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Dietary *trans* octadecenoic acids upregulate the liver gene encoding Peroxisome Proliferator-Activated Receptor-α in transition dairy cows

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Effects of feeding calcium salts of conjugated linoleic acid (CLA) or *trans* octadecenoic acids (*trans* 18 : 1) on lipid metabolism and hepatic contents of mRNA encoding carnitine palmitoyltransferase 1 (CPT1), microsomal triglyceride transfer protein (MTP) and peroxisome proliferator-activated receptor alpha (PPARα) were examined in 15 early *post-partum* Holstein cows. Dietary treatments were initiated at approximately 4 weeks prior to expected calving dates and continued for 7 weeks *post partum*. Treatments *prepartum* consisted of 1) a basal diet (Control), 2) basal diet+150 g/d of CLA mix (CLA), or 3) basal diet+150 g/d of *trans* 18 : 1 mix (TRANS). Intakes of calcium salts of CLA and *trans* 18 : 1 mixes were adjusted to 225 g/d during the 7-week *post-partum* treatment period. Blood samples were collected at weeks 1, 2 and 4 *post partum* and plasma was harvested immediately for subsequent hormone and metabolite assays. Concentrations of insulin, insulin-like growth factor-I (IGF-I), and leptin in blood did not vary among cows fed the three diets. Plasma nonesterified fatty acid (NEFA) concentrations decreased between weeks 1 and 4 of lactation and were lower in cows fed the diet supplemented with *trans* 18 : 1 than in those fed a control diet at week 2 *post partum*. Periparturient fat supplementation had no detectable effects on CPT1 mRNA content in the liver. Steady-state concentration of MTP mRNA in the liver was greater in the TRANS treatment group than in the control group at week 1 *post partum*. Feeding *trans* 18 : 1 supplements to transition dairy cows upregulated hepatic PPARα mRNA content during the first month of lactation. Under the present experimental conditions, dietary CLA had minimal effects on plasma and hepatic lipid metabolite concentrations in early lactation Holstein cows. Results indicate that dietary *trans* fatty acids may affect liver lipid metabolism in *post-partum* dairy cows through alterations in PPARα gene expression.

Keywords: CLA, *trans* fatty acid, liver genes.

In *post-partum* dairy cows, a key metabolic adjustment in support of lactation is extensive mobilization of body fat to meet the increased demand for energy (Bell, 1995; Drackley, 1999). However, if the rate at which fatty acids are mobilized from adipose tissue exceeds their rate of oxidation and export out of the liver, triacylglycerol (TAG) accumulates within the hepatocytes, and the acetyl-CoA that is not incorporated into the tricarboxylic acid cycle is converted to ketones (Grummer, 1993; Goff & Horst, 1997; Drackley, 1999).

Factors controlling the flux of free fatty acids between hepatic oxidative and esterification pathways are not fully understood in dairy cattle. The translocation of long-chain fatty acids into the mitochondrion, a step that is required for β-oxidation, is regulated by the enzyme carnitine palmitoyltransferase-1 (CPT1; EC 2.3.1.21; Grummer, 1993; Drackley, 1999). In ruminants, CPT1 activity is inhibited by malonyl coenzyme A and methylmalonyl coenzyme A (Brindle et al. 1985; Knapp et al. 1992). As suggested by Zammit (1990), inhibition of CPT1 by methylmalonyl coenzyme A may be a mechanism to link supply of propionate from ruminal fermentation with the need for NEFA oxidation. The nature and extent to which dietary management of the dairy cow during the transition period affects hepatic CPT1 and other metabolic enzyme activities or synthesis remain to be elucidated.

Microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein composed of a unique large subunit (MW 97 kDa) and the multifunctional

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enzyme, protein disulphide isomerase (Wetterau & Zilversmit, 1985; Wetterau et al. 1990; Wetterau et al. 1991). MTP transfers lipids into apolipoprotein B-containing lipoproteins (Gordon et al. 1994; Patel & Grundy, 1996; Davidson & Shelness, 2000), and therefore may play a crucial role in the assembly and secretion of very low density lipoprotein (VLDL) in the liver (Bremmer et al. 2000a). In periparturient dairy cows, hepatic MTP mRNA increases between day −27 to 2, but without detectable correlations between hepatic MTP activity, mass, or mRNA with either TAG or plasma NEFA (Bremmer et al. 2000b). Other studies report significant alterations in hepatic lipid metabolism as a result of dietary fat supplementation during the *pre-partum* (Grum et al. 1996) or early *post-partum* (Grum et al. 2002) period in dairy cattle. In both studies, hepatic peroxisomal oxidation of palmitate was increased by dietary fat supplementation. The observation that both total and peroxisomal β-oxidations correlate negatively with hepatic lipid accumulation (Grum et al. 2002) would suggest that dietary fat-induced alteration in liver lipid metabolism may play a role in the aetiology of the fatty liver that occurs in dairy cows at parturition.

