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Expression of α-tocopherol–associated genes and α-tocopherol accumulation in Japanese Black (Wagyu) calves with and without α-tocopherol supplementation[1]

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ABSTRACT: The aim of the study was to clarify 1) the distribution of 6 α-tocopherol (α-Toc)–associated gene expressions in 20 major tissues, including metabolic, reproductive, endocrine, immune, and digestive and absorptive tissues, in relation to α-Toc status and 2) the change in expression patterns of the genes induced when α-Toc was orally administered to Japanese Black (JB) calves. This study examined weaned male JB calves ($n = 10$), of which 5 calves were orally administered α-Toc for 2 wk (30 IU·kg^{-1} BW·d^{-1}; TOC group). The others did not receive the α-Toc supplement and were the control (CONT) group. The 20 tissues and venous blood (serum) were sampled on the final day. In both groups, the mean mRNA expression levels for α-Toc transfer protein, afamin (AFM), ATP-binding cassette transporter A1, and tocopherol-associated protein were greatest in the liver ($P < 0.05$), whereas scavenger receptor class B, Type I (SR-BI) mRNA was greatest in the adrenal gland ($P < 0.05$). The gene for cytochrome P450 family 4, subfamily F, polypeptide 2 was most highly expressed in the liver, testes, and adrenal gland. The α-Toc content was greatest ($P < 0.05$) in the testes of the 20 sampled tissues in the CONT group. However, the levels in the testes and jejunum were similar and greater ($P < 0.05$) than the levels in the other 18 tissues in the TOC group. The mean increase in α-Toc levels after oral α-Toc administration (mean α-Toc content for the TOC group divided by the CONT group content) were greater ($P < 0.05$) in the jejunum (40.7-fold) and duodenum and liver (26.3- and 23.1-fold) than in the serum (7.8-fold). In the liver, α-Toc administration significantly increased ($P < 0.05$) the AFM and SR-BI mRNA expression levels. The results show that the liver may play an important role in the regulation of α-Toc disposition, but other peripheral tissues that accumulate large amounts of α-Toc could moderate the local α-Toc status and functions, as inferred from the high expressions of the α-Toc–associated genes in JB calves.

Key words: α-tocopherol binding protein, cattle, mRNA expression, vitamin E

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INTRODUCTION

Japanese Black (**JB**) beef cattle (Wagyu) are an improved beef production breed in Japan, but JB calves have greater susceptibility to infectious diseases compared with Holstein calves because they produce fewer immune cells (Ohtsuka et al., 2011). Oral administration of α-tocopherol (**α-Toc**), the most biologically active form of vitamin E, to JB calves has been shown to improve the immune system (Otomaru et al., 2013). Therefore, increasing α-Toc status in the early growth stages of JB calves may reduce the morbidity and mortality costs during beef production. However, the molecular mechanisms underlying α-Toc partitioning

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and accumulation in various tissues are unknown in JB calves. Some pioneering studies conducted with humans and rodents have identified and characterized some α-Toc–associated molecules, such as α-Toc transfer protein (**α-TTP**), afamin (**AFM**), scavenger receptor class B, Type I (**SR-BI**; a high-density lipoprotein [**HDL**] receptor), ATP-binding cassette transporter A1 (**ABCA1**; a HDL transporter), tocopherol-associated protein (**TAP**), and cytochrome P450 family 4, subfamily F, polypeptide 2 (**CYP4F2**; Yamauchi et al., 2001; Mardones et al., 2002; Voegele et al., 2002; Traber, 2007). Our hypotheses were that tissue-specific distribution and accumulation of α-Toc was associated with these genes' expression patterns and that the expression levels would change when α-Toc was administered to JB calves. Therefore, the aim of the study was to clarify the tissue distribution of 6 α-Toc–associated gene expressions in 20 major tissues, including the liver (metabolic), testis (reproductive), adrenal gland (endocrine), thymus, spleen, lymph node (immune), and gastrointestinal (**GI**) tract (digestive and absorptive) tissues, and to elucidate the changes in gene expression levels in JB calves by comparing calves that had and had not received orally administered α-Toc.

MATERIALS AND METHODS

This study was conducted according to the animal experimentation guidelines of the National Agriculture and Food Research Organization (NARO, 2011) and was approved by the Animal Care Committee of NARO Institute of Livestock and Grassland Science.

Animals and Management

Ten 3-mo-old male JB calves were used. Five calves were orally administered α-Toc (mixed form of D-α-Toc 10 IU·kg^{-1} BW·d^{-1} and DL-α-Toc acetate 20 IU·kg^{-1} BW·d^{-1}) for 2 wk (**TOC** group). Five other calves did not receive α-Toc supplement and acted as a control (**CONT**) group. All the calves were weaned from their dams at 3 mo old. Thereafter, they were kept in individual stanchions for 2 wk to become habituated to the experimental diet before starting the α-Toc administration. The amount of feed was calculated according to the Japanese feeding standard for beef cattle (MAFF, 2000) based on the expected 0.7 kg ADG. Calves were limit-fed the growth concentrate (ZEN-NOH, Tokyo, Japan) and timothy hay. The growth concentrate contained on average 19.1% CP and 32.8 IU α-Toc/kg DM basis. The ingredients were 39% grain (corn, milo, and wheat), 36% chaff and bran (bran, corn gluten meal, corn distillers' grain solubles, and draff), 11% vegetable oil cake (soybean and rapeseed), and 14% other components (alfalfa, alfalfa meal, molasses, and minerals), on a raw materials basis. Timothy hay contained on average 8.7% CP and 13.4 IU α-Toc/kg DM basis. This feed was given to each animal in the morning (0830–0900 h) and afternoon (1600–1630 h). Alpha-tocopherol mix was added to the concentrate and given to the calves in the TOC group every morning. The mean DMI of the concentrate were 20.0 and 20.4 g DM·kg^{-1} BW·d^{-1} and for timothy hay were 5.0 and 5.4 g DM·kg^{-1} BW·d^{-1}, respectively, for the CONT and TOC groups. All calves had free access to water and mineral salt blocks. The mineral salt blocks contained Fe, Cu, Co, Zn, Mn, I, Se, and Na.

