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Effects of Protocatechuic Acid on Trans Fat Induced Hepatic Steatosis in Mice

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The effects of protocatechuic acid (PCA) on hepatic activity and/or mRNA expression of lipogenic enzymes and sterol regulatory element-binding proteins (SREBPs) in mice fed a trans fatty acid (TFA)-rich diet were examined. PCA at 1, 2, or 4% was provided for 10 weeks. Results showed that TFA diet significantly enhanced hepatic activity and mRNA expression of fatty acid synthase, 3-hydroxy-3-methylglutaryl coenzyme A reductase, stearoyl-CoA desaturase-1, and SREBP-1c ($P < 0.05$); however, the intake of PCA significantly diminished the activity and mRNA expression of these lipogenic factors and decreased hepatic lipid accumulation ($P < 0.05$). TFA diet significantly increased hepatic levels of TFA and pro-inflammatory cytokines ($P < 0.05$). However, PCA intake significantly lowered hepatic content of 18:1 trans and 18:2 trans, as well as reduced the level of test cytokines ($P < 0.05$). These results indicate that PCA is a potent agent for attenuating TFA-induced hepatic steatosis.

KEYWORDS: Trans fatty acid; protocatechuic acid; hepatic steatosis; stearoyl-CoA desaturase-1

INTRODUCTION

It has been documented that consumption of a trans fatty acid-rich diet enhanced lipogenesis and increased tumor necrosis factor (TNF)-α secretion in liver, which consequently caused liver injury (*1*−*3*). Although it is advised to decrease trans fatty acids intake to avoid the occurrence of steatohepatitis, this suggestion may not be practical because the intake of trans fatty acids in Western society has remarkably increased in past decades due to easily available foods containing trans fat. Thus, other dietary strategies need to be developed to counteract the adverse impact of dietary trans fatty acids. One possibility is to consume natural and edible agents with antilipogenic and anti-inflammatory effects.

Protocatechuic acid (3,4-dihydroxybenzoic acid) is a phenolic compound found in many plant foods such as *Hibiscus sabdariffa* (roselle) and *Eucommia ulmoides* (du-zhong) (*4*, *5*). Our previous animal study reported that this compound could reduce triglyceride (TG) and total cholesterol (TC) levels in plasma and liver in diabetic mice (*6*). However, it is uncertain whether this compound is able to attenuate lipogenesis in animals fed a diet rich in trans fatty acids. Also, the effect of this compound upon activity and mRNA expression of lipogenic enzymes such as fatty acid synthase (FAS) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase remains unclear. Stearoyl-CoA desaturase (SCD)-1 catalyzes the rate-limiting step in the cellular biosynthesis of monounsaturated fatty acids, primarily oleate and palmitoleate, which could be incorporated into and stored as TG in the liver (*7*). It is indicated that the decrease of SCD-1 mRNA expression alleviated the severity of fatty liver (*8*, *9*). Thus, this enzyme has been considered as a promising target for the treatment of metabolic diseases. On the other hand, sterol regulatory element-binding proteins (SREBPs) are important transcription factors responsible for fatty acid and cholesterol metabolism (*10*), in which SREBP-1c could modulate genes involved in fatty acid synthesis, whereas SREBP-2 is predominant in mediating genes associated with cholesterol synthesis (*11*, *12*). If protocatechuic acid could regulate SCD-1 and SREBPs, this compound may attenuate hepatic steatosis via multiple actions.

Another hallmark of trans fatty acids induced liver injury is enhanced inflammatory stress characterized by increasing hepatic level of pro-inflammatory cytokines such as interleukin (IL)-6 and TNF-α (*13*, *14*). Our previous study found that protocatechuic acid intake effectively lowered the cardiac and renal levels of IL-6 and TNF-α in diabetic mice (*6*). Thus, it is hypothesized that this compound could protect the liver against inflammatory damage. In the present study, a diet rich in trans fatty acids was used to induce hepatic steatosis and inflammatory stress, and the effects and possible actions of protocatechuic acid on activity and mRNA expression of lipogenic factors and the level of inflammatory cytokines in liver were investigated.

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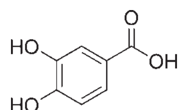

Figure 1. Structure of protocatechuic acid.

Table 1. Composition (Grams per Kilogram) of Normal Diet (ND) and Trans Fatty Acid Diet (TFA)

	ND	TFA
cornstarch	415	415
sucrose	205	205
casein	200	200
fiber	20	20
vitamin mix	10	10
mineral mix	35	35
olive oil	90	0
soybean oil	25	25
shortening	0	90
protein (%)	20.0	20.0
carbohydrate (%)	62.6	62.6
total fat (%)	11.7	11.7

MATERIALS AND METHODS

Materials. Protocatechuic acid (PCA, 99.5%) was purchased from Sigma Chemical Co. (St. Louis, MO). The structure of PCA is shown in **Figure 1**. All chemicals used in these measurements were of the highest purity commercially available.

