ELSEVIER

Nutrition Research 23 (2003) 549–566

NUTRITION RESEARCH

www.elsevier.com/locate/nutres

Effects of modified tall oil on body composition and serum and tissue levels of cholesterol, phospholipids, and ∝-tocopherol in adult ovariectomized rats

Patrick R. O'Quinn[a], Sung I. Koo[b], Sang K. Noh[b], Jim L. Nelssen[a], Robert D. Goodband[a,*], Mike D. Tokach[a]

[a]*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506, USA*
[b]*Human Nutrition, Kansas State University, Manhattan, KS 66506, USA*

Received 26 June 2002; received in revised form 3 February 2003; accepted 3 February 2003

Abstract

Adult ovariectomized rats were assigned by weight (initially 257 g) to an AIN-93G diet containing 8% ∝-tocopherol (∝TP)-stripped soybean oil or the same diet containing 1% modified tall oil (MTO) at the expense of soybean oil. Rats fed MTO had lower body weight, total body fat, and more lean tissue ($P < 0.05$). Rats fed MTO had lower serum ∝TP levels ($P < 0.005$) at 4 and 6 wk and higher ($P < 0.05$) total cholesterol in the liver, kidneys, and abdominal and retroperitoneal fat. Rats fed MTO had enhanced abdominal and retroperitoneal fat ∝TP (134.11 vs 81.66 nmol/g and 128.51 vs 92.87 nmol/g for rats fed MTO and the control diet, respectively $P \leq 0.005$). These data suggest that adult ovariectomized rats fed a diet containing MTO gain less weight, are leaner, have less total body fat, and preferentially shift deposition of ∝TP to adipose tissues.

Keywords: Modified tall oil; Body composition; Cholesterol; Phospholipids; ∝-tocopherol

1. Introduction

Conjugated linoleic acid (CLA) was first positively identified in 1987 [1], and is an imprecise collective term describing any of the positional and geometric conjugated dienoic isomers of linoleic acid (*cis* 9, *cis* 12-octadecadienoic acid). Feeding CLA to laboratory animals and livestock species (swine and poultry) has been shown to increase rate and(or) efficiency of gain [2,3,4], as well as reduce fat deposition and increase lean content [3,4,5].

* Corresponding author. Tel.: +1-785-532-1228; fax: +1-785-532-7079.
E-mail address: Goodband@ksu.edu (R.D. Goodband).

0271-5317/03/$ – see front matter
doi:10.1016/S0271-5317(03)00020-4

Modified tall oil (MTO) is derived from further processing of crude tall oil which is a by-product from the kraft pulping of pine wood in the pulp and paper industry [6]. Modified tall oil is a rich source (~70%) of CLA. Numerous feeding studies have been conducted with MTO and growing-finishing swine [7,8,9,10,11]. These studies conclusively demonstrated that MTO reduces adiposity and increases belly firmness with only marginal improvements in growth performance or lean mass. Recently, MTO was shown to interact with vitamin E to increase display color stability and decrease oxidative deterioration of longissimus muscle pork chops [8].

Little information exists concerning the biological effects of MTO in animal species other than young, rapidly growing swine. Further exploration of MTO is needed with other animal models, especially given its ability to reduce adiposity in growing swine as well as the fact that much research with MTO has focused only on growth and carcass characteristics. The objective of the present research was to determine the effects of MTO on the body composition and serum and tissue levels of cholesterol, PL, and ∝TP in adult ovariectomized rats, which are often used as a surrogate for post-menopausal women.

2. Materials and methods

2.1. Animals and diets

Twenty-six adult female Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN USA) with an initial weight of 224 ± 6 g were individually placed in plastic cages with stainless-steel wire bottoms in a windowless room maintained at 24 to 26°C and 70% relative humidity. A 12-hr light/dark cycle with the light period from 0915 to 2115 hr was maintained. The rats were housed in an animal care facility at Kansas State University, approved by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and all experimental procedures were approved by the Kansas State University Animal Care and Use Committee (Protocol No. 1531). Upon arrival, rats were given free access to a nutritionally adequate diet (Table 1) formulated to the AIN-93 recommendations [12] by Dyets Inc., (Bethlehem, PA USA) and deionized water until assigned to dietary treatment 12 d later. The deionized water was obtained from a water purification system (Millipore Corp., Marlboro, MA USA) and delivered through a stainless-steel nipple watering system.

At 5 d, rats weighing 243 ± 11 g were ovariectomized under halothane anesthesia, and allowed 7 d to recover. Ovariectomized rats were used to mimic the state of ovarian hormone deficiency in postmenopausal women, in which metabolism of lipids and lipid-soluble vitamins are markedly altered. Using this model, this study was conducted to determine if MTO favorably modifies lipid parameters. During this recovery time, rats were trained to meal feed according to methods previously described [13,14]. Briefly, rats were fed 90% of the average free access intake, which was determined by averaging the intake of the prior 5 d. This equated to a daily feed intake during the experiment of 15 g that was fed in meals of 6 g at 0900 hr and 9 g at 1600 hr. Twelve days after arrival, rats weighing 257 ± 9 g were randomly assigned by body weight to one of two dietary treatment groups and fed their respective diets for the remainder of the 42 d study. The MTO used in this experiment

Table 1
Composition of basal diet[1]

Ingredient	Basal diet, g/kg
Egg white	200
Corn starch	396.486
Dextrinized corn starch	132
Dextrose	100
α-tocopherol-stripped soybean oil	70.014
Cellulose	50
Mineral mix[2]	35
Vitamin mix	10
Biotin (1 mg/g biotin sucrose mix)	4
Choline bitartrate	2.5

[1] Formulated by Dyets, Inc., Bethlehem, PA USA, according to the recommendations of the American Institute of Nutrition [12].

[2] As purchased, the mineral mix was zinc-free. Zinc carbonate was added to the diet to achieve a zinc level of 32 mg/kg.

contained 70.44% conjugated linoleic acid with a similar isomeric profile as previously reported (Table 2) [7,15]. Over 90% of the CLA isomers in the MTO were comprised of the following four isomers: *cis* 9, *trans* 11 (34.46%); *trans* 10, *cis* 12 (25.27%); *cis* 10, *cis* 12 (16.38%); and *trans* 9, *trans* 11 (14.25%). The oil mixes (1% dietary inclusion) used to create the experimental diets contained the fatty acids necessary to equalize the diets in fatty acid profiles, thus the diet with MTO contained .5544% actual conjugated linoleic acid isomers.