Sampling Procedure

Blood samples (each 10 mL) were taken from the calves on the first and final day of the experiment by jugular venipuncture using a vacuum tube that did not contain an anticoagulant for serum collection. The samples were kept at 37°C for 2 h in an incubator. After centrifugation (1,000 × *g* for 20 min at 4°C), the serum was stored at –30°C until α-Toc analyses. The calves were killed by exsanguination after an overdose of anesthetic (pentobarbital sodium, Somnopentyl; Kyoritsu Seiyaku Corp., Tokyo, Japan), which induced complete loss of consciousness. Immediately after death, the 20 tissues were taken, trimmed, and cut into 0.5 by 0.5 by 0.5 cm samples (Supplemental Table S1; see the online version of the article at http://journalofanimalscience.org). The mucosal specimens from the lower GI tissues were exfoliated using a slide glass. At least 10 samples of each tissue were washed in cold saline, frozen in liquid nitrogen, and stored at –80°C until measurement of the mRNA expression levels of the genes and their α-Toc contents. The BW of the calves was measured on the first and final days using a cattle weigh bridge.

Total RNA Extraction and cDNA Cloning

Two to 3 specimens per tissue were weighed and crushed in liquid nitrogen using a crushing machine (multibead shocker; Yasui Kikai Corp., Tokyo, Japan). Total RNA was isolated by the acid guanidinium thiocyanate–phenol–chloroform method using an extraction reagent (TRIzol; Life Technologies Inc., Carlsbad, CA). The absence of RNA degradation was confirmed by electrophoresis on 2% agarose gel plates. The concentration of isolated total RNA was determined by optical density measurement at 260 nm and its purity was measured at a wavelength ratio of 260:280 nm (1.8–2.1 was the acceptable value range) using a spectrophotometer (NanoDrop ND-1000; Life Technologies Inc.). For cDNA cloning, 2 μg of total RNA was reverse transcribed with deoxyribonuclease

Table 1. Primers and dual-labeled probe designs and product sizes used in the quantitative real-time PCR analyses of bovine α-tocopherol–associated genes and the housekeeping gene

Gene[1]	Sequence (5′ to 3′)[2]	Size, bp	R^2	Eff.,[3] %	Accession no.
α-TTP	F: CACCGGCAGCAAAGTTCTGA	123	0.99	101	NM_001206676
	P: AAGCTCCGACGTGATGAGACTCACACGA				
	R: TGTGTCTCTACCTCCTGGACAA				
AFM	F: TCTCCACTGAAGAACTGACCTTTC	119	0.99	98	NM_001192175
	P: TCAACGCAGGCAAACTCTTCGCTCAGG				
	R: CTCTCCAAGAACTAAATCCACCAAA				
SR-BI	F: CTCTCCCATCCTCACTTCTACAAC	142	0.99	100	NM_174597
	P: ACCCAGTCCTGGCAGAGGCAGTGTC				
	R: GCAGTTTCACAGAGCAGTTCATG				
ABCA1	F: TGAGCACCCAGGAACTCTTAAAC	98	0.99	101	NM_001024693
	P: TCCTTCCATACATCGGGTCCCAAAGCCA				
	R: CACCAAGCAGGGCGTTTCTG				
TAP	F: AGCAGGACTTGTTCAAGACCAA	149	0.99	98	NM_177943
	P: CCCCATCTTCTCGGTCTGGCGGACAC				AF432353
	R: CGGCTTCCAGAGATGCTTGAG				
CYP4F2	F: CCGGAGCATCACCAACGC	81	0.99	103	NM_001075322
	P: CAGCTGCCATTGCACCCAAAGACATGC				
	R: TCCCCAAGCCAGGGTTTTAGA				
ACTB	F: TCGACACCGCAACCAGTTC	70	0.99	99	NM_173979
	P: ACCACGAGCGCAGCAATATCATCATCCA				BC102948
	R: CATGCCGGAGCCGTTGTC				

[1]α-TTP = α-tocopherol transfer protein; AFM = afamin; SR-BI = scavenger receptor class B, Type I; ABCA1 = ATP-binding cassette transporter A1; TAP = tocopherol-associated protein; CYP4F2 = cytochrome P450, family 4, subfamily F, polypeptide 2; ACTB = β-actin.

[2]F = forward primer; P = dual-labeled probe; R = reverse primer.

[3]Eff. = PCR efficiency.

using a PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions.