Animals and Diets. Three-week-old male C57BL/6 mice were obtained from the National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). Use of the mice was reviewed and approved by both Chung Shan Medical University and China Medical University animal care committees. Mice were housed on a 12 h light−12 h dark schedule and fed mouse standard diet for a 1 week acclimation. Mice were then divided into two groups; one continuously consumed normal diet, and the other was switched to a diet prepared with shortening, rich in trans fatty acids, and defined as TFA diet. Diet composition is presented in **Table 1**.

Experimental Design. The TFA diet group was further divided into four subgroups, in which PCA at 0, 1, 2, or 4 g was mixed with 100, 99, 98, or 96 g of TFA diet. Body weight was measured every week. After 10 weeks of feeding, mice were killed with carbon dioxide. Blood was collected, and plasma was immediately separated from erythrocytes. Liver and epididymal fat from each mouse were collected and weighed. Then, 0.1 g of liver tissue was homogenized in 2 mL of phosphate-buffered saline (PBS, pH 7.2) on ice, and the homogenate was collected. The protein concentration of homogenate was determined according to the method of Lowry et al. (*15*) using bovine serum albumin as a standard. Sample was diluted to 1 mg of protein/mL and used for measurements.

Fatty Acid Composition Analysis. After lipid was extracted from diet or liver, fatty acid composition was analyzed by using a HP5890 gas chromatograph equipped with a FID and 30 m Omegawax capillary column (Supelco Chromatography Products, Bellefonte, PA). Fatty acids were quantified by determining areas under identified peaks, and heptadecanoic acid was used as an internal control. Results are reported as percentage of total fatty acids.

Blood Analyses. Serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined by using commercial assay kits (Randox Laboratories Ltd., Crumlin, U.K.). Commercial enzymatic colorimetric kits were used to determine plasma concentrations of TG and TC (Sigma Diagnostics, Madrid, Spain).

Steatosis Grade Assay. Resected liver specimens were used to determine the grade of steatosis by a licensed pathologist. According to the grading system of Pekow et al. (*16*), steatosis was assayed by a percentage of hepatocytes containing fat droplets: grade 0 = absent, grade 1 = 1−5%, grade 2 = 6−32%, grade 3 = 33−66%, and grade 4 = >66% of hepatocytes affected.

Hepatic TG and TC Measurement. The methods described in Miura et al. (*17*) were used to measure hepatic TG and TC content. Briefly, 1 mL of liver homogenate was mixed with 2.5 mL of chloroform/methanol (2:1, v/v). The chloroform layer was collected and concentrated by a rotary evaporator. After mixing with 10% Triton X-100 in isopropanol, the sample was assayed by Wako Triglyceride E-Test and Total Cholesterol E-Test kits according to the manufacturer's instructions (Wako Pure Chemical, Osaka, Japan). Hepatic TG and TC levels were expressed as milligrams per gram of wet tissue.

Assay for Activity of Malic Enzyme (ME), FAS, HMG-CoA Reductase, and SCD-1. The activity of ME and FAS was measured according to the methods of Stelmanska et al. (*18*) and Nepokroeff et al. (*19*). Both were determined by spectrophotometric assays. FAS activity was determined from the rate of malonyl-CoA-dependent NADPH oxidation and calculated as nanomoles of NADPH formed or oxidized per minute per milligram of protein. HMG-CoA reductase activity was measured by a radiochemical method as described in Kita et al. (*20*). [3-^{14}C]HMG-CoA was used as a substrate, and [3-^{14}C]mevalonone synthesized during the assay was isolated by anion-exchange column chromatography. The SCD-1 activity index was calculated from a ratio of product-to-precursor, 18:1n-9/18:0 (*21*).

