Tocopherols and tocotrienols in serum and liver of dairy cows receiving conjugated linoleic acids or a control fat supplement during early lactation

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ABSTRACT

The fat-soluble vitamin E comprises the 8 structurally related compounds (congeners) α -, β -, γ -, and δ -tocopherol (with a saturated side chain) and α -, β -, γ -, and δ -tocotrienol (with a 3-fold unsaturated side chain). Little is known regarding the blood and liver concentrations of the 8 vitamin E congeners during the transition from pregnancy to lactation in dairy cows. We thus quantified tocopherols (T) and tocotrienols (T3) in serum and liver and hepatic expression of genes involved in vitamin E metabolism in pluriparous German Holstein cows during late gestation and early lactation and investigated whether dietary supplementation (from d 1 in milk) with conjugated linoleic acids (CLA; 100 g/d; each 12% of *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA; $n = 11$) altered these compared with control-fat supplemented cows (CTR; $n = 10$). Blood samples and liver biopsies were collected on d -21, 1, 21, 70, and 105 (liver only) relative to calving. In both groups, the serum concentrations of α T, γ T, β T3, and δ T3 increased from d -21 to d 21 and remained unchanged between d 21 and 70, but were unaffected by CLA. The concentrations of the different congeners of vitamin E in liver did not differ between the CTR and the CLA groups. In both groups, the concentrations of the vitamin E forms in liver changed during the course of the study. The hepatic mRNA abundance of genes controlling vitamin E status did not differ between groups, but α -tocopherol transfer protein and tocopherol-associated protein mRNA increased with time of lactation in both. In conclusion, the concentrations of vitamin E congeners and the expression of genes related to vitamin E status follow characteristic time-related changes during the transition from late gestation to early lactation but are unaffected by CLA supplementation at the dosage used.

Key words: tocopherol, tocotrienol, conjugated linoleic acid, dairy cow

INTRODUCTION

The term “vitamin E” comprises 8 different natural compounds: α -, β -, γ -, and δ -tocopherol (**T**) and α -, β -, γ -, and δ -tocotrienol (**T3**), of which α T is the most abundant form in mammals (Frank et al., 2012). Tocopherols have a saturated phytyl side chain and T3 a 3-fold unsaturated isoprenoid side chain. In plasma and tissues of humans and animals, T, and particularly α T, are the predominant congeners and T3 are usually found at very low concentrations, if at all (Frank et al., 2012). The selective retention of α T over all other congeners and the particularly low concentrations of T3 are a consequence of the preferential metabolism (initiated by cytochrome P450 monooxygenases) of the non- α T forms to side-chain shortened water-soluble carboxyethyl hydroxychromanol (**CEHC**) metabolites in combination with the activity of the hepatic α -tocopherol transfer protein (**TTP**; Grebenstein et al., 2014).

α -Tocopherol is the only congener presently used as vitamin E supplement for dairy cows. Plasma vitamin E concentrations decrease gradually throughout the antepartum period, with nadir concentrations around calving and a gradual increase thereafter (Weiss, 1998). The depletion of serum vitamin E concentrations during early lactation may be related to increased incidences of postpartum diseases, such as left displaced abomasum (Qu et al., 2013). Supplementation of dairy cows with vitamin E during the dry period can partially attenuate the decline in plasma vitamin E concentrations around calving (Politis, 2012). Furthermore, beneficial effects of vitamin E supplementation during the dry period have also been reported; for example, enhancing immune function (Politis et al., 1995, 1996) and decreasing the incidence of clinical mastitis in dairy cows (Smith et al., 1984; Weiss et al., 1997). Such effects seem to depend on the plasma α T concentration

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achieved and on the infection pressure present in the herds studied (Politis, 2012).

Supplementation with *trans*-10,*cis*-12 CLA is used to reduce milk fat content in dairy cows as a dietary strategy aimed to improve energy balance, especially during early lactation (Sippel et al., 2009; Schlegel et al., 2012). The observation that CLA-fed laboratory animals have improved vitamin E status attracted interest in the interaction between vitamin E and CLA (Liao et al., 2008; Chao et al., 2010). Liver vitamin E concentrations were 15-fold higher in CLA-fed mice than in control animals (Liao et al., 2008). The improved vitamin E status of CLA-treated animals might be due to the induction of TTP expression in the liver (Chen et al., 2012). An increase in longissimus muscle α T concentration as a result of feeding rumen-protected CLA was also recently reported in young Simmental heifers (Schlegel et al., 2012).