Quantitative Real-Time PCR Analysis

Quantitative real-time PCR analysis was performed with a PCR reagent (TaqMan Gene Expression Master Mix; Life Technologies Inc.) using a real-time PCR system (StepOnePlus; Life Technologies Inc.). The primers and dual-labeled (FAM-TAMRA) probe were newly designed and created (Sigma-Aldrich Corp., St. Louis, MO). The design and their expected amplicon sizes are presented in Table 1. The PCR conditions were 50 cycles of the following protocol: 15 s denaturation at 95°C and 1 min annealing and extension at 60°C. All primers and the dual-labeled (FAM-TAMRA) probe set were tested for PCR reaction suitability by measuring the R^2 values and PCR efficiencies (Table 1). The relative mRNA expression was calculated by the 2-ΔΔCt method using β-actin (**ACTB**) as a housekeeping gene. To mitigate any bias related to potential differential expressions of the genes used for data normalization, the variation in the expressions of 3 housekeeping genes (ACTB, glycerol-3-phosphate dehydrogenase, and 18S rRNA) were checked in the 20 tissues (data not shown) and ACTB was chosen as the most suitable reference gene for this study. The relative mRNA expression for each gene was determined by calculating the difference in the cycle threshold value to that of ACTB. In the tissue distribution analysis, the values in the jejunum (for AFM, SR-BI, and ABCA1) and colon (for α-TTP, TAP, and CYP4F2) were set as the reference values. The mean value of the CONT group was set as the reference value when comparing the 2 groups.

Alpha-Tocopherol Analysis

The α-Toc concentration in the serum was measured using reversed-phase HPLC, as described in a previous report (Haga et al., 2014). The 20 tissue samples were mixed with PBS, 0.1 *M* SDS solution, ethanol, and *n*-heptane (Merck and Co., Inc., Kenilworth, NJ). The phases were separated by centrifugation (200 × *g* for 10 min at 20°C), and the heptane layer was transferred and dried under reduced pressure in a concentrator (SpeedVac; Thermo Fisher Scientific Inc., Waltham, MA). Methanol was added to dissolve the residue. All extractions were performed under low light. A HPLC system (Shimadzu Corp., Kyoto, Japan) and an Octadecylsilyl 6.0 × 150 mm column packed

Table 2. The tissue distributions of the mRNA expressions of α-tocopherol-associated genes in 20 different tissue types for the no α-tocopherol supplement (control [CONT]) group

	mRNA expression level, arbitrary units[1]					
Tissue	α-TTP	AFM	SR-BI	ABCA1	TAP	CYP4F2
Liver	294.31[a]	208,652.9[a]	3.60[c]	19.69[a]	4,238.19[a]	10,357.22[a]
Testis	3.80[b]	16.0[b]	31.99[b]	1.69[b]	99.84[b]	8,995.88[a]
Adrenal gland	0.36[b]	ND[2]	55.15[a]	3.51[b]	8.49[b]	6,007.56[b]
Thymus	0.01[b]	0.9[b]	0.55[c]	2.04[b]	168.61[b]	3.00[c]
Spleen	0.47[b]	ND	1.37[c]	3.66[b]	6.79[b]	0.51[c]
Lymph node	0.04[b]	ND	0.70[c]	1.81[b]	3.04[b]	ND
Rumen	11.26[b]	ND	0.34[c]	1.66[b]	1.87[b]	193.65[c]
Reticulum	6.60[b]	ND	0.23[c]	1.18[b]	2.75[b]	132.04[c]
Omasum	1.73[b]	ND	0.68[c]	1.51[b]	4.71[b]	5.34[c]
Abomasum	0.14[b]	ND	0.43[c]	1.50[b]	45.18[b]	0.40[c]
Duodenum	0.04[b]	0.8[b]	0.92[c]	0.57[b]	153.91[b]	920.63[c]
Jejunum	0.02[b]	1.0[b]	1.00[c]	1.00[b]	136.13[b]	776.50[c]
Ileum	0.06[b]	ND	0.52[c]	1.41[b]	39.25[b]	63.68[c]
Cecum	0.36[b]	ND	0.25[c]	0.63[b]	1.22[b]	0.35[c]
Colon	1.00[b]	ND	0.35[c]	1.08[b]	1.00[b]	1.00[c]
Renal cortex	0.55[b]	156.3[b]	0.88[c]	2.39[b]	10.76[b]	93.72[c]
Lung	3.74[b]	ND	0.47[c]	3.51[b]	0.77[b]	0.09[c]
Skeletal muscle	0.34[b]	ND	2.11[c]	3.61[b]	2.52[b]	97.63[c]
Heart muscle	0.72[b]	ND	0.96[c]	5.47[b]	17.68[b]	0.47[c]
Adipose	32.6[b]	ND	1.30[c]	4.93[b]	3.40[b]	7.50[c]
SEM	6.75	15,204.6	1.42	0.46	95.38	337.80

[a–c]Significant differences (adjusted $P < 0.05$) among the values for each gene in the 20 tissues of the CONT group ($n = 5$).

[1]Messenger RNA expression levels of all genes were normalized to β-actin mRNA levels and expressed relative to the mRNA abundance in the jejunum (for afamin [AFM], scavenger receptor class B, Type I [SR-BI], and ATP-binding cassette transporter A1 [ABCA1]) and colon (for α-tocopherol transfer protein [α-TTP], tocopherol-associated protein [TAP], and cytochrome P450, family 4, subfamily F, polypeptide 2 [CYP4F2]).