Dietary polyunsaturated fatty acids (PUFA) lower blood triglyceride, decrease intramuscular lipid droplet size, improve insulin sensitivity, and enhance nonhepatic glucose utilization in the rat (Xu et al. 2002). PUFA elicit these metabolic changes through either inducing the transcription of genes encoding proteins involved in lipid oxidation or suppressing the expression of genes encoding proteins involved with lipid synthesis (Clarke et al. 2002). Peroxisome proliferator-activated receptor alpha (PPARα), a member of the nuclear receptor superfamily, is a key transcription regulator of many genes involved in lipid transport, oxidation and thermogenesis (Clarke et al. 2002). Because PPARα coordinates fatty acid metabolism in the liver, and PPARα knock-out mice develop fatty liver when fasted (Hashimoto et al. 2000), we hypothesized that this transcription factor may play a role in supplemental fat-induced alteration in lipid metabolism in transition dairy cows.

The objective of this study was to examine the effect of feeding calcium salts of conjugated linoleic acid (CLA) and *trans* octadecenoic acids (TRANS) on lipid metabolism and hepatic contents of mRNA encoding CPT1, MTP and PPARα in early *post-partum* Holstein cows.

Materials and Methods

Animals, diets and sampling

Thirty-eight multiparous Holstein cows were used in a completely randomized design to examine the effect of feeding calcium salts of CLA and *trans* octadecenoic acids (*trans* 18:1) on production and metabolic responses during the transition to lactation (Selberg et al. 2004). Plasma and liver samples from a subset of these cows ($n=5$ for each treatment group) were used in the current study to elucidate further the effect of dietary CLA and *trans* 18:1 on liver genes involved in lipid oxidation during the early *post-partum* period. As described previously (Selberg et al. 2004), Holstein cows were assigned randomly to receive a control (basal diet), CLA-supplemented (basal diet+CLA mix) or *trans* 18:1-supplemented (basal diet+ *trans* 18:1 mix) diet from approximately 4 weeks before calving until 7 weeks *post partum*. Experimental diets were formulated to meet or exceed NRC (2001) recommendations for energy and crude protein during the early *post-partum* period (Table 1; Selberg et al. 2004). Amounts of calcium salts of CLA and *trans* 18:1 mixes were 150 g/d *prepartum* and 225 g/d during the 7-week *post-partum* treatment period. The CLA fat supplement contained 9·18% (w/w) *cis*-8, *trans*-10, 6·34% *cis*-9, *trans*-11, 8·19% *cis*-11, *trans*-13, and 7·88% *trans*-10, *cis*-12 CLA isomers (Bioproducts Inc., Fairlawn, OH, USA). The *trans* octadecenoic acid mix contained 20·62% (w/w) *trans*-6–8, 10·47% *trans*-9, 10·62% *trans*-10, 7·05% *trans*-11, and 8·73% *trans*-12 (Bioproducts Inc.). Complete fatty acid profiles of dietary fat supplements are presented in Table 2 (Selberg et al. 2004). Doses of CLA and *trans* fatty acids used in this study were based on a previous study which showed no deleterious effects of 225 g/d of ruminally inert CLA on dry matter intake in early lactation dairy cows (Dhiman et al. 2003). Fat supplements were mixed with the concentrates and offered as part of a total mixed ration to experimental animals. Blood samples were collected via coccygeal vessel at weeks 1, 2 and 4 of lactation and plasma was immediately prepared for subsequent hormone and metabolite analyses. On days 2, 14±2 and 28±2 *post partum*, liver samples were collected via biopsy, rinsed with sterile saline and stored at −80 °C until analysed for TAG and mRNA abundance. These samples were selected based on our previous observation that most of plasma and liver metabolites were back to calving levels by 4 weeks *post partum* (Selberg et al. 2004).

Quantification of plasma hormones

Concentrations of insulin in plasma were measured using the double antibody radioimmunoassay (RIA) procedure described by Malven et al. (1987). Sensitivity of the assay was 0·1 ng/ml, and intra- and interassay CV was 2·7 and 6·9%, respectively. Concentrations of insulin-like growth factor-I (IGF-I) in plasma were determined using a double antibody RIA (Simmen et al. 1989). Plasma samples were extracted with acid ethanol and neutralized with Tris–HCl prior to RIA to remove IGF binding proteins that may interfere with the assay. Human recombinant IGF-I (Upstate Biotechnology, Lake Placid, NY, USA) was used as the standard and monoiodinated tracer for the IGF-I RIA. Extracted plasma samples (10 µl) were incubated with human IGF-I antiserum (1:20 000 dilution) and [^{125}I]IGF-I (adjusted to 20 000 cpm/tube) for 24 h at 4 °C. Antigen-antibody complexes were precipitated by addition of sheep anti-rabbit gamma globulin at a dilution of 1:4.