To the best of our knowledge, the effect of dietary CLA on blood and liver concentrations of all vitamin E congeners and on the hepatic expression of genes involved in the regulation of vitamin E concentrations has not yet been investigated in dairy cows. The current study was designed to characterize potential effects of CLA supplementation on vitamin E status during the transition from pregnancy to lactation in dairy cows.

MATERIALS AND METHODS

Animals, Treatment, and Experimental Design

The experiment was conducted at the experimental station of the Friedrich-Loeffler-Institute (Braunschweig, Germany). The experimental procedures performed in this study were in accordance with European Community regulations concerning the protection of experimental animals and the guidelines of the LAVES (Lower Saxony State office for Consumer Protection and Food Safety, Germany, File Number 33.14.42502-04-071/07). Cows, experimental design, and treatments were described in detail by Pappritz et al. (2011). A subset of animals and samples from that study; that is, only pluriparous cows, was considered for the present study. Briefly, 21 German Holstein cows in late gestation were housed in freestall barns and fed *ad libitum* with a partial mixed ration (6.8 MJ of NE_L/kg of DM) consisting of 37.8% corn silage, 25.2% grass silage, and 37% concentrate (DM basis) during the study period. The concentrate portion of the ration contained 2% mineral feed (per kg of mineral feed: 140 g of Ca, 120 g of Na, 70 g of P, 40 g of Mg, 6 g of Zn, 5.4 g of Mn, 1 g of Cu, 100 mg of I, 40 mg of Se, 5 mg of Co, 1,000,000 IU of vitamin A, 100,000 IU of vitamin D₃, and 1,500 mg of vitamin E). Diets were

formulated according to the recommendation of the German Society of Nutrition Physiology (GfE, 2001). At 1 DIM, cows were assigned to either the CLA group (n = 11) or control group (CTR; n = 10) and fed 100 g of the respective fat supplements daily until 182 DIM. The fat supplements were incorporated into 4 kg of additional concentrate (8.8 MJ of NE_L/kg DM) consisting of the same components as the concentrate used in the partial mixed ration. Cows in the CLA group received 100 g/d of rumen-protected CLA fat (Lutrell Pure, BASF SE, Ludwigshafen, Germany) supplying 7.6 g of *cis*-9,*trans*-11 CLA and 7.6 g of *trans*-10,*cis*-12 CLA per day. The CLA supplement contained (% of total FAME): C_{16:0} (10.9%), C_{18:0} (50.3%), C_{18:1 cis}-9 (10.7%); C_{18:2 cis}-9,*trans*-11 (12.0%), C_{18:2 trans}-10,*cis*-12 (11.9%), other CLA (0.95%), and other FA (3.3%). The cows in the CTR group received 100 g/d of rumen-protected control fat (Silafat, BASF SE) in which CLA was substituted by stearic acid. The control fat supplement contained (% of total FAME): C_{16:0} (10.9%), C_{18:0} (87.3%), C_{18:1 cis}-9 (<0.01%); C_{18:2 cis}-9,*trans*-11 (0.06%), C_{18:2 trans}-10,*cis*-12 (0.02%), other CLA (0.15%), and other FA (1.58%).

Blood and Liver Tissue Sampling

Blood samples were collected via the jugular vein using evacuated tubes. Samples collected on d -21, 1, 21, and 70 relative to parturition were used. All tubes were centrifuged at 4°C and 3,000 × g for 10 min and the serum obtained was kept frozen (-80°C) until analyzed. Liver tissue samples were obtained on d -21, 1, 21, 70, and 105 relative to calving by transcutaneous biopsy using an automatic device (BARD, Tempe, AZ) under ultrasound control. Tissue samples were snap-frozen in liquid nitrogen and stored at -80°C.

