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The messenger RNA profiles in liver, hypothalamus, white adipose tissue, and skeletal muscle of female Zucker diabetic fatty rats after topiramate treatment

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Abstract

Topiramate (TPM) is a novel neurotherapeutic agent approved for the treatment of epilepsy and for migraine prophylaxis. It has been observed that in obese-associated, type 2 diabetic rodent models, TPM treatment reduced the body weight gain, improved insulin sensitivity, and enhanced glucose-regulated insulin release. A long-term treatment with TPM thus ameliorated obesity and diabetic syndromes in female Zucker diabetic fatty rats and *db/db* mice. The molecular mechanisms of TPM antiobesity and antidiabetic effects remain unknown. We have applied DNA microarray technology to explore genes that might be involved in the mechanisms by which TPM improves insulin sensitivity and blood glucose handling, as well as body weight control. In female Zucker diabetic fatty rats, 7-day TPM treatment significantly reduced the plasma levels of glucose and triglyceride in a dose-dependent manner. The DNA microarray data revealed that TPM treatment altered messenger RNA profiles in liver, hypothalamus, white adipose tissue, and skeletal muscle. The most marked effect of TPM on gene expression occurred in liver with those genes related with metabolic enzymes and signaling regulatory proteins involved in energy metabolism. TPM treatment decreased messenger RNA amounts for sterol regulatory element binding protein-1c, stearoyl-coenzyme A (CoA) desaturase-1, choline kinase, and fatty acid CoA ligase, long chain 4. TPM also up-regulated 3 cholesterol synthesis genes. In addition, the short-term effect of TPM on gene expression was examined at 16 hours after a single administration. TPM markedly reduced hepatic expression of genes related with fatty acid synthesis, eg, stearoyl-CoA desaturase and acetyl-CoA carboxylase. TPM also changed genes related with fatty acid β-oxidation, increased 3-2-*trans*-enoyl-CoA isomerase and mitochondrial acyl-CoA thioesterase, and decreased fatty acid CoA ligase (long chain 2 and long chain 5). These gene expression changes were independent of food intake as shown by pair feeding. Our results suggest that TPM regulates hepatic expression of genes involved in lipid metabolism, which could be part of the mechanisms by which TPM reduces plasma triglyceride levels in obese diabetic rodents.

1. Introduction

Topiramate (TPM) is a novel neurotherapeutic agent approved for the treatment of epilepsy and for migraine prophylaxis. It is synthesized from D-fructose and contains a sulfamate moiety that is essential for its pharmacologic activity [1]. TPM has been shown to exert several pharmacologic activities in the nervous system [1]. In contrast to other antiepileptic drugs, TPM treatment was associated with body weight loss in humans [2-4].

In our previous studies, we have shown that TPM treatment markedly reduced blood glucose and triglyceride levels in both Zucker diabetic fatty (ZDF) rats and *db/db* mice without a significant reduction in body weight gain [5]. In these TPM-treated ZDF rats and *db/db* mice, glucose-stimulated insulin release was increased by 2- to 3-fold during an oral glucose tolerance test (OGTT) compared with that in controls. A hyperinsulinemic-euglycemic clamp study showed that TPM improved insulin sensitivity in female ZDF rats [6]. We also observed a 1.4-fold increase of pancreatic insulin content and heightened insulin immunostaining in pancreatic beta cells in *db/db* mice treated with TPM. TPM has also been reported by other research laboratories to reduce energy efficiency and fat gains in lean (fa/?) and obese (fa/fa) Zucker rats [7]. All these observations suggested that TPM might be able to improve glycemic and lipid metabolism in obese-associated

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diabetes; however, the molecular mechanisms underlying this apparent effect of TPM are unclear.

The liver plays an essential role in carbohydrate and lipid metabolism and is a key target for insulin action on fuel metabolism. Reduced insulin sensitivity and deregulation of fuel metabolism in hepatocytes contribute significantly to the pathogenesis of obesity and diabetes. Hepatic lipid metabolism includes fatty acid oxidation, fatty acid and cholesterol de novo synthesis, triglyceride and bile acid synthesis, as well as lipoprotein assembly. These metabolic pathways are coordinately regulated to maintain lipid homeostasis under physiologic conditions.

As an initial step to elucidate the molecular and cellular mechanisms of TPM action on obesity and diabetes, we have used a microarray-based gene expression profiling approach to identify genes affected by TPM in 4 major insulin-sensitive tissues involved in energy metabolism (liver, hypothalamus, white adipose tissue, and skeletal muscle) of female ZDF rats, with emphasis on hepatic lipid metabolism. We found that TPM down-regulates the expression of fatty acid/triglyceride synthesis genes but up-regulates fatty acid oxidation and cholesterol synthesis genes in the liver. TPM affected fewer genes in the hypothalamus, white adipose tissue, and skeletal muscle. Our results suggested that TPM regulates hepatic gene expression of lipid metabolism, which could be part of the mechanisms by which TPM reduces plasma triglyceride levels in obese diabetic rodents.