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Table 1. Ingredients and chemical composition of experimental diets

Ingredient g/kg DM	*Prepartum*[1]	*Post partum*[2]
Alfalfa hay	…0	102
Bermuda grass hay	132	0
Corn silage	392	289
Cottonseed hulls	0…	51
Citrus pulp	111	67
Prolak[3]	13	14
Corn meal	200	199
Soybean meal	42	136
Whole cottonseed	50	99
Mineral mix[4]	55	0…
Mineral mix[5]	…0	40
Trace mineralized salt[6]	6	2
Feed phosphorus[7]	…0	2
Chemical composition		
CP, g/kg DM	121	180
ADF, g/kg DM	218	221
NDF, g/kg DM	362	322
NFC, g/kg DM	391	367
Ether extract, g/kg DM	43	52
NE_L, Mcal/kg DM[8]	1·54	1·65
Ca, g/kg DM	17·5	3·5
Mg, g/kg DM	3·0	3·2
K, g/kg DM	11·2	13·6
Na, g/kg DM	2·5	4·0
S, g/kg DM	2·2	2·0
Fe, mg/kg DM	402	406
Zn, mg/kg DM	92	106
Cu, mg/kg DM	18	29
Mn, mg/kg DM	63	76
Mo, mg/kg DM	1	1

[1] Diets contained 0 or 14·4 g/kg of Ca salts of conjugated linoleic acids (CLA), or 14·4 g/kg of Ca salts of *trans* octadecenoic fatty acid mixture (DM basis). The Ca salt products partially replaced cornmeal

[2] Diets contained 0 or 12·9 g/kg of Ca salts of CLA, or 12·9 g/kg of Ca salts of *trans* octadecenoic fatty acid mixture (DM basis). The Ca salt products partially replaced cornmeal

[3] A marine and animal protein supplement containing 680 g/kg rumen-undegraded protein (H J Baker & Bro., Inc., Stamford, CT, USA)

[4] Mineral and vitamin mix contained 228 g/kg CP, 21 g/kg fat, 228·9 g/kg Ca, 1·6 g/kg P, 27·7 g/kg Mg, 7·5 g/kg Na, 2·0 g/kg K, 24·2 g/kg S, 80·3 g/kg Cl, 146·7 mg/kg of Mn, 95·0 mg/kg of Zn, 26·6 mg/kg of Fe, 112·5 mg/kg of Cu, 10·7 mg/kg of Co, 7·9 mg/kg of I, 6·9 mg/kg of Se, 268,130 i.u./kg of vitamin A, 40 000 i.u./kg of vitamin D, and 1129 i.u./kg of vitamin E (DM basis)

[5] Mineral and vitamin mix contained 264 g/kg CP, 17·4 g/kg fat, 101·5 g/kg Ca, 9·0 g/kg P, 31 g/kg Mg, 86 g/kg Na, 51 g/kg K, 15 g/kg S, 41 g/kg Cl, 2231 mg/kg of Mn, 1698 mg/kg of Zn, 339 mg/kg of Fe, 512 mg/kg of Cu, 31 mg/kg of Co, 26 mg/kg of I, 7·9 mg/kg of Se, 147,756 i.u./kg of vitamin A, 43 750 i.u./kg of vitamin D, and 787 i.u./kg of vitamin E (DM basis)

[6] Minimum concentrations of 400 g/kg Na, 550 g/kg Cl, 2·5 g/kg Mn, 2 g/kg Fe, 0·33 g/kg Cu, 0·07 g/kg I, 0·05 g/kg Zn, 0·025 g/kg Co (DM basis). Manufactured by Flint River Mills, Inc., Bainbridge, GA, USA

[7] Contains 210 g/kg P and 150 to 180 g/kg Ca. Manufactured by Southeastern Minerals, Inc., Bainbridge, GA, USA

[8] Net Energy for lactation values for *prepartum* and *post-partum* diets containing calcium salts were 6·61 and 7·07 MJ/kg DM, respectively

Table 2. Fatty acid profile of calcium salts of conjugated linoleic acid (CLA) and *trans*-octadecenoic isomers (TRANS)