Quantification of Vitamin E Congeners in Serum Samples

Tocopherols and T3 were quantified by a validated method described elsewhere (Greibenstein and Frank, 2012). Briefly, serum was thawed on ice and 100 µL was transferred to a glass tube. Two milliliters of ethanol containing 1% ascorbic acid (wt/vol), 900 µL of deionized water, and 25 µL of butylated hydroxytoluene (BHT; 1 mg/mL dissolved in ethanol) were added. n-Hexane (2 mL) was added, hand-mixed by inversion for 1 min, and centrifuged (5 min, ambient temperature, 800 × g). A 1.5-mL volume of the supernatant was transferred to a fresh test tube and the extraction repeated. The pooled supernatants were evaporated under vacuum using a centrifugal evaporator (Christ SpeedDry; Christ, Osterode Germany) and the dried residues resuspended

in 100 μ L of methanol:water (85:15; vol/vol); then, 10 μ L of the suspension was injected into a Jasco HPLC system (AS-950 Plus autosampler, PU-980 Plus pump, FP-950 Plus fluorescence detector, LG-980-02 gradient unit, and a 3-line degasser; Jasco, Groß-Umstadt, Germany). Congeners were separated on a Kinetex PFP column (2.6 μ m, 150 $\times$ 4.6 mm; Phenomenex, Aschaffenburg, Germany) with a methanol:water (85:15, vol/vol) mobile phase delivered at a flow rate of 0.8 mL/min. The fluorescence detector was operated at an excitation wavelength of 296 nm and emission wavelength of 325 nm. Peaks were recorded and integrated using ChromPass version 1.8.6.1 (Jasco). The concentrations of T and T3 were quantified against external standard curves with authentic compounds (Sigma Aldrich, St. Louis, MO).

Quantification of Vitamin E Congeners in Liver Samples

Liver vitamin E concentrations were determined as described for serum with the exception of an additional saponification step added before extraction (Grebenstein and Frank, 2012). For this purpose, 2 mL of ethanol containing 1% ascorbic acid (wt/vol), 900 μ L of deionized water, and 300 μ L of saturated KOH were added to 200 mg of liver tissue. Samples were saponified for 30 min at 70°C in a shaking water bath and then cooled on ice. Butylated hydroxytoluene (25 μ L of a 1 mg/mL ethanolic solution), 1 mL of deionized water, and 300 μ L of glacial acetic acid were added and the samples subsequently treated as described above for serum.

In the CLA group, the analyses had to be limited to samples from liver collected on DIM 21 and 105 because the liver samples collected on DIM 1 and 70 in this group had been used for other analyses and the amount of tissue remaining was insufficient for a reliable analysis.

RNA Extraction and Quantitative Real-Time Reverse Transcription-PCR

Total RNA was extracted from the liver homogenates using Trizol (Invitrogen, Karlsruhe, Germany) and purified using spin columns (RNeasy Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions and with DNase treatment. The concentration and purity of RNA was assessed by measuring the absorbance at 260 and 280 nm using a Nanodrop 1000 (peQLab Biotechnology, Erlangen, Germany). The integrity of the RNA was assessed using ethidium bromide-denaturing RNA electrophoresis and rechecked

in selected samples using an Agilent 2100 Bioanalyzer and RNA 6000 Nano Kit system (Agilent, Waldbronn, Germany) according to the manufacturer's protocol. Reverse transcription (**RT**) was carried out with 250 ng of total RNA per 20- μ L reaction using 200 U of Revert Aid reverse transcriptase, 500 μ M of each deoxynucleotide triphosphate, and 20 U of RiboLock ribonuclease inhibitor (Fermentas, St. Leon-Rot, Germany), with 200 pmol of random hexamer primers (Sigma-Aldrich, Nümbrecht, Germany) for 10 min at 27°C, 60 min at 42°C, and 1 min at 99°C. A negative template control and a no-reverse-transcriptase control were included in each run. Reverse transcription was carried out in duplicate for each sample.