Experimental diets were formulated by adding 1% of either an ∝-TP-stripped soybean oil mix or an MTO mix, both being matched in fatty acid profiles (Table 3), to the standard basal

Table 2
Analyzed composition of modified tall oil

Fatty acid	%
C16:0, palmitic acid	0.66
C16:1, palmitoleic acid	0.61
C18:0, stearic acid	—
C18:1, oleic acid[1]	23.46
C18:2, alpha linoleic acid	4.83
C18:2, conjugated linoleic acid	70.44
Total	100.00
Isomeric profile of CLA	
CLA isomer	% of total CLA
cis 9, *trans* 11	34.46
trans 9, *trans* 11	14.25
cis 10, *cis* 12	16.38
trans 10, *cis* 12	25.27
3 unidentified CLA isomers	9.64
Total	100.00

[1] Includes less than 2% C18:1, elaidic acid.

Table 3
Fatty acid composition of supplemental oil mixes[1]

Item	Soybean oil mix, g/100g	Modified tall oil mix, g/100g
Modified tall oil	—	78.70
α-tocopherol-stripped soybean oil	79.84	—
C16:0, palmitic acid	—	9.0
C18:0, stearic acid	—	4.5
C18:1, oleic acid	2.98	—
C18:2, linoleic acid	17.18	—
C18:3, linolenic acid	—	7.8

[1] Pure α-tocopherol was added to both oil mixes at the rate of 6.9 mg/100g of oil mixture.

diet. Thus, because of equal feed intake and matching of fatty acid profiles, any observed biological responses could be attributed to the conjugated isomers of linoleic acid found in MTO. The supplemental linoleic acid present in the diet containing soybean oil was alpha linoleic acid, and the supplemental linoleic acid present in the diet containing MTO was predominantly conjugated linoleic acid. Experimental diets were mixed bi-weekly and stored in sealed and air-evacuated plastic containers in the dark at 6°C to maintain freshness.

Table 4
Growth rate and feed conversion efficiency of rats fed control or MTO diets

Item	Dietary group	
	Control	MTO
d 0 to 7		
Daily gain, g	1.55 ± .66	1.23 ± .43
Gain:feed, g:g	.10 ± .04	.08 ± .03
d 7 to 14		
Daily gain, g	2.67 ± .43	2.42 ± .43
Gain:feed, g:g	.18 ± .03	.16 ± .03
d 14 to 21		
Daily gain, g	1.88 ± .41	1.53 ± .73
Gain:feed, g:g	.13 ± .03	.10 ± .05
d 21 to 28		
Daily gain, g	1.79 ± .35	1.70 ± .40
Gain:feed, g:g	.12 ± .02	.11 ± .03
d 28 to 35		
Daily gain, g	.84 ± .42	.89 ± .31
Gain:feed, g:g	.06 ± .03	.06 ± .02
d 35 to 42		
Daily gain, g	1.21 ± .44	1.15 ± .34
Gain:feed, g:g	.08 ± .03	.08 ± .02
Overall (d 0 to 42)		
ADG, g	1.66 ± .22[a]	1.49 ± .26[b]
Gain:feed, g:g	.11 ± .01[a]	.10 ± .02[b]

n = 13; values not sharing a common superscript are significantly different ($P < 0.05$). Feed intake was held constant at 15 g/d.

2.2. Determination of growth performance and blood and tissue sampling

Individual rats were weighed weekly for determination of gain and feed conversion efficiency. Initially (just prior to initiation of the study) and at 4 wk and 6 wk, serum samples were obtained by placing a capillary tube into the orbital sinus [16] from the six heaviest rats per dietary group. Rats were bled following an 18-hr fast. The serum was allowed to clot for 1-hr before it was centrifuged for 30-min. Serum was stored at −20°C until analyzed for cholesterol, PL, and ∝TP. Upon completion of the study (42-d), six randomly selected rats per dietary group were humanely sacrificed and the following tissues were collected: heart, brain, liver, kidneys, and retroperitoneal fat, and samples of the gastrocnemius muscle and abdominal fat depot. These tissues were weighed and stored in plastic vials in the dark at −70°C until analyzed for cholesterol, PL, and ∝TP.

2.3. Determination of body composition

At the end of the growth trial randomly selected rats (n = 6, and different from those samples for tissue collection) were euthanized by overdose of CO_2 prior to being scanned via DEXA for the determination of body composition. The validity of using DEXA for body composition analysis has recently been verified for rats weighing over 200 g [17]. A Hologic QDR-1000 instrument (Hologic, Waltham, MA USA) was used to determine bone mineral content, bone mineral density, and fat and lean contents of each rat.

2.4. Determination of serum and tissue lipids

Serum total cholesterol levels were determined using a commercially available enzymatic diagnostic kit (Catalog No. 352-20, Sigma Chemical, St. Louis, MO USA). For the analysis, 25 μL of serum was used, and absorbance was read at 500 nm with a UV-1201 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD USA). Values were determined by substituting absorbance readings into a standard curve equation. Whole tissues were minced finely with razor blades. Tissue subsamples, ranging from 300 (heart, brain, and adipose tissues) to 500 mg (liver and kidneys), were used for lipid extraction [18]. Briefly, lipids were extracted from the tissues with a mixture of chloroform:methanol 2:1 (v/v) containing 10 mg of BHT (10 mg per 100 mL of methanol). Tissue total cholesterol levels were determined from the lipid extracts colorimetrically using *o*-phthalaldehyde as previously described [19]. Serum and tissue PL levels were colorimetrically determined [20]. Serum PL levels were determined on 100 μL aliquots of the lipid extract, as prepared above.