Fatty acid	CLA†	TRANS†
	g/100 g fatty acids	
12 : 0	0·15	0·04
14 : 0	0·37	0·31
16 : 0	15·38	12·21
16 : 1	0·14	0·15
18 : 0	2·24	6·70
18 : 1, *trans* 6–8	…	20·62
18 : 1, *trans*-9	…	10·47
18 : 1, *trans*-10	…	10·62
18 : 1, *trans*-11	…	7·05
18 : 1, *trans*-12	…	8·73
18 : 1, *cis*-9	…	10·04
18 : 2, *cis*-9, *cis*-12	34·86	1·97
CLA, *cis*-8, *trans*-10	9·18	…
CLA, *cis*-9, *trans*-11	6·34	…
CLA, *cis*-11, *trans*-13	8·19	…
CLA, *trans*-10, *cis*-12	7·88	…
CLA, others	12·19	…
Unknown‡	3·06	11·07

† Fatty acid composition provided by Bioproducts Inc. (Fairlawn, OH, USA)

‡ Numbers representing fractions of total peak area that have not been identified

Sample tubes were counted for 1 min in the gamma counter. Sensitivity of the assay was 2·5 ng/ml, and intra- and interassay CV was 5·1 and 7·6%, respectively. Plasma leptin concentration was measured using a double antibody RIA (Multi-species leptin RIA kit, Linco Research Inc., St. Charles, MO, USA). Detailed characteristics of this assay for measuring bovine plasma leptin concentration were described previously (Delavaud et al. 2002). The minimum detectable concentration was 1·0 ng/ml and intra-assay CV was 3·3%.

Plasma and liver metabolite analyses

Concentrations of NEFA (NEFA-C kit; Wako Fine Chemical Industries USA, Inc., Dallas, TX, USA) and β-hydroxybutyric acid (BHBA; Sigma procedure No. 319, Sigma Chemical Co., St. Louis, MO, USA) in plasma were measured enzymically with commercial kits. Hepatic lipid was extracted from finely homogenized liver with a 2 : 1 (vol/vol) mixture of chloroform and methanol, and concentrations of total lipids were determined gravimetrically after drying under N_2 (Drackley et al. 1991). The lipid extract was redissolved in 3 : 2 (vol/vol) mixture of hexane and isopropanol, and concentrations of TAG were measured colourimetrically as described by Foster & Dunn (1973).

RNA isolation and dot blot analysis

Total liver cellular RNA was isolated using TRIzol reagent (Life Technologies, Grand Island, NY, USA) following the

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Table 3. Effects of conjugated linoleic acid (CLA) and *trans*-octadecenoic acids (TRANS) on plasma hormone concentrations in *post-partum* Holstein cows

Values are least squares means with SEM for $n=5$ per treatment

	Day of lactation				*P* value		
	2	14	28	SEM	T[a]	D[b]	T × D[c]
Insulin, ng/ml					0·57	0·04	0·61
Control	0·28	0·32	0·38	0·06			
CLA	0·31	0·23	0·33	0·06			
TRANS	0·33	0·36	0·42	0·06			
IGF-I, ng/ml					0·76	0·23	0·64
Control	109·6	116·0	99·1	15·8			
CLA	94·0	99·8	92·0	15·8			
TRANS	83·4	102·6	101·0	15·8			
Leptin, ng/ml					0·15	0·02	0·05
Control	3·8	4·2	6·8	0·8			
CLA	5·0	5·2	6·7	0·8			
TRANS	3·8	4·0	3·9*	0·8			

* Value was significantly different ($P<0·05$) from that of the control or CLA group at day 28
[a] Effect of treatment; [b] Effect of day; [c] Effect of treatment × day interaction

manufacturer's instructions. Samples of total RNA (10 μg) were immobilized on Biotrans nylon membranes (ICN, Irvine, CA, USA) inserted in a microsample filtration unit (Schleicher & Schuell, Keene, NH, USA). The RNA was cross-linked to the membrane by u.v. irradiation and baked at 80 °C for 1 h. Complementary DNA probes were generated using primers designed from human CPT1 (forward: 5′ ATG GCA GAA GCT CAC CAA GCT 3′; reverse: 5′ GTA GAT GTA CTC CTC CCA CCA 3′), human MTP1 (forward: 5′ CCG TTT CTC CAG GAG TGG ATC; reverse: 5′ GGC TGA CAT ACC AGC ATA GGA 3′) and bovine PPARα (forward: 5′ CAG ATG GCT CCG TTA TTA CAG; reverse: 5′ TCT GTA GGT GGA GTT TGA GCA 3′) sequences. The RNA filters were prehybridized in Rapid-Hyb® buffer (Amersham-Pharmacia Biotech., Piscataway, NJ) at 60 °C for 30 min. The filters then were hybridized with random primer-labelled bovine CTP1, MTP and PPAR cDNA probes for 18 h. After hybridization, RNA filters were washed for 20 min in 50 ml 2X SSC, 0·1% (v/v) SDS at room temperature followed by two 15-min washes in 0·1X SSC, 0·1% SDS at 42 °C. The target gene transcripts were identified by autoradiography at −80 °C. Hybridization signals for each gene were quantified by densitometric analysis. Equal loading of total RNA and specificity of treatment effects were verified by subsequent re-hybridization of RNA membranes with 18S ribosomal RNA probe.