Analysis by quantitative real-time RT-PCR was performed in an Mx3000P cycler (Stratagene, Amsterdam, the Netherlands). The characteristics of the primers and the real-time PCR conditions are shown in Table 1. The reaction was performed in triplicate in a total volume of 10 μ L composed of 2 μ L of cDNA (diluted 1:4) as template, 1 μ L of the assay-specific primer mix, 5 μ L of the SYBR Green JumpStart Taq Readymix (Sigma-Aldrich, Steinheim, Germany), and 2 μ L of water. A negative-template control for quantitative PCR, as well as a negative-template control and no-reverse-transcriptase control of cDNA were included in each run. Inter-run variation was corrected by including inter-run calibrator. Melting curve analysis confirmed the presence of a single product for each real-time RT-PCR assay. For each PCR reaction, a standard curve was generated using serial dilution of cDNA to calculate efficiency-corrected relative quantities of the targets. The real-time PCR efficiency rates of the investigated transcripts ranged from 1.94 to 2.05.

Three reference genes; namely, eukaryotic translation initiation factor 3, subunit K (*EIF3K*), low-density lipoprotein receptor-related protein 10 (*LRP10*), and RNA polymerase II (*POLR2A*) were used to normalize gene expression data (Saremi et al., 2012). The expression stability of the reference genes was validated using geNorm (Vandesompele et al., 2002).

Statistical Analyses

Statistical analysis of the data was carried out using SAS software (version 9.3; SAS Institute Inc., Cary, NC). The data were tested for normality before analysis using the UNIVARIATE procedure. When the data were not normally distributed, they were transformed using a log₁₀ transformation before analysis. The data were analyzed using repeated measures in the MIXED procedure of SAS. The model included treatment, time (sampling date), and interaction of treatment $\times$ time

Table 1. Characteristics of primers and real-time PCR conditions

Gene ¹	Sequence (5 to 3')	NCBI accession no.	Length (bp)	Concentration (nM)	Mean Cq ²	Annealing conditions ³ (s/°C)
<i>TTP</i>						
Forward	GCCACCAAGCTAGTTCACCT	NM_001206676.1	113	200	21.8	60/59
Reverse	CCTAGAGACCTGATGCAGCG					
<i>TAP</i>						
Forward	GCAGCGATCCTGGCATCTAT	NM_177943.2	109	200	20.3	30/61
Reverse	TCTGAGGCTTTGTCTGGAAGC					
<i>CYP4F2</i>						
Forward	ACAAGCCGCTCTTGACAGAA	NM_001035042.1	196	1,000	22.6	60/58
Reverse	GAGGCGACGAGAGTTGTTGT					
<i>EIF3K</i>						
Forward	CCAGGCCCAACCAAGAAGAA	NM_001034489	125	400	22.2	45/59
Reverse	TTATACCTTCCAGGAGGTCCATGT					
<i>LRP10</i>						
Forward	CCAGAGGATGAGGACGATGT	BC149232	139	400	21.1	30/61
Reverse	ATAGGGTTGCTGTCCCTGTG					
<i>POLR2A</i>						
Forward	GAAGGGGGGAGAGACAAACTG	X63564	86	800	20.3	60/60
Reverse	GGGAGGAAGAAGAAAAAGGG					

¹*TTP* = α -tocopherol transfer protein; *TAP* = tocopherol-associated protein; *CYP4F2* = cytochrome P₄₅₀ 4F2; *EIF3K* = eukaryotic translation initiation factor 3, subunit K; *LRP10* = lipoprotein receptor-related protein 10; *POLR2A* = RNA polymerase II.

²Mean quantification cycle.

³Initial denaturation for 10 min at 90°C; denaturation for 30 s at 95°C; extension for 30 s at 72°C, except for *TTP* and *CYP4F2* (60 s at 72°C).

as the fixed effects and cow as the random effect. The compound symmetry was determined as the most appropriate covariance structure for all repeated state-ments according to the Akaike information criterion. Prepartum data were included in the statistical model as a covariate. The Tukey-Kramer adjustment was used to account for multiple comparisons. Data presented in this paper show the nontransformed values of the data; however, all *P*-values were calculated using the transformed data where necessary. The threshold of significance was set at $P < 0.05$; trends were declared at $0.05 < P < 0.10$.

RESULTS

Milk Fat Content and DMI

The dietary supplementation of CLA during early lactation gradually decreased milk fat percentage and resulted in a significant 12% reduction from wk 8 of supplementation onward, thus confirming the effective-ness of the CLA treatment, as described previously (Pappritz et al., 2011).