Real-Time Polymerase Chain Reaction (RT-PCR) for mRNA Expression. RT-PCR was used to examine the hepatic mRNA expression. Liver tissue was homogenized in guanidinethiocyanate, and RNA was extracted using TRIizol reagent and further digested with DNase. Total RNA was isolated using the SV Total RNA Isolation kit (Promega, Madison, WI) according to the manufacturer's protocol. Two micrograms of total RNA was used to generate cDNA. Reverse transcription was performed in a one-step protocol using the iScript cDNA Synthesis Kit (Bio-Rad Co., Hercules, CA) according to the manufacturer's instructions. The primers for PCR were synthesized on the basis of previously published primer sequences. ME: forward, 5′-CCA CCA GCG CGG CTA CCT GCT GAC GCG GGA-3′; reverse, 5′-CCT CTG ACT CGC CGG TGC CGC AGC CCG ATG-3′. FAS: forward, 5′-CAT GAC CTC GTG ATG AAC GTG T-3′; reverse, 5′-CGG GTG AGG ACG TTT ACA AAG-3′. HMG-CoA reductase: forward, 5′-CCT GAC ACT GAA CTG AAG CG-3′; reverse, 5′-TCT TTC CAG AAC ACA GCA CG-3′. SCD-1: forward, 5′-CCT CCG GAA ATG AAC GAG AG-3′; reverse, 5′-CAG GAC GGA TGT CTT CTT CCA-3′. SREBP-1a: forward, 5′-TAG TCC GAA GCC GGG TGG GCG CCG GCG CCAT-3′; reverse, 5′-GAT GTC GTT CAA AAC CGC TGT GTG TCC AGT TC-3′. SREBP-1c: forward, 5′-ATC GGC GCG GAA GCT GTC GGG GTA GCG TC-3′; reverse, 5′-ACT GTC TTG GTT GTT GAT GAG CTG GAG CAT-3′. SREBP-2: forward, 5′-CAT GGA CAC CCT CAC GGA GCT GGG CGA CGA-3′; reverse, 5′-TGC ATC ATC CAA TAG AGG GCT TCC TGG CTC-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward, 5′-TGA TGA CAT CAA GAA GGT GGT GAA G-3′; reverse, 5′-CCT TGG AGG CCA TGT AGG CCA T-3′. The target concentration was expressed relative to the concentration of a reference housekeeping gene, GAPDH. The PCR was conducted using the following parameters: 50 °C for 2 min, 95 °C for 10 min, and 40 cycles at 94 °C for 20 s and 60 °C for 1 min. Generated fluorescence from each cycle was quantitatively analyzed by using the Taqman system based on real-time sequence detection system (ABI Prism 7700; Perkin-Elmer Inc., Foster City, CA). In this study, the mRNA level was calculated as a percentage of the normal diet group.

Hepatic Inflammatory Factors Determination. Liver tissue was homogenized in 10 mM Tris-HCl buffered solution (pH 7.4) containing 2 M NaCl, 1 mM ethylenediaminetetraacetic acid, 0.01% Tween 80, and 1 mM phenylmethanesulfonyl fluoride and centrifuged at 9000*g* for 30 min at 4 °C. The supernatant was used for cytokine determination. The levels of IL-1β, IL-6, TNF-α, and monocyte chemoattractant protein (MCP)-1 were measured by ELISA using cytoscreen immunoassay kits (BioSource International, Camarillo, CA). Samples were assayed in duplicates according to the manufacturer's instructions. The sensitivity of the assay, that is, the lower limit of detection, was 5 nmol/L for IL-1β, and IL-6 and 10 nmol/L for TNF-α and MCP-1.

Statistical Analysis. The effect of each treatment was analyzed from 10 mice ($n = 10$) in each group. All data were expressed as mean ± standard deviation (SD). Statistical analysis was done using one-way analysis of variance (ANOVA), and post hoc comparisons were carried out using Dunnet's t test. Differences with $P < 0.05$ were considered to be statistically significant.

RESULTS

As presented in **Table 2**, the TFA diet had significantly higher 16:1 trans, 18:1 trans, 18:2 trans, and total trans levels than the normal diet ($P < 0.05$). Compared with normal diet groups, mice consuming the TFA diet had significantly higher final body weight, liver weight, epididymal fat, water intake, and feed intake (**Table 3**, $P < 0.05$). However, PCA treatments at 2 and 4% significantly lowered mice final body weight, liver weight, and epididymal fat ($P < 0.05$). As shown in **Table 4**, consumption of the TFA diet significantly increased hepatic content of three trans fatty acids, 16:1, 18:1, and 18:2 ($P < 0.05$). However, PCA intake at 2 and 4% significantly lowered hepatic content of 18:1 trans and 18:2 trans ($P < 0.05$) and increased hepatic levels of 20:4, 20:5, and 22:6 ($P < 0.05$).

The TFA diet significantly elevated ALT, AST, and plasma and hepatic levels of TG and TC (**Table 5**, $P < 0.05$). PCA treatments dose-dependently decreased TFA diet induced ALT and AST elevation ($P < 0.05$), and these treatments significantly, but not dose-dependently, lowered plasma and hepatic TG levels ($P < 0.05$). PCA intake at 2 and 4% significantly reduced plasma and hepatic TC levels ($P < 0.05$). TFA diet led to eight mice exhibiting hepatic steatosis at grades 3 and 4. PCA intake at 2 and 4% decreased mouse number at grade 3. No mice showed hepatic steatosis at grade 4 in these two diet groups.

