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Trans-Fatty Acid-Stimulated Mammary Gland Growth in Ovariectomized Mice is Fatty Acid Type and Isomer Specific

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Abstract

We previously reported that the *trans-18:2* fatty acid *trans*-10, *cis*-12 conjugated linoleic acid (t10,c12-CLA) stimulates mammary gland development independent of estrogen and its receptor. Given the negative consequences of dietary *trans*-fatty acids on various aspects of human health, we sought to establish whether other *trans*-fatty acids could similarly induce ovary-independent mammary gland growth in mice. Prepubertal BALB/cJ mice were ovariectomized at 21 days of age then were fed diets enriched with *cis*-9, *trans*-11 CLA (c9,t11-CLA), or mixtures of *trans*-18:1 fatty acids supplied by partially hydrogenated sunflower, safflower, or linseed oil. The resultant mammary phenotype was evaluated 3 weeks later and compared to the growth response elicited by t10,c12-CLA, or the defined control diet. Whereas partially hydrogenated safflower oil increased mammary gland weight, none of the partially hydrogenated vegetable oils promoted mammary ductal growth. Similarly, the c9,t11-CLA supplemented diet was without effect on mammary development. Taken together, our data emphasize a unique effect of t10,c12-CLA in stimulating estrogen-independent mammary gland growth manifest as increased mammary ductal area and elongation that was not recapitulated by c9,t11-CLA or the partially hydrogenated vegetable oil diets.

Keywords

Mammary gland; *Trans*-fatty acids; Conjugated linoleic acid; Partially hydrogenated vegetable oil

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Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest to disclose.

Introduction

The level of *trans*-fatty acids in the modern food supply has increased dramatically over the last century [1]. Partially hydrogenated plant and marine oils have served as an inexpensive substitute for butterfat while improving the texture of processed foods, and increasing product shelf life [2]. Despite these many advantages, epidemiologic evidence has revealed that *trans*-fatty acids significantly elevate the risk of atherosclerosis [3], where a mere 2% increase in energy intake from *trans*-fatty acids increased risk for developing cardiovascular disease by 23% [4]. *Trans*-fatty acids also negatively affect serum lipids by elevating low density lipoprotein and lowering high density lipoprotein cholesterol [5], while the isomeric profile of dietary *trans*-fatty acids can differentially impact blood lipoprotein profiles [6]. Moreover, the results from multiple studies indicate that *trans*-fatty acids have a negative effect on systemic inflammation leading to increased levels of C-reactive protein, interleukin-6, and tumor necrosis factor-α [7], while also exacerbating insulin resistance in populations with comorbidities such as obesity [8].

The majority of dietary *trans*-fatty acids are *trans*-18:1 isomers produced during the partial hydrogenation of plant oils [9]. In some cases, *trans*-fatty acids can account for 60% of total fatty acids in partially hydrogenated vegetable oils (PHVO) [10]. An alternative source of dietary *trans*-fatty acids is fat from ruminant animals, where the level of *trans*-fatty acids in typical ruminant-derived foods is approximately 2–4% of total fatty acids [10]. The predominant *trans*-fatty acid in ruminant-derived fats is *trans*-18:1, with smaller amounts of *trans*-18:2 *cis*-9, *trans*-11 conjugated linoleic acid (c9,t11-CLA) [10]. Minor amounts of the *trans*-18:2 fatty acid *trans*-10, *cis*-12 CLA (t10,c12- CLA) are present in ruminant-derived foods, whereas this isomer accounts for approximately half of the CLA in mixed CLA supplements marketed for weight loss [11].

Dietary fats have long been implicated in modifying development of the normal mammary gland [12–14] and its susceptibility to tumorigenesis [15]. Generally speaking, unsaturated fatty acids stimulate the growth of normal and transformed mammary epithelial cells *in vitro* [16] and *in vivo* [17], whereas saturated fatty acids restrict their growth [16]. Moreover, we recently identified that a diet supplemented with t10,c12-CLA stimulated the mammary glands of ovariectomized (OVX) prepubertal mice to undergo allometric growth, independent of any requirement for estrogen (E) and its receptor (ER) [17]. Our experiments further indicated a role for altered insulin/IGF-1 signaling in conjunction with concomitant metabolic dysregulation in mediating this response [17]. In addition to these findings, we and others have shown that dietary t10,c12-CLA promotes hyperinsulinemia [18], hepatic steatosis [19], inflammation [20], and lipoatrophy [21] while also stimulating precocious lobuloalveolar development in the mammary glands [22], and accelerating tumorigenesis in mice overexpressing ErbB2 [23].

Policies to minimize the consumption of *trans*-fatty acids have only been implemented in recent times [24], leaving in place questions about the long-standing impacts of their intake on human health and disease. Given parallels between t10,c12-CLA and other dietary *trans*-fatty acids, we sought to compare the ability of various mixtures of *trans*-fatty acid isomers to promote E-independent mammary growth. Our findings reveal that this response is

specific to t10,c12-CLA, and does not occur in response to dietary c9,t11-CLA or mixtures of different *trans*-18:1 isomers derived from PHVO. These data further highlight the varying effects of diet on the developing mammary gland.

Materials and Methods

Animals

All experiments were conducted with the approval of the University of California, Davis Institutional Animal Care and Use Committee. BALB/cJ mice (Jackson Laboratory, Bar Harbor, ME, USA) were used in all experiments. Mice were housed under a 14:10 h light-dark cycle, and allowed *ad libitum* feed and water. Mice were bilaterally ovariectomized (OVX) at 21 days of age, prior to the onset of puberty. Mice were anesthetized with ketamine/xylazine (IP, 60 and 10 mg/kg, respectively) and received post-operative buprenorphine (SC, 0.05 mg/kg) analgesia. Mice were fed a control diet after OVX then were assigned to the experimental diets the following day.

Diets

The control (CON) and t10,c12-CLA diets used in Experiment 1 were as described [17]. The c9,t11-CLA diet used in Experiment 1 was the CON diet with 1% fat by weight as c9,t11-CLA (Table 1). All PHVO diets (Experiment 2) were formulated from an AIN-93G base diet and contained 15% total fat by weight, where 10% of the fat was soybean oil. The PHVO diets contained 5% fat as either partially hydrogenated sunflower (PH-SUN), partially hydrogenated safflower (PH-SAF), or partially hydrogenated linseed (PH-LIN) oil by weight. The control diet (TFA-CON) contained 5% stearic acid by weight. The c9,t11/t10,c12-CLA diet in Experiment 2 contained 2.3% stearic acid and 2.7% Clarinol-G80 that supplied approximately 1% 9,11 and 1% t10,c12-CLA by weight. The level of t10,c12-CLA in this diet was approximately equivalent to that used in the diet that contained t10,c12-CLA in Experiment 1.

All PHVO diets were enriched for *trans*-18:1 fatty acids containing between 11.37 g and 13.58 g *trans*-18:1/100 g fatty acids (Table 2). Both the TFA-CON and c9,t11/t10,c12-CLA diet were low in total *trans*-18:1, containing 0.18 g and 0.10 g *trans*-18:1/100 g fatty acids respectively (Table 2). All PHVO diets contained similar amounts of total saturated fatty acids, *cis*-monounsaturated fatty acids, and *cis*-polyunsaturated fatty acids, while both the TFA-CON and c9,t11/t10,c12-CLA diets contained a slightly higher amount of total fatty acids in these classes (Table 2). The TFA-CON and PHVO diets contained no CLA (Table 2) [6].