During the postpartum period, DMI did not differ between groups (Figure 1) and was 22.3 ± 0.24 kg/d (mean $\pm$ SEM) and 21.2 ± 0.24 kg/d in the CTR and CLA groups, respectively. The DMI increased with time ($P < 0.01$), and an interaction of treatment $\times$ time was observed ($P < 0.01$).

Serum and Liver Concentrations of Vitamin E Congeners

We detected no significant differences between the CTR and CLA groups with respect to the serum con-centrations of any of the vitamin E congeners (Figure 2). The serum concentrations of α T, γ T, β T3, and δ T3

Figure 1. Dry matter intake (means $\pm$ SEM) in dairy cows receiving CLA or a control fat supplement (CTR) from d 1 of lactation. Asterisks indicate a significant difference ($P < 0.01$) between CLA and CTR at a given time point.

changed over time ($P < 0.01$) and followed a similar pattern in both groups; that is, they increased from d -21 to d 21 and remained largely unchanged between d 21 and 70. No time-related differences were found for the serum concentrations of α T3 and γ T3 during the course of the study. For the serum concentrations of γ T3, we observed a trend for a treatment $\times$ time interaction ($P = 0.07$). No treatment $\times$ time interactions were observed for the serum concentrations of any other vitamin E forms.

No significant differences were observed between the CTR and CLA groups for liver concentrations of any of the vitamin E congeners (Figure 3). Time-related changes in the liver concentrations of the vitamin E forms were noted in both experimental groups ($P < 0.05$; $P = 0.07$ in case of γ T3). There was no treatment $\times$ time interaction for any of the vitamin E forms assessed in liver.

Hepatic Expression of Genes Related to Vitamin E Transport and Metabolism

The mRNA abundance of *TTP* was not affected by CLA supplementation but increased 1.62-fold in the CTR group from d -21 to 105 ($P < 0.01$; Figure 4A). A trend was observed for the interaction between treatment $\times$ time for the mRNA abundance of *TTP* ($P = 0.10$). The abundance of tocopherol-associated protein (*TAP*) mRNA did not differ between the groups, but changed over time and followed a similar pattern in both groups postpartum ($P < 0.001$; Figure 4B). In the postpartum period, *TAP* mRNA abundance was greater on d 105 than on d 70 (2.80- and 2.70-fold for the CTR and CLA groups, respectively). No treatment $\times$ time interaction was observed for *TAP* mRNA abundance. The mRNA abundance of cytochrome P₄₅₀ 4F2 (*CYP4F2*) was not affected by treatment or time and there was no treatment $\times$ time interaction.

DISCUSSION

Little is known regarding the changes in total vitamin E (T+T3) concentrations in dairy cows during the period between pregnancy and early lactation, because previous studies only quantified α T alone or together with γ T, but not T3. However, T3 might also significantly contribute to vitamin E status. The blood concentrations of α T in dairy cows decrease gradually throughout the antepartum period, reaching a nadir at parturition and increase thereafter (Weiss, 1998; Weiss et al., 2009; Qu et al., 2013). Our observations confirm this time course in the postpartum period only, as the serum concentrations of α T in the both groups were increased within 3 wk postpartum to relatively con-

stant concentrations that were higher than before and at calving (d 1). However, due to the study design, we were unable to assess potential changes during the first days after calving. In the present study, the unchanged serum α T concentrations between d -21 and d 1 are not in agreement with previous studies (Weiss, 1998; Qu et al., 2013), which reported a decrease in circulating α T concentrations in the antepartum period in dairy cows. At present, we have no explanation for this unexpected result.

In the present study, the serum concentrations of γ T, β T3, and δ T3 were far below those of α T (on average 93/6,527, 1/6,527, and 2/6,527, respectively), but showed mostly similar patterns across time as α T.

