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Dietary *trans*-vaccenic acid (*trans*11-18:1) increases concentration of *cis*9,*trans*11-conjugated linoleic acid (rumenic acid) in tissues of lactating mice and suckling pups

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Abstract — Lactating mice were fed *trans*-vaccenic acid (*trans*11-18:1, TVA) to assess desaturation of TVA to *cis*9,*trans*11- conjugated linoleic acid (9/11CLA). Diets contained 30 g·kg⁻¹ 18:2n-6 (LA) or 20 g LA plus 10 g 18:0 (SA), TVA, or a CLA mixture (MCLA). Compared with SA, feeding TVA increased 9/11CLA concentrations in blood plasma phospholipid, triglyceride, and free fatty acid fractions. However, concentrations of 9/11CLA in plasma fractions were greater when MCLA was fed compared with SA or TVA. No 9/11CLA was detected in liver of mice fed SA, and it was only 1 mg·g⁻¹ of total fatty acids in the carcass. In contrast, 9/11CLA content of liver (5 mg·g⁻¹) and carcass (6 mg·g⁻¹) of mice fed TVA was similar to liver (5 mg·g⁻¹) and carcass (7 mg·g⁻¹) of mice fed MCLA. Mammary tissue of SA-fed mice had no detectable 9/11CLA, compared with 5 or 14 mg·g⁻¹ for TVA or MCLA-fed mice. Stearoyl-CoA desaturase activity in mammary tissue from TVA-fed dams was 14% greater compared with SA. Activity of this enzyme in liver tissue was similar among treatments. In pups nursing TVA-fed dams, 9/11CLA accounted for 3 mg·g⁻¹ in liver but no 9/11CLA was detected in the carcass. In pups nursing MCLA-fed dams, however, 9/11CLA accounted for 8 and 6 mg·g⁻¹ in liver and carcass. Results indicated TVA desaturation enhanced 9/11CLA in tissues and milk fat.

stearoyl-CoA desaturase / *trans*-vaccenic acid / rumenic acid / milk fat

Résumé — L'acide *trans*-vaccénique (*trans*11-18:1) alimentaire augmente la concentration de l'acide linoléique conjugué (acide ruménique, *cis*9,*trans*11-18:2) dans les tissus de la souris allaitante et du sourceau. Des souris allaitantes ont reçu de l'acide *trans*-vaccénique (*trans*11-18:1, TVA) afin de démontrer la désaturation du TVA en acide linoléique conjugué *cis*9,*trans*11-18:2 (9/11CLA). Des régimes contenant 30 g·kg⁻¹ d'acide linoléique (18:2n-6, LA) ou 20 g de LA plus

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10 g d'acide stéarique (18:0, SA), de TVA ou d'un mélange de CLA (MCLA), ont été distribués. Par rapport à la consommation de SA, celle de TVA a accru les concentrations plasmatiques de 9/11CLA dans les fractions phospholipides, triglycérides et acides gras libres. Par ailleurs, les concentrations du 9/11CLA dans ces fractions ont été plus élevées avec l'apport de MCLA qu'avec celui de SA ou de TVA. Le 9/11CLA n'a pas été détecté dans le foie de souris recevant du SA, et a représenté $1 \text{ mg}\cdot\text{g}^{-1}$ d'acides gras totaux de la carcasse. En revanche, les teneurs en 9/11CLA dans le foie ($5 \text{ mg}\cdot\text{g}^{-1}$) et la carcasse ($6 \text{ mg}\cdot\text{g}^{-1}$) de souris ayant reçu du TVA ont été similaires à celles mesurées dans le foie ($5 \text{ mg}\cdot\text{g}^{-1}$) et la carcasse ($7 \text{ mg}\cdot\text{g}^{-1}$) de souris ayant reçu du MCLA. Le tissu mammaire de souris ayant reçu du SA ne contenait pas de quantité détectable de 9/11CLA, contrairement au tissu mammaire de souris ayant reçu du TVA ($5 \text{ mg}\cdot\text{g}^{-1}$) ou du MCLA ($14 \text{ mg}\cdot\text{g}^{-1}$). L'activité stéaroyl-CoA désaturase a été plus élevée de 14 % dans le tissu mammaire des souris recevant du TVA que dans celui des souris recevant du MCLA. L'activité de cette enzyme dans le tissu hépatique a été similaire pour tous les traitements. Chez les souriceaux allaités par les souris ayant reçu du TVA, le 9/11CLA a représenté $3 \text{ mg}\cdot\text{g}^{-1}$ d'acides gras dans le foie mais n'a pas été détecté dans la carcasse. Cependant, chez les souriceaux allaités par les souris ayant reçu du MCLA, le 9/11CLA a représenté 8 et $6 \text{ mg}\cdot\text{g}^{-1}$ d'acides gras, respectivement dans le foie et la carcasse. Ces résultats indiquent que la désaturation du TVA a accru le 9/11CLA dans les tissus et la matière grasse du lait.

stéaroyl-CoA désaturase / acide *trans*-vaccénique / acide ruménique / matière grasse du lait

1. INTRODUCTION

Cis9,trans11-18:2 (9/11CLA), naturally found in ruminant meat or milk, is a potent anticarcinogen in various rodent models [25] and human mammary cancer cells [24]. Bovine milk contains substantial amounts of 9/11CLA (2 to $28 \text{ mg}\cdot\text{g}^{-1}$ total fatty acids) and *trans11-18:1* (TVA) (12 to $75 \text{ mg}\cdot\text{g}^{-1}$), due to extensive biohydrogenation of 18:2n-6 and 18:3n-3 in the rumen, especially when unsaturated oils are fed [6]. Dietary intake of 9/11CLA in a mixed population of individuals in USA ranged between 79 and $133 \text{ mg}\cdot\text{d}^{-1}$ [30] and, although lower, was comparable to intakes in European countries [16, 29, 31]. However, extrapolating from animal data [14], it seems the required intake of 9/11CLA to exhibit a cancer protective effect must be at least $500 \text{ mg}\cdot\text{d}^{-1}$ [30].

Consumption of dairy products by lactating women increased the concentration of 9/11CLA in their milk when compared with a conventional diet [11, 27]. Feeding a diet with elevated TVA concentration also increased the amount of 9/11CLA in human serum [31]. Total intake of *trans-18:1* from

ruminant fats in the European Union was estimated to range from 0.8 to $1.8 \text{ g}\cdot\text{d}^{-1}$ [36]. Thus, intake of TVA from ruminant products may exceed $1 \text{ g}\cdot\text{d}^{-1}$.