[2]ND = not detected (after amplification in almost all samples).

with 5-μm particles (Shim-pack CLC; Shimadzu GLC Ltd., Tokyo, Japan) were used for the analysis. The column temperature was controlled at 40°C, the mobile phase was methanol–ethanol (9:1, vol/vol), and fluorescence was detected at an excitation wavelength of 285 nm and an emission wavelength of 330 nm. The α-Toc contents in the tissues were normalized to the wet tissue weight. For this study, the α-Toc level increases after oral α-Toc administration were defined by

$$\text{increase value} = \alpha\text{-Toc level of } N_1 \text{ in } N_2/\text{mean } \alpha\text{-Toc level of } N_1 \text{ in the CONT group,}$$

in which N_1 is each tissue or serum type out of 20 tissues or serums and N_2 is the individual in the TOC group ($n = 5$).

Statistics

The differences between mRNA expressions and α-Toc levels among the 20 tissues within the same group were statistically analyzed using the MIXED model procedure, with tissues as fixed effects and the animal as a random effect. If a significant difference was detected (adjusted $P < 0.05$) between tissues, then differences among least squares means were analyzed using Bonferroni's multiple range test. The differences between the increase values for α-Toc levels among the 21 samples (20 tissues and serum) were also statistically analyzed using the MIXED model procedure and Bonferroni's multiple range test. Comparisons of the ADG and mRNA expressions between the CONT and TOC groups were performed using Student's t test at the $P < 0.05$ significance level. The not-detected data were not used in the statistical analysis. All analyses were conducted using SAS software (version Add-In 6.1 for Microsoft Office; SAS Institute Japan Ltd., Tokyo, Japan).

RESULTS

Body Weight and ADG

The mean BW of the calves in the CONT and TOC group on the first day of the experiment were 112.6 and 112.1 kg, respectively (SEM = 5.9). No significant differences were found in the ADG between the 2 groups (0.95 vs. 0.82 kg/d for the CONT vs. the TOC group, respectively; SEM = 0.06) after 2 wk.

Table 3. The tissue distributions of the mRNA expressions of α-tocopherol–associated genes in 20 tissue types for the α-tocopherol–supplemented group (TOC[1])

	mRNA expression level, arbitrary units[2]					
Tissue	α-TTP	AFM	SR-BI	ABCA1	TAP	CYP4F2
Liver	333.57[a]	392,790.5[a]	3.59[c]	19.85[a]	4,833.31[a]	8,591.14[a]
Testis	1.70[b]	9.0[b]	19.19[b]	1.82[def]	97.90[b]	5,412.32[b]
Adrenal gland	0.15[b]	ND[3]	53.91[a]	4.12[be]	7.51[b]	3,769.89[b]
Thymus	0.02[b]	2.3[b]	0.38[c]	2.20[cdef]	172.48[b]	1.99[c]
Spleen	0.50[b]	ND	1.01[c]	4.13[be]	7.72[b]	0.28[c]
Lymph node	0.02[b]	ND	0.46[c]	2.63[cdef]	3.86[b]	ND
Rumen	11.03[b]	ND	0.27[c]	1.76[def]	2.46[b]	148.02[c]
Reticulum	7.91[b]	ND	0.16[c]	1.10[ef]	3.00[b]	98.92[c]
Omasum	1.87[b]	ND	0.83[c]	1.63[def]	6.81[b]	4.78[c]
Abomasum	0.18[b]	ND	0.30[c]	2.14[cdef]	82.72[b]	0.79[c]
Duodenum	0.09[b]	1.1[b]	0.48[c]	0.94[ef]	96.92[b]	208.54[c]
Jejunum	0.01[b]	1.0[b]	1.00[c]	1.00[ef]	204.92[b]	1,177.01[c]
Ileum	0.04[b]	ND	0.49[c]	2.01[def]	15.13[b]	24.88[c]
Cecum	0.31[b]	ND	0.19[c]	0.60[f]	1.34[b]	0.40[c]
Colon	1.00[b]	ND	0.25[c]	1.08[ef]	1.00[b]	1.00[c]
Renal cortex	1.13[b]	418.1[b]	0.69[c]	2.39[cde]	4.02[b]	60.80[c]
Lung	2.63[b]	ND	0.61[c]	5.40[bc]	1.32[b]	0.11[c]
Skeletal muscle	0.26[b]	ND	1.64[c]	1.84[de]	2.24[b]	74.66[c]
Heart muscle	0.98[b]	ND	0.76[c]	6.31[b]	17.76[b]	0.39[c]
Adipose	28.03[b]	ND	0.88[c]	4.54[bd]	2.88[b]	1.82[c]
SEM	7.55	27,970.1	1.28	0.43	107.74	246.16

[a–f]Significant differences (adjusted $P < 0.05$) among the values for each gene in the 20 tissues of the TOC group ($n = 5$).

[1]TOC group, in which calves were orally administered α-tocopherol (30 IU·kg–1 BW·d–1) for 2 wk ($n = 5$).

[2]Messenger RNA expression levels of all genes were normalized to β-actin mRNA levels and expressed relative to the mRNA abundance in the jejunum (for afamin [AFM], scavenger receptor class B, Type I [SR-BI], and ATP-binding cassette transporter A1 [ABCA1]) and colon (for α-tocopherol transfer protein [α-TTP], tocopherol-associated protein [TAP], and cytochrome P450, family 4, subfamily F, polypeptide 2 [CYP4F2]).

[3]ND = not detected (after amplification in almost all samples).