In the present study, feed samples were no longer available for laboratory analyses. Therefore, one limitation of our study is the absence of compositional data and subsequently intake data for T and T3. The absence of these data makes it difficult to determine whether the higher concentration of α T3 compared with other T3 isomers in the serum reflected the dietary source or a potential bio-discrimination in the absorption of the different T3. The passage of digesta through the ruminant gastrointestinal tract is a complex process involving ruminal fermentation processes, selective retention, mixing, degradation, and escape of particles and liquid from the rumen before passing to the small and large intestines. Furthermore, in ruminants, because of the continuous flow of digesta to the small intestine, the secretion of bile, enzymes, and digestive juices is continuous and not subjected to large cyclic changes as observed in nonruminants. This may lead to differences in the metabolism and thus retention (and ultimately tissue concentrations) of vitamin E in ruminants compared with nonruminants. These results suggest that T3 may follow alternative metabolic pathways that warrant further investigation.

The selective deposition of α T and its secretion to the circulation results from preferential postabsorptive elimination of congeners of vitamin E other than α T. Consequently, α T is retained to the greatest extent, followed by γ T (2–20% of total vitamin E), whereas the T3 are eliminated rapidly (Frank et al., 2012). To date, 2 mechanisms are known to contribute to the selective retention of α T and discrimination against the non- α T congeners. One is a 32-kDa cytosolic protein, *TTP*, expressed predominantly in liver, with different relative binding affinities for T and T3 as follows: natural α T, 100%; β T, 38%; γ T, 9%; δ T, 2%, and α T3, 12% (Hosomi et al., 1997). There is no information available regarding the relative binding affinities of *TTP* toward the other T3. The second mechanism is related to *CYP4F2*, which is expressed predominantly in liver and catalyzes the hydroxylation of the terminal methyl group of the

Figure 2. Time course of the serum concentrations (means $\pm$ SEM) of α -tocopherol (A), γ -tocopherol (B), α -tocotrienol (C), β -tocotrienol (D), γ -tocotrienol (E), and δ -tocotrienol (F) in dairy cows during late gestation and early lactation. Supplementation with CLA or control fat (CTR) was initiated at d 1 of lactation. Different letters indicate differences ($P < 0.05$) between the time points in the control (CTR) (a, b), and in the CLA (A, B) group, respectively. When summing all tocopherols ($\alpha\text{T} + \gamma\text{T} = \Sigma\text{T}$), all tocotrienols ($\alpha\text{T3} + \beta\text{T3} + \gamma\text{T3} + \delta\text{T3} = \Sigma\text{T3}$), and all vitamin E congeners ($\Sigma\text{T} + \Sigma\text{T3}$), the P -values for treatment were 0.28 (ΣT), 0.83 (ΣT3), and 0.28 ($\Sigma\text{T} + \Sigma\text{T3}$); for time, the P -values were <0.0001 [ΣT and ($\Sigma\text{T} + \Sigma\text{T3}$)] and 0.06 (ΣT3); there was no treatment $\times$ time interaction in any of the summed variables.

Figure 3. Time course of the hepatic concentrations (means $\pm$ SEM) of α -tocopherol (A), γ -tocopherol (B), α -tocotrienol (C), β -tocotrienol (D), γ -tocotrienol (E), and δ -tocotrienol (F) in dairy cows during late gestation and early lactation. Supplementation with CLA or control fat (CTR) was initiated at d 1 of lactation. Different letters indicate differences ($P < 0.05$) between the time points in the control (CTR; a–d) and in the CLA (A, B) group, respectively.

Figure 4. Time course of hepatic mRNA abundance (means $\pm$ SEM) of α -tocopherol transfer protein (TTP, A), tocopherol-associated protein (TAP, B), and cytochrome P450 4F2 (*CYP4F2*, C) in dairy cows during late gestation and early lactation. Supplementation with CLA or control fat (CTR) was initiated at d 1 of lactation. Different letters indicate differences ($P < 0.05$) between the time points in the control (CTR) (a, b) and in the CLA (A, B) group, respectively.