Pollard et al. [28] showed that isolated microsomes from rat liver desaturated up to 65% of exogenous TVA to 9/11CLA via stearoyl-CoA desaturase (SCD). Liver SCD activity in rats is expressed during the early suckling period (7 to 13 d of age), and it peaked at 6 months of age [35]. Enterocytes in the rodent small intestine also possess SCD, but this activity was 50% lower than in liver microsomes [12]. Concentration of 9/11CLA in the whole carcass of growing female mice fed TVA ($10 \text{ g}\cdot\text{kg}^{-1}$ of diet) was $23 \text{ mg}\cdot\text{g}^{-1}$ compared with $7 \text{ mg}\cdot\text{g}^{-1}$ for controls [32]. It was suggested that adipose tissue was the major site of bioconversion of TVA to 9/11CLA.

The content (12 to $28 \text{ mg}\cdot\text{g}^{-1}$) of 9/11CLA in mouse mammary epithelial cell cultures increased linearly in response to increasing TVA in the culture medium, due to greater SCD mRNA abundance and activity [15]. Rat pups nursing dams fed a diet supplemented with a CLA isomer mixture had

improved body weight gain for d 10 postpartum [7]. Recent evidence indicated 9/11CLA, not *trans*10,*cis*12-18:2, enhances growth and feed conversion efficiency in young rodents [25]. Since the lactating mammary gland is capable of extensive desaturation, it may be an important source of dietary 9/11CLA for the neonate during the suckling period.

The objective of this study was to evaluate the extent to which dietary TVA may contribute to 9/11CLA content of tissues from lactating mice and their pups. Activity of stearoyl-CoA desaturase in mammary gland and liver was determined to assess the relative importance of each tissue in the bioconversion of TVA to 9/11CLA. To ensure that a homogenous amount of treatment fatty acids was available to lactating mice given our experimental design, linoleic

acid (the primary polyunsaturated fatty acid in foodstuffs) was chosen as a carrier for 18:0 (the primary substrate for stearoyl-CoA desaturase), TVA, and the conjugated linoleic acid mixture.

2. MATERIALS AND METHODS

2.1. Animals and experimental design

Twenty-four lactating CD-1 mice were assigned to one of four dietary groups from d 3 to 14 postpartum. A Harlan Teklad (Harlan, Madison, WI, USA) mouse-breeder diet containing the required complement [23] of nutrients for lactation was used as a carrier for one of four fatty acid treatments (Tab. I). Diets were prepared the day before parturition and stored at 4 °C. Supplemental fatty acids included 30 g 18:2n-6 (LA;

Table I. Fatty acid composition of diets¹ fed to lactating mice.

Fatty acid	LA	SA	TVA	MCLA
	mg·g ⁻¹ total fatty acids			
4:0	1	1	1	1
6:0	1	0	1	0
8:0	1	1	1	1
10:0	2	2	2	2
12:0	7	7	7	7
14:0	13	13	13	13
16:0	209	206	202	210
<i>cis</i> 9-16:1	21	20	20	21
18:0	101	175	98	102
<i>trans</i> 11-18:1	4	4	90	4
<i>cis</i> 9-18:1	323	303	296	315
18:2n-6	289	243	247	252
<i>cis</i> 9, <i>trans</i> 11-18:2	1	1	1	25
<i>trans</i> 10, <i>cis</i> 12-18:2	0	0	0	26
18:3n-3	24	19	19	19
20:3n-6	1	1	1	1
20:4n-6	3	3	2	2
Total, mg·g ⁻¹ diet as fed	109	109	102	101

¹ LA = 30 g·kg⁻¹ 18:2n-6, SA = 20 g·kg⁻¹ LA + 10 g·kg⁻¹ 18:0, TVA = 20 g·kg⁻¹ LA + 10 g·kg⁻¹ *trans*11-18:1, MCLA = 20 g·kg⁻¹ LA + 10 g·kg⁻¹ CLA mixture.

control)·kg⁻¹ diet (United States Biochemical Corp., Cleveland, OH, USA) or 20 g LA plus 10 g 18:0 (SA) (> 99% purity; Nu-Check Prep, Elysian, MN, USA), 10 g *trans*11-18:1 (TVA) (> 99% purity; Nu-Check Prep, Elysian, MN, USA), or 10 g of a mixture of conjugated linoleic acid isomers (MCLA) (41% *cis*9,*trans*11-18:2 and 44% *trans*10,*cis*12-18:2) (Natural Lipids, Norway). Dams were fed the control diet during the first 2 d of lactation at 18:00 h. On d 3 postpartum, six lactating mice were randomly assigned to each dietary group and fed 9 g·d⁻¹ of their assigned diets for the remainder of the experiment. Litters were standardized to six pups on d 3. Mice were kept in a mouse colony, maintained at 23 °C with a 12 h light:dark cycle daily, and housed in individual polypropylene cages with access to water at all times. The experimental protocol was reviewed and approved by the Virginia Polytechnic Institute and State University Animal Care Committee and conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* [23].

2.2. Measurements and sampling

Diet samples were collected on d 3, 8, and 15 and stored at 4 °C. At the end of the experiment, samples from each treatment were composited prior to fatty acid analysis. Dams and their litters were weighed prior to feeding on d 3, 8, and 15 postpartum to estimate initial and final weights. On d 14 postpartum, pups were separated from the dams for 2 h prior to feeding to insure maximal accumulation of milk in the mammary gland. Subsequently, one dam from each dietary group was randomly selected for collection of milk (1 mL) by suction. Milk fat was isolated by centrifugation at 3000 × *g* for 10 min, and stored at -20 °C until fatty acid analysis.

Lactating mice and their pups were sacrificed by cervical dislocation in the morning of d 15 postpartum. Blood samples (1 mL) from lactating mice were obtained by

heart puncture immediately after death. Blood was transferred to tubes containing 150 IU heparin in 100 μL of sterile saline, then centrifuged at 3000 × *g* for 15 min for harvesting plasma. Plasma was stored at -20 °C until lipid extraction and fatty acid analysis.

The liver from lactating mice and their pups was excised and weighed. Mammary gland tissue from dams also was dissected and weighed. In addition, a portion (400 mg) of liver and mammary tissue was immediately placed in liquid nitrogen prior to storage at -80 °C, before determination of stearoyl-CoA desaturase activity. The remaining portion of liver and mammary tissue was stored at -20 °C.

Dam and pup carcasses were stripped of skin, head, and remaining organs. The empty carcass was weighed prior to storage at -20 °C. Subsequently, empty carcasses were lyophilized (Dura-Top freeze dryer, FTS Systems, Inc., Stone Ridge, NY, USA).