Alpha-Tocopherol–Associated Genes mRNA Levels in the 20 Tissues

The expression of the α-Toc–associated genes (α-TTP, AFM, SR-BI, ABCA1, TAP, and CYP4F2 mRNA) in the 20 tissue samples are shown in Tables 2 (the CONT group) and 3 (the TOC group). In both groups, the levels of α-TTP, AFM, ABCA1, and TAP mRNA were significantly greater in the liver than in the other 19 tissues ($P < 0.05$). The SR-BI mRNA levels were greatest in the adrenal gland ($P < 0.05$) followed by the testis. The CYP4F2 mRNA level values for the liver and the testis were similar in the CONT group, but the value for the liver was significantly greater than that for the testis in the TOC group. In the GI tract (rumen to colon region) tissues, the mean mRNA levels for AFM, TAP, and CYP4F2 peaked in the jejunum and duodenum in both groups.

All the comparison data between the 2 groups for each mRNA expression in the tissues are shown in Tables 4 through 6. In the liver, the expressions of AFM and SR-BI mRNA were significantly greater ($P = 0.004$ and $P = 0.048$, respectively) in the TOC group than in the CONT group, although other mRNA levels were not significantly different between the 2 groups. The expressions of α-TTP mRNA in the reticulum, duodenum, and heart muscle in the TOC group were significantly greater ($P = 0.047$, $P = 0.008$, and $P = 0.030$, respectively) than in the CONT group. In the renal cortex, the α-TTP mRNA level in the TOC group was more than 2-fold greater ($P = 0.020$) and the TAP mRNA level in the TOC group was about 3-fold lower ($P = 0.010$) than in the CONT group.

Alpha-Tocopherol Levels in the Serum and the 20 Tissue Types

The mean levels of α-Toc and the mean increase values for α-Toc levels in the serum and the 20 tissues are shown in Table 7. The mean concentration of serum α-Toc was similar in the CONT and TOC groups (0.66 vs. 0.57 μg/mL, respectively; SEM = 0.14, $P = 0.76$; not shown) at the beginning of the experiment. However, it was about 7.8-fold greater in the TOC group than in the CONT group on the final day of the experiment. In the CONT group, the α-Toc content was greatest in the testis ($P < 0.05$). However, in the TOC group, the contents in the testis and the jejunum were greater ($P < 0.05$) than in

Table 4. Comparison values for the relative mRNA levels of α-tocopherol transfer protein (α-TTP) and afamin (AFM) in 20 tissue types with and without α-tocopherol supplement

	α-TTP mRNA, arbitrary units[1]				AFM mRNA, arbitrary units			
Tissue	CONT[2]	TOC[3]	SEM	*P*-value[4]	CONT	TOC	SEM	*P*-value
Liver	1.00	1.38	0.13	0.15	1.00	1.58	0.12	0.004
Testis	1.00	0.55	0.18	0.23	1.00	0.47	0.15	0.08
Adrenal gland	1.00	0.50	0.18	0.16	ND[5]	ND		
Thymus	1.00	1.61	0.26	0.26	1.00	2.09	0.38	0.16
Spleen	1.00	1.28	0.10	0.18	ND	ND		
Lymph node	1.00	0.80	0.15	0.53	ND	ND		
Rumen	1.00	1.20	0.07	0.20	ND	ND		
Reticulum	1.00	1.46	0.12	0.047	ND	ND		
Omasum	1.00	1.32	0.16	0.35	ND	ND		
Abomasum	1.00	1.54	0.16	0.08	ND	ND		
Duodenum	1.00	3.12	0.45	0.008	1.00	1.18	0.25	0.73
Jejunum	1.00	0.96	0.15	0.91	1.00	0.84	0.16	0.64
Ileum	1.00	0.70	0.21	0.52	ND	ND		
Cecum	1.00	1.04	0.15	0.90	ND	ND		
Colon	1.00	1.22	0.11	0.36	ND	ND		
Renal cortex	1.00	2.50	0.35	0.02	1.00	2.24	0.36	0.11
Lung	1.00	0.86	0.13	0.62	ND	ND		
Skeletal muscle	1.00	0.96	0.15	0.89	ND	ND		
Heart muscle	1.00	1.67	0.16	0.03	ND	ND		
Adipose	1.00	1.05	0.17	0.90	ND	ND		

[1]Gene mRNA levels are expressed relative to the mRNA abundance in the CONT group.

[2]CONT group = control; calves did not received α-tocopherol supplement ($n = 5$).

[3]TOC group, in which calves were orally administered α-tocopherol (30 IU·kg–1 BW·d–1) for 2 wk ($n = 5$).

[4]*P*-value of the comparison between 2 groups by Student's *t* test.

[5]ND = not detected (after amplification in almost all samples).

the other 18 tissues. The mean α-Toc level increase values were significantly greater ($P < 0.05$; more than 40-fold) in the jejunum than in the serum and the other 19 tissues. The value for the duodenum was second greatest (more than 25-fold) and significantly greater ($P < 0.05$) than that for the serum and the other 17 tissues, except the liver, where it was more than 20-fold greater. In the TOC group immune tissues, the α-Toc content in the lymph node was significantly greater ($P < 0.05$) than in the thymus. In fact, the mean α-Toc level increase value for the lymph node was 2 times greater than that for the thymus, although this was not statistically significant.