While the total level of *trans*-18:1 fatty acids in each PHVO diet was similar, the isomeric profile of these fatty acids differed. The PH-LIN diet was lowest in *trans*-6–8 18:1, while levels were highest in the PH-SUN diet (Table 2). In parallel, the PH-LIN diet contained more *trans*-9–10 18:1 than the PH-SAF diet (Table 2). The PH- SUN diet had the highest content of *trans*-6–8 18:1 and was lower in *trans*-11–15 18:1 (Table 2). The PH-SAF diet contained higher amounts of *trans*-11–15 18:1 (Table 2).

Experiment 1: Effect of c9,t11-CLA on Ovary-Independent Postnatal Mammary Growth

Mice OVX at 21 days of age were assigned to the CON diet or that with 1% fat as either 9,11 or t10,c12-CLA. Mice were maintained on these diets for 21 days and then euthanized by CO_2 inhalation followed by exsanguination. Experiments were repeated in three independent cohorts (n = 4–7/group/cohort).

Experiment 2: Effect of PHVO on Ovary-Independent Postnatal Mammary Growth

Mice OVX at 21 days of age were assigned to the TFA-CON diet or that with 5% fat as PH-SUN, PH-SAF, PH-LIN, or c9,t11/t10,c12-CLA. Mice were fed these diets for 21 days *ad libitum* then were euthanized by CO_2 inhalation followed by exsanguination.

Mammary Gland Whole Mount Preparation and Analysis

Whole mounts were prepared from the 4th inguinal mammary glands [25]. Briefly, mammary glands were excised, spread on a glass slide, and air-dried prior to fixation in either Carnoy's (Experiment 1, cohort 1) or Tellyesniczky's (Experiment 1, cohort 2 and 3; Experiment 2) fixative. Samples were dehydrated in graded ethanol, cleared in xylene, and coverslipped with Cytoseal 280 (Fisher Scientific, Waltham, MA, USA). Digital images were captured (Qimaging QiCAM RGB Cooled CCD) using an Olympus SZX16 stereomicroscope.

Ductal elongation was measured as the distance from the teat to the furthest ductal terminus using either digital calipers (Experiment 1) or the line tool in the open-source software FIJI [26]. Ductal area was determined using the magic wand tool to select only the ductal network following background subtraction, color deconvolution, and image binarization using FIJI [26]. To quantify ductal branchpoints, images were first adjusted by performing background subtraction, color deconvolution, and local contrast enhancement [26]. Ductal branchpoints were then quantified as the number of ductal bifurcations that were manually tagged using FIJI [26] (Experiment 1), or as the number of junctions detected using Angiotool (Experiment 2) [27]. Branchpoint density was calculated by expressing branchpoint number per ductal area.

Fatty Acid Analysis

The fatty acid profile of the diets was analyzed using a modified method of the direct transesterification method of Sukhija and Palmquist [28] as we have described previously [29]. An aliquot of the solution, containing the fatty acid methyl esters (FAME), was taken for GLC analysis. Total lipids were extracted from epithelium-free mammary fat pad and omental fat according to the method of Bligh and Dyer [30] using a mixture of methanol:chloroform:water (2:2:1.8, by vol). The lipids were transesterified with methanolic sodium methoxide (0.5 M $NaOCH_3$/methanol, 15 min at room temperature). The GLC analysis of all FAME extracts was performed on a GC-2010 gas chromatograph (Shimadzu) equipped with a flame ionization detector using a CP-Sil 88 capillary column (100 m × 0.25 mm I.D. × 0.25 μm film thickness, Varian). GLC conditions were described previously [29]. Fatty acid results were expressed as percentages (g/100 g fatty acids) of fatty acids detected with a chain length between 10 and 24 carbon atoms. The lower limit of detection was <0.001/100 g fatty acids.

Statistics

Data were analyzed using the Proc GLM procedure in SAS (Cary, NC, USA). Experiment 1 was a Random Complete Block Design and was analyzed by one-way ANOVA with blocking by experimental cohort. Experiment 2 was a completely randomized design and was analyzed by one-way ANOVA. Body weight data was analyzed using the Proc GLM procedure with repeated measures. Means were significant when $P < 0.05$. Data were transformed where appropriate.

Results

Experiment 1: Effect of c9,t11-CLA on Ovary-Independent Mammary Growth

We previously showed that t10,c12-CLA stimulated mammary growth independent of E and the ER [17]. Given that both isomers of CLA (t10,c12-CLA and c9,t11-CLA) are present in CLA dietary supplements [31], as well as in ruminant-derived meat and dairy products [32], we sought to determine if the effect of 10,12 was recapitulated by dietary c9,t11-CLA. As anticipated, t10,c12-CLA increased ductal elongation in OVX mice, whereas dietary c9,t11-CLA had no effect (Fig. 1d). Furthermore, t10,c12-CLA increased ductal area, whereas the glands from mice fed c9,t11-CLA were unchanged (Fig. 1e). There was no increase in branchpoint density in response to c9,t11-CLA, whereas branchpoint density was reduced in mice fed t10,c12-CLA. These findings emphasize that the primary effect of t10,c12-CLA on the mammary glands is increased ductal elongation (Fig. 1f). From these data we conclude that despite similarities between these CLA isomers, only t10,c12-CLA evokes a proliferative response in the mammary gland.

Effect of PHVO Diets on Ovary-Independent Mammary Growth (Experiment 2)

We next investigated the effects of different PHVO on ovary-independent mammary gland growth. Given that one hallmark of t10,c12-CLA supplementation is reduced adiposity [20] and increased liver mass [18], we measured the wet mass of both the liver and the lymph node-free 4th inguinal mammary gland. As anticipated, the diet containing the CLA isomer mixture (c9,t11/t10,c12-CLA) reduced the mass of the mammary glands, whereas the mammary glands of mice fed the PH-SAF diet were heavier than those from mice fed TFA-CON- and c9,t11/t10,c12-CLA (Fig. 2a). In parallel, the c9,t11/t10,c12-CLA diet, but not the PHVO diets, increased liver mass (Fig. 2b). Uterine mass was unchanged across the different diet groups (Fig. 2c). There was no effect of the various diets on body weight (Fig. 2d).

We also determined the fatty acid profile of omental adipose tissue (Table 4) and the 4th inguinal epithelium-free mammary fat pad (distal to the teat and supramammary lymph node, Table 3). The fatty acid profile of both the omental and epithelium-free mammary fat pad depots generally reflected that of the diet. In parallel, the total level of *cis*-polyunsaturated fatty acids was greatest in fat depots from c9,t11/t10,c12-CLA-fed mice (Tables 3, 4).

Fat depots from mice fed the various PHVO had higher levels of *trans*-18:1 than TFA-CON or c9,t11/t10,c12-CLA-fed mice, where mice fed PH-SUN had a significantly higher

trans-18:1 content in the mammary fat pads than mice fed TFA-CON, c9,t11/t10,c12-CLA, or PH-LIN diet (Table 3). Consumption of the PH-SUN diet resulted in greater accumulation of *trans*-6–8 18:1 in the mammary fat pad compared to all other diets (Fig. 3a; Table 3).