2.3. Sample analyses

Lyophilized carcasses were ground through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden) prior to analyses. Total N and crude fat content were analyzed using standard procedures [3]. Lipids were extracted from plasma (0.5 mL), liver, carcass, and mammary gland tissue with chloroform/methanol (2:1, vol/vol). Blood plasma lipid fractions (free fatty acids, FFA; phospholipids, PL; cholesterol esters, CE and triglycerides, TG) were isolated [17] using Bond Elut[®] aminopropyl disposable columns (500 mg) in a Vac Elut[®] system (Analytichem International, Harbor City, CA, USA).

Fatty acids in diets, blood plasma lipid fractions, liver, carcass, mammary gland, and milk fat were methylated by in situ transesterification with 0.5 N methanolic NaOH at 50 °C for 30 min, followed by 14% boron trifluoride in methanol also

at 50 °C for 30 min [26]. Undecenoate (Nu-Check Prep, Elysian, MN, USA) was used as the internal standard. Samples were injected by auto-sampler into a Hewlett Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett Packard, Sunnyvale, CA, USA). Methyl esters of fatty acids were separated on a 30 m × 0.25 mm i.d. fused silica capillary column (SP-2380, Supelco, Inc., Bellefonte, PA, USA). Pure methyl ester standards (NuCheck Prep, Elysian, MN, USA) were used to identify peaks, and determine correction factors for individual fatty acids.

The injector temperature was maintained at 225 °C and the detector temperature at 275 °C. The initial column temperature was 205 °C (held for 12 min), and was programmed to increase 2 °C·min⁻¹ to a final temperature of 220 °C (held for 2 min). Ultra pure helium was the carrier gas.

Stearoyl-CoA desaturase activity in liver and mammary gland tissue was determined by incubation of isolated microsomal protein (2 mg) with 70 μM [¹⁴C]-stearoyl-CoA (0.025 μCi) (American Radiolabeled Chemicals, St. Louis, MO, USA) and 1.2 mM NADH at 37 °C for 20 min. The reaction was stopped by addition of 0.5 mL 10% KOH in methanol (wt/vol). Fatty acids were extracted with hexane and methylated with 14% boron trifluoride in methanol prior to separation by thin layer chromatography and scintillation counting. Activity is expressed as pmole *cis*⁹-18:1·min⁻¹·mg⁻¹ microsomal protein.

2.4. Statistical analysis

Data are reported as Least squares means ± pooled SEM. All data were analyzed as a completely randomized block design using the MIXED procedure of SAS [33] Tukey's studentized range test was used to determine differences between treatments. Overall differences between treatment means were considered to be significant when $P \leq 0.05$.

3. RESULTS

3.1. Body weight and carcass composition

Results on body weight and carcass composition have been discussed previously [19]. Briefly, body weight, liver, and mammary tissue weights of lactating mice on d 15 postpartum averaged 31.4 ± 0.7, 2.1 ± 0.1, and 1.3 ± 0.1 g, respectively, across treatments. Crude protein and fat concentration of the carcass averaged 68.0 ± 0.8 and 7.0 ± 0.4%, and were not affected by diet. Body weight of individual pups averaged 5.9 ± 0.4 g. Liver weight of pups nursing dams fed LA, TVA, or MCLA averaged 0.23 ± 0.03 g compared with 0.16 ± 0.03 g for pups nursing SA-fed dams. Crude protein concentration in the carcass was not different across treatments and averaged 58.7 ± 1.2%. Crude fat concentration, however, was lower in the carcass of pups nursing SA or MCLA-fed dams (16.0 ± 1.3%) compared with LA or TVA (21.3 ± 1.3%).

3.2. *Trans*¹¹-18:1 (TVA) and *cis*⁹,*trans*¹¹-18:2 (9/11CLA) distribution in blood plasma lipid fractions

To determine the blood plasma lipid fractions in which treatment fatty acids were distributed for transport to tissues, free fatty acid (FFA), phospholipid (PL), cholesterol ester (CE), and triglyceride (TG) fractions were isolated from blood plasma. Despite differences in percentages of individual fatty acids, lactating dams did not differ (data not shown) significantly in concentration of total fatty acids in their FFA (122 ± 40 μg·mL⁻¹), PL (573 ± 83 μg·mL⁻¹), TG (155 ± 45 μg·mL⁻¹), and CE (290 ± 48 μg·mL⁻¹) fractions.

The total amount of 9/11CLA in blood plasma was significantly greater when MCLA (5.8 ± 0.1 μg·mL⁻¹) was fed compared with other treatments. Data indicated, however, that a portion of dietary TVA was

desaturated because feeding TVA ($3.7 \pm 0.1 \mu\text{g}\cdot\text{mL}^{-1}$) increased 9/11CLA in blood plasma by $1.9 \mu\text{g}\cdot\text{mL}^{-1}$ compared with LA or SA ($1.5 \pm 0.1 \mu\text{g}\cdot\text{mL}^{-1}$). Among lipid fractions, dietary MCLA supplementation increased the concentration of 9/11CLA in the FFA ($12 \text{ mg}\cdot\text{g}^{-1}$ total fatty acids) and TG ($14 \text{ mg}\cdot\text{g}^{-1}$) fractions to a greater extent than TVA (2 and $4 \text{ mg}\cdot\text{g}^{-1}$ for FFA and TG) or LA plus SA (1 and $1 \text{ mg}\cdot\text{g}^{-1}$ for FFA and TG) (Fig. 1). Feeding TVA ($2 \text{ mg}\cdot\text{g}^{-1}$) or MCLA ($3 \text{ mg}\cdot\text{g}^{-1}$) resulted in similar 9/11CLA concentration in PL, but feeding TVA ($3 \text{ mg}\cdot\text{g}^{-1}$) increased 9/11CLA in CE to a greater extent than MCLA ($1 \text{ mg}\cdot\text{g}^{-1}$). Concentrations of *trans*10,*cis*12-18:2 in total blood plasma were only detectable when MCLA was fed ($3 \text{ mg}\cdot\text{g}^{-1}$ total fatty acids). Despite containing only 14% of total fatty acids in plasma, the TG fraction of MCLA-fed dams contained similar amounts of

9/11CLA ($1.9 \mu\text{g}\cdot\text{mL}^{-1}$) compared with phospholipids ($1.7 \mu\text{g}\cdot\text{mL}^{-1}$), which contained 51% of total plasma fatty acids.