The TFA diet enhanced hepatic activity of ME, FAS, HMG-CoA reductase, and SCD-1 (**Table 6**, $P < 0.05$). PCA treatments dose-dependently mitigated TFA-induced increase in hepatic FAS and SCD-1 activities ($P < 0.05$). Hepatic HMG-CoA reductase activity was significantly reduced by 4% PCA treatment ($P < 0.05$). Hepatic mRNA expression of lipogenic factors is shown in **Figure 2**. The TFA diet significantly up-regulated mRNA expression of four test enzymes and three test SREBPs ($P < 0.05$). The intake of PCA dose-dependently attenuated TFA diet induced increase in FAS, SCD-1, and SREBP-1c mRNA expression ($P < 0.05$). PCA treatments at 2 and 4% significantly

Table 2. Fatty Acid Composition (Percent) in Normal Diet (ND) and Trans Fatty Acid Diet (TFA)[a]

	ND	TFA
lauric acid, 12:0	0.47 ± 0.12 a	0.55 ± 0.16 a
mirystic acid, 14:0	1.32 ± 0.21 a	0.84 ± 0.18 a
palmitic acid, 16:0	21.12 ± 2.15 b	15.29 ± 1.70 a
palmitoleic acid, 16:1 cis	0.92 ± 0.14 a	0.31 ± 0.07 a
palmitelaidic acid, 16:1 trans	–[b] a	1.66 ± 0.22 b
stearic acid, 18:0	24.53 ± 2.38 b	17.58 ± 1.95 a
oleic acid, 18:1 cis	28.30 ± 2.02 b	24.81 ± 1.84 a
elaidic acid, 18:1 trans	0.62 ± 0.09 a	31.76 ± 2.23 b
linoleic acid, 18:2 cis	19.56 ± 1.32 b	1.80 ± 0.25 a
linoelaidic acid, 18:2 trans	– a	3.65 ± 1.38 b
linolenic acid, 18:3 cis	1.06 ± 0.40 b	– a
arachidonic acid, 20:4	0.57 ± 0.32 b	– a
total SFA	47.44 ± 3.27 b	34.26 ± 2.11 a
total MFA	29.84 ± 3.04 a	58.54 ± 3.81 b
total PUFA	21.19 ± 1.49 b	5.45 ± 0.17 a
total trans	0.62 ± 0.06 a	37.07 ± 2.35 b

[a] Values are mean ± SD, $n = 10$. Means in a row without a common letter differ, $P < 0.05$. [b] –, too low to be detected.

Table 3. Body Weight, Water Intake, Feed Intake, Liver Weight, and Epididymal Fat in Mice Treated with Normal Diet (ND) or Trans Fatty Acid Diet (TFA) with Protocatechuic Acid (PCA) at 1, 2, or 4% for 10 Weeks[a]

	ND	TFA	TFA + PCA, 1%	TFA + PCA, 2%	TFA + PCA, 4%
body weight, g					
initial	16.3 ± 0.9 a	15.7 ± 1.1 a	15.5 ± 1.2 a	16.2 ± 0.8 a	16.0 ± 0.6 a
final	25.8 ± 2.0 a	38.1 ± 2.9 c	36.8 ± 2.5 c	32.5 ± 2.1 b	31.6 ± 2.3 b
water intake, mL/mouse/day	2.3 ± 0.7 a	5.5 ± 1.0 b	5.3 ± 0.9 b	5.8 ± 1.2 b	5.2 ± 1.3 b
feed intake, g/mouse/day	2.1 ± 0.6 a	5.0 ± 0.9 b	4.5 ± 0.6 b	4.7 ± 0.5 b	4.5 ± 1.0 b
liver weight, g	1.21 ± 0.18 a	2.13 ± 0.23 c	1.97 ± 0.20 c	1.60 ± 0.15 b	1.56 ± 0.19 b
epididymal fat, g	0.21 ± 0.13 a	1.92 ± 0.31 c	1.84 ± 0.28 c	1.10 ± 0.20 b	0.93 ± 0.22 b

[a] Values are mean ± SD, $n = 10$. Means in a row without a common letter differ, $P < 0.05$.