Statistical analysis

Hormone, metabolite and mRNA responses to dietary treatments were evaluated using the MIXED procedure of SAS for repeated measures (Littell et al. 1998). Fixed effects included diet, day of lactation and diet × day interaction. The variance for cow nested within diet was used as the random error term to test the main effect of diet. When diet × day interactions were detected ($P<0·05$), means within each collection day were compared using the SLICE option of the MIXED procedure. In mRNA analyses, the densitometric values for target genes were adjusted using the values for 18S ribosomal RNA as covariate.

Results

Plasma hormones

Dietary treatment had no detectable effect on plasma insulin concentration within the first month of lactation (Table 3). Mean plasma insulin concentration was greater ($P<0·05$) at week 4 than at weeks 1 and 2 *post partum* (Table 3). There were no differences between the effects of diet or blood collection time on plasma IGF-I concentration (Table 3). A treatment × week interaction was detected ($P<0·05$) for blood leptin concentration (Table 3). Plasma leptin concentrations were greater at week 4 than at weeks 1 and 2 in control cows and those fed CLA. Conversely, in cows fed the TRANS diet, plasma leptin concentration was lower and significantly different from that of the control or CLA diet at week 4 (Table 3).

Plasma and liver metabolites

In all treatment groups, plasma NEFA concentrations were greater ($P<0·01$) at week 1 than at week 4 *post partum* (Table 4). Compared with control cows, supplemental *trans* fatty acids lowered ($P<0·01$) plasma NEFA concentration at week 2 *post partum* (Table 4). Blood NEFA concentrations did not differ significantly between the control cows and those fed CLA (Table 4). The concentration of BHBA in plasma was lower ($P<0·05$) at week 4 than at

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Table 4. Effects of conjugated linoleic acid (CLA) and *trans*-octadecenoic acids (TRANS) on plasma metabolite concentrations in *post-partum* Holstein cows

Values are least squares with SEM for $n=5$ per treatment

	Day of lactation				*P* value		
	2	14	28	SEM	T[a]	D[b]	T × D[c]
NEFA, μeq/l					0·21	0·01	0·04
Control	1001	947	368	160			
CLA	1068	752	474	160			
TRANS	694	356**	382	160			
BHBA, mg/l					0·59	0·02	0·15
Control	72	79	49	15			
CLA	82	107	56	15			
TRANS	80	65	61	15			

** Value significantly different ($P<0·01$) from that of the control at day 14
[a] Effect of treatment; [b] Effect of day; [c] Effect of treatment × day interaction

Table 5. Effects of conjugated linoleic acid (CLA) and *trans*-octadecenoic acids (TRANS) on liver metabolite concentrations in postpartum Holstein cows

Values are least squares means with SEM for $n=5$ per treatment

	Day of lactation				*P* value		
	2	14	28	SEM	T[a]	D[b]	T × D[c]
Total lipid, % wet liver weight					0·97	0·23	0·97
Control	5·8	7·6	6·5	1·8			
CLA	6·8	7·7	6·8	1·8			
TRANS	6·9	7·7	6·7	1·8			
TAG, % wet liver weight					0·97	0·01	0·05
Control	3·3	5·6	3·2	1·3			
CLA	3·3	5·1	3·7	1·3			
TRANS	4·0	4·1*	3·0	1·3			

* Value was significantly different ($P<0·05$) from that of control or CLA group at day 14
[a] Effect of treatment; [b] Effect of day; [c] Effect of treatment × day interaction

weeks 1 and 2 *post partum* but did not differ among the dietary treatments (Table 4). Total liver lipid concentrations were similar across the diets and blood collection periods (Table 5). A treatment × week interaction was detected ($P<0·05$) for liver TAG concentration (Table 5). Hepatic TAG concentration increased between weeks 1 and 2 and then decreased by week 4 of lactation in control cows and those fed CLA. Liver TAG concentrations did not differ among weeks of lactation in cows fed the *trans* 18 : 1 supplement (Table 5).

