challenging situations (Kessel et al., 2008; van Dorland et al., 2009; Gross and Bruckmaier, 2015). Therefore, the present study aims to identify currently unconsidered factors involved in the achievement of metabolic adaptation in transition dairy cows.

MATERIALS AND METHODS

Characterization of Cows and Liver Sampling

Experimental procedures and sampling followed the guidelines of the Swiss Law on Animal Protection and were approved by the Veterinary Office of the Canton Fribourg, Switzerland. For our analysis, we collected liver and blood samples from 6 dairy cows (Red Holstein, parity 2 to 7, mean ± SD: 3.7 ± 2.3) at 3 stages: 3 wk before expected calving (**T1**, 22 ± 4 d antepartum) and 2 wk (**T2**, 10 ± 2 d postpartum) and 3 wk (**T3**, 17 ± 2 d postpartum) after calving. Cows were kept at an experimental station with individual daily recording of feed intake and milk yield. Liver sampling was performed by blind percutaneous needle biopsy under local anesthesia as described by van Dorland et al. (2009). Liver samples were directly put in RNAlater (Ambion, Applied Biosystems, Austin, TX) and stored at −80°C until extraction. We calculated energy balance (**EB**) of the animals weekly using milk yield, DMI, and development of BW. Blood samples obtained before liver sampling were analyzed for concentrations of glucose, fatty acids, and BHB to characterize the metabolic changes occurring during the transition period as described previously (Gross et al., 2011a). Apart from the liver samples, the 6 cows were also phenotyped for various traits and metabolic characteristics, shown in Table 1. Only 5 samples were collected at T1; one cow (cow 1) has no sample at T1 because of technical reasons.

RNA Isolation and Sequencing

RNA was isolated from liver samples using RNeasy Mini kit (Qiagen, Hombrechtikon, Switzerland). The quality and quantity of the isolated RNA was measured with an Agilent 2100 Bioanalyzer (Agilent Technologies, Basel, Switzerland) and Qubit 2.0 Fluorometer (Life Technologies, Zug, Switzerland). The RNA integrity number (RIN) of all samples was between 8.1 and 9.0. Approximately 800 ng of high-quality RNA was used for strand-specific paired-end RNA library preparation (TruSeq stranded mRNA sample preparation guide Part #15031047 Rev.D, Illumina, Zürich, Switzerland). Total mRNA libraries were multiplexed (pool of 4 μL of each sample after dilution to 2 n*M*, adding ∼1% PhiX control) and sequenced each in 3 lanes (in the same run) on the Illumina HiSeq2000 platform using 2 × 100-bp paired-end sequencing cycles. The Illumina BCL output files with base calls and qualities were converted into FASTQ file format and demultiplexed with CASAVA (v1.8.2) software (Illumina).

RNA Read Alignment and Counting

To avoid bias in the subsequent analyses, we conducted comprehensive quality control using FastQC (version 0.11.2; Andrews, 2010) for the forward and reverse reads of the 3 lanes for all 17 samples. In general, none of the read sequences failed overall quality control; nevertheless, as a matter of course, some sequences contained bases with low quality scores (<10 Sanger Quality Score). To assess whether these outliers affected our analyses, we trimmed the bad bases for all samples using the program Trimmomatic (version 0.33; Bolger et al., 2014). After quality control, we combined the different lanes for each sample before