Table 4. Hepatic Fatty Acid Composition (Percent) in Mice Treated with Normal Diet (ND) or Trans Fatty Acid Diet (TFA) with Protocatechuic Acid (PCA) at 1, 2, or 4% for 10 Weeks[a]

	ND	TFA	TFA + PCA, 1%	TFA + PCA, 2%	TFA + PCA, 4%
mirystic acid, 14:0	0.72 ± 0.11 a	0.93 ± 0.18 a	0.41 ± 0.08 a	0.35 ± 0.10 a	0.29 ± 0.06 a
palmitic acid, 16:0	17.28 ± 1.72 c	9.53 ± 0.86 a	10.22 ± 1.19 a	12.45 ± 1.05 b	13.59 ± 1.40 b
palmitoleic acid, 16:1 cis	1.03 ± 0.31 a	0.62 ± 0.09 a	0.58 ± 0.22 a	0.90 ± 0.16 a	0.71 ± 0.24 a
palmitelaidic acid, 16:1 trans	–[b] a	2.45 ± 0.21 c	2.51 ± 0.17 c	1.48 ± 0.15 b	0.79 ± 0.12 b
stearic acid, 18:0	23.74 ± 1.80 c	15.82 ± 1.54 a	19.53 ± 1.71 b	20.31 ± 1.16 b	20.26 ± 1.03 b
oleic acid, 18:1 cis	26.30 ± 2.02 b	18.37 ± 1.47 a	17.57 ± 1.77 a	16.62 ± 1.92 a	16.38 ± 1.70 a
elaidic acid, 18:1 trans	1.06 ± 0.08 a	23.61 ± 2.23 e	19.06 ± 1.92 d	13.69 ± 1.18 c	10.41 ± 1.24 b
linoleic acid, 18:2 cis	21.06 ± 1.32 d	10.79 ± 1.64 a	14.10 ± 1.55 b	17.75 ± 2.06 c	18.63 ± 1.93 c
linoelaidic acid, 18:2 trans	1.53 ± 0.25 a	13.57 ± 1.38 d	10.24 ± 1.06 c	5.12 ± 0.72 b	6.93 ± 0.28 b
linolenic acid, 18:3 cis	2.01 ± 0.33 a	1.56 ± 0.42 a	1.37 ± 0.14 a	2.08 ± 0.46 a	1.74 ± 0.10 a
arachidonic acid, 20:4	4.38 ± 0.52 b	2.05 ± 0.61 a	2.34 ± 0.35 a	6.67 ± 0.59 c	7.35 ± 0.67 c
eicosapentaenoic acid, 20:5	– a	– a	0.35 ± 0.08 b	0.96 ± 0.09 b	1.14 ± 0.08 b
docosahexaenoic acid, 22:6	– a	– a	– a	0.81 ± 0.11 b	0.92 ± 0.13 b
total SFA	41.74 ± 3.05 c	27.18 ± 2.27 a	30.16 ± 2.57 a	33.51 ± 3.10 b	34.14 ± 3.18 b
total MFA	29.39 ± 1.90 a	43.55 ± 3.48 d	39.72 ± 3.20 c	32.76 ± 2.62 b	28.27 ± 2.06 a
total PUFA	27.98 ± 2.61 a	28.47 ± 1.88 a	28.05 ± 2.19 a	33.39 ± 2.54 b	36.71 ± 2.89 c
total trans	2.59 ± 0.33 a	39.23 ± 2.93 d	32.81 ± 2.75 c	20.86 ± 1.37 b	18.11 ± 1.44 b

[a] Values are mean ± SD, $n = 10$. Means in a row without a common letter differ, $P < 0.05$. [b] –, too low to be detected.

Table 5. Serum Level of ALT and AST, Plasma Level of TG and TC, Hepatic Level of TG and TC, and Steatosis Grade in Mice Treated with Normal Diet (ND) or Trans Fatty Acid Diet (TFA) with Protocatechuic Acid (PCA) at 1, 2, or 4% for 10 Weeks[a]

	ND	TFA	TFA + PCA, 1%	TFA + PCA, 2%	TFA + PCA, 4%
ALT, U/L	41 ± 5 a	156 ± 21 e	119 ± 18 d	90 ± 8 c	75 ± 10 b
AST, U/L	52 ± 7 a	174 ± 18 e	141 ± 20 d	100 ± 13 c	77 ± 10 b
plasma TG, g/L	1.83 ± 0.25 a	5.57 ± 0.52 d	4.62 ± 0.41 c	2.79 ± 0.37 b	2.58 ± 0.40 b
plasma TC, g/L	1.33 ± 0.31 a	3.42 ± 0.48 c	3.35 ± 0.43 c	2.41 ± 0.19 b	2.29 ± 0.27 b
hepatic TG, mg/g of wet tissue	25.8 ± 1.5 a	50.3 ± 4.2 e	43.2 ± 3.8 d	36.1 ± 2.1 c	30.2 ± 2.3 b
hepatic TC, mg/g of wet tissue	2.6 ± 0.7 a	7.1 ± 1.8 c	6.7 ± 1.4 c	5.4 ± 0.8 b	5.0 ± 1.2 b
steatosis grade, 0/1/2/3/4	10/0/0/0/0	0/0/2/5/3	0/0/5/4/1	0/3/5/2/0	0/5/4/1/0

[a] Values are mean ± SD, n = 10. Means in a row without a common letter differ, $P < 0.05$.