Feeding TVA caused a 2.5-fold increase in the total amount of TVA ($48 \pm 1.0 \mu\text{g}\cdot\text{mL}^{-1}$) in blood plasma compared with other treatments ($8 \pm 1.0 \mu\text{g}\cdot\text{mL}^{-1}$). Except for CE, all other plasma lipid fractions contained greater concentrations of TVA when it was fed. As opposed to the distribution of 9/11CLA, 56% of total plasma TVA was found in PL ($47 \text{ mg}\cdot\text{g}^{-1}$) compared with other fractions.

3.3. Fatty acid profiles in liver, mammary gland, and carcass of lactating mice

Concentrations ($\text{mg}\cdot\text{g}^{-1}$ total fatty acids, Tab. II) of 14:0 and 16:0 in the carcass of

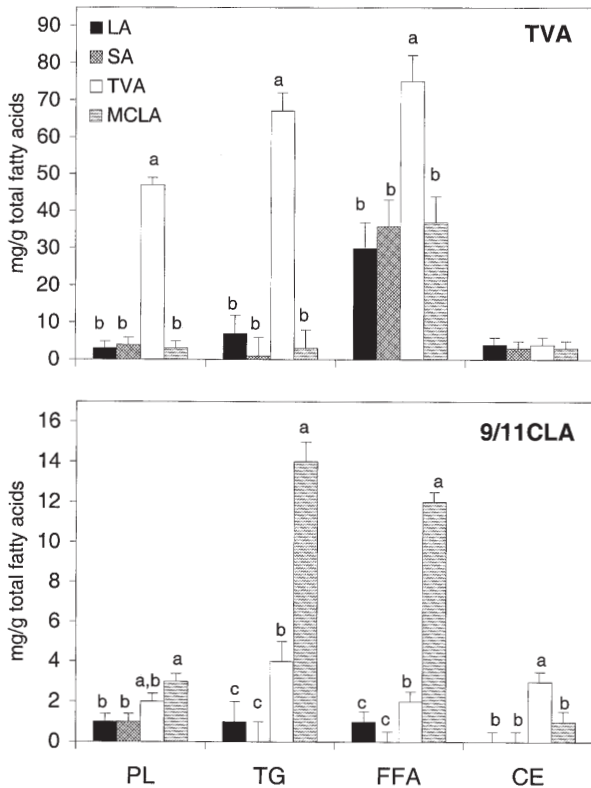


Figure 1. Concentration of *trans*11-18:1 (TVA) and *cis*9,*trans*11-18:2 (9/11CLA) in blood plasma phospholipids (PL), triglycerides (TG), free fatty acids (FFA), and cholesterol esters (CE) of lactating mice fed a diet containing $30 \text{ g}\cdot\text{kg}^{-1}$ 18:2n-6 (LA) or 20 g LA plus 10 g 18:0 (SA), *trans*11-18:1 (TVA), or a conjugated linoleic acid mixture (MCLA). Superscripts denote significant ($P < 0.05$) differences due to treatments.

Table II. Fatty acid profiles in tissues of lactating mice fed a diet containing 30 g·kg⁻¹ 18:2n-6 (LA) or 20 g LA plus 10 g 18:0 (SA), *trans*11-18:1 (TVA), or a conjugated linoleic acid mixture (MCLA).