Mice fed the PHVO treatments had a higher level of c9,t11-CLA in both the omental (Table 4) and mammary fat pads (Table 3), despite the absence of c9,t11-CLA in the diet. The content of vaccenic acid (*trans*-11 18:1), the precursor to c9,t11-CLA, was greater in the mammary fat pads (Table 3; Fig. 3a) and omental fat (Table 4; Fig. 3b) of PHVO-fed mice compared to mice fed the control or c9,t11/t10,c12-CLA diets. This distribution closely reflected the diet composition, where the PHVO diets contained more *trans*-11 18:1 than the control and c9,t11/t10,c12-CLA diets (Table 2). As anticipated, the accumulation of CLA was greatest in tissues from mice fed the c9,t11/t10,c12-CLA diet (Tables 3, 4). However, the content of c9,t11-CLA was higher than t10,c12-CLA in the mammary fat pads and omental fat from mice fed the c9,t11/t10,c12-CLA diet (Tables 3, 4), despite there being approximately equal amounts of c9,t11 and t10,c12-CLA in the diet (Table 2).

While the c9,t11/t10,c12-CLA mixture stimulated ovary-independent mammary growth, there was no effect of the various PHVO diets on ductal elongation compared to that in mice fed the TFA-CON diet (Fig. 4f). Similarly, mammary ductal area was increased by dietary c9,t11/t10,c12-CLA, but was unaffected by the PH-LIN, PH-SAF, or PH-SUN diets (Fig. 5a). Moreover, whereas dietary c9,t11/t10,c12-CLA increased total branchpoint number (Fig. 5b), there was no effect of diet on branchpoint density once branchpoint number was expressed relative to ductal area (Fig. 5c).

Discussion

The Ability of *Trans*-Fatty Acids to Stimulate E-Independent Mammary Growth is Type-Specific and Isomer-Specific

Multiple lines of evidence have highlighted the deleterious effects of dietary *trans*-fatty acids on human health [3, 5, 9, 33]. While the contribution of individual fatty acids is often difficult to parse in epidemiological studies, considering the effects of different *trans*-fatty acid isomers in controlled experiments is crucial. We previously showed that dietary t10,c12-CLA, a *trans-18:2* isomer, stimulates allometric mammary growth and tumorigenesis in OVX mice [17]. Those experiments supported that t10,c12-CLA-induced mammary growth independent of E and ER given that inhibition of E biosynthesis or antagonism of ER failed to block t10,c12-CLA-mediated mammary growth [17]. In parallel, we highlighted that metabolic dysregulation including well- documented insulin resistance could be implicated in t10,c12-CLA-stimulated mammary growth, and that administering the insulin-sensitizer rosiglitazone abrogated this response [17]. Here we establish that diet-stimulated mammary gland growth is specific to t10,c12-CLA and cannot be recapitulated by c9,t11-CLA or diets enriched with different mixtures of *trans*-18:1 isomers derived from PHVO. Although we cannot exclude the potential for an effect of different *trans*-fatty acid isomers on the mammary glands after a longer exposure period or when fed at different levels, we conclude that E-independent mammary gland growth is t10,c12-CLA-specific within the confines of this defined model system.

Dietary *Trans*-18:1 Isomers Are Metabolized to *Trans*-18:2 Fatty Acids *In Vivo*

The c9,t11-CLA content in the mammary fat pads and omental fat from mice fed PH-LIN, PH-SAF, and PH-SUN was approximately 10-fold greater than in control-fed mice. Given that the PH-LIN, PH-SAF, or PH-SUN diets were devoid of c9,t11-CLA, its accumulation in adipose tissue likely reflects conversion of *trans*-11 18:1 to c9,t11-CLA by delta-9-desaturase [34, 35]. Along these lines, Loor *et al*. found that the CLA content in the livers and whole carcasses of lactating mice was approximately equal when they were fed diets containing either a CLA mixture or *trans*-18:1 fatty acids [34]. Separately, CLA accumulated in the triacylglycerides but not the phospholipid fraction of adipose tissue in mice fed a diet containing *trans*-11 18:1, indicating that desaturation of *trans*-11 18:1 likely occurred in adipose tissue [36]. Moreover, inhibition of delta-9 desaturase with cyclopropenoic fatty acids prevented consversion of *trans*-11 18:1 to c9,t11-CLA in rats [37]. Given that the amount of *trans-11* 18:1 in the PH-LIN, PH-SAF, and PH-SUN diets was similar, it follows that the extent of c9,t11-CLA accumulation did not differ across the diet groups.

Our data also indicate that compared to t10,c12, c9,t11-CLA is preferentially incorporated into both the mammary fat pad and omental fat, given that the content of c9,t11- CLA in both fat depots was elevated despite approximately equal amounts of c9,t11 and t10,c12-CLA in the c9,t11/ t10,c12-CLA mixed isomer diet. Given the magnitude of the difference and the low content of *trans*-11 18:1 in the c9,t11/t10,c12-CLA diet preparation, conversion of *trans*-11 18:1 to c9,t11-CLA by delta-9 desaturase is likely only partially responsible for the difference. Given that mice are coprophagous, some CLA in the tissues may have come from the feces. Along these lines, the content of t10,c12-CLA in lipids extracted from the livers and hearts of pigs fed a mixed CLA diet was lower than that supplied by the diet, while incorporation of 9,11 into liver phospholipids was significantly higher than t10,c12-CLA [38]. In parallel, layer hens fed a diet containing mixed CLA had less t10,c12-CLA accumulate in tissues relative to the amount present in the diet [39]. Taken together, these data emphasize that c9,t11-CLA selectively incorporated into tissues across various monogastric species.

A preponderance of evidence points to the deleterious effects of dietary *trans*-fatty acids on human health that has led to broad policy initiatives limiting their presence in the human diet [33]. While our previous findings highlighted a novel effect of dietary t10,c12-CLA on estrogen-independent mammary gland growth [17], our data herein do not support a similar effect of diets high in *trans*-18:1 fatty acids supplied by various PHVO. At the same time, c9,t11-CLA, which is most common in dietary supplements, as well as is a minor constituent of ruminant-derived foodstuffs, also had no effect. Combined, these data indicate a specific effect of t10,c12-CLA on mammary gland development.