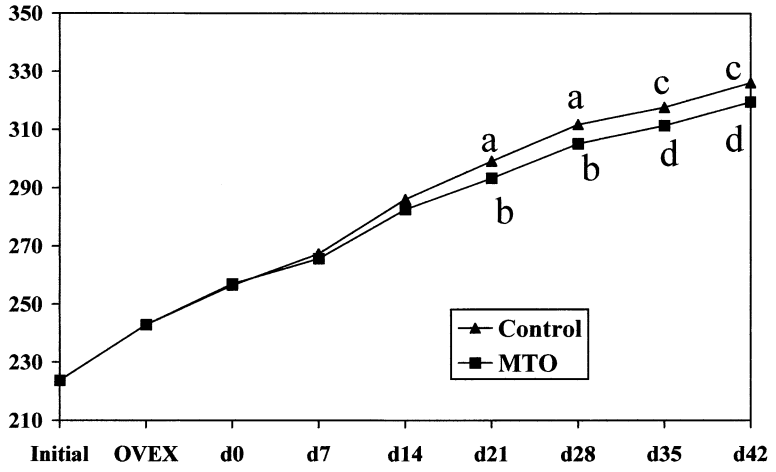
Serum and tissue ∝TP were determined according to previously described methods [21]. Briefly, 80 μL of serum was placed in test tubes containing 150 mg of Na_2SO_4 and 1 mL of acetone. An internal standard (100 μL of ∝TP acetate) was added to each tube to verify recovery. This mixture was centrifuged (10-min at 1360 × g), and the resultant supernatant was then filtered through a microfilter membrane (0.45 μm PTFE, Alltech Associates, Inc., Deerfield, IL USA), dried under N_2, and redissolved in 150 μL of chloroform:methanol 1:3 (v/v) prior to injection (15 μL) into the HPLC. The αTP levels were determined using a reverse-phase high performance liquid chromatography (HPLC) column (Alltima C18, 5

μm, 4.6 × 150 mm, Alltech Associates, Inc.) and Beckman System Gold software (Beckman Instruments, Inc., Fullerton, CA USA) as previously described [13,14]. Methanol was used as the mobile phase [22] and was propelled at 2 mL/min. Detection was monitored at 292 nm (Module 166, Beckman Instruments, Inc.). Tissue ∝TP analyses were identical to those described for serum except 100 (brain and kidneys) to 200 mg (liver) of tissue was used for homogenization in acetone. Additionally, 400 μL of internal standard was added and 600 μL of chloroform:methanol 1:3 (v/v) was used to redissolve the mixture. Under these conditions, serum and tissue ∝TP were eluted at 4.05 min. The standard curve (peak area vs ng of αTP) was constructed by using αTP standards. Concentrations of αTP from 75 to 300 ng yielded a linear curve ($r = 0.999$).

Fatty acid profiles were determined on 200 μL aliquots of the lipid extract from each tissue collected. Analyses were conducted using a Hewlett-Packard gas chromatograph (Hewlett-Packard, Co., 5890 Series II Gas Chromatograph, Palo Alto, CA USA) equipped with a 100-m polar capillary column designed to elute fatty acids in the range of 14 to 20 carbons.

2.5. Statistics

Values are presented as means ± SD. Statistical analyses for body composition and serum and tissue measurements were performed using paired *t* tests. Final body weight was used as a covariate in the analysis of organ weights [23]. Differences were considered significant at $P < 0.05$, unless otherwise noted.



Values are means of 13 rats per dietary group.
[a,b]Significantly different at $P = .04$.
[c,d]Significantly different at $P \leq .07$.

Fig. 1. Weekly mean body weights (g).

Table 5
Organ weights of rats fed control or MTO diets

Item, g	Dietary group	
	Control	MTO
Body weight	331.10 ± 13.56[a]	317.07 ± 10.83[b]
Liver	7.01 ± .41	7.28 ± .69
Heart	1.06 ± .06	1.07 ± .06
Kidneys	1.70 ± .06	1.62 ± .09
Brain	1.83 ± .05[a]	1.72 ± .02[b]
Retroperitoneal fat	2.39 ± .26[a]	1.56 ± .29[b]

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$). Live weight was used as a covariate for the tissue analysis.

3. Results

3.1. Body and organ weights

All rats were observed to be healthy for the duration of this study. All meals were consumed within 1 hr of feedings. Rats fed the diet containing MTO had small reductions in daily weight gain and increased gain:feed ratio during each of the weekly time intervals (Table 4). This translated into a significant reduction in both growth parameters when determined for the duration of the trial (d 0 to 42). Weekly body weights were not affected by diet until week 3 (Fig. 1). Body weights of rats fed the diet containing MTO were reduced in wk-3 and wk-4 of the study; with only a nonsignificant decrease for the duration of the trial.

Heart, liver, and kidney weights were similar between the dietary groups (Table 5); however, brain weight from rats fed the diet containing MTO was reduced, and rats fed MTO had less retroperitoneal fat. This reduction in retroperitoneal fat was about 35% and the reduction in brain weight was about 6%.

Table 6
Body composition analysis of rats fed control or MTO diets

Item	Dietary group	
	Control	MTO
Body weight, g	293.82 ± 4.22	295.90 ± 12.37
Bone mineral content (BMC), g	7.31 ± .26	7.24 ± .21
Bone mineral density, g/cm^2	.137 ± .003	.136 ± .002
Fat, g	54.02 ± 2.22[a]	43.05 ± 3.93[b]
Fat-free, g	232.50 ± 6.09[a]	245.72 ± 14.50[b]
Fat-free + BMC, g	239.80 ± 6.13	252.97 ± 14.59
Fat, %	18.40 ± .96[a]	14.57 ± 1.72[b]
Fat-free, %	79.12 ± 1.00[a]	82.96 ± 1.75[b]

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

Table 7
Total lipid content of selected tissues of rats fed control or MTO diets

	Dietary group	
	Control	MPO
Tissue lipid, mg/whole tissue		
Liver	95.20 ± 7.94	104.34 ± 14.74
Heart	177.24 ± 8.67	168.35 ± 9.36
Brain	107.28 ± 5.50	108.45 ± 3.67
Kidneys	126.16 ± 12.15	123.04 ± 6.98
Retroperitoneal fat	853.86 ± 31.89	857.36 ± 34.78
Tissue lipid, mg/g tissue		
Gastrocnemius muscle	47.77 ± 2.84	46.56 ± .66
Abdominal fat	884.65 ± 22.10[a]	841.28 ± 28.91[b]

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

3.2. Body composition measurements

Dual energy X-ray absorptiometry scans of the rats revealed no differences in bone mineral content, bone mineral density, or in the combination of bone mineral content plus fat-free mass (Table 6). However, rats fed the diet containing MTO had significantly reduced body fat (expressed either as total grams or as a percentage) and increased fat-free body mass (expressed either as total grams or as a percentage). Total body fat decreased approximately 21% and the fat-free body mass increased approximately 5% in rats fed the diet containing MTO. Improvements in body composition from feeding MTO are primarily due to reductions in adiposity, although the increases in lean mass were appreciable.

