Data in Brief

Transcript profiling in the liver of early-lactating dairy cows fed conjugated linoleic acid

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ABSTRACT

In the present study, transcript profiling was carried out in liver biopsies from high-yielding dairy cows at week 5 of lactation in order to identify genes and pathways regulated by feeding rumen-protected conjugated linoleic acid (CLA) during the transition period. Analysis of a bovine whole genome microarray revealed a total number of 130 annotated differentially expressed genes (DEGs) in the liver between cows of the CLA group and the control group (filter: P < 0.05 and fold change (FC) ≥ 1.3 or ≤− 1.3). The number of DEGs in the liver being up-regulated was markedly higher than that being down-regulated (86 vs. 44). Gene set enrichment analysis revealed that the most enriched biological processes and molecular functions assigned to the 86 up-regulated genes were S-methyltransferase activity, ribonucleoprotein complex biogenesis, homocysteine S-methyltransferase activity, methionine biosynthetic process and spliceosome assembly, while the most enriched biological processes and molecular functions assigned to the 44 down-regulated genes were exopeptidase activity, cytokinesis after mitosis, cytokinesis during cell cycle, protein serine/threonine kinase activity and cytokinesis. The microarray dataset from this study has been deposited in NCBI's Gene Expression Omnibus under the accession number GSE87391.

Specifications

Organism/cell line/tissue	Bos taurus (Holstein cows)/liver
Sex	Female
Sequencer or array type	Affymetrix GeneChip® Bovine Genome Array
Data format	Raw CEL files, RMA normalized data
Experimental factors	Conjugated linoleic acid (CLA) fat vs. Control fat
Experimental features	Liver biopsy samples were taken at week 5 of lactation from Holstein cows fed either a CLA fat or a control fat from week 3 prepartum to week 14 postpartum
Consent	Data are publicly available from NCBI GEO
Sample source location	Gießen, Germany

1. Direct link to deposited data

The microarray data are available at: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE87391

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2. Experimental design, materials and methods

2.1. Experimental design

A total of 40 primi- and multiparous Holstein cows with an average parity number of 2.6 were used for this study. The study was carried out at the Agricultural Experimental Station Hirschau of the Technical University of Munich (Germany) and was approved by the Bavarian state animal care and use committee. The experimental period lasted from week 3 prepartum to week 14 postpartum. Details on feeding regimen and husbandry conditions can be found in a recent publication [1]. In brief, the cows were allotted to two groups of 20 cows each: control group and CLA group. Cows of both groups received an identical partial mixed ration (PMR) ad libitum, which was calculated to meet the energy and protein demand of a cow weighing 650 kg and producing 21 kg milk/d. In addition, cows of both groups were individually offered a limited amount of concentrate via transponder-access feeding stations in order to cover the individual extra requirements for milk production. Calculations for energy and protein supply followed the recommendations of the German Society of Nutrition Physiology [2]. The composition and the nutrient and energy concentrations of both, PMR and concentrate, and the amount of concentrate offered are shown in [1]. The rumen-protected fat supplements, either control fat or CLA fat, were fed via an extra portion of concentrate (supplemental concentrate) containing 27.3% fat supplement (either control fat or CLA fat) for both

groups. The supplemental concentrate was fed during the experimental period at a constant amount of 630 g of DM/d and cow by hand once daily. Through the supplemental concentrate the cows of the CLA group received 4.3 g of cis-9,trans-11 CLA and 3.8 g trans-10,cis-12 CLA per day, which represents a CLA dose that is commonly used in the feeding of dairy cows. The composition and the nutrient and energy concentrations of the supplemental concentrate and the contents of major fatty acids in the fat supplements are also given in [1]. Cows were milked twice daily and milk yields of each cow were recorded automatically. Data on milk yield and milk composition have been reported in [1]. At week 1, 5 and 14 of lactation, blood samples and liver biopsies were taken. The liver biopsy procedure has been described in detail in [1]. The liver biopsy samples were immediately snap-frozen in liquid nitrogen and stored at −80 °C until analysis. In the present study, transcript profiling was carried out in the liver samples from week 5 of lactation in order to identify genes and pathways regulated by feeding rumen-protected CLA during the transition period. The first

weeks after parturition represent a critical phase in the productive cycle of high-yielding dairy cows because the liver experiences pronounced metabolic and inflammatory stress which increases the risk to develop liver-associated diseases, such as fatty liver and ketosis [3].

2.2. RNA isolation

Total RNA was isolated from liver biopsy samples using Trizol reagent (Invitrogen, Karlsruhe, Germany) according the manufacturer's protocol within one week after finishing the trial. Concentration and purity of the RNA were estimated from the optical density at 260 and 280 nm, respectively, using an Infinite 200 M microplate reader and a NanoQuant plateTM (both from Tecan, Männerdorf, Switzerland). The integrity of total RNA was checked by 1% agarose gel electrophoresis and the total RNA was judged as suitable if intact bands corresponding to the 18S and 28S ribosomal RNA subunits were visible. The isolated RNA was stored at −80 °C until microarray analysis.