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Abbreviations

ANOVA	Analysis of variance
c9,t11-CLA	*Cis*-9, *trans*-11 conjugated linoleic acid
CLA	Conjugated linoleic acid
CON	Control diet experiment 1
TFA-CON	Control diet experiment 2
E	Estrogen
ER	Estrogen receptor
IP	Intraperitoneal
OVX	Ovariectomized
PH-LIN	Partially hydrogenated linseed oil
PH-SAF	Partially hydrogenated safflower oil
PH-SUN	Partially hydrogenated sunflower oil
PHVO	Partially hydrogenated vegetable oil
SC	Subcutaneous
t10,c12-CLA	*Trans*-10, *cis*-12 conjugated linoleic acid

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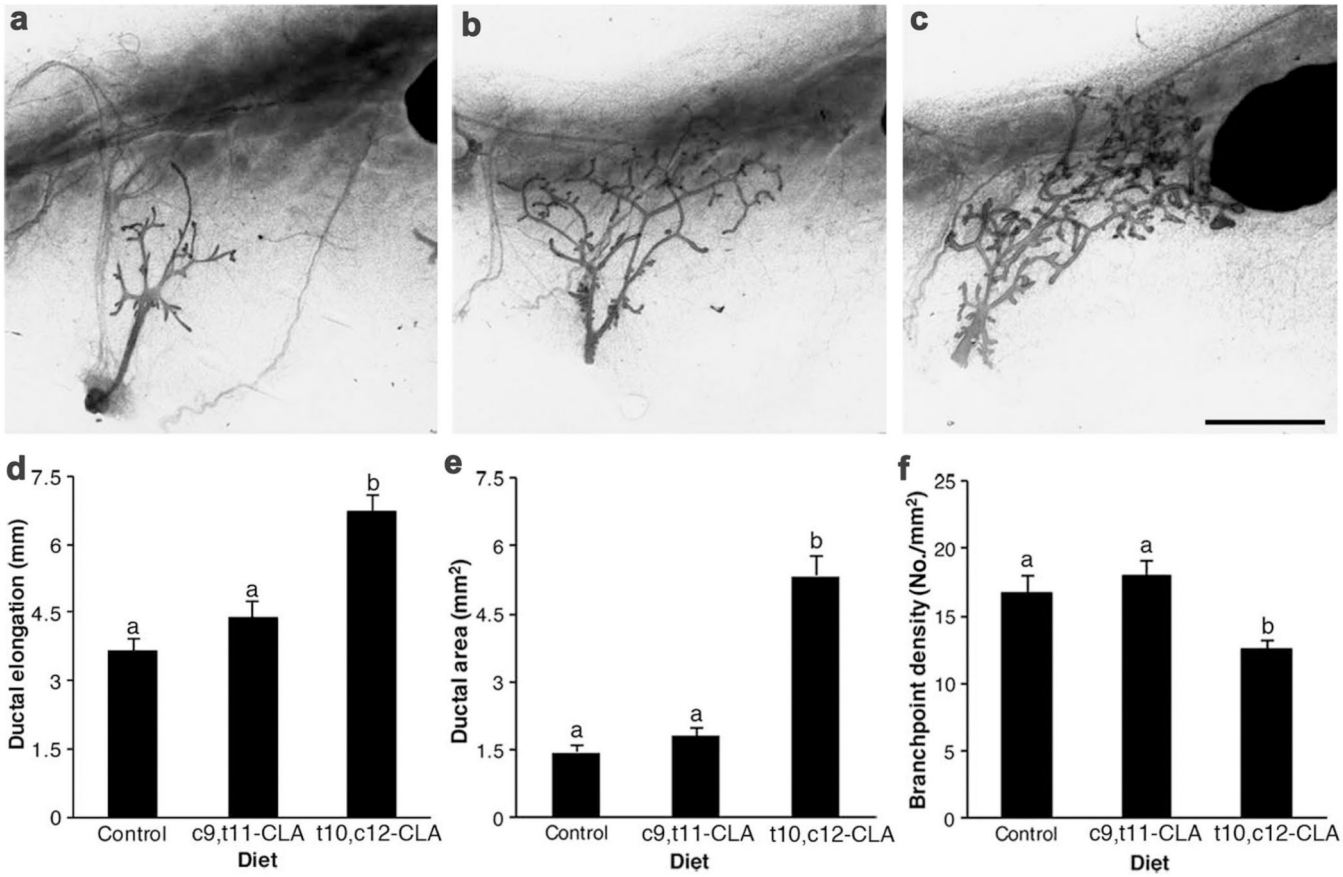

Fig. 1.

Dietary *trans*-10*, cis*-12 conjugated linoleic acid (*t*10,*c*12 CLA), but not *cis*-9*, trans*-11 conjugated Linoleic acid (*c*9,*t*11 CLA), stimulates mammary gland growth in ovariectomized mice. Representative images of whole mounts from inguinal mammary glands of 42d old mice fed either the (**a**) control diet or that with 1% fat as (**b**) c9,t11 or (**c**) t10,c12 CLA. Mice were ovariectomized at 21 days, and fed the experimental diet from 22 days until euthanasia at 42 days of age. *Scale bar* 2 mm. **d** Mammary ductal elongation was the distance from the teat to the furthest-reaching ductal terminus. **e** Ductal area was measured using FIJI. **f** Branchpoint density was the number of ductal bifurcations counted using FIJI normalized for ductal area. Data are mean ± SEM from three experimental cohorts (n = 4–7/diet/cohort). [a,b]Means with *different letters* are different ($P < 0.05$)

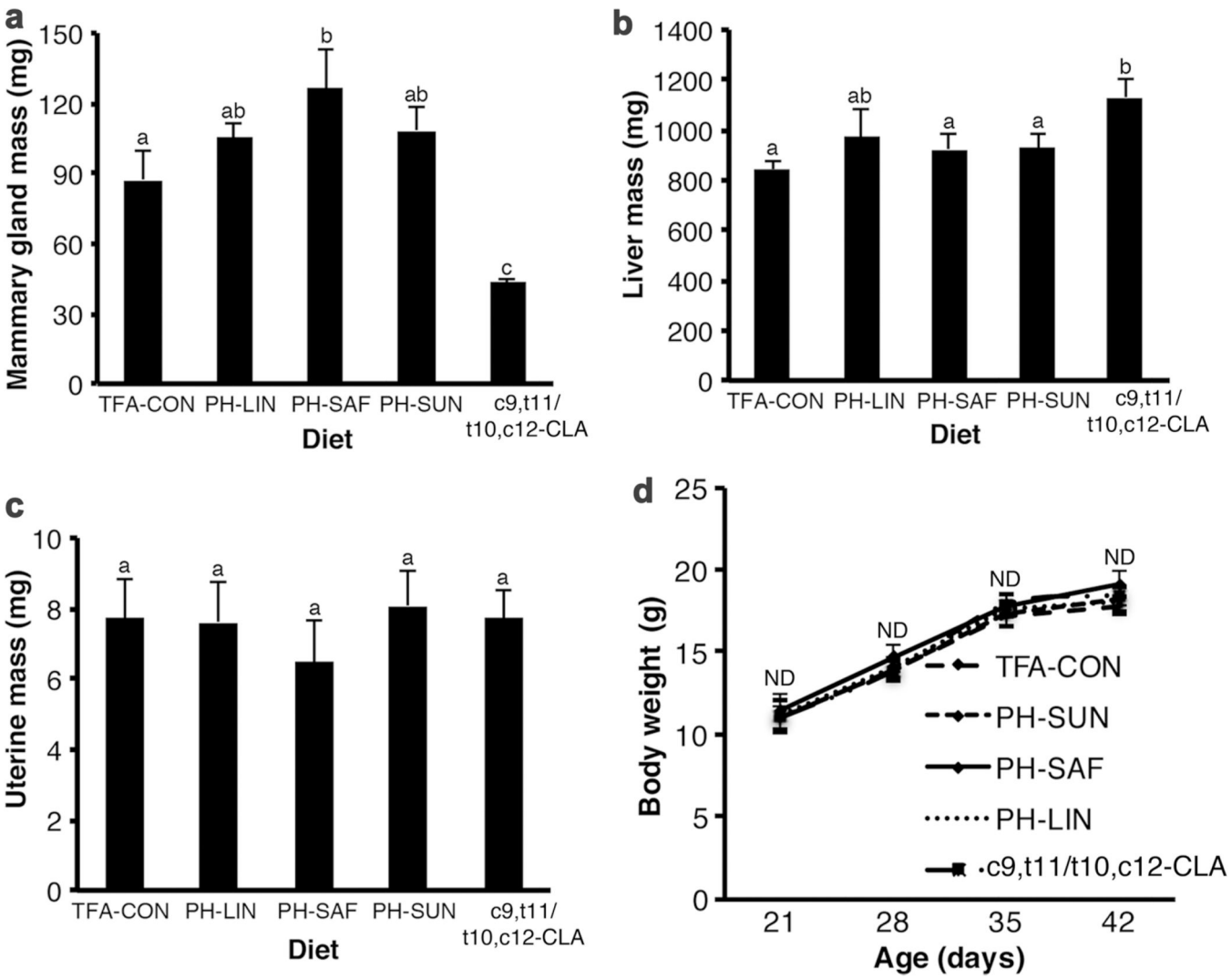

Fig. 2.