DISCUSSION

Alpha-TTP, purified and cloned from liver (Sato et al., 1991; Arita et al., 1995), is known to bind specifically to α-Toc and to play an important role in regulating the plasma α-Toc level (Ouahchi et al., 1995). Furthermore, α-TTP is also expressed in other peripheral tissues, such as leukocytes (Misaki et al., 2003), placental trophoblasts (Kaempf-Rotzoll et al., 2003), and the lungs (Valacchi et al., 2007). Another potential candidate protein for α-Toc–specific binding and transport to plasma and extravascular fluids is AFM (Voegele et al., 2002). Afamin is a member of the albumin family and is expressed in the liver (Lichenstein et al., 1994) and brain (Kratzer et al., 2009). Lipoproteins are the major carriers of α-Toc in the bloodstream. The results of recent studies demonstrated that SR-BI (HDL receptor) and ABCA1 (HDL transporter) mediated cellular α-Toc uptake and secretion (Mardones et al., 2002; Shichiri et al., 2010). Tocopherol-associated protein can bind to α-Toc (Stocker et al., 1999) and has α-Toc–dependent nuclear translocation and transcriptional activation properties (Yamauchi et al., 2001). Finally, the vitamin E family is metabolized by side chain degradation initiated by CYP4F2-catalyzed ω-hydroxylation, which occurs mainly in the liver (Sontag and Parker, 2002). As described above, the expression of the 6 α-Toc–associated genes in various tissues may play a key role in regulating the transportation, tissue distribution, metabolism, and physiological function of α-Toc in animals. There is limited information available about the expression patterns of these α-Toc–associated genes in bovine species (Rajapaksha et al., 1997; Farke et al., 2006). However, we hypothesized that the expressions of these α-Toc–associated genes have functional roles in the complex, systemic, and local molecular mechanisms that control α-Toc disposition in JB calves.

Table 5. Comparison values for the relative mRNA levels of scavenger receptor class B, Type I (SR-BI) and ATP-binding cassette transporter A1 (ABCA1) in 20 tissue types with and without α-tocopherol supplement

	SR-BI mRNA, arbitrary units[1]				ABCA1 mRNA, arbitrary units			
Tissue	CONT[2]	TOC[3]	SEM	*P*-value[4]	CONT	TOC	SEM	*P*-value
Liver	1.00	1.28	0.07	0.048	1.00	0.90	0.11	0.67
Testis	1.00	0.77	0.21	0.61	1.00	0.96	0.15	0.90
Adrenal gland	1.00	1.26	0.08	0.10	1.00	1.05	0.09	0.81
Thymus	1.00	0.89	0.05	0.29	1.00	0.96	0.09	0.84
Spleen	1.00	0.95	0.03	0.46	1.00	1.01	0.06	0.95
Lymph node	1.00	0.84	0.07	0.28	1.00	1.04	0.18	0.37
Rumen	1.00	1.02	0.09	0.94	1.00	0.95	0.08	0.76
Reticulum	1.00	0.89	0.06	0.41	1.00	0.83	0.10	0.41
Omasum	1.00	1.57	0.26	0.29	1.00	0.97	0.13	0.91
Abomasum	1.00	0.88	0.07	0.43	1.00	1.28	0.20	0.52
Duodenum	1.00	0.67	0.12	0.16	1.00	1.46	0.15	0.13
Jejunum	1.00	1.28	0.25	0.60	1.00	0.89	0.14	0.74
Ileum	1.00	1.19	0.16	0.57	1.00	1.28	0.15	0.39
Cecum	1.00	0.98	0.13	0.94	1.00	0.85	0.15	0.66
Colon	1.00	0.92	0.07	0.59	1.00	0.90	0.13	0.71
Renal cortex	1.00	1.02	0.04	0.82	1.00	0.89	0.06	0.42
Lung	1.00	1.66	0.21	0.12	1.00	1.30	0.14	0.34
Skeletal muscle	1.00	1.00	0.06	0.99	1.00	0.46	0.24	0.29
Heart muscle	1.00	1.01	0.05	0.89	1.00	1.03	0.07	0.84
Adipose	1.00	0.86	0.11	0.56	1.00	0.82	0.14	0.57

[1]Gene mRNA levels are expressed relative to the mRNA abundance in the CONT group.

[2]CONT group = control; calves did not received α-tocopherol supplement (n = 5).

[3]TOC group, in which calves were orally administered α-tocopherol (30 IU·kg–1 BW·d–1) for 2 wk (n = 5).

[4]*P*-value of the comparison between 2 groups by Student's t test.

Dietary α-Toc may be absorbed by the small intestine and secreted with chylomicrons into the lymph vessels toward the liver (Traber, 2007). In the calf GI tract (rumen to the colon), the jejunum and duodenum expressed high levels of AFM, TAP, and CYP4F2 mRNA and had a high α-Toc content. These 2 regions may be major oral α-Toc absorption sites in JB calves. Furthermore, the mRNA expression data suggested that AFM might be a backup transfer mechanism across the intestinal barrier, which is similar to the AFM functional role in the porcine blood–brain barrier (Kratzer et al., 2009). In addition, the absorption of dietary α-Toc may be regulated by CYP4F2 metabolism in the small intestine. It is particularly interesting that the mRNA levels of 2 intracellular α-Toc–specific binding proteins, α-TTP and TAP, in the GI tract had unique expression patterns: 1) the gene expressions showed opposite trends and 2) α-TTP mRNA expressions in the reticulum and duodenum were upregulated by oral α-Toc administration. The functions of α-TTP and TAP in the GI tract have not been clarified, but these results suggest that the distribution of mRNA expressions for intracellular α-Toc specific binding proteins show their involvement in the regulation of absorbed dietary α-Toc in the GI tract.