Table 1
The 25 most strongly up- and down-regulated genes in the liver of cows of the CLA group compared to the control group.

Up-regulated in the CLA group		Down-regulated in the CLA group	
Gene symbol (gene title)	FC	Gene symbol (gene title)	FC
FXYD2 (FXYD domain containing ion transport regulator 2)	2.21	SLC2A5 (solute carrier family 2/facilitated glucose/fructose transporter), member 5)	−2.69
RNF144B (ring finger protein 144B)	1.59	MYH10 (myosin, heavy chain 10, non-muscle)	−1.73
LGALS3 (lectin, galactoside-binding, soluble, 3)	1.56	MT2A (metallothionein 2A)	−1.70
LAMP1 (lysosomal-associated membrane protein 1)	1.56	CA13 (carbonic anhydrase XIII)	−1.65
CDH11 (cadherin 11, type 2, OB-cadherin)	1.54	LOC100140338 (similar to nuclear antigen Sp100)	−1.63
ASPN (aspirin)	1.51	AK3L1 (adenylate kinase 3-like 1)	−1.60
GPM6A (glycoprotein M6A)	1.51	CATSPERG (cation channel, sperm-associated, gamma)	−1.57
TMEM167A (transmembrane protein 167A)	1.51	CPA1 (carboxypeptidase A1)	−1.48
EIF2A (eukaryotic translation initiation factor 2A, 65 kDa)	1.49	LOC781494 (similar to Myeloid-associated differentiation marker)	−1.39
IRX3 (iroquois homeobox 3)	1.47	ARF6 (ADP-ribosylation factor 6)	−1.38
KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog)	1.47	PGCP (plasma glutamate carboxypeptidase)	−1.36
LOC100137763 (hypothetical protein LOC100137763)	1.46	NFKBIE (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon)	−1.36
CRP (C-reactive protein, pentraxin-related)	1.44	RGS9BP (regulator of G protein signaling 9 binding protein)	−1.36
ACYP2 (acylphosphatase 2, muscle type)	1.43	ZAP70 (zeta-chain (TCR) associated protein kinase 70 kDa)	−1.35
ACSL3 (acyl-CoA synthetase long-chain family member 3)	1.42	SKAP1 (src kinase associated phosphoprotein 1)	−1.35
BHMT (betaine–homocysteine S-methyltransferase)	1.42	AARSD1 (alanyl-tRNA synthetase domain containing 1)	−1.34
MTR (5-methyltetrahydrofolate-homocysteine methyltransferase)	1.42	KLHDC4 (kelch domain containing 4)	−1.34
CA4 (carbonic anhydrase IV)	1.40	CEP110 (centrosomal protein 110 kDa)	−1.34
NRP1 (neuropilin 1)	1.40	C9orf100 (vav-like protein C9orf100 homolog)	−1.34
SMEK2 (SMEK homolog 2, suppressor of mek1)	1.39	CYP17A1 (steroid 17-alpha-hydroxylase/17,20 lyase)	−1.33
FBP1 (fructose-1,6-bisphosphatase 1)	1.39	SERPINB6 (serpin peptidase inhibitor, clade B (ovalbumin), member 6)	−1.33
ATE1 (arginyltransferase 1)	1.39	LOC100139934 (similar to zinc finger protein 90)	−1.32
ACSM3 (acyl-CoA synthetase medium-chain family member 3)	1.38	CSNK1G2 (casein kinase 1, gamma 2)	−1.32
SCOC (short coiled-coil protein)	1.38	B3GALT2 (UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2)	−1.32
AHSG (alpha-2-HS-glycoprotein)	1.38	OLFML1 (olfactomedin-like 1)	−1.32

2.3. Microarray analysis

For microarray analysis, RNA pools (n = 5) each for the CLA group and the control group were prepared. Each RNA pool comprised RNA from 4 different cows. The RNA pools were sent to the Kompetenzzentrum Fluoreszente Bioanalytik (KFB) at the University of Regensburg for hybridization to the GeneChip® Bovine Genome Array (Affymetrix, High Wycombe, UK) representing approximately 23,000 bovine transcripts. Prior to hybridization, the concentration and the integrity of the RNA pools were assessed using a Bioanalyzer 2100 (Agilent, Böblingen, Germany). The A260/A280 ratios and the RNA integrity number (RIN) of the RNA pools were 2.02 ± 0.05 and 7.4 ± 0.4 (mean $\pm$ SD, n = 10), respectively. Subsequently, total RNA was transcribed to first- and second-strand cDNA. After purification and testing on the Bioanalyzer 2100, the double-stranded cDNA served as a template for the *in vitro*-transcription reaction for cRNA synthesis. The cRNA was labeled with biotin using a GeneChip labeling kit (Affymetrix). After checking the quality and quantity of the labeled cRNA, cRNA was fractionated and hybridized with the bovine gene chips. Finally, gene chips were washed and stained in a GeneChip Fluidics station 450 (Affymetrix), and scanned at high resolution (GeneChip scanner 3000, Affymetrix). All procedures were performed according to Affymetrix protocols (GeneChip expression analysis, Technical manual from Affymetrix). The quality of hybridization was assessed in all samples following the manufacturer's recommendations. After scanning the gene chips, cell intensity (CEL) files containing a single intensity value for each probe cell were generated from the raw image data with GeneChip Operating Software v1.2 (Affymetrix). CEL files were imported into Expression Console v1.1 (Affymetrix) and subjected to global normalization using the Robust Multiarray Average (RMA) algorithm. The RMA normalized data were used to statistically evaluate differences between the two groups by Student's *t*-test. Genes were considered as differentially expressed genes (DEGs) at P < 0.05 and fold-change (FC) ≥ 1.3 or ≤ -1.3.