Ovariectomized mice fed a diet containing partially hydrogenated safflower oil have increased mammary gland mass. Mice were ovariectomized at 21 days and fed either the control diet (TFA-CON) or that with 5% fat as partially hydrogenated (PH) Linseed oil (PH-LIN), PH-SAF, partially hydrogenated sunflower oil (PH-SUN), or 2.7% conjugated linoleic acid (*c*9,*t*11/*t*10,*c*12 CLA) from 22 days of age until euthanasia at 42 days of age. Wet organ mass was measured for (**a**) lymph node-free mammary gland, (**b**) liver, and (**c**) uterus. **d** Body weight (g) was measured at 21, 28, 35, and 42 days of age. Data are mean ± SEM *(n* = 6–8/diet). [a, b, c]Means with *different letters* are different ($P < 0.05$). *ND* no difference

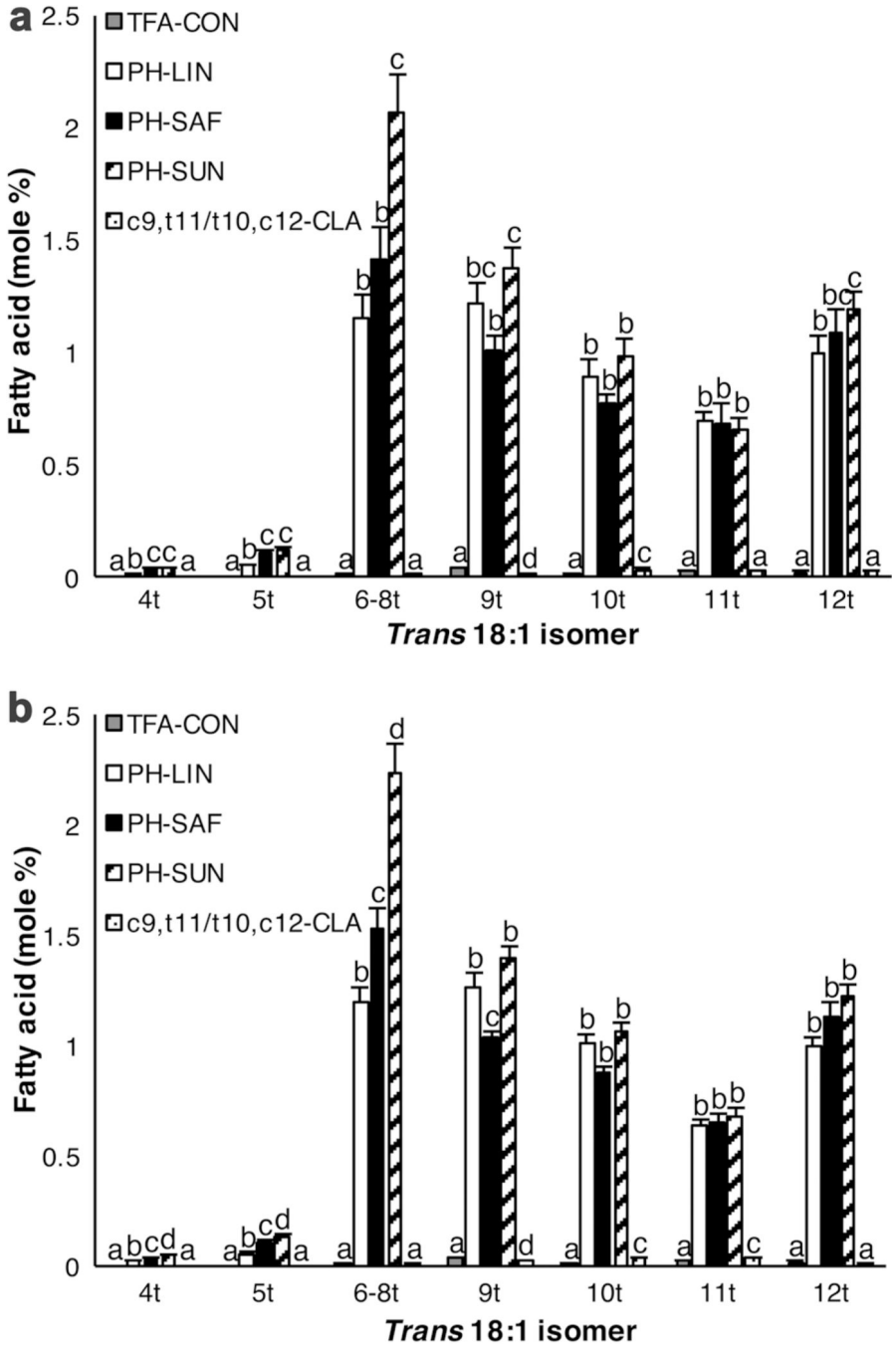

Fig. 3.

Distribution of different *trans*-18:1 isomers in adipose tissue from ovariectomized mice fed the control diet (TFA-CON) or that with 5% fat as partially hydrogenated linseed oil (PH-LIN), partially hydrogenated safflower oil (PH-SAF), partially hydrogenated sunflower oil (PH-SUN), or 2.7% conjugated linoleic acids (*c*9,*t*11/*t*10,*c*12 CLA). Mice were ovariectomized at 21 days and fed the experimental diets from 22 days of age until euthanasia at 42 days of age. Fatty acid methyl esters from (**a**) epithelium-free mammary fat pad and (**b**) omental fat were analyzed by gas-liquid chromatography. *X*-axis indicates

position of *trans* double bond of 18:1 fatty acids. Data are mean ± SEM (n = 4–5/diet).
[a b c d]Means with *different letters* are different ($P < 0.05$) for a given isomer