Treatment	Fatty acid																																																																																																																																																																																																				
	14:0	16:0	c9-16:1	18:0	c9-18:1	18:2n-6	t10,c12-18:2	18:3n-3	20:3n-6	20:4n-6																																																																																																																																																																																											
	mg·g ⁻¹ total fatty acids																																																																																																																																																																																																				
Liver											LA	5 ^{ab}	358 ^a	13 ^b	239 ^a	169	100	0 ^b	0.8	10 ^a	93	SA	5 ^b	332 ^{ab}	14 ^b	208 ^{ab}	181	120	0 ^b	1.4	12 ^a	119	TVA	5 ^{ab}	295 ^b	17 ^{ab}	176 ^b	194	139	0 ^b	1.4	13 ^a	131	MCLA	6 ^a	351 ^a	18 ^a	203 ^b	185	107	2 ^a	1	8 ^b	106	SEM	0.4	16	1	13	11	14	0.1	0.4	1	17	Carcass											LA	27 ^b	315 ^b	46	131 ^b	265 ^a	134 ^a	0 ^b	4	4	58 ^a	SA	24 ^b	314 ^b	42	145 ^a	281 ^a	120 ^b	0 ^b	4	4	51 ^{ab}	TVA	25 ^b	312 ^b	44	129 ^b	275 ^a	118 ^b	0 ^b	3	4	46 ^b	MCLA	31 ^a	363 ^a	39	126 ^b	231 ^b	125 ^{ab}	5 ^a	4	4	53 ^{ab}	SEM	2	6	4	5	7	4	0.2	0.2	0.3	3	Mammary gland											LA	76	261	24 ^a	76 ^c	261 ^b	166 ^a	0 ^b	8	5 ^a	18 ^{ab}	SA	68	258	24 ^a	94 ^a	281 ^a	150 ^{bc}	0 ^b	6	5 ^a	17 ^b	TVA	67	252	24 ^a	79 ^{bc}	257 ^b	147 ^c	0 ^b	7	4 ^b	16 ^b	MCLA	65	252	19 ^b	88 ^{ab}	253 ^b	162 ^{ab}	9 ^a	8	3 ^c	21 ^a	SEM	4	5	1	3	6	4	0.2	0.5	0.2	1
LA	5 ^{ab}	358 ^a	13 ^b	239 ^a	169	100	0 ^b	0.8	10 ^a	93	SA	5 ^b	332 ^{ab}	14 ^b	208 ^{ab}	181	120	0 ^b	1.4	12 ^a	119	TVA	5 ^{ab}	295 ^b	17 ^{ab}	176 ^b	194	139	0 ^b	1.4	13 ^a	131	MCLA	6 ^a	351 ^a	18 ^a	203 ^b	185	107	2 ^a	1	8 ^b	106	SEM	0.4	16	1	13	11	14	0.1	0.4	1	17	Carcass											LA	27 ^b	315 ^b	46	131 ^b	265 ^a	134 ^a	0 ^b	4	4	58 ^a	SA	24 ^b	314 ^b	42	145 ^a	281 ^a	120 ^b	0 ^b	4	4	51 ^{ab}	TVA	25 ^b	312 ^b	44	129 ^b	275 ^a	118 ^b	0 ^b	3	4	46 ^b	MCLA	31 ^a	363 ^a	39	126 ^b	231 ^b	125 ^{ab}	5 ^a	4	4	53 ^{ab}	SEM	2	6	4	5	7	4	0.2	0.2	0.3	3	Mammary gland											LA	76	261	24 ^a	76 ^c	261 ^b	166 ^a	0 ^b	8	5 ^a	18 ^{ab}	SA	68	258	24 ^a	94 ^a	281 ^a	150 ^{bc}	0 ^b	6	5 ^a	17 ^b	TVA	67	252	24 ^a	79 ^{bc}	257 ^b	147 ^c	0 ^b	7	4 ^b	16 ^b	MCLA	65	252	19 ^b	88 ^{ab}	253 ^b	162 ^{ab}	9 ^a	8	3 ^c	21 ^a	SEM	4	5	1	3	6	4	0.2	0.5	0.2	1											
SA	5 ^b	332 ^{ab}	14 ^b	208 ^{ab}	181	120	0 ^b	1.4	12 ^a	119	TVA	5 ^{ab}	295 ^b	17 ^{ab}	176 ^b	194	139	0 ^b	1.4	13 ^a	131	MCLA	6 ^a	351 ^a	18 ^a	203 ^b	185	107	2 ^a	1	8 ^b	106	SEM	0.4	16	1	13	11	14	0.1	0.4	1	17	Carcass											LA	27 ^b	315 ^b	46	131 ^b	265 ^a	134 ^a	0 ^b	4	4	58 ^a	SA	24 ^b	314 ^b	42	145 ^a	281 ^a	120 ^b	0 ^b	4	4	51 ^{ab}	TVA	25 ^b	312 ^b	44	129 ^b	275 ^a	118 ^b	0 ^b	3	4	46 ^b	MCLA	31 ^a	363 ^a	39	126 ^b	231 ^b	125 ^{ab}	5 ^a	4	4	53 ^{ab}	SEM	2	6	4	5	7	4	0.2	0.2	0.3	3	Mammary gland											LA	76	261	24 ^a	76 ^c	261 ^b	166 ^a	0 ^b	8	5 ^a	18 ^{ab}	SA	68	258	24 ^a	94 ^a	281 ^a	150 ^{bc}	0 ^b	6	5 ^a	17 ^b	TVA	67	252	24 ^a	79 ^{bc}	257 ^b	147 ^c	0 ^b	7	4 ^b	16 ^b	MCLA	65	252	19 ^b	88 ^{ab}	253 ^b	162 ^{ab}	9 ^a	8	3 ^c	21 ^a	SEM	4	5	1	3	6	4	0.2	0.5	0.2	1																						
TVA	5 ^{ab}	295 ^b	17 ^{ab}	176 ^b	194	139	0 ^b	1.4	13 ^a	131	MCLA	6 ^a	351 ^a	18 ^a	203 ^b	185	107	2 ^a	1	8 ^b	106	SEM	0.4	16	1	13	11	14	0.1	0.4	1	17	Carcass											LA	27 ^b	315 ^b	46	131 ^b	265 ^a	134 ^a	0 ^b	4	4	58 ^a	SA	24 ^b	314 ^b	42	145 ^a	281 ^a	120 ^b	0 ^b	4	4	51 ^{ab}	TVA	25 ^b	312 ^b	44	129 ^b	275 ^a	118 ^b	0 ^b	3	4	46 ^b	MCLA	31 ^a	363 ^a	39	126 ^b	231 ^b	125 ^{ab}	5 ^a	4	4	53 ^{ab}	SEM	2	6	4	5	7	4	0.2	0.2	0.3	3	Mammary gland											LA	76	261	24 ^a	76 ^c	261 ^b	166 ^a	0 ^b	8	5 ^a	18 ^{ab}	SA	68	258	24 ^a	94 ^a	281 ^a	150 ^{bc}	0 ^b	6	5 ^a	17 ^b	TVA	67	252	24 ^a	79 ^{bc}	257 ^b	147 ^c	0 ^b	7	4 ^b	16 ^b	MCLA	65	252	19 ^b	88 ^{ab}	253 ^b	162 ^{ab}	9 ^a	8	3 ^c	21 ^a	SEM	4	5	1	3	6	4	0.2	0.5	0.2	1																																	
MCLA	6 ^a	351 ^a	18 ^a	203 ^b	185	107	2 ^a	1	8 ^b	106	SEM	0.4	16	1	13	11	14	0.1	0.4	1	17	Carcass											LA	27 ^b	315 ^b	46	131 ^b	265 ^a	134 ^a	0 ^b	4	4	58 ^a	SA	24 ^b	314 ^b	42	145 ^a	281 ^a	120 ^b	0 ^b	4	4	51 ^{ab}	TVA	25 ^b	312 ^b	44	129 ^b	275 ^a	118 ^b	0 ^b	3	4	46 ^b	MCLA	31 ^a	363 ^a	39	126 ^b	231 ^b	125 ^{ab}	5 ^a	4	4	53 ^{ab}	SEM	2	6	4	5	7	4	0.2	0.2	0.3	3	Mammary gland											LA	76	261	24 ^a	76 ^c	261 ^b	166 ^a	0 ^b	8	5 ^a	18 ^{ab}	SA	68	258	24 ^a	94 ^a	281 ^a	150 ^{bc}	0 ^b	6	5 ^a	17 ^b	TVA	67	252	24 ^a	79 ^{bc}	257 ^b	147 ^c	0 ^b	7	4 ^b	16 ^b	MCLA	65	252	19 ^b	88 ^{ab}	253 ^b	162 ^{ab}	9 ^a	8	3 ^c	21 ^a	SEM	4	5	1	3	6	4	0.2	0.5	0.2	1																																												
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a, b, c Least squares means within column and treatment category with different superscripts differ ($P < 0.05$).

dams fed MCLA were greater compared with other treatments. In liver tissue, however, feeding LA and MCLA resulted in greater 16:0 concentration compared with TVA. Palmitoleic acid concentration in the liver of MCLA-fed mice was greater compared with LA or SA. In mammary tissue, however, palmitoleic acid concentration was lower in response to feeding MCLA compared with LA, SA, or TVA.

Stearic acid concentration in the carcass and mammary tissue was greater due to feeding SA compared with LA or TVA. Feeding MCLA, however, resulted in intermediate concentrations of 18:0 in mammary tissue compared with SA or TVA. Oleic acid concentration in this tissue was greater when SA was fed compared with other treatments. In contrast, concentration of oleic acid in the carcass of mice fed MCLA was lower compared with other treatments.