Gene expressions for α-TTP, AFM, ABCA1, TAP, and CYP4F2 mRNA were highest in the liver. The increase value for α-Toc level in the liver was 23.1-fold, whereas that for serum concentration was 7.8-fold. This difference in the degree of increase might be attributable to the regulation of α-Toc metabolism and secretion from the liver into the blood circulation by the highly expressed hepatic α-Toc–associated genes. The administration of α-Toc significantly increased the expression of SR-BI and AFM in the liver. Furthermore, the hepatic uptake of α-Toc and/or α-Toc secretion, which depend on SR-BI and AFM, may be promoted by the increase in oral α-Toc intake. These results suggest that the liver of JB calves, as in humans and rodents, might be the "commander" tissue that defines systemic α-Toc status by controlling secretion into the bloodstream, transportation to peripheral tissues, and enzymatic metabolism of α-Toc. However, Farke et al. (2006) reported that the mRNA level for ABCA1 in cattle was higher in the lung, spleen, and skeletal muscles than in the liver, which did not agree with our data. This discrepancy might be attributable to the differences in the bovine breeds and the life stage of the animals used in the 2 studies. Three-month-old, male JB beef calves were used in our study, but Farke et al. (2006) used an adult lactating Holstein–Friesian cow in their study. The different breeds (beef or dairy cattle) and life stages (calves or lactating cow) might

Table 6. Comparison values for the relative mRNA levels of tocopherol-associated protein (TAP) and cytochrome P450, family 4, subfamily F, polypeptide 2 (CYP4F2) in 20 tissue types with and without α-tocopherol supplement

	TAP mRNA, arbitrary units[1]				CYP4F2 mRNA, arbitrary units			
Tissue	CONT[2]	TOC[3]	SEM	*P*-value[4]	CONT	TOC	SEM	P-value
Liver	1.00	1.14	0.08	0.45	1.00	1.19	0.08	0.27
Testis	1.00	0.98	0.12	0.94	1.00	0.86	0.08	0.42
Adrenal gland	1.00	0.88	0.18	0.76	1.00	0.90	0.12	0.70
Thymus	1.00	1.02	0.06	0.88	1.00	0.95	0.12	0.86
Spleen	1.00	1.14	0.06	0.32	1.00	0.80	0.13	0.49
Lymph node	1.00	1.27	0.10	0.20	ND[5]	ND		
Rumen	1.00	1.31	0.09	0.08	1.00	1.10	0.13	0.73
Reticulum	1.00	1.09	0.04	0.25	1.00	1.08	0.12	0.77
Omasum	1.00	1.45	0.12	0.07	1.00	1.28	0.29	0.66
Abomasum	1.00	1.83	0.28	0.14	1.00	2.86	0.79	0.30
Duodenum	1.00	0.63	0.12	0.11	1.00	0.33	0.21	0.11
Jejunum	1.00	1.50	0.23	0.30	1.00	2.18	0.57	0.33
Ileum	1.00	0.39	0.22	0.18	1.00	0.56	0.20	0.30
Cecum	1.00	1.10	0.14	0.74	1.00	1.67	0.31	0.30
Colon	1.00	1.00	0.14	1.00	1.00	1.44	0.28	0.46
Renal cortex	1.00	0.37	0.14	0.01	1.00	0.93	0.10	0.75
Lung	1.00	1.70	0.20	0.07	1.00	1.77	0.30	0.25
Skeletal muscle	1.00	0.89	0.10	0.59	1.00	1.10	0.22	0.84
Heart muscle	1.00	1.00	0.12	0.99	1.00	1.19	0.33	0.79
Adipose	1.00	0.85	0.06	0.21	1.00	0.35	0.42	0.47

[1]Gene mRNA levels are expressed relative to the mRNA abundance in the CONT group.

[2]CONT group = control; calves did not received α-tocopherol supplement (n = 5).

[3]TOC group in which calves were orally administered **α-**tocopherol (30 IU·kg–1 BW·d–1) for 2 wk (n = 5).

[4]*P*-value of the comparison between 2 groups by Student's *t* test.

[5]ND = not detected (after amplification in almost all samples).

have different lipid metabolisms. In fact, in typical lactating Holstein cows, the plasma or serum total cholesterol levels (more than 170 mg/dL; Kweon et al., 1986) are significantly higher than those in weaned JB calves (average 70.0 mg/dL [SEM = 7.1], data not shown in this study, and 76.2 mg/dL [SEM = 4.0]; Yoshida et al., 2010). These differences in cholesterol status may affect the distribution of ABCA1 mRNA expression in tissues because the ABCA1 transcript activity is reportedly under regulation of the liver X receptor and sterol regulatory element-binding protein 2, which are key proteins in cholesterol metabolism (Knight, 2004; Wong et al., 2006). This relationship indicates the possibility that α-Toc secretion from the liver via ABCA1 might be altered by differences in breeds and life stages.

The adrenal glands and testes take up cholesterol to synthesize steroid hormones, which explains why the SR-BI mRNA levels in the adrenal gland and testis were highest among the 20 tissues, and were capable of taking up large amounts of cholesterol from the bloodstream HDL (Acton et al., 1996). A previous study investigating the distribution of SR-BI mRNA in 6 tissues from cows demonstrated that their levels were high in the adrenal cortex and corpus luteum (Rajapaksha et al., 1997). In addition, Higuchi et al. (2013) reported that serum α-Toc is mainly distributed in the HDL fraction of lipoproteins in calves. These results suggest the high-expressed SR-BI in these tissues may take up some α-Toc along with HDL. The excessive accumulation of α-Toc in these tissues might be regulated by its metabolism via the highly expressed CYP4F2 as well as the liver. The expressions of these α-Toc–associated genes in the adrenal gland and testes were high, regardless of α-Toc supplementation. Furthermore, in a previous study (H. Ishizaki, S. Haga, and M. Nakano. National Agriculture and Food Research Organization Institute of Livestock and Grassland Science, Nasushiobara, Japan, personal communication), α-Toc oral administration for 2 wk prevented the plasma cortisol surge in steer calves stressed by truck transportation. Supplementing α-Toc in goats significantly improved reproductive organ development (Hong et al., 2009). These results imply that high expressions of these genes in the adrenal gland and testis might help alleviate the response to physical stress and the functional development of reproductive organs in JB calves through high α-Toc accumulation.