Table 6. Hepatic Activity of Malic Enzyme (ME), Fatty Acid Synthase (FAS), 3-Hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA Reductase), and Stearoyl-CoA Desaturase (SCD)-1 in Mice Treated with Normal Diet (ND) or Trans Fatty Acid Diet (TFA) with Protocatechuic Acid (PCA) at 1, 2, or 4% for 10 Weeks[a]

	ND	TFA	TFA + PCA, 1%	TFA + PCA, 2%	TFA + PCA, 4%
ME, mmol/min/mg of protein	3.2 ± 0.4 a	5.5 ± 0.7 c	5.2 ± 0.8 c	4.6 ± 1.0 b	4.3 ± 0.5 b
FAS, mmol/min/mg of protein	2.3 ± 0.6 a	6.5 ± 0.9 e	5.7 ± 0.6 d	4.6 ± 0.7 c	3.6 ± 0.8 b
HMG-CoA reductase, pmol/min/mg of protein	21.0 ± 1.1 a	30.2 ± 1.3 c	29.5 ± 1.0 c	28.7 ± 0.9 c	25.3 ± 1.2 b
SCD-1 index, 18:1n-9/18:0	0.05 ± 0.01 a	1.32 ± 0.08 e	1.01 ± 0.05 d	0.71 ± 0.04 c	0.43 ± 0.03 b

[a] Values are mean ± SD, n = 10. Means in a row without a common letter differ, $P < 0.05$.

down-regulated mRNA expression of ME and HMG-CoA reductase ($P < 0.05$). SREBP2 expression was significantly lowered only by the 4% PCA treatment ($P < 0.05$). PCA treatments failed to affect hepatic SREBP-1a expression ($P > 0.05$).

As shown in **Table 7**, the TFA diet significantly increased IL-1β, IL-6, TNF-α, and MCP-1 levels found in liver ($P < 0.05$). PCA intake dose-dependently reduced the detected levels of IL-1β, IL-6, and TNF-α in this tissue ($P < 0.05$); however, the MCP-1 level was significantly reduced by PCA treatments at 2 and 4% ($P < 0.05$).

DISCUSSION

It has been reported that intake of TFA up-regulated hepatic mRNA expression of SREBP-1c and caused fatty liver (*22*). In our present study, TFA intake increased hepatic content of TFA and up-regulated hepatic mRNA expression of upstream lipogenic factors, SREBP-1c and SREBP-2, which subsequently promoted mRNA expression and activity of FAS and HMG-CoA reductase and finally enhanced downstream lipid synthesis and accumulation in liver. Our data of hepatic TG and TC content also suggested that the TFA diet favored lipid synthesis and/or storage in this tissue. These findings not only corroborated that TFA favored the development of fatty liver but also provided evidence to explain the possible mechanism for TFA-caused steatosis. On the other hand, we found that PCA intake markedly lowered hepatic TFA content and diminished hepatic mRNA expression of SREBP-1c and SREBP-2 in TFA-treated mice. Then, the less available SREBP-1c and SREBP-2 further reduced the hepatic activity and expression of FAS and HMG-CoA reductase and, consequently, contributed to attenuate lipogenesis and/or reduce lipid accumulation in the liver. Our results of steatosis grade also showed that PCA treatments alleviated TFA-induced fatty liver. In addition, it is noted that PCA intake also markedly reduced epididymal fat in TFA-treated mice. Apparently, this compound could provide an antilipogenic effect for other tissues. These results suggested that PCA was a potent agent to prevent or improve TFA-induced steatosis and obesity.