*Trans*11-18:1 concentration in tissues from mice fed LA, SA, or MCLA averaged 1 mg·g⁻¹ (Fig. 2). However, feeding TVA increased *trans*11-18:1 in liver (19 ± 0.2 mg·g⁻¹), carcass (26 ± 0.6 mg·g⁻¹), and mammary tissue (44 ± 0.4 mg·g⁻¹). An increase in 9/11CLA in tissues of mice fed TVA also was observed. Liver (6 ± 0.4 mg·g⁻¹) and carcass (6 ± 0.4 mg·g⁻¹) from mice fed TVA contained similar amounts of 9/11CLA compared with MCLA-fed mice (5 ± 0.4 and 7 ± 0.4 mg·g⁻¹, for liver and carcass) (Fig. 2). In mammary tissue, however, concentration of 9/11CLA averaged 5 ± 0.3 mg·g⁻¹ due to feeding TVA, but it increased to 14 ± 0.3 mg·g⁻¹ in response to feeding MCLA.

*Trans*10,*cis*12-18:2 was only detected in tissues of mice fed MCLA (Tab. II). Mammary gland tissue contained the highest

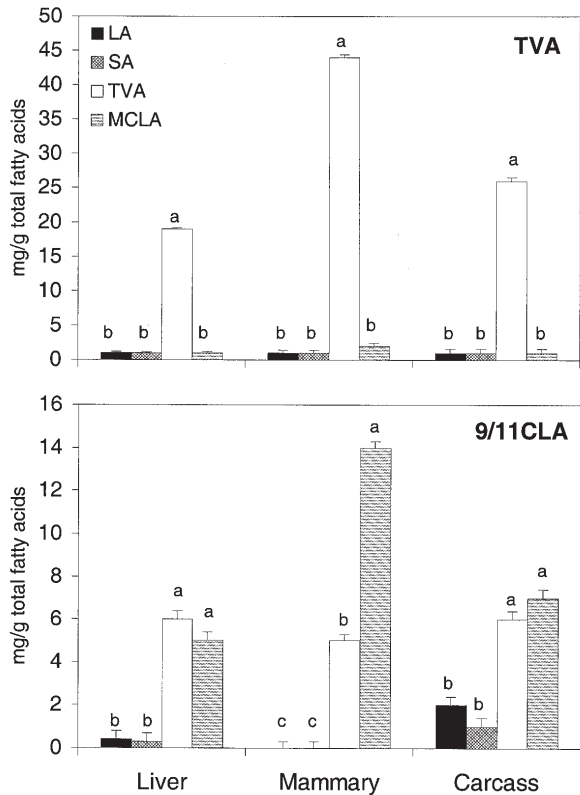


Figure 2. Concentration of *trans*11-18:1 (TVA) and *cis*9,*trans*11-18:2 (9/11CLA) in liver, mammary, and carcass tissue of lactating mice fed a diet containing 30 g·kg⁻¹ 18:2n-6 (LA) or 20 g LA plus 10 g 18:0 (SA), *trans*11-18:1 (TVA), or a conjugated linoleic acid mixture (MCLA). Superscripts denote significant (*P* < 0.05) differences due to treatments.

concentration of *trans*10,*cis*12-18:2, followed by carcass and liver. Despite similar consumption of both CLA isomers from MCLA, 9/11CLA accumulated to a greater extent.

Linoleic acid concentrations in the carcass and mammary tissue were greater when LA was fed compared with other treatments. Concentrations of 20:3n-6 in liver and mammary tissue of MCLA-fed mice were lower compared with other treatments. In contrast, 20:4n-6 concentration was greater in mammary tissue due to feeding MCLA compared with SA or TVA.

3.4. Fatty acid profiles in liver and carcass of suckling pups

The concentration of 16:0 in the carcass of pups nursing SA-fed dams was lower compared with that of pups nursing LA-fed dams (Tab. III). Pups nursing TVA and MCLA-fed dams contained an intermediate concentration of 16:0 in the carcass.

Stearic acid concentration in the carcass of pups nursing MCLA-fed dams was higher compared with pups in LA (Tab. II). In contrast, 18:0 concentration in the liver was greater in pups suckling dams fed LA, SA, or MCLA compared with TVA. Oleic acid concentration was higher in liver of pups nursing SA, TVA, and MCLA-fed dams compared with LA.

Concentration of TVA in liver ($24 \pm 2 \text{ mg}\cdot\text{g}^{-1}$) and carcass ($29 \pm 2 \text{ mg}\cdot\text{g}^{-1}$) of pups nursing dams fed TVA was higher compared with other treatments ($< 2 \text{ mg}\cdot\text{g}^{-1}$, for liver or carcass) (Fig. 3). The elevated content of TVA in mammary tissue (Tab. II) and milk fat (Tab. V) due to feeding TVA suggests this fatty acid was readily transferred to pups via milk. Pups nursing TVA-fed dams also had greater concentrations of 9/11CLA in the liver ($3 \pm 0.4 \text{ mg}\cdot\text{g}^{-1}$), but not carcass, compared with pups nursing LA or SA-fed dams (Fig. 3). Nursing dams fed MCLA, however, resulted in greater

concentrations of 9/11CLA in the liver ($8 \pm 0.4 \text{ mg}\cdot\text{g}^{-1}$) and carcass ($6 \pm 0.1 \text{ mg}\cdot\text{g}^{-1}$). Concentrations of *trans*10,*cis*12-18:2 were only detectable in liver and carcass of pups nursing MCLA-fed dams (Tab. III).

3.5. Stearoyl-CoA desaturase activity in liver and mammary gland tissue of lactating mice

Activity of SCD in liver tissue did not differ due to treatments, and averaged $24.5 \text{ pmole } cis9-18:1\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ protein (Tab. IV). In mammary tissue, however, activity was 14% greater in response to feeding TVA ($33.8 \text{ pmole } cis9-18:1$) compared with other treatments ($29.5 \text{ pmole } cis9-18:1$). Greater concentrations of 9/11CLA in mammary tissue and milk fat of lactating mice fed TVA may be linked to enhanced SCD activity.

4. DISCUSSION

Amounts and concentrations of 9/11CLA and TVA in blood plasma were substantially elevated in response to feeding MCLA and TVA (Fig. 1). *Trans*10,*cis*12-18:2, however, was detectable exclusively when MCLA was fed. Feeding TVA resulted in a similar concentration of 9/11CLA in PL, but a higher concentration in CE when compared with MCLA. Compared with basal levels (LA and SA), concentration of 9/11CLA in plasma TG was nearly tripled due to feeding TVA. Although liver SCD activity was similar across diets (Tab. IV), a portion of dietary TVA may have been converted to 9/11CLA in this tissue because cultures of liver microsomes desaturated TVA to 9/11CLA [28]. Liver desaturase activity increases progressively during lactation in rats [5]. Normal VLDL-triglyceride synthesis and secretion depend on SCD [22], and both were enhanced in lactating compared with virgin rats [4]. Hence, rather than storing endogenously synthesized

Table III. Fatty acid profiles in tissues of pups suckling lactating mice fed a diet containing 30 g·kg⁻¹ 18:2n-6 (LA) or 20 g LA plus 10 g 18:0 (SA), *trans*11-18:1 (TVA), or a conjugated linoleic acid mixture (MCLA).