side-chain of vitamin E. This enzyme displays a higher activity toward non- α T congeners (Sontag and Parker, 2002, 2007). Inhibition of tocopherol- ω -hydroxylation by lignans or alkylresorcinols leads to increased plasma concentrations of γ T (Parker et al., 2000; Ross et al., 2004; Grebenstein et al., 2014) and reduced excretion of its short-chain metabolite γ CEHC (Frank et al., 2008), suggesting an important role of this pathway in the regulation of vitamin E status. In support of this, Grebenstein et al. (2014) recently reported that vitamin E metabolism, not TTP, is responsible for the selective retention of α T and discrimination against γ T. Interestingly, Chen et al. (2012) reported that the liver α T concentrations were 7 times higher in CLA-supplemented mice than in the control group. They found that neither absorption nor degradation of α T to α CEHC was affected by CLA supplementation. Induction of TTP mRNA and protein expression was demonstrated as the crucial factor for the improved vitamin E status in the CLA-fed mice (Chen et al., 2012). In the present study, in the CTR group, liver α T concentrations changed inversely over time compared with that of serum α T. On the other hand, the expression of *TTP* mRNA changed during the course of the study, being higher (1.62-fold) on d 105 compared with d -21. This indicates an involvement of TTP in the increase of α T concentrations in the serum. It has already been documented that TTP binds α T, facilitates its secretion via lipoproteins, and thus determines its concentration in circulation (Hosomi et al., 1997). Thus, the more α T is secreted, the less α T is retained in the liver. Our results indicate an improvement in hepatic release of α T into the circulation to support target tissues postpartum. The increase in serum α T concentrations on d 21 and 70 compared with d 1 and d -21 might also be in response to increased feed intake postpartum. Therefore, we cannot determine whether the increase in serum α T concentrations 3 wk after parturition in the present study was mainly a consequence of increased feed intake, upregulation of TTP expression, or a combination of both, although the latter seems most likely.

The involvement of *CYP4F2* in the observed changes in serum and liver vitamin E is unlikely, as its mRNA abundance did not change over time. Alternatively, it may indicate that posttranscriptional modifications of this factor came into play and may have concealed an involvement, as it has recently been shown that vitamin E metabolism, not TTP, is required for the selective retention of α T and discrimination against γ T (Grebenstein et al., 2014). Further studies including protein expression and activity of selected factors may provide additional clues to the involvement of factors in vitamin E metabolism during the periparturient period in dairy cows.

The increase in vitamin E status of laboratory animals supplemented with CLA has focused attention on the interactions between vitamin E and CLA (Liao et al., 2008; Chao et al., 2010). Thus, in the current study, we hypothesized that dietary supplementation with CLA during early lactation might influence serum and liver concentrations of the various vitamin E congeners by altering the hepatic mRNA abundance of genes related to vitamin E status in dairy cows. In addition to α T and γ T, all 4 T3 were detected in serum and liver of dairy cows during late gestation and early lactation. In spite of lower DMI during the first days of lactation (significantly on d 21 and numerically on d 7, 14, and 28; Figure 1) and thus presumably lower vitamin E intake in the CLA group, serum α T concentrations did not differ between the CLA-supplemented and CTR cows (Figure 2A). Thus, CLA might help to retain α T in serum.

Tocopherol-associated protein, also named SEC14-like 2 (*SEC14L2*), is a cytosolic protein that is expressed in many human tissues, with higher expression in the liver, brain, and prostate (Zimmer et al., 2000). In contrast to TTP, TAP seems to have a broad affinity for small lipid molecules, including various vitamin E isoforms and some phospholipids (Kempná et al., 2003). In the present study, upregulation of *TAP* mRNA abundance on d 105 compared with d 70 in the CTR and CLA groups coincided with the greater hepatic concentrations of β T3 and δ T3. We thus speculate that TAP might be selectively involved in the accumulation of β T3 and δ T3 in the liver, perhaps by limiting access to bound tocotrienols of the degrading enzymes. However, the mechanism and function of the *TAP* upregulation observed herein remains to be elucidated.

CONCLUSIONS

All 4 congeners of T3 were detected in serum and liver of dairy cows during the transition from pregnancy to lactation, albeit at distinctively lower concentrations than α T and γ T. The increase in *TTP* mRNA abundance with DIM in the CTR group may show an involvement of TTP in the increase of α T concentrations in the serum. The data indicate time-related changes in the serum and liver concentrations of the vitamin E congeners and in the hepatic expression of genes involved in the regulation of vitamin E concentrations, but they were not affected by the CLA supplement at the dosage used in the present study.

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