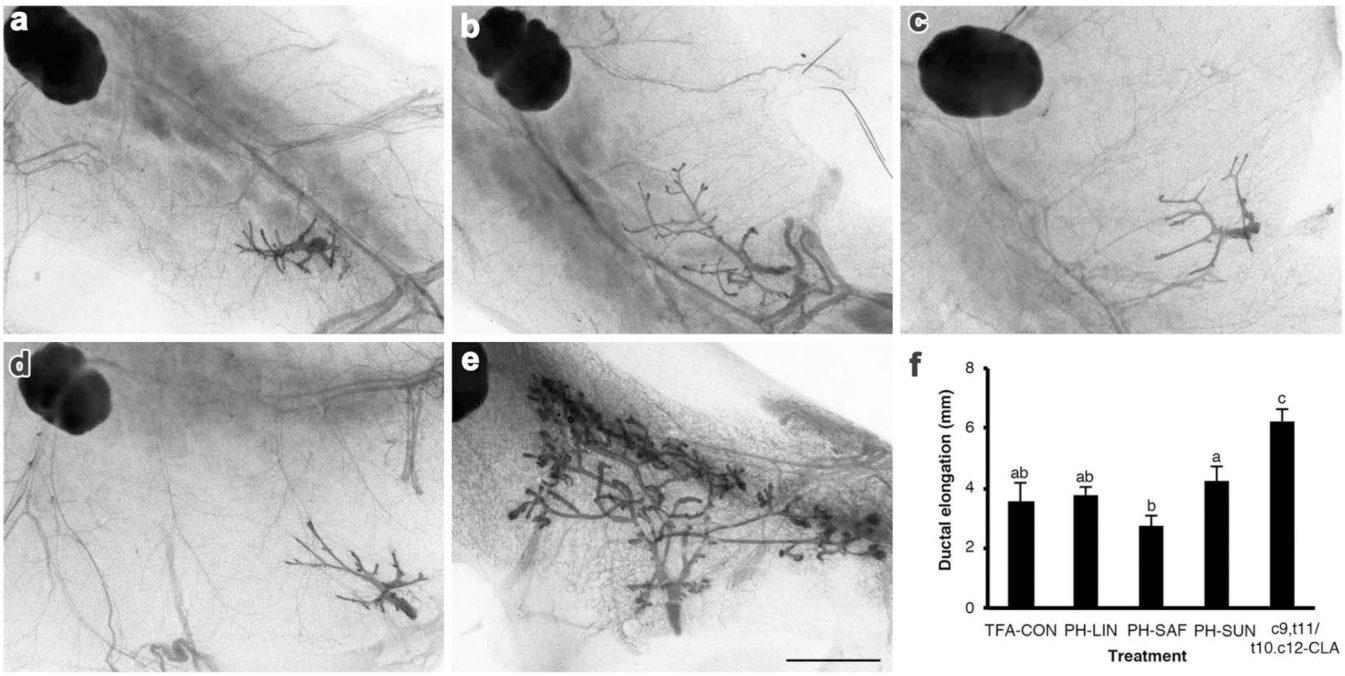

Fig. 4.

Dietary conjugated linoleic acid (*c*9,*t*11/*t*10,*c*12-CLA), but not various dietary *trans*-18:1 isomers, stimulates mammary gland growth in ovariectomized mice. Representative images of whole mounts from inguinal mammary glands of 42 days old mice fed either the (**a**) control diet (TFA-CON) or that with 5% fat as (**b**) partially hydrogenated linseed oil (PH-LIN), (**c**) partially hydrogenated safflower oil (PH-SAF), (**d**) partially hydrogenated sunflower oil (PH-SUN), or (**e**) 2.7% conjugated linoleic acids (*c*9,*t*11/*t*10,*c*12 CLA). Mice were ovariectomized at 21 days, then fed the experimental diets from 22 days until euthanasia at 42 days of age. *Scale bar* 2 mm. **f** Mammary ductal elongation was the distance from the teat to the furthest-reaching ductal terminus. Data are mean ± SEM (*n* = 6–8/diet). [a, b, c]Means with *different letters* are different ($P < 0.05$)

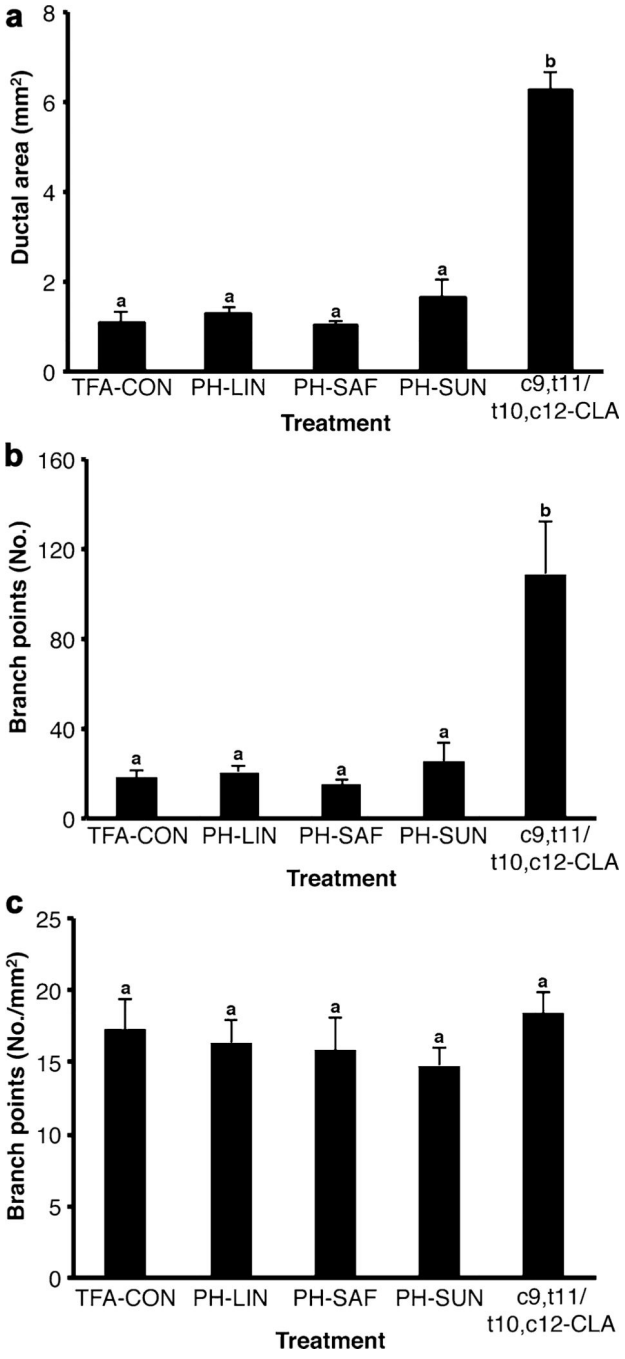

Fig. 5.