Liver genes

Periparturient fat supplementation had no detectable effect on CPT1 mRNA concentration in the liver (Fig. 1). A treatment × day interaction was detected ($P<0·05$) for hepatic MTP mRNA concentration (Fig. 2). The relative

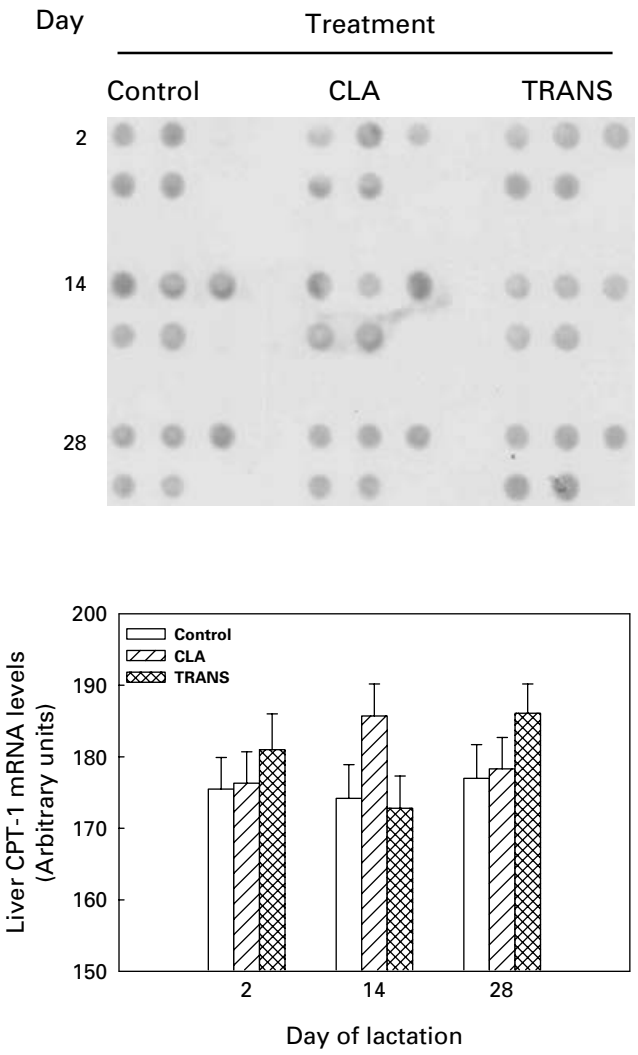


Fig. 1. Dot-blot hybridization of bovine CPT1 cDNA to hepatic mRNA from lactating Holstein cows fed a control, CLA-supplemented (CLA), or *trans* 18 : 1 (TRANS)-supplemented diet. The upper panel is an autoradiogram of a filter probed with CPT1 cDNA and the bottom panel depicts the relative abundance of liver CPT1 gene adjusted for 18S rRNA hybridization signals.

abundance of hepatic MTP mRNA transcript was greater for cows in the TRANS treatment group than for those in the control group at day 2 *post partum* (Fig. 2). Steady-state concentrations of liver PPARα mRNA were greater ($P<0·01$) in cows fed the *trans* 18 : 1-supplemented diet than in those receiving the control or CLA-supplemented diet (Fig. 3). Hepatic PPARα mRNA concentration did not differ among cows receiving the control or CLA-supplemented diet (Fig. 3) at any of the liver sampling periods.

Discussion

The present results provide no evidence for significant alteration of plasma insulin, IGF-I or leptin concentrations

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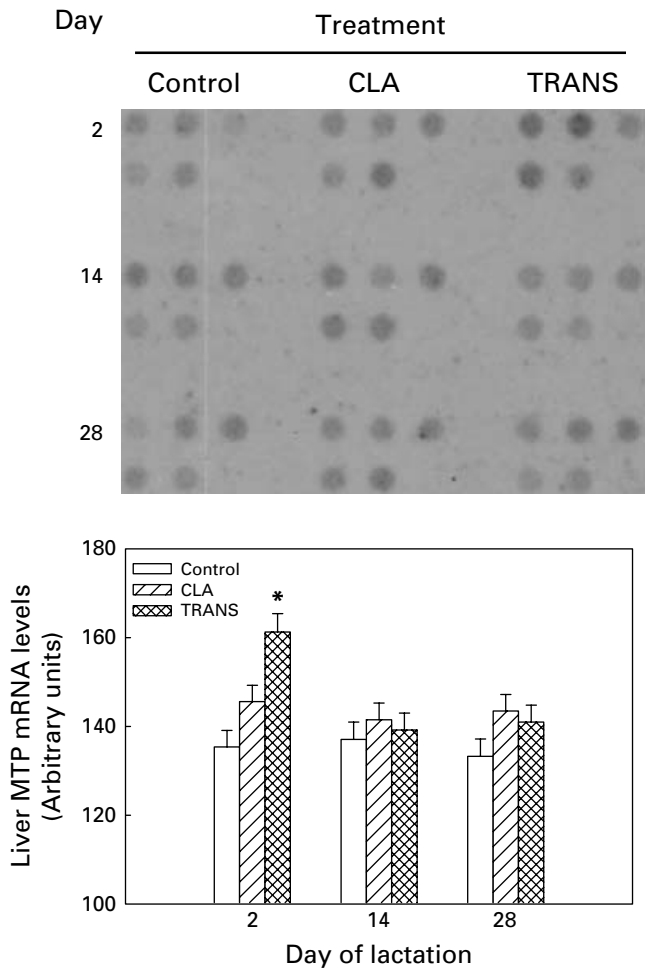