Table 8
Tissue and serum cholesterol levels of rats fed control or MTO diets

Item	Dietary group	
	Control	MTO
Tissue, μmol/g		
Tissue, μmol/g		
Liver	7.13 ± .75[a]	7.85 ± .66[b]
Heart	2.92 ± .76	3.04 ± .51
Brain	35.49 ± 3.48	36.66 ± 3.00
Kidneys	9.21 ± .89[a]	10.56 ± .89[b]
Gastrocnemius muscle	2.57 ± .57	2.87 ± .44
Abdominal fat	2.34 ± .46[a]	3.14 ± .46[b]
Retroperitoneal fat	2.21 ± .55[a]	3.61 ± .62[b]
Serum, mM		
Initial	1.64 ± .09	
4-wk	1.65 ± .16	1.60 ± .07
6-wk	1.68 ± .13	1.59 ± .07

n = 6; values without a common superscript are significantly different ($P < 0.05$).

Table 9
Tissue and serum phospholipid levels of rats fed control or MTO diets

Item	Dietary group	
	Control	MTO
Tissue, μmol/g		
Liver	33.71 ± 2.13[a]	36.09 ± 1.61[b]
Heart	28.40 ± 4.37	26.37 ± 2.14
Brain	51.74 ± 1.93	52.14 ± 2.69
Kidneys	30.15 ± 1.95	30.29 ± 1.75
Gastrocnemius muscle	20.48 ± 2.11	20.20 ± 1.10
Abdominal fat	1.52 ± .34	1.26 ± .78
Retroperitoneal fat	1.14 ± .83	.86 ± .34
Serum, mM		
Initial	1.93 ± .21	
4-wk	1.45 ± .18	1.44 ± .15
6-wk	1.42 ± .13	1.33 ± .06

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

3.3. Serum and tissue lipids

The total lipid content (mg lipid/whole tissue) of each tissue analyzed (Table 7) was similar except for the abdominal fat depot where the lipid concentration per gram of tissue increased compared to rats fed MTO.

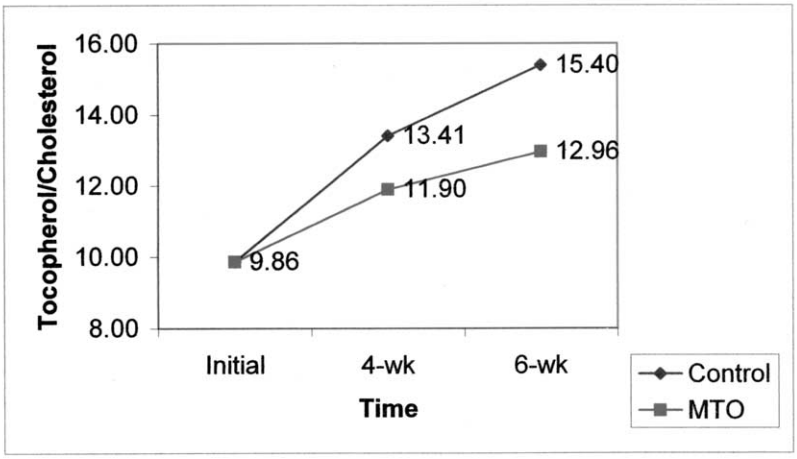
Total cholesterol contents were increased in the liver, kidneys, and abdominal and retroperitoneal fat depots from feeding the diet containing MTO (Table 8). Cholesterol levels in the brain, heart, and gastrocnemius muscle were not affected by dietary treatment. Feeding the diet containing MTO numerically lowered 6-wk serum cholesterol levels.

Phospholipid content increased in the liver of rats fed the diet containing MTO (Table 9),

Table 10
Tissue and serum ∝-tocopherol levels of rats fed control or MTO diets

Item	Dietary group	
	Control	MTO
Tissue, nmol/mg total lipid		
Liver	141.95 ± 14.34	146.72 ± 13.34
Heart	122.23 ± 6.86	116.67 ± 5.25
Brain	59.04 ± 3.58	56.89 ± 3.77
Kidneys	79.47 ± 13.59	74.67 ± 7.84
Gastrocnemius muscle	35.12 ± 2.86[a]	30.89 ± 2.95[b]
Abdominal fat	81.66 ± 17.94[a]	134.11 ± 14.55[b]
Retroperitoneal fat	92.87 ± 13.92[a]	128.51 ± 10.48[b]
Serum, μM		
Initial	19.06 ± 4.86	
4-wk	22.04 ± .91[a]	19.18 ± 1.78[b]
6-wk	25.37 ± 2.40[a]	20.64 ± 1.86[b]

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).



Values are means of six rats per dietary group.