Dietary conjugated linoleic acid (c9,*t*11/*t*10,*c*12-CLA), but not various dietary *trans*-18:1 fatty acids, increases mammary gland branch point number in ovariectomized mice. Mice were ovariectomized at 21 days and fed the either the control diet (TFA-CON) or that with 5% fat as partially hydrogenated linseed oil (PH-LIN), PH-SAF, partially hydrogenated sunflower oil (PH-SUN), or 2.7% conjugated linoleic acids (*c*9,*t*11/*t*10,*c*12-CLA) from 22 days of age until euthanasia at 42 days of age. **a** Mammary ductal area was measured using ImageJ. **b** Mammary gland branch point number was measured using AngioTool. **c**

Branchpoint density was branchpoint number normalized to ductal area. Data are mean ± SEM (n = 6–8/diet). [a, b]Means with *different letters* are different ($P < 0.05$)

Table 1

Fatty acid composition (g/100 g fatty acids) of experimental diets (Experiment 1)

Fatty acid	Diet		
	Control	c9,t11-CLA	t10,c12-CLA
16:0	10.98	10.18	10.49
18:0	3.81	3.63	3.79
18:1 c9	20.62	20.42	19.25
18:2 n6	54.15	48.25	48.36
18:3 n3	6.60	5.95	5.92
c9,t11-CLA	0.00	6.39	1.19
t10,c12-CLA	0.00	0.99	6.81
Σ[a] SFA	15.87	14.95	15.31
Σ MUFA	22.11	21.85	20.59
Σ PUFA	60.77	54.24	54.31
Σ CLA	0.00	7.80	8.47
Σ TFA	0.42	0.34	0.35

[a]Indicates sum of fatty acid class

Table 2

Fatty acid profile (g/100 g fatty acid) of experimental diets (Experiment 2)

Fatty acid	Diet				
	TFA-CON	PH-LIN	PH-SAF	PH-SUN	c9,t11/t10,c12-CLA
14:0	0.46	0.19	0.20	0.18	0.32
16:0	12.75	9.33	9.46	8.43	10.98
18:0	16.51	14.46	13.95	14.35	9.76
18:1 t4	0.00	0.11	0.19	0.21	0.00
18:1 t5	0.00	0.26	0.45	0.52	0.00
18:1 t6-t8	0.03	2.87	3.60	4.79	0.01
18:1 t9	0.03	1.77	1.48	1.83	0.01
18:1 t10	0.03	1.98	1.66	2.05	0.02
18:1 t11	0.06	1.62	1.57	1.47	0.04
18:1 t12	0.02	1.72	1.81	1.70	0.01
18:1 13/t14	0.00	2.24	2.59	1.78	0.00
18:1 c9	24.74	18.19	17.68	18.13	23.35
18:1 t15	0.00	1.06	1.21	1.02	0.00
18:1 c11	1.05	1.45	1.45	1.41	1.15
18:1 c12	0.05	0.47	0.51	0.43	0.03
18:1 c13	0.04	0.37	0.42	0.32	0.03
18:1 c14	0.01	0.56	0.78	0.45	0.00
18:2 c9,c12 (n-6)	37.49	35.23	34.87	34.71	36.81
20:0	0.31	0.25	0.29	0.30	0.30
18:3 9c,12c,15c (n-3)	4.52	4.26	4.26	4.33	4.31
c9,t11-CLA	0.00	0.00	0.00	0.00	5.28
t10,c12-CLA	0.00	0.00	0.00	0.00	5.30
22:0	0.31	0.21	0.27	0.45	0.27
Σ^{a} Others	1.58	1.42	1.29	1.14	2.04
Σ SFA	30.35	24.44	24.17	23.72	21.63
Σ MUFA *cis*	25.89	23.27	23.44	22.51	24.54
Σ PUFA *cis*	42.01	39.49	39.14	39.05	41.12

Fatty acid	Diet				
	TFA-CON	PH-LIN	PH-SAF	PH-SUN	c9,t11/t10,c12-CLA
Σ C18:1 *trans*	0.18	11.37	11.97	13.58	0.10
Total FA content (%)	13.55	13.10	12.89	13.91	12.76

[a]Indicates sum of fatty acid class

Table 3

Fatty acid profile (mol%) of epithelium-free mammary fat pads collected from ovariectomized mice fed the control diet (TFA-CON), or the control diet with 5% fat as partially-hydrogenated linseed oil (PH-LIN), partially-hydrogenated safflower oil (PH-SAF), or partially hydrogenated sunflower oil (PH-SUN), or the control diet with 2.7% fat as conjugated linoleic acid (c9,t11/t10,c12-CLA)

Fatty acid	Diet										*P* value
	TFA-CON		PH-LIN		PH-SAF		PH-SUN		c9,t11/t10,c12-CLA		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
14:0	2.54^{a}	0.34	2.54^{a}	0.57	2.61^{a}	0.47	2.21^{a}	0.44	0.96^{b}	0.13	0.050
16:0	18.56^{a}	0.33	18.27^{ab}	0.37	18.22^{abc}	0.59	16.84^{c}	0.24	17.12^{bc}	0.65	0.046
18:0	5.34^{a}	0.49	4.24^{a}	0.32	4.34^{a}	0.53	4.30^{a}	0.34	5.40^{a}	0.43	0.135
18:1 4t	0.00^{a}	0.00	0.02^{b}	0.00	0.04^{c}	0.01	0.04^{c}	0.00	0.00^{a}	0.00	<0.0001
18:1 5t	0.00^{a}	0.00	0.06^{b}	0.00	0.10^{c}	0.01	0.12^{c}	0.01	0.00^{a}	0.00	<0.0001
18:1 6–8t	0.01^{a}	0.00	1.15^{b}	0.10	1.41^{b}	0.15	2.06^{c}	0.17	0.01^{a}	0.00	<0.0001
18:1 9t	0.04^{a}	0.00	1.22^{bc}	0.09	1.00^{b}	0.08	1.38^{c}	0.08	0.02^{d}	0.00	<0.0001
18:1 10t	0.02^{a}	0.00	0.89^{b}	0.07	0.77^{b}	0.05	0.99^{b}	0.07	0.03^{c}	0.00	<0.0001
18:1 11t	0.03^{a}	0.00	0.69^{b}	0.05	0.69^{b}	0.08	0.66^{b}	0.04	0.03^{a}	0.00	<0.0001
18:1 12t	0.02^{a}	0.01	1.00^{b}	0.07	1.09^{bc}	0.10	1.19^{c}	0.08	0.02^{a}	0.00	<0.0001
18:1 9c	35.81^{a}	0.24	31.96^{b}	0.51	31.39^{b}	0.98	31.58^{b}	0.54	30.92^{b}	1.00	0.0004
18:1 11c	1.49^{a}	0.08	1.84^{b}	0.11	1.83^{b}	0.13	1.81^{b}	0.11	1.46^{a}	0.10	0.0279
18:1 12c	0.04^{a}	0.01	0.28^{bc}	0.01	0.30^{b}	0.02	0.26^{c}	0.01	0.06^{a}	0.00	<0.0001
18:1 13c	0.03^{a}	0.00	0.20^{b}	0.01	0.25^{c}	0.01	0.22^{bc}	0.01	0.02^{a}	0.00	<0.0001
18:1 14c/16t	0.01^{a}	0.00	0.20^{b}	0.01	0.27^{c}	0.02	0.16^{d}	0.01	0.01^{a}	0.00	<0.0001
18:2 9c,12c (n-6)	27.10^{a}	0.81	26.13^{a}	1.14	26.46^{a}	1.36	27.49^{a}	0.92	31.89^{b}	1.09	0.0063
20:0	0.10^{ab}	0.01	0.08^{b}	0.01	0.08^{b}	0.01	0.08^{b}	0.01	0.11^{a}	0.01	0.0562
18:3 9c,12c,15c (n-3)	2.16^{a}	0.06	2.20^{a}	0.11	2.23^{a}	0.11	2.28^{a}	0.09	2.42^{a}	0.09	0.3368