Fig. 2. Dot-blot hybridization of bovine MTP cDNA to hepatic mRNA from lactating Holstein cows fed a control, CLA-supplemented (CLA), or *trans* 18 : 1 (TRANS)-supplemented diet. The upper panel is an autoradiogram of a filter probed with MTP cDNA and the bottom panel depicts the relative abundance of liver MTP gene adjusted for 18S rRNA hybridization signals. Asterisks indicate treatment differences ($P<0{\cdot}01$) at day 2 *post partum*.

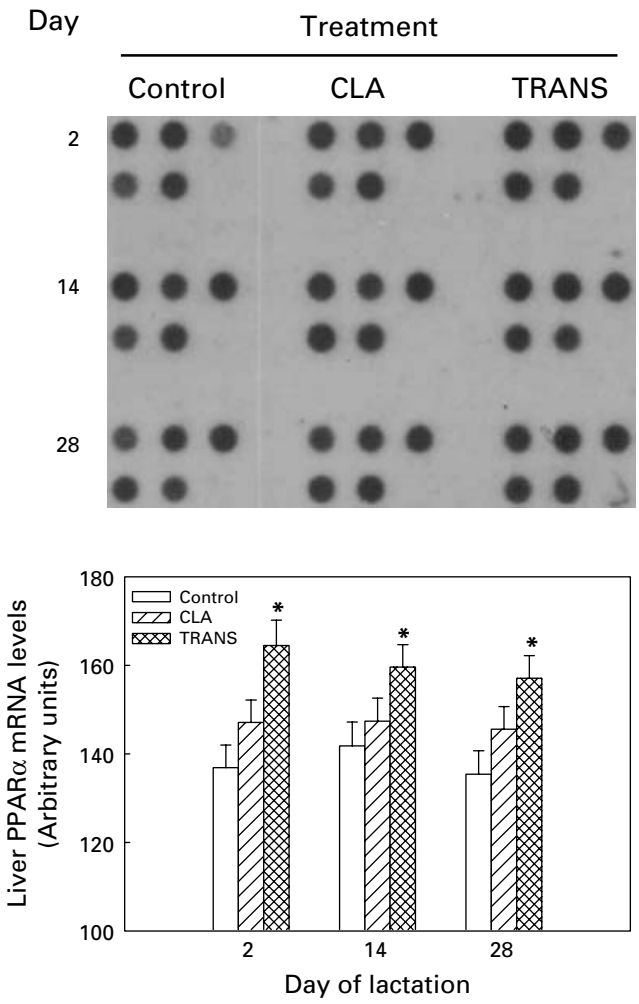


Fig. 3. Dot-blot hybridization of bovine PPARα cDNA to hepatic mRNA from lactating Holstein cows fed a control, CLA-supplemented (CLA), or *trans* 18 : 1 (TRANS)-supplemented diet. The upper panel is an autoradiogram of a filter probed with PPARα cDNA and the bottom panel depicts the relative abundance of liver PPARα gene adjusted for 18S rRNA hybridization signals. Asterisks indicate treatment differences ($P<0{\cdot}01$) at days 2, 14 and 28 *post partum*.

following dietary supplementation of CLA or *trans* fatty acids to *post-partum* Holstein cows. These results are consistent with previous reports showing abomasal infusion of CLA to have no detectable effect on plasma insulin or leptin concentration in late-lactation dairy cows (Baumgard et al. 2002). In contrast with the present findings, Baumgard et al. (2000) report a significant increase in plasma IGF-I concentration in mid-lactation dairy cows receiving abomasal infusion of the *cis*-9, *trans*-11 CLA isomer. In dairy cows, plasma IGF-I concentration is at its nadir during early lactation when growth hormone concentration and milk yield are greatest (Vega et al. 1991; Vicini et al. 1991). Conversely, the concentration of IGF-I in plasma increases and that of growth hormone decreases as the lactating animal regains a positive energy status after calving. We surmise that the net effect of *cis* or *trans* fatty acids on blood IGF-I concentration may vary depending on the physiological state of the animal at the time of treatment implementation.