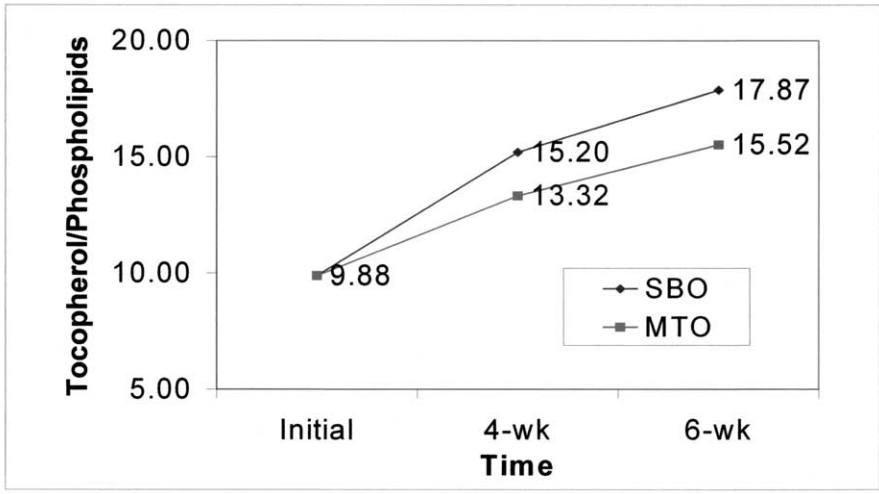
Ratios are significantly different ($P < .02$) at 4- and 6-wk.

Fig. 2. Average serum ratios of ∝-tocopherol to cholesterol (μM:mM).

but other tissue and serum levels were not affected by dietary treatment group. Similar to cholesterol, there was a trend toward lowered serum PL levels by 6 wk for rats fed the diet containing MTO.

Levels of ∝TP were not affected by dietary treatment group in the brain, liver, and kidneys

Values are means of six rats per dietary group.



Ratios are significantly different ($P = .03$) at 6-wk.

Fig. 3. Average serum ratios of ∝-tocopherol to phospholipids (μM:mM).

Table 11
Liver fatty acid composition (percent) of rats fed control or MTO diets

Fatty acid, %	Control	MTO
C14:0, myristic acid	2.88 ± 1.71	6.09 ± 3.07
C16:0, palmitic acid	2.58 ± 1.59[a]	23.98 ± 4.66[b]
C16:1, palmitoleic acid	3.88 ± 1.49	2.22 ± .54
C18:0, stearic acid	23.64 ± 6.08[a]	15.03 ± 5.98[b]
C18:1, oleic acid	17.33 ± 4.33	13.99 ± 2.04
C18:1, elaidic acid	2.20 ± .62	1.51 ± .10
C18:2, linoleic acid	34.13 ± 1.69[a]	24.21 ± 1.24[b]
C18:3, linolenic acid	—	—
C20:1, eicosanoic acid	1.84 ± .36	1.42 ± .50
C20:4, arachidonic acid	11.52 ± 2.78	11.56 ± 2.82

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

(Table 10). Feeding the MTO diet resulted in a small decrease in ∝TP levels in the heart, and this diet significantly reduced ∝TP in the gastrocnemius muscle. However, feeding the MTO diet significantly increased ∝TP levels of both fat depots (Table 10). In addition, serum ∝TP levels were reduced at both 4 and 6 wk from feeding the MTO diet. This reduction in serum ∝TP, combined with the nonsignificant reduction in serum cholesterol and phospholipids, led to significantly reduced ∝TP to cholesterol and ∝TP to phospholipid ratios in serum in rats receiving the diet containing MTO (Figs. 2 and 3, respectively).

No consistent effect on tissue fatty acid profiles was observed due to dietary supplementation with MTO (Tables 11–17). However, agreeing with work in pigs [7], MTO did increase the amounts of C16:0 present in select tissues (liver, kidneys, gastrocnemius muscle, and retroperitoneal fat).

4. Discussion

The overall reduction in body weight from feeding MTO is in general agreement with earlier data where CLA reduced body weight (ADG) in mice [24,25] and swine [7,26].

Table 12
Heart fatty acid composition (percent) of rats fed control or MTO diets

Fatty acid, %	Control	MTO
C14:0, myristic acid	6.04 ± .83[a]	2.43 ± .40[b]
C16:0, palmitic acid	18.71 ± 4.63	20.85 ± 4.32
C16:1, palmitoleic acid	6.02 ± 1.30[a]	2.58 ± .40[b]
C18:0, stearic acid	18.47 ± 5.69	23.09 ± 5.03
C18:1, oleic acid	6.60 ± 2.97[a]	12.08 ± 1.83[b]
C18:1, elaidic acid	11.78 ± 11.96	5.45 ± 2.45
C18:2, linoleic acid	19.10 ± 4.29	21.60 ± 3.52
C18:3, linolenic acid	—	—
C20:1, eicosanoic acid	—	—
C20:4, arachidonic acid	13.27 ± 4.07	11.92 ± 3.10

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

Table 13
Brain fatty acid composition (percent) of rats fed control or MTO diets

Fatty acid, %	Control	MTO
C14:0, myristic acid	4.38 ± 1.33[a]	14.20 ± 4.18[b]
C16:0, palmitic acid	15.63 ± 3.41[a]	12.18 ± 2.03[b]
C16:1, palmitoleic acid	5.11 ± 1.30	6.21 ± 1.04
C18:0, stearic acid	22.20 ± 3.45[a]	19.12 ± 2.21[b]
C18:1, oleic acid	8.98 ± 1.00	13.29 ± .84
C18:1, elaidic acid	9.49 ± .59[a]	8.84 ± 2.09[b]
C18:2, linoleic acid	1.22 ± .00	6.64 ± 2.71
C18:3, linolenic acid	12.41 ± 3.12	11.18 ± .00
C20:1, eicosanoic acid	10.51 ± 2.81	1.80 ± .00
C20:4, arachidonic acid	10.08 ± 4.62[a]	6.53 ± .52[b]

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

However, MTO has improved body weight gain (ADG) in several studies with swine [8,9,11]. The decrease in G:F in the present study agrees with data in mice where feed disappearance per unit of body weight gain increased with increasing dietary CLA [25]. However, MTO has been shown to increase gain:feed ratio in swine [8,11].