Paton and Ntambi (*23*) reported that SCD-1 catalyzed the D(9)-cis desaturation of a range of fatty acyl-CoA substrates, and the preferred substrates were palmitoyl- and stearoyl-CoA. Our present study found that TFA enhanced the activity and mRNA expression of SCD-1 in the liver, which could further promote the biosynthesis of monounsaturated fatty acid (MFA), especially 18:1 trans. These findings indicate that the contribution of TFA to steatotic development was partially due to TFA up-regulation of hepatic SCD-1. Thus, the increased hepatic TFA content in TFA-treated mice as we observed could be explained. Furthermore, we found PCA intake effectively diminished hepatic SCD-1 activity and mRNA expression in mice fed the TFA diet, which subsequently lowered hepatic MFA production or accumulation. These results revealed that PCA could counteract the adverse effect of dietary TFA via regulating SCD-1 expression and reducing the formation of 18:1 trans and 18:2 trans. It should be pointed out that 18:1 trans, not 18:1 cis, was the major MFA affected by PCA treatments. This finding implied that this compound might be able to affect hepatic synthesis of fatty acid with certain structure. It is known that SREBP-1c could mediate subsequent activation of SCD-1 gene promoter (*24*). The lower hepatic SCD-1 expression we observed in PCA-treated mice could be partially ascribed to this compound already attenuating the expression of SREBP-1c, an upstream regulator of SCD-1. In addition, we noted that PCA intake increased hepatic content of 20:4, 20:5, and 22:6. It seems that this compound favored hepatic polyunsaturated fatty acid (PUFA) synthesis. Although the mechanism remains unknown, this finding implied that PCA might regulate fatty acid metabolism via elongation and/or desaturation. It is reported that PUFA could suppress SCD-1 gene expression (*25*). Thus, it is possible that PCA elevated hepatic PUFA synthesis, which further limited hepatic SCD-1 expression.

Teegala et al. (*26*) reported that TFA consumption enhanced systemic inflammation. Harvey et al. (*27*) observed that TFA treatment induced the release of TNF-α and MCP-1 in human aortic endothelial cells and concluded that TFA played a role in the induction of pro-inflammatory responses. In our present study, the TFA diet markedly increased the levels of IL-1β, IL-6, TNF-α, and MCP-1 found in the liver, which indicated that the TFA diet elevated inflammatory stress via raising the concentration of pro-inflammatory cytokines and chemotactic factor in this tissue and/or circulation. Furthermore, we found that PCA intake effectively reduced the level of four test cytokines detected in liver, which revealed that this compound could attenuate TFA-induced inflammation via diminishing pro-inflammatory cytokines in the liver and/or circulation. It is interesting to find that PCA treatments