2.4. Functional interpretation of microarray data

To extract biological meaning from the identified DEGs, the bioinformatic tools from the Database for Annotation, Visualization and Integrated Discovery (DAVID) were applied [4]. The DAVID Functional Annotation Chart tool was used for gene set enrichment analysis (GSEA) with a modified Fisher's exact test (EASE score) in order to identify enriched (overrepresented) Gene Ontology (GO) terms within GO categories biological process and molecular function and enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways assigned to the DEGs, separately for the up- and down-regulated genes.

3. Results

Considering the filter criteria P < 0.05 and FC ≥ 1.3 or ≤ -1.3 a total number of 130 annotated DEGs could be identified in the liver between cows of the CLA group and the control group. The number of DEGs in the liver being up-regulated was markedly higher than that being down-regulated (86 vs. 44). The 25 most strongly up- and down-regulated genes in the liver of cows of the CLA group compared to the control group are presented in Table 1. The FC of the most strongly up-regulated genes ranged between 2.21 (FXYD2) and 1.38 (AHSG), while those of the most strongly down-regulated genes ranged between −2.69 (SLC2A5) and −1.32 (OLFML1).

GSEA revealed that the top 5 enriched GO terms (biological process and molecular function) assigned to the 86 up-regulated genes were *S*-methyltransferase activity, ribonucleoprotein complex biogenesis, homocysteine *S*-methyltransferase activity, methionine biosynthetic process and spliceosome assembly, while the top 5 enriched GO terms assigned to the 44 down-regulated genes were exopeptidase activity, cytokinesis after mitosis, cytokinesis during cell cycle, protein serine/threonine kinase activity and cytokinesis. Table 2 shows all enriched

Table 2
The most enriched Gene Ontology (GO) terms within categories biological process and molecular function and KEGG pathways assigned to the 86 up- and 44 down-regulated genes in the liver of cows of the CLA group compared to the control group.

Up-regulated in the CLA group	Down-regulated in the CLA group
GO terms	
S-methyltransferase activity	Exopeptidase activity
Ribonucleoprotein complex biogenesis	Cytokinesis after mitosis
Homocysteine *S*-methyltransferase activity	Cytokinesis during cell cycle
Methionine biosynthetic process	Protein serine/threonine kinase activity
Spliceosome assembly	Cytokinesis
Methionine metabolic process	Metallopeptidase activity
Sulfur amino acid biosynthetic process	Retina development in camera-type eye
Regulation of cellular protein metabolic process	Establishment of organelle localization
RNA binding	
Aspartate family amino acid biosynthetic process	
KEGG pathways	
One carbon pool by folate	T cell receptor signaling pathway
Histidine metabolism	Steroid hormone biosynthesis
Cysteine and methionine metabolism	B cell receptor signaling pathway
Drug metabolism	Natural killer cell mediated cytotoxicity
PPAR signaling pathway	Neurotrophin signaling pathway
Spliceosome	Endocytosis

The GO terms were sorted by their enrichment P-values (EASE score) (top: lowest P-value, bottom: highest P-value) within the GO categories biological process and molecular function. Only GO terms with P-values < 0.05 are shown.

GO terms (P < 0.05) assigned to the up- and down-regulated genes. In addition, Table 2 summarizes the most enriched KEGG pathways assigned to the 86 up-regulated and the 44 down-regulated genes.

4. Conclusion

Considering the present results from microarray analysis, in particular the small number of DEGs (130) and the weak regulation of DEGs (greatest FC of 2.21 and −2.69, respectively) in the liver of cows, indicates that feeding of rumen-protected CLA to high-yielding dairy cows during the transition period has, with few exceptions, only little impact on metabolic and regulatory pathways in the liver. One of these exceptions in favour of a small impact of CLA on liver metabolism in dairy cows is that hepatic genes up-regulated in the CLA group are obviously involved in the metabolism of amino acids, namely cysteine, methionine and histidine. The biological significance of this finding remains to be elucidated in the future.

References

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