An additional aim of this study was to determine the effects of MTO supplementation on body composition when feed intake was not a contributing factor. Reduced feed intake has consistently been reported with CLA supplementation [3,5,7,24,26]. West et al. (1998) [24] speculated that CLA may invoke palatability or postingestive effects that lead to a food aversion or that CLA could modulate appetite without adversely affecting the animal. This proposed modulation of appetite was related to the ability of CLA to modulate energy metabolism. Recently, CLA was shown to dose-dependently activate the peroxisome proliferator-activated receptor-$_{\gamma}$ gene [27], which partially controls transcription of the leptin gene [28]. Thus, CLA may reduce appetite through the actions of leptin. However, it was concluded that the reductions in feed intake from CLA supplementation are not large enough to account for the observed changes in body composition [5,24]. In contrast, MTO has been shown to stimulate feed intake in swine [7,9]. The confounding effects of feed intake were

Table 14
Kidney fatty acid composition (percent) of rats fed control or MTO diets

Fatty acid, %	Control	MTO
C14:0, myristic acid	11.00 ± 2.03[a]	6.66 ± .04[b]
C16:0, palmitic acid	10.11 ± 2.55[a]	25.62 ± 3.97[b]
C16:1, palmitoleic acid	7.52 ± 1.69	6.44 ± 1.27
C18:0, stearic acid	13.34 ± 5.37	13.77 ± 1.76
C18:1, oleic acid	24.08 ± 5.51	20.08 ± 2.65
C18:1, elaidic acid	—	—
C18:2, linoleic acid	19.30 ± 4.59	13.74 ± 4.50
C18:3, linolenic acid	—	—
C20:1, eicosanoic acid	—	—
C20:4, arachidonic acid	14.65 ± 4.23	13.69 ± 3.42

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

Table 15
Gastrocnemius muscle fatty acid composition (percent) of rats fed control or MTO diets

Fatty acid, %	Control	MTO
C14:0, myristic acid	2.10 ± .44	2.23 ± .44
C16:0, palmitic acid	27.29 ± 1.84[a]	30.57 ± 3.58[b]
C16:1, palmitoleic acid	4.25 ± 1.15	4.55 ± 1.15
C18:0, stearic acid	16.39 ± 1.89	16.41 ± 3.25
C18:1, oleic acid	9.61 ± 3.72	9.10 ± 3.57
C18:1, elaidic acid	2.24 ± .70	2.39 ± .43
C18:2, linoleic acid	19.05 ± 2.80	20.80 ± 3.34
C18:3, linolenic acid	11.32 ± 6.69	4.31 ± 3.32
C20:1, eicosanoic acid	—[a]	.43 ± .29[b]
C20:4, arachidonic acid	7.73 ± 1.43	9.23 ± 3.39

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

removed in the present study to allow for a more accurate interpretation of the data. Hence, the overall reduction in body weight gain is due to the conjugated linoleic acid content of the MTO used in this experiment.

The reduction in brain weights from MTO supplementation is not readily explainable. If MTO was acting as a peroxisome proliferator, as has been implicated for CLA, then increases in liver and(or) kidney weights might have been expected [29]. Instead liver weights were not affected and kidney weights were slightly and nonsignificantly reduced by MTO supplementation. Supplementation of CLA to rats has been shown to increase liver weights [30]. Peroxisome proliferators enhance cell proliferation in affected tissues including kidneys [29], liver, testes, and pancreas [31,32]. However, in the current study organ weights were not affected in the liver or kidneys by MTO supplementation. The reduction in retroperitoneal fat depot weight from MTO supplementation is consistent with overall reductions in adiposity from dietary supplementation of CLA in mice [5,24,33], chickens [33], rats [34], and pigs [3,4], with and MTO supplementation in pigs [7,8,10,11].

The decrease in fat mass from MTO supplementation agrees with prior data on MTO in pigs and CLA in laboratory animals and livestock species. The increase in lean mass also agrees with work on MTO in pigs [7] and CLA in mice [5]. It is apparent from the observed changes in body composition in the present study that the increasing leanness is predominantly through alterations in fat mass, although increases in fat-free mass were greater on a total weight basis. Feeding CLA is reported to enhance norepinephrine-induced lipolysis and hormone sensitive lipase activity and increased total carnitine palmitoyltransferase activity [35]. Additionally, CLA reduces lipoprotein lipase activity while enhancing lipolysis, and stimulates fatty acid β-oxidation in skeletal muscle and fat pad, but not liver [5]. However, these results have been questioned. It has instead been suggested that CLA depressed body fat accumulation by reducing preadipocyte number when given during a period(s) of hyperplastic growth [36]. These researchers observed increased adipocyte size and lipid content in response to CLA supplementation; an unlikely event if lipolysis was favored. Reductions in adipocyte volume were also linked to reductions in adipose tissue mass by CLA [34]. Differing methodologies and CLA concentrations *in vitro* undoubtedly contribute to the confusion over the exact mode(s) of action of CLA in reducing adiposity. It is likely

Table 16
Abdominal fat fatty acid composition (percent) of rats fed control or MTO diets

Fatty acid, %	Control	MTO
C14:0, myristic acid	1.63 ± .63	1.79 ± .61
C16:0, palmitic acid	25.32 ± 2.98	28.88 ± 4.38
C16:1, palmitoleic acid	6.94 ± 1.15[a]	5.80 ± .78[b]
C18:0, stearic acid	3.59 ± .72	3.24 ± .22
C18:1, oleic acid	29.36 ± 4.11[a]	26.28 ± 1.96[b]
C18:1, elaidic acid	2.44 ± .38	2.25 ± .37
C18:2, linoleic acid	27.82 ± 4.11	28.34 ± 2.99
C18:3, linolenic acid	.32 ± .30	.24 ± .06
C20:1, eicosanoic acid	2.31 ± .63	2.37 ± .43
C20:4, arachidonic acid	.25 ± .03[a]	.81 ± .10[b]

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

that a combination of these mechanisms result in reduced adiposity with CLA supplementation.

Li and Watkins (1998) [37] postulated that CLA had the potential to influence bone formation and resorption through modulation of *ex vivo* PGE_2 production. However, MTO did not affect either bone mineral density or bone mineral content in the present study. The 6 wk duration of the study may have not been long enough to detect differences in bone mineral density.