This study has revealed the different expression patterns of α-Toc–associated genes and the accumulation of α-Toc in 3 immune tissues. The α-Toc content in the mesenteric lymph node was the highest. The

Table 7. Serum α-tocopherol (α-Toc) concentrations, α-Toc contents in 20 tissue types and mean increase value of α-Toc after oral α-Toc administration

Tissue	α-Toc levels		Mean increase value,[3] -fold
	CONT[1]	TOC[2]	
Serum, μg/mL	0.70	5.45	7.8^{DE}
Tissues, μg/g of wet tissue			
Liver	1.00^{b}	23.16^{b}	23.1^{BC}
Testis	5.20^{a}	41.11^{a}	7.9^{DE}
Adrenal gland	1.71^{b}	21.52^{b}	12.6^{CD}
Thymus	0.38^{b}	1.99^{de}	5.2^{DE}
Spleen	0.64^{b}	8.35^{cde}	13.1^{CD}
Lymph node	1.21^{b}	14.93^{bc}	12.3^{CD}
Rumen	0.33^{b}	2.13^{de}	6.4^{DE}
Reticulum	0.34^{b}	2.35^{de}	6.8^{DE}
Omasum	0.37^{b}	1.78^{de}	4.8^{DE}
Abomasum	1.01^{b}	4.46^{cde}	4.4^{DE}
Duodenum	0.52^{b}	13.79^{bcd}	26.3^{B}
Jejunum	0.92^{b}	37.61^{a}	40.7^{A}
Ileum	0.61^{b}	4.33^{cde}	7.1^{DE}
Cecum	0.63^{b}	5.25^{cde}	8.4^{DE}
Colon	0.54^{b}	4.37^{cde}	8.0^{DE}
Renal cortex	0.99^{b}	5.34^{cde}	5.4^{DE}
Lung	1.22^{b}	6.46^{cde}	5.3^{DE}
Skeletal muscle	0.55^{b}	0.77^{e}	1.4^{E}
Heart muscle	1.18^{b}	2.61^{cde}	2.2^{DE}
Adipose	0.69^{b}	1.85^{de}	2.7^{DE}
SEM	0.14	1.25	1.0

$^{a–e}$Significant differences ($P < 0.05$) among values in each tissue within the same group.

$^{A–E}$Significant differences ($P < 0.05$) among the mean increase values for each tissue and serum.

[1]CONT group = control; calves did not received α-tocopherol supplement ($n = 5$).

[2]TOC group, in which calves were orally administered α-Toc (30 IU·kg–1 BW·d–1) for 2 wk ($n = 5$).

[3]Increase in value = α-Toc content of N_1 in N_2/mean α-Toc content of N_1 in the CONT group, in which N_1 is each tissue species of 20 or serum and N_2 is the individual in the TOC group ($n = 5$).

reasons for this may be that 1) high dietary α-Toc absorbed by the small intestine goes through the mesenteric lymph node with chylomicrons before transport to the liver and 2) the metabolic level of α-Toc is quite low in lymph nodes because of the low CYP4F2 mRNA expression. These points suggest that oral α-Toc administration in JB calves might have some effects on immune function because of the large accumulation of α-Toc in mesenteric lymph node lymphocytes. However, the accumulation of α-Toc in the thymus was lowest and the expressions of AFM and TAP were very high compared with the spleen and lymph nodes. The relationship between these α-Toc–associated gene expressions and α-Toc accumulation in the immune tissues remains unclear. Further studies need to investigate other candidate genes related to α-Toc transport to immune tissues.

The mRNA expression of α-TTP and TAP in calf kidneys changed significantly in response to oral α-Toc administration. The kidney seems to play an important role in systemic retinol (fat-soluble vitamin A) homeostasis in rats (Blomhoff et al., 1991). Recent studies have shown that this mechanism is regulated by the reuptake of retinol via binding of its carrier retinol-binding protein (**RBP**) to the endocytic receptor (Raila et al., 2005). The RBP mRNA has been detected in the rat kidney (Makover et al., 1989), which suggests that the complexing of the fat-soluble vitamins with vitamin-specific binding proteins expressed in the kidney might be involved in the regulation of the vitamin homeostasis. Unfortunately, the relationship between α-Toc (fat-soluble vitamin E) homeostasis and the recycling functions of the kidney is unclear. These results suggest the possibility of a novel function for the kidney in vitamin E homeostasis mediated by α-Toc specific binding proteins in JB calves.

In conclusion, this study has demonstrated that the liver may play an important role in the regulation of systemic α-Toc status because of the high expression α-Toc–associated genes, but other peripheral tissues showed unique expression patterns for α-Toc–associated genes and α-Toc accumulation properties. They may also have the ability to modify the local α-Toc status. These results will help clarify the complex, systemic, and local molecular mechanisms involved in the tissue distribution of α-Toc and its physiological effects in each tissue, which should improve the effective application of α-Toc to increase the performance of JB calves.

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