Treatment	Fatty acid									
	14:0	16:0	c9-16:1	18:0	c9-18:1	18:2n-6	10,c12-18:2	18:3n-3	20:3n-6	20:4n-6
	mg·g ⁻¹ total fatty acids									
Liver										
LA	8 ^b	239	6 ^b	202 ^a	124 ^b	176	0 ^b	6 ^a	10 ^a	215
SA	8 ^b	246	8 ^a	204 ^a	167 ^a	169	0 ^b	3 ^b	9 ^{ab}	174
TVA	12 ^a	243	8 ^a	176 ^b	165 ^a	169	0 ^b	3 ^b	10 ^a	172
MCLA	7 ^b	244	6 ^b	204 ^a	158 ^a	179	3 ^a	4 ^{ab}	7 ^b	179
SEM	1	8	0.6	9	11	5	0.1	1	0.7	21
Carcass										
LA	67	282 ^a	20	110 ^b	297	113 ^{ab}	0 ^b	3 ^{ab}	6 ^b	42 ^b
SA	56	256 ^b	21	132 ^{ab}	306	125 ^a	0 ^b	2 ^{bc}	8 ^a	57 ^a
TVA	61	267 ^{ab}	23	118 ^{ab}	300	111 ^b	0 ^b	3 ^a	6 ^b	42 ^b
MCLA	54	268 ^{ab}	17	140 ^a	308	109 ^b	3 ^a	2 ^c	5 ^c	48 ^{ab}
SEM	5	7	2	10	5	5	0.1	0.2	0.5	6

a, b, c Least squares means within column and treatment category with different superscripts differ ($P < 0.05$).

9/11CLA, the liver may provide this fatty acid to the lactating mammary gland. Intestinal desaturation [12] also could have contributed to the greater 9/11CLA concentration in blood when TVA was fed.

Dietary TVA is not incorporated into CE, due to low specificity of lecithin:cholesterol acyl transferase (LCAT) for this fatty acid

[10]. This effect may explain the greater 9/11CLA concentration in CE due to feeding TVA, because linoleic acid is the main substrate for LCAT [10]. In contrast, rapid incorporation and clearance of TVA from plasma TG suggests tissues preferentially metabolize it when esterified in the triglyceride moiety [10]. Hence, TVA and

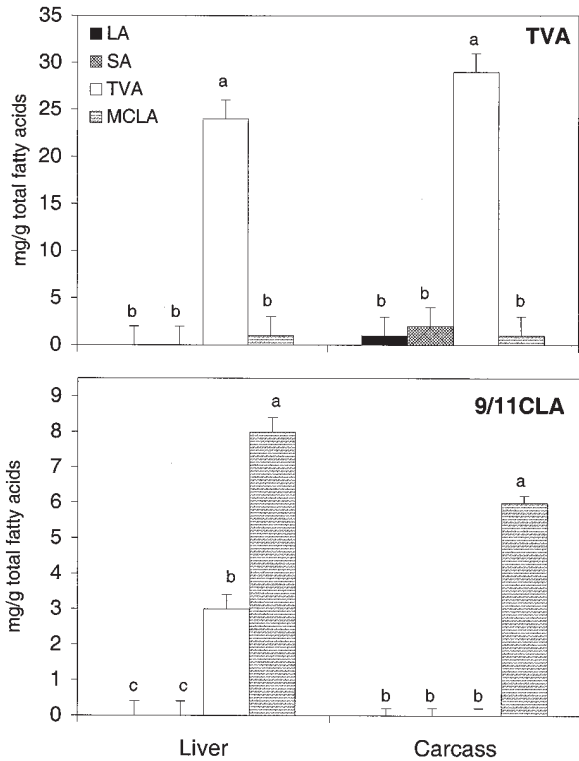


Figure 3. Concentration of *trans*11-18:1 (TVA) and *cis*9,*trans*11-18:2 (9/11CLA) in liver and carcass tissue of pups suckling lactating mice fed a diet containing 30 g·kg⁻¹ 18:2n-6 (LA) or 20 g LA plus 10 g 18:0 (SA), *trans*11-18:1 (TVA), or a conjugated linoleic acid mixture (MCLA). Superscripts denote significant ($P < 0.05$) differences due to treatments.

Table IV. Stearoyl-CoA desaturase activity in liver and mammary gland tissue from lactating mice fed a diet containing 30 g·kg⁻¹ 18:2n-6 (LA) or 20 g LA plus 10 g 18:0 (SA), *trans*11-18:1 (TVA), or a conjugated linoleic acid mixture (MCLA).

Tissue	LA	SA	TVA	MCLA	SEM
	pmole·min ⁻¹ ·mg ⁻¹ protein				
Liver	25.52	22.67	24.15	25.52	1.46
Mammary gland	28.94 ^b	30.69 ^b	33.80 ^a	29.12 ^b	0.95

^{a, b} Least squares means within row and treatment category with different superscripts differ ($P < 0.05$).

Table V. Fatty acid profiles in milk fat from one lactating mouse fed a diet containing 30 g·kg⁻¹ 18:2n-6 (LA) or 20 g LA plus 10 g 18:0 (SA), *trans*11-18:1 (TVA), or a conjugated linoleic acid mixture (MCLA).

Fatty acid	LA	SA	TVA	MCLA
	mg·g ⁻¹ total fatty acids			
8:0	3	3	6	3
10:0	39	53	61	47
12:0	60	74	61	60
14:0	14	101	42	68
16:0	241	250	254	228
<i>cis</i> 9-16:1	26	17	20	17
18:0	48	65	59	64
<i>trans</i> 11-18:1	1	1	37	1
<i>cis</i> 9-18:1	293	248	254	260
18:2n-6	195	175	191	192
<i>cis</i> 9, <i>trans</i> 11-18:2	1	1	6	26
<i>trans</i> 10, <i>cis</i> 12-18:2	0	0	0	23
18:3n-3	8	8	6	9
20:3n-6	5	4	3	2
20:4n-6	12	9	17	7

9/11CLA may be selectively incorporated into plasma PL and TG for rapid utilization by tissues.