The marginal reductions in serum cholesterol from MTO supplementation are in general agreement with prior work on CLA in hamsters [38]and rabbits [39], respectively. Tissue lipid enrichment has also been reported from dietary supplementation of CLA [25,40] and MTO [10]. suggested a tocopherol-sparing effect from feeding CLA, as evidenced by higher plasma tocopherol to cholesterol ratios in CLA-supplemented hamsters. In the current study, serum levels of αTP and cholesterol were reduced. Because of the greater magnitude of response in the αTP levels from MTO supplementation, the ratio of αTP to cholesterol actually increased in rats fed the control diet rather than in rats fed the MTO-supplemented diet. Tissue tocopherol levels were not determined by Nicolosi et al. (1997) [38], thus, comparisons can not be made with the present study.

Table 17
Retroperitoneal fat fatty acid composition (percent) of rats fed control or MTO diets

Fatty acid, %	Control	MTO
C14:0, myristic acid	1.35 ± .37[a]	2.13 ± .49[b]
C16:0, palmitic acid	23.46 ± 2.57[a]	29.59 ± 1.93[b]
C16:1, palmitoleic acid	5.81 ± .75	5.56 ± .86
C18:0, stearic acid	3.54 ± .61	2.98 ± .72
C18:1, oleic acid	28.84 ± 4.78	25.16 ± 4.21
C18:1, elaidic acid	2.32 ± .70	2.34 ± .61
C18:2, linoleic acid	31.46 ± 3.93	28.96 ± 3.25
C18:3, linolenic acid	.41 ± .20	.37 ± .00
C20:1, eicosanoic acid	2.51 ± .43	2.57 ± .55
C20:4, arachidonic acid	.31 ± .12	.34 ± .10

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

The preferential shift in site of αTP deposition from feeding MTO suggests a link to the improvements observed in color stability and decreases in oxidative deterioration from feeding swine vitamin E and MTO together [8]. The decreases in oxidative deterioration were determined by analyzing thiobarbituric acid (TBA) levels. Other research with CLA did not observe differences in tissue TBA values [40]. The data from the current study indicate that MTO could protect membrane lipids (such as PL) from oxidative damage by altering the deposition of αTP.

It has been demonstrated in swine that feeding CLA or MTO has a fat firming effect [7,26]. This effect is primarily due to a saturation of the fatty acids present in the adipose tissue. In the present study with rats, the amounts of unsaturated fatty acids (C16:1 and C18:1) decreased in the abdominal fat depot while the amounts of saturated fatty acids (C14:0 and C16:0) increased in the retroperitoneal fat depot. This observation lends support to a saturating effect of MTO on adipose tissues and helps explain increases in fat firmness. However, this observation raises the question of whether or not MTO reduces adiposity or just increases the density of the adipose tissues. Lipid contents of adipose tissues were largely unaffected in the present study.

Earlier work in swine [7] demonstrated that the proposed biologically active isomer in CLA (*trans* 10, *cis* 12) [41] may not be the same as for MTO. Swine fed diets containing similar amounts of the *trans* 10, *cis* 12 and *cis* 9, *trans* 11 isomers had significantly different growth performance, with pigs fed diets containing MTO having increased ADG and ADFI compared to pigs fed diets containing CLA. This warrants further investigation into differences between CLA (either synthetically produced or derived from sunflower oil) and MTO. Since MTO is derived from plants (southern yellow pine; primarily *Pinus taeda*), the sterol portion could exert some biological influence. Recently, administration of tall oil phytosterols was shown to reduce cholesterol in human patients [42].

These data add to the depth of knowledge on CLA supplementation, but also create questions as to the exact mode(s) of action. Modified tall oil appears to elicit many of the same biological responses in laboratory animals as previously reported for CLA. Future work must be done with MTO to determine potential reasons for observed differences in feed intakes and metabolism of α-tocopherol between CLA and MTO. The isomeric profile of MTO is different than that of CLA, and the precursors differ as well. Thus, some currently unidentified component of MTO may contribute to the observed differences between it and sources of CLA. However, this remains to be determined.

5. Conclusion

These data demonstrate for the first time that feeding modified tall oil elicits beneficial biological responses in a rat model used to emulate post-menopausal women. Specifically, modified tall oil slowed body weight gain, reduced adiposity, and slightly increased actual lean content. These data further demonstrate that modified tall oil alters the metabolism of $\propto$-tocopherol in a manner that concentrates it in the adipose tissues. Modified tall oil may reduce serum cholesterol levels and does not appear to affect bone mineral content or bone mineral density. Modified tall oil has been identified as a potent feed additive in the livestock

production area. It now offers promise to beneficially alter the body and serum and tissue compositions in adult ovariectomized rats, which are commonly used as a model for post-menopausal women.

Acknowledgments

Supported in part by the Kansas Agricultural Experiment Station (KAES). Contribution No. 02-470-J from the KAES. Appreciation is extended to Dr. Robert Teeter of Oklahoma State University, Stillwater, OK 74078, USA for assistance in collecting the body composition measurements.