Fatty acid	Diet										*P* value
	TFA-CON		PH-LIN		PH-SAF		PH-SUN		c9,t11/t10,c12-CLA		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
CLA 9c,11t	0.04^{a}	0.00	0.37^{b}	0.03	0.30^{b}	0.02	0.36^{b}	0.03	3.63^{c}	0.21	<0.0001
CLA 10t,12c	0.00^{a}	0.00	0.00^{a}	0.00	0.00^{a}	0.00	0.00^{a}	0.00	2.23^{b}	0.23	<0.0001
22:0	0.16^{a}	0.01	0.14^{a}	0.01	0.15^{a}	0.01	0.14^{a}	0.01	0.08^{b}	0.00	0.0003
∑^{e} SFA	28.65^{a}	0.47	27.00^{ab}	1.05	27.17^{ab}	0.87	25.11^{bc}	0.64	24.48^{c}	0.34	0.0031
∑ MUFA *cis*	41.06^{a}	0.41	38.41^{b}	0.67	37.84^{b}	1.17	37.49^{b}	0.81	33.97^{c}	1.27	0.0005
∑ PUFA *cis*	30.12^{a}	0.80	29.11^{a}	1.17	29.52^{a}	1.39	30.53^{a}	0.94	35.39^{b}	1.13	0.0035
∑ CLA	0.04^{a}	0.00	0.39^{b}	0.03	0.31^{b}	0.02	0.37^{b}	0.03	6.12^{c}	0.45	<0.0001
∑ 18:1 *trans*	0.12^{a}	0.01	5.23^{b}	0.38	5.37^{bc}	0.48	6.60^{c}	0.46	0.12^{a}	0.01	<0.0001

Data are mean and SE (n = 4–5/diet)

$^{a, b, c, d}$Least squares means are different $P < 0.05$

^{e}Indicates sum of fatty acid class

Table 4

Fatty acid profile (mol%) of omental fat samples collected from ovariectomized mice fed the control diet (TFA-CON), or the control diet with 5% fat as partially hydrogenated linseed oil (PH-LIN) partially hydrogenated safflower oil (PH-SAF), or partially hydrogenated sunflower oil (PH-SUN), or the control diet with 2.7% fat as conjugated linoleic acid (c9,t11/t10,c12-CLA)

Fatty acid	Diet										*P* value
	TFA-CON		PH-LIN		PH-SAF		PH-SUN		c9,t11/t10,c12-CLA		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
14:0	1.76^{a}	0.22	1.85^{a}	0.33	1.90^{a}	0.19	1.51^{a}	0.25	0.73^{b}	0.08	0.0003
16:0	20.44^{a}	0.42	20.06^{a}	0.44	20.22^{a}	0.41	18.34^{b}	0.38	17.80^{b}	0.64	0.0014
18:0	5.44^{a}	0.40	4.22^{c}	0.18	4.34^{bc}	0.30	4.40^{bc}	0.18	5.10^{ab}	0.32	0.0260
18:1 4t	0.00^{a}	0.00	0.02^{b}	0.00	0.04^{c}	0.00	0.05^{d}	0.00	0.00^{a}	0.00	<0.0001
18:1 5t	0.00^{a}	0.00	0.06^{b}	0.00	0.11^{c}	0.01	0.13^{d}	0.01	0.00^{a}	0.00	<0.0001
18:1 6–8t	0.01^{a}	0.00	1.20^{b}	0.06	1.53^{c}	0.10	2.24^{d}	0.13	0.01^{a}	0.00	<0.0001
18:1 9t	0.04^{a}	0.00	1.27^{b}	0.06	1.04^{c}	0.03	1.40^{b}	0.05	0.02^{d}	0.00	<0.0001
18:1 10t	0.01^{a}	0.00	1.01^{b}	0.04	0.87^{b}	0.03	1.06^{b}	0.04	0.03^{c}	0.00	<0.0001
18:1 11t	0.03^{a}	0.00	0.64^{b}	0.02	0.65^{b}	0.04	0.67^{b}	0.04	0.03^{c}	0.00	<0.0001
18:1 12t	0.02^{a}	0.00	0.99^{b}	0.04	1.13^{b}	0.06	1.23^{b}	0.05	0.01^{a}	0.00	<0.0001
18:1 9c	34.34^{a}	0.46	30.00^{bc}	0.47	29.38^{b}	0.47	29.78^{bc}	0.56	31.29^{c}	0.67	<0.0001
18:1 11c	1.22^{a}	0.04	1.64^{b}	0.10	1.58^{b}	0.07	1.63^{b}	0.09	1.45^{ab}	0.07	0.0051
18:1 12c	0.03^{a}	0.00	0.27^{b}	0.01	0.30^{c}	0.01	0.26^{b}	0.01	0.06^{d}	0.00	<0.0001
18:1 13c	0.02^{a}	0.00	0.21^{b}	0.01	0.27^{c}	0.01	0.24^{d}	0.01	0.03^{a}	0.00	<0.0001
18:1 14c/16t	0.00^{a}	0.00	0.21^{b}	0.01	0.31^{c}	0.01	0.18^{d}	0.01	0.01^{a}	0.00	<0.0001
18:2 9c,12c (n-6)	27.49^{a}	0.64	26.60^{a}	0.82	26.53^{a}	0.63	27.99^{a}	0.51	32.29^{b}	0.71	<0.0001
20:0	0.11^{a}	0.01	0.09^{bc}	0.01	0.10^{ac}	0.01	0.09^{bc}	0.01	0.08^{c}	0.01	0.0151
18:3 9c,12c,15c (n-3)	2.43^{ab}	0.09	2.51^{a}	0.08	2.51^{a}	0.07	2.64^{a}	0.09	2.26^{b}	0.08	0.0381

Fatty acid	Diet										*P* value
	TFA-CON		PH-LIN		PH-SAF		PH-SUN		c9,t11/t10,c12-CLA		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
c9,t11-CLA	0.03^{a}	0.00	0.40^{b}	0.03	0.35^{b}	0.03	0.35^{b}	0.03	3.68^{c}	0.15	<0.0001
t10,c12-CLA	0.00^{a}	0.00	0.01^{a}	0.01	0.00^{a}	0.00	0.00^{a}	0.00	2.09^{b}	0.19	<0.0001
22:0	0.16^{a}	0.01	0.15^{a}	0.01	0.15^{a}	0.01	0.16^{a}	0.01	0.09^{b}	0.00	<0.0001
∑^{e} SFA	29.25^{a}	0.47	27.61^{a}	0.89	27.91^{a}	0.41	25.59^{b}	0.55	24.46^{b}	0.38	<0.0001
∑ MUFA *cis*	39.79^{a}	0.33	36.74^{b}	0.37	36.36^{b}	0.51	35.64^{bc}	0.64	34.16^{c}	0.85	<0.0001
∑ PUFA *cis*	30.79^{a}	0.67	29.98^{a}	0.86	29.95^{a}	0.65	31.57^{a}	0.55	35.20^{b}	0.78	0.0002
∑ CLA	0.03^{a}	0.00	0.42^{b}	0.03	0.37^{b}	0.03	0.36^{b}	0.03	6.05^{c}	0.35	<0.0001
∑ 18:1 *trans*	0.11^{a}	0.00	5.40^{b}	0.23	5.66^{b}	0.28	6.97^{c}	0.29	0.12^{a}	0.00	<0.0001

Data are mean and SE (n = 5/diet)

*a, b, c, d*Least squares means are different $P < 0.05$

^{e}Indicates sum of fatty acid class

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