This selectivity may be important during lactation when an increase in mammary gland lipoprotein lipase (LPL) activity in rodents [13] is accompanied by extensive hydrolysis and uptake of fatty acids from plasma TG and PL by the mammary gland for milk fat synthesis [34]. Data from the present study indicated that 78% of total TVA and 82% of total 9/11CLA in blood plasma were found in PL plus TG, and these fractions contained 64% of total fatty acids in plasma. Rat mammary gland LPL removes fatty acids esterified in the sn-1 position of plasma TG and PL prior to removal of fatty acids at the sn-3 position of the TG [34]. *Trans*11-18:1 is preferentially concentrated in the sn-1 position of plasma TG [9] and PL [10]. Feeding TVA and MCLA increased TVA and 9/11CLA content in mammary tissue by 2.3 and 1.1 mg·g⁻¹ tissue, respectively. Thus, similar

to TVA, 9/11CLA bound to plasma TG and PL was readily absorbed by mammary cells.

The concentration of 18:0 in mammary tissue was similar when MCLA or SA were fed. Oleic acid concentration, however, was greater due to feeding SA only. Despite similar activity of SCD in mammary tissue when mice were fed MCLA or SA, responses seem to indicate that feeding MCLA may have affected desaturation of 18:0. For example, the ratio (data not shown) of 18:0/*cis*9-18:1 was greater in response to MCLA (0.35) compared with CO or TVA (0.30). Transcription of the SCD gene in mouse mammary cell cultures was reduced by *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 [19]. In vivo, however, feeding *trans*10, *cis*12-18:2 to lactating mice reduced SCD mRNA abundance and activity to a greater extent than *cis*9,*trans*11-18:2 [20]. In the present study, apparent intakes of *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 averaged 22 and 23 mg·d⁻¹ compared with 124 and 110 mg·d⁻¹ [21], respectively, in

the study of Lin et al. [20]. Differences in mammary SCD activity may be a function of CLA isomer availability.

Compared with SA or LA, feeding TVA increased mammary gland concentration of 9/11CLA from not detectable levels to 5 mg·g⁻¹ total fatty acids (Fig. 2). Milk fat concentration of 9/11CLA also was greater in response to feeding TVA (Tab. V). Greater activity of SCD (Tab. IV) in mammary tissue may have enhanced desaturation of dietary TVA to 9/11CLA. Previous results showed TVA increased cellular 9/11CLA concentration in mouse mammary cell cultures as a result of greater SCD mRNA abundance and activity [15]. This response was linear between 0, 12.5, 25, and 50 μM TVA, but decreased at 100 μM when compared with 18:0. At high concentrations (due to greater dietary intake), TVA may influence SCD mRNA processing or stability in the nucleus after transcription and reduce mRNA abundance and activity [19].

During lactation in mice, desaturation of dietary linoleic acid enhances the concentration of arachidonic acid in the liver and mammary gland [1]. Linoleic acid was used as carrier for treatment fatty acids in the present study, and concentrations in tissues or carcass were five times greater than previously reported in lactating mice fed pure 18:0, *cis9*-18:1, or 18:3n-3 [1]. Thus, dietary linoleic acid was available for desaturation in tissues. Although feeding LA resulted in greater 18:2n-6 in carcass and mammary tissue, arachidonic acid concentrations did not increase accordingly. Large variation in animal response and (or) similar availability of 18:2n-6 with all diets, may have precluded clear differences in arachidonic acid concentrations between treatments. In liver tissue of rats fed a CLA mixture, concentration of 20:3n-6 was reduced [18]. Feeding MCLA in the present study reduced the concentration of 20:3n-6 in liver and mammary tissue, but it increased 20:4n-6 only in the mammary gland (Tab. II). Lower 20:3n-6

may be due to a reduction in the extent of 18:3n-3 elongation. Both isomers could potentially inhibit the amount or activity of elongases, because dietary *cis9,trans11*-18:2 or *trans10,cis12*-18:2 (compared with *cis9*-18:1) reduced 20:3n-6 concentration in liver and mammary tissue of lactating mice [21]. In contrast, only feeding *trans10,cis12*-18:2 increased 20:4n-6 concentration in milk fat and mammary tissue [21]. From the available data, it is not possible to explain the observed increase in 20:4n-6 concentration in response to MCLA.

When compared with LA, the carcass from pups nursing MCLA-fed dams contained higher 18:0 and CLA isomer concentrations (Tab. III; Fig. 3). Oleic acid concentration, however, was similar regardless of treatment. Feeding *trans10,cis12*-18:2, but not *cis9,trans11*-18:2, to lactating mice was associated with higher 18:0 concentration in the carcass of nursing pups [20]. Desaturation of 18:0 in 3T3L1 adipocytes was reduced by *trans10,cis12*-18:2, increasing 18:0 concentration, due to lower SCD mRNA abundance and activity [8].

Concentrations of TVA in pup liver and carcass reflected that of mammary gland tissue or milk fat from TVA-fed mice. *Cis9,trans11*-18:2 concentration was only measurable in the liver of pups nursing TVA-fed dams. As suggested previously, desaturation of milk fat-derived TVA may have occurred primarily in the liver as opposed to adipose tissue [32]. However, transfer of endogenously synthesized 9/11CLA via milk fat certainly contributed to the amount found in liver tissue from pups nursing TVA-fed mice.

5. CONCLUSIONS

Dietary *trans11*-18:1 increases the amount of *cis9,trans11*-18:2 in blood plasma, milk, and tissue lipids during lactation. Greater availability of *trans11*-18:1 in the diet may increase the circulating

levels of *cis9,trans11-18:2* (via desaturation in the liver) in lactating females to a similar extent than CLA supplements. Endogenous synthesis of *cis9,trans11-18:2* from *trans11-18:1* in mammary tissue may be due in part to greater stearoyl-CoA desaturase activity. *Cis9,trans11-CLA* derived from *trans11-18:1* is available for secretion in milk fat. Furthermore, dietary or endogenous *cis9,trans11-18:2* are readily transferred to suckling offspring via milk.

The maternal diet and tissue stores are key factors in determining the supply of long-chain fatty acids secreted in human milk. By demonstrating efficient bioconversion of dietary *trans*-vaccenic acid to *cis9,trans11-* conjugated linoleic acid via desaturation during lactation, our data imply that *trans*-vaccenic acid could play an important role in maternal and neonatal health. Particularly because humans also are capable of desaturating *trans11-18:1* to *cis9,trans11-18:2* [2], which is considered a functional food component with a range of potential health benefits [25].

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