References

[1] Ha YL, Grimm NK, Pariza MW. Anticarcinogens from fried ground beef: altered derivatives of linoleic acid. Carcinogenesis 1987;8:1881–7.
[2] Chin SF, Storkson JM, Albright KJ, Cook ME, Pariza MW. Conjugated linoleic acid is a growth factor as shown by enhanced weight gain and improved feed efficiency. J Nutr 1994;124:2344–9.
[3] Dugan MER, Aalhus JL, Schaefer AL, Kramer JKG. The effect of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. Can J Anim Sci 1997;77:723–5.
[4] Thiel RL, Sparks JC, Wiegand BR, Parrish FC Jr, Ewan RC. Conjugated linoleic acid improves performance and body composition in swine. J Anim Sci 1998;76(Suppl 1):127 (Abstract).
[5] Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. Lipids 1997;32:853–8.
[6] Huibers DTA. Tall oil. In: Kroschwitz JI, Howe-Grant M, editors. Encyclopedia of Chemical Technology. 4th Ed. New York, NY USA: Wiley-Interscience Publications, 1997. 616–22.
[7] O'Quinn PR, Nelssen JL, Goodband RD, Unruh JA, Woodworth JC, Smith JS, Tokach MD. Effects of modified tall oil versus a commercial source of conjugated linoleic acid and increasing levels of modified tall oil on growth performance and carcass characteristics of growing-finishing pigs. J Anim Sci 2000;78: 2359–68.
[8] Waylan AT, O'Quinn PR, Nelssen JL, Unruh JA, Goodband RD, Woodworth JC, Tokach MD, Koo SI. Effects of modified tall oil and vitamin E on growth performance, carcass characteristics, and meat quality of growing-finishing pigs. J Anim Sci 2002;80:1575–85.
[9] Waylan AT, O'Quinn PR, Nelssen JL, Unruh JA, Goodband RD, Woodworth JC, Tokach MD. Effects of modified tall oil, chromium nicotinate, and L-carnitine on growth performance, carcass characteristics, and meat quality of growing-finishing pigs. Can J Anim Sci 2003 (Submitted).
[10] O'Quinn PR, Andrews BS, Goodband RD, Unruh JA, Nelssen JL, Woodworth JC, Tokach MD. Effects of modified tall oil and creatine monohydrate on growth performance, carcass characteristics, and meat quality of growing-finishing pigs. J Anim Sci 2000;78:2376–82.
[11] Woodworth JC, Goodband RD, Unruh JA, Nelssen JL, Tokach MD, Waylan AT, O'Quinn PR. Interactive effects between fat source and modified tall oil on growth performance and carcass characteristics of finishing pigs. J Anim Sci 1999;77(Suppl 1):5 (Abstract).
[12] Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.
[13] Noh SK, Koo SI. The lymphatic absorption of lipids is normalized by enteral phosphatidylcholine infusion in ovariectomized rats with estrogen replacement. J Nutr Biochem 1997;8:152–61.
[14] Kim E, Noh SK, Koo SI. Marginal zinc deficiency lowers the lymphatic absorption of ∝-tocopherol in rats. J Nutr 1998;128:265–70.

[15] AOAC. Official Methods of Analysis (16th Ed.). Association of Official Analytical Chemists, Arlington, VA USA. 1995.
[16] Riley V. Adaptation of orbital bleeding technique to rapid serial blood studies. Proc Soc Exp Biol Med 1960;104:751–4.
[17] Bertin E, Ruiz J, Mourot J, Peiniau P, Portha B. Evaluation of dual-energy X-ray absorptiometry for body-composition assessment in rats. J Nutr 1998;128:1550–4.
[18] Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
[19] Rudel LL, Morris MD. Determination of cholesterol using o-phthalaldehyde. J Lipid Res 1973;14:364–6.
[20] Raheja RK, Kaur C, Singh A, Bhatia IS. New colorimetric method for the quantitative estimation of phospholipids without acid digestion. J Lipid Res 1973;14:695–7.
[21] Zaspel BJ, Csallany AS. Determination of alpha-tocopherol in tissues and plasma by high-performance liquid chromatography. Anal Biochem 1983;130:146–50.
[22] Liu JF, Huang CJ. Tissue ∝-tocopherol retention in male rats is compromised by feeding diets containing oxidized frying oil. J Nutr 1995;125:3071–80.
[23] SAS Institute Inc. SAS User's Guide: Release 6.03. SAS Institute, Cary, NC USA, 1988.
[24] West DB, Delany JP, Camet PM, Blohm F, Truett AA, Scimeca J. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. Am J Physiol 1998;275:R667–72.
[25] Belury MA, Kempa-Steczko A. Conjugated linoleic acid modulates hepatic lipid composition in mice. Lipids 1997;32:199–204.
[26] Eggert JM, Belury MA, Kempa-Steczko A, Schinkel AP. Effects of conjugated linoleic acid (CLA) on growth and composition of lean gilts. J Anim Sci 1999;77(Suppl 1):117 (Abstract).
[27] Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, Portocarrero CP, Peck LW, Nickel KP, Belury MA. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fa/fa rat. Biochem Biophys Res Comm 1998;244:678–82.
[28] Houseknecht KL, Baile CA, Matteri RL, Spurlock ME. The biology of leptin: a review. J Anim Sci 1998;76:1405–20.
[29] Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. Biochim Biophys Acta 1996;1302:93–109.
[30] Sugano M, Tsujita A, Yamasaki M, Noguchi M, Yamada K. Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. Lipids 1998;33:521–7.
[31] Belury MA, Moya-Camarena SY, Liu K, Vanden Heuvel JP. Dietary conjugated linoleic acid induces peroxisome-specific enzyme accumulation and ornithine decarboxylase activity in mouse liver. J Nutr Biochem 1997;8:579–84.
[32] Reddy JK. Carcinogenicity of peroxisome proliferators: evaluation and mechanisms. Biochem Soc Trans 1990;18:92–4.
[33] Park Y, Albright KJ, Liu W, Cook ME, Pariza MW. Dietary conjugated linoleic acid (CLA) reduces body fat content and isomers of CLA are incorporated into phospholipid fraction. 1995; IFT Book of Abstracts 183.
[34] Sisk M, Azain MJ, Hausman DB. Effect of conjugated linoleic acid on fat pad weights and cellularity in Sprague-Dawley and Zucker rats. FASEB J 1998;12:A536.
[35] Pariza M, Park Y, Kim S, Sugimoto K, Albright K, Liu W, Storkson J, Cook M. Mechanism of body fat reduction by conjugated linoleic acid. FASEB J 1997;11:A139.
[36] Satory DL, Smith SB. Conjugated linoleic acid inhibits proliferation but stimulates lipid filling of murine 3T3-L1 preadipocytes. J Nutr 1999;129:92–7.
[37] Li Y, Watkins BA. Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo Prostaglandin E_2 biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids 1998;33:417–25.
[38] Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery 1997;22:266–77.

[39] Lee KN, Kritchevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 1994;108:19–25.
[40] Dugan MER, Aalhus JL, Jeremiah LE, Kramer JKG, Schaefer AL. The effects of feeding conjugated linoleic acid on subsequent pork quality. Can J Anim Sci 1999;79:45–51.
[41] Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW. Evidence that the trans-10, cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids 1999;34:235–41.
[42] Jones PJH, Howell T, MacDougall DE, Feng JY, Parsons W. Short-term administration of tall oil phytosterols improves plasma lipid profiles in subjects with different cholesterol levels. Metabolism 1998;47:751–6.