Plasma NEFA concentration decreased between weeks 1 and 4 of lactation and was lower in cows fed the *trans* 18 : 1-supplemented diet than in those receiving a control diet at 2 weeks *post partum*. Because plasma NEFA concentration is a reliable index of the magnitude of fat mobilization (Bauman et al. 1988), the present finding on blood NEFA concentration would indicate that periparturient *trans* fatty acid supplementation may have prevented excessive lipolysis in dairy cows in early lactation. The lack of effect of CLA on plasma NEFA and BHBA

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concentrations in the present study is consistent with previous studies showing no detectable effects of periparturient feeding of CLA on plasma lipid metabolites during early (Bernal-Santos et al. 2003) or established (Perfield et al. 2002) lactation in cattle. The observation that hepatic lipid concentrations did not differ between cows fed the control diet and those fed the CLA-supplemented diet agrees with our earlier report (Selberg et al. 2004) and would indicate that dietary CLA may have had minimal effects on lipid metabolism within the bovine liver.

Factors controlling the flux of free fatty acids between hepatic oxidation and esterification pathways are not fully understood in dairy cattle. The translocation of long-chain fatty acids into the mitochondrion, a step that is required for β-oxidation, is regulated by CPTI (Drackley, 1991; Grummer, 1993). In the present study, fat supplementation in periparturient Holstein cows had no detectable effect on CPTI mRNA abundance in the liver, suggesting that dietary CLA or *trans* fatty acids had minimal effects on mitochondrial fatty acid oxidation in early *post-partum* dairy cows. These results are consistent with an earlier report of no correlations between hepatic CPT activity and serum phospholipid, free fatty acid, triglyceride or total cholesterol concentrations in healthy cattle (Mizutani et al. 1997). It has been suggested that, in dairy cows, day relative to parturition may have a more pronounced effect on CPT activity than the diet composition or feeding level (Dann et al. 2000).

Results of this study provide the first direct evidence of up-regulation of hepatic MTP transcript by dietary *trans* fatty acids following calving and, thus, suggest that supplemental *trans* fatty acids may have reduced liver lipidosis in transition dairy cows through enhancement of TAG export out of the liver as very low density lipoprotein (VLDL). However, this does not rule out the possibility that the lower hepatic TAG content detected at week 2 in cows fed the TRANS diet may have resulted from the decreased circulating NEFA concentration in *trans* fatty acid-supplemented cows at week 2 *post partum*. Additionally, whether and how dietary *trans* fatty acids affect liver apolipoprotein B100 availability and the MPT activity remains to be documented.

PPARα is a key transcriptional regulator of many genes involved in lipid transport, oxidation and thermogenesis (Clarke et al. 2002). In the present study, the steady-state concentration of PPARα mRNA in the liver was greater in the TRANS treatment group than in the control group in all three liver biopsy periods. Because PPARα regulates the transcription of genes encoding peroxisomal enzymes involved in long-chain fatty acid oxidation (Clarke et al. 2002), the current findings suggest that dietary *trans* fatty acids enhanced peroxisomal fat oxidation in early *post-partum* dairy cows. In support of this hypothesis, previous studies report significant alterations in hepatic lipid metabolism as a result of dietary fat supplementation *pre-partum* (Grum et al. 1996) or *post partum* (Grum et al. 2002). In addition, these earlier reports show both total and peroxisomal β-oxidation to be correlated negatively to liver lipid accumulation (Grum et al. 2002), suggesting that dietary fats may play a role in the aetiology of the fatty liver that occurs in dairy cows at parturition.

The observation that supplemental CLA failed to alter hepatic PPARα mRNA concentration is difficult to reconcile with previous reports that indicate that several isomers of CLA are high-affinity ligands and activators of PPARα (Moya-Camarena et al. 1999). The discrepancy between our findings and previous rodent data may be attributed to the level and duration of CLA as well as to species-specific responses to CLA (Moya-Camarena & Belury, 1999). Additionally, the ability of a PPAR ligand such as CLA to elicit differential effects across animal species may be related to species-specific differences in absorption, metabolism, and optimal dose of the test compound (Lake, 1995).

In summary, feeding selected *trans* fatty acids to transition Holstein cows decreased plasma NEFA concentration within 2 weeks of calving, suggesting that periparturient supplements of *trans* fatty acid may prevent excessive lipolysis in early-lactation dairy cows. Under the present experimental conditions, dietary CLA had minimal effects on plasma and hepatic lipid metabolite concentrations in early-lactation Holstein cows. The observation that dietary *trans* fatty acids up-regulated PPARα mRNA transcript within the first month of lactation raises the possibility that supplemental *trans* fatty acids may enhance peroxisomal fat oxidation in early *post-partum* dairy cows.

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