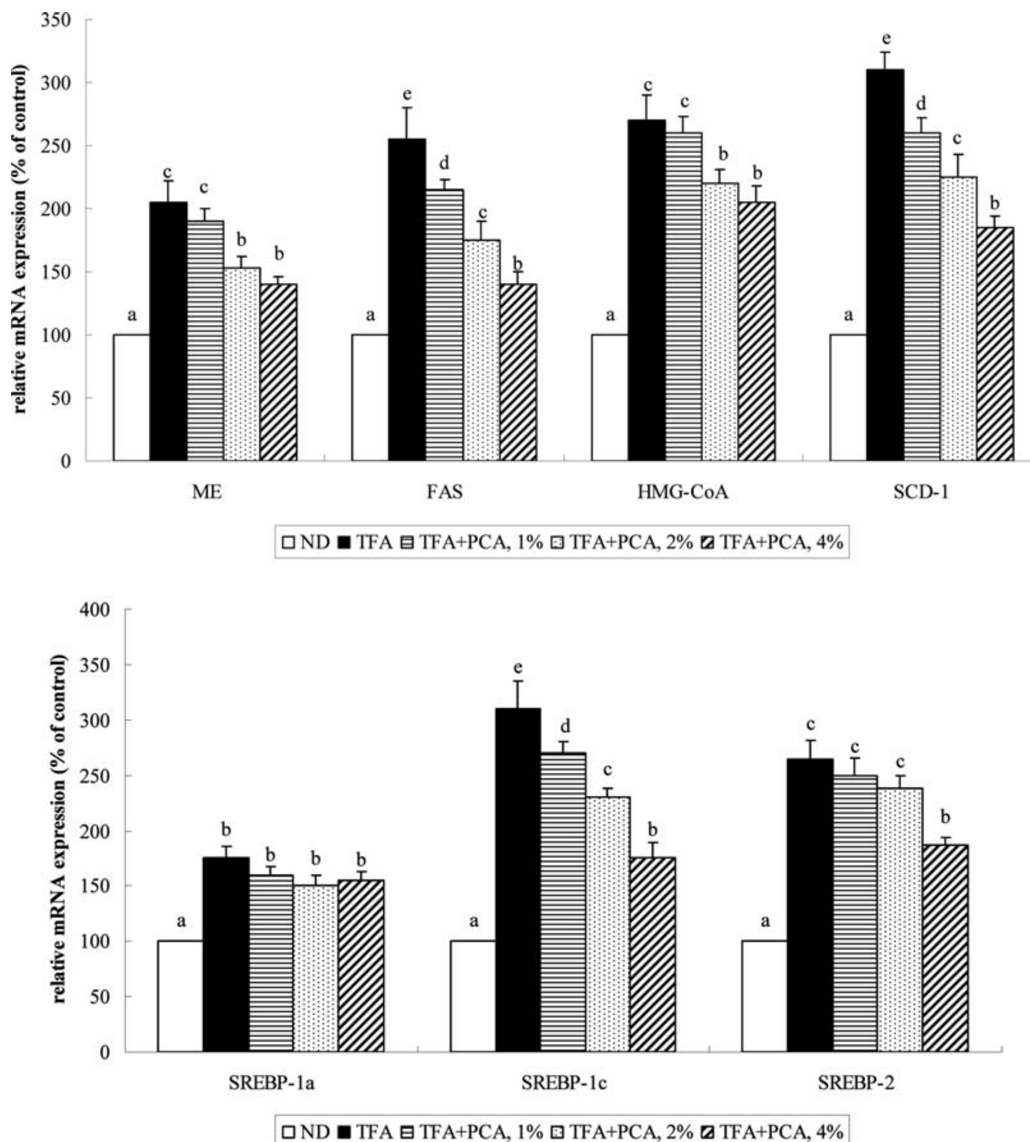

Figure 2. Hepatic mRNA expression of malic enzyme (ME), fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA reductase), stearoyl-CoA desaturase (SCD)-1, sterol regulatory element-binding protein (SREBP)-1a, SREBP-1c, and SREBP-2 in mice treated with normal diet (ND) or trans fatty acid diet (TFA) with protocatechuic acid (PCA) at 1, 2, or 4% for 10 weeks. Data are mean ± SD (n = 10). Means among bars without a common letter differ, $P < 0.05$.

Table 7. Hepatic Level (Nanomoles per Liter) of IL-1β, IL-6, TNF-α, and MCP-1 in Mice Treated with Normal Diet (ND) or Trans Fatty Acid Diet (TFA) with Protocatechuic Acid (PCA) at 1, 2, or 4% for 10 Weeks[a]

	ND	TFA	TFA + PCA, 1%	TFA + PCA, 2%	TFA + PCA, 4%
IL-1β	24 ± 5 a	238 ± 26 e	199 ± 20 d	152 ± 17 c	128 ± 20 b
IL-6	19 ± 4 a	205 ± 21 e	171 ± 18 d	123 ± 15 c	95 ± 8 b
TNF-α	16 ± 3 a	307 ± 30 e	266 ± 23 d	212 ± 19 c	173 ± 24 b
MCP-1	10 ± 3 a	311 ± 25 c	302 ± 27 c	259 ± 18 b	246 ± 21 b

[a] Values are mean ± SD, n = 10. Means in a row without a common letter differ, $P < 0.05$.

dose-dependently reduced ALT, AST, and pro-inflammatory cytokines but decreased activity and mRNA expression of lipogenic enzymes only at doses ≥2%. These findings implied that the lower ALT, AST, and inflammatory stress from PCA treatments might be simply due to the anti-inflammatory action of this compound. However, to exert antihepatosteatotic effects, higher doses of this compound were required.

After calculation and justification, the daily TFA consumed by mice in our present study was equal to 4 g/day for a 70 kg human male. Although this amount is lower than the 5.9 g/day consumed by some of the American population (*28*), it caused severe hepatic steatosis in mice. Obviously, dietary TFA should be more carefully managed to minimize its adverse impact upon human health. On the other hand, the daily PCA intake of mice was equal to 110−440 mg/day for a 70-kg human male. PCA is a natural phenolic compound; however, further study is necessary to ensure the safety of this compound at these doses before it is used in humans. PCA content in roselle calyx was 280 ± 47 mg/100 g of dry weight (*29*). Thus, it seems not practical to consume foods such as roselle and du-zhong to obtain this compound.

In conclusion, trans fatty acids caused hyperlipidemia and steatohepatitis. The intake of protocatechuic acid effectively attenuated hepatic lipid accumulation via reducing the activity and/or mRNA expression of FAS, HMG-CoA reductase, SCD-1, and SREBP-1c. This compound also diminished inflammatory stress via lowering the level of IL-1β, IL-6, TNF-α, and MCP-1 in the liver. These results suggest that protocatechuic acid could be

considered as a potent agent for alleviating trans fatty acids induced hepatic steatosis.

ABBREVIATIONS USED

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FAS, fatty acid synthase; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; ME, malic enzyme; MFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acid; RT-PCR, real time-Polymerase Chain Reaction; SCD-1, stearoyl-CoA desaturase-1; SFA, saturated fatty acids; SREBPs, sterol regulatory element-binding proteins; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α.

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