2. Materials and methods

2.1. Animals

Female ZDF rats, 8 to 10 weeks old (ZDF/Gmi-*fa/fa*, Genetics Models, Indianapolis, IN), were used in the study. Upon arrival, they were housed individually in hanging metal cages and acclimated for 5 days before the study. These rats were maintained at an ambient temperature of 21° to 23°C on a 12-hour light/dark cycle with free access to water and diet chow (Purina 5008, Purina Mills, St Louis, MO). The animal study protocols were approved by the Johnson & Johnson Pharmaceutical Research & Development, LLC (Spring House, PA) Institutional Animal Care and Use Committee.

2.2. Experimental design

2.2.1. Effects of TPM on blood glucose, plasma lipid, and hormone levels, and energy metabolism in female ZDF rats

Four groups of rats, vehicle (0.5% Methocel) and TPM (10, 50, and 100 mg/kg per day) treated (n = 8 of each group), were used in this study. Rats were dosed orally via gavage for 14 days. The body weight and food intake were monitored. After the 14-day treatment, rats were fasted overnight and killed by decapitation the next day to collect trunk blood samples for measurement of plasma concentrations of major metabolites and hormones. In a separate study, 2 groups of rats were treated with vehicle or TPM at 100 mg/kg per day for 14 days and these rats were used for either an OGTT or an energy expenditure study.

2.2.2. Effects of TPM on gene expression in liver, hypothalamus, white adipose tissue, and skeletal muscle

First, we used 2 groups of rats, vehicle or TPM treated (100 mg/kg per day, via gavage dosing), to study the effects of TPM treatment on gene expression after 7 days' multiple-dosing treatment (n = 3 for each treatment group). Next, we conducted a pair-feeding study to analyze the short-term effect of TPM on gene expression (16 hours after a single dose). In this pair-feeding study, we used 4 groups (n = 3 of each group) of rats, namely, vehicle (Veh), TPM, vehicle pair-feeding (Veh-PF), and TPM pair-feeding (TPM-PF) groups. The use of the TPM-PF group in this study was based on the consideration of the statistical method applied in data analysis. In this study, rats in the Veh or TPM groups had free access to food, whereas rats in the Veh-PF or TPM-PF groups were fed with a limited amount of food (calculated from the first study) similar to TPM-treated rats. Samples from liver, white adipose tissue (periovarian), skeletal muscle (soleus), and whole hypothalamus were collected with a freeze-clamp in liquid nitrogen for RNA isolation.

2.3. DNA microarray and quantitative reverse transcription-polymerase chain reaction

Total RNA was isolated from tissue samples by using Trizol (Invitrogen, Carlsbad, CA) and an RNeasy Mini kit (Qiagen, Valencia, CA). Probes for microarray analysis were

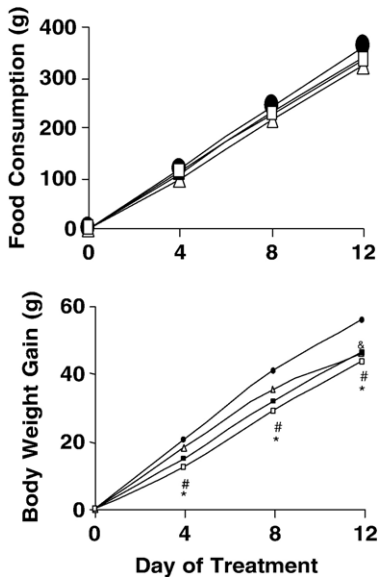


Fig. 1. Food intake and body weight gain in ZDF rats treated with TPM for 14 days. Accumulative food consumption and body weight of each rat were measured on days 4, 8, and 12 of treatment. (–●–) indicates vehicle; (–△–), TPM, 10 mg/kg per day; (–■–), TPM, 50 mg/kg per day; (–□–), TPM, 100 mg/kg per day. $^{*}P < .05$, TPM 100 vs vehicle; $^{\#}P < .05$, TPM 50 vs vehicle, $^{\&}P < .05$, TPM 10 vs vehicle. n = 8, repeated-measures ANOVA.

Table 1
Plasma glucose, lipid, and hormone profiles in female ZDF rats treated with TPM for 14 days

	Vehicle	TPM (mg/kg per day)		
		10	50	100
Glucose (mg/dL)	156 ± 5	145 ± 4	132 ± 4**	126 ± 3**
Triglyceride (mg/dL)	1031 ± 92	1009 ± 139	959 ± 138	763 ± 68*
FFA (μmol/L)	1250 ± 135	1237 ± 169	1105 ± 230	1156 ± 139
Total cholesterol (mg/dL)	97 ± 3	95 ± 5	119 ± 13	113 ± 4**
HDL cholesterol (mg/dL)	39 ± 1	nd	nd	55 ± 5**
Plasma urine nitrogen (mg/dL)	15 ± 1	13 ± 1	15 ± 1	15 ± 1
β-Hydroxybutyrate (mg/dL)	10.3 ± 0.6	10.9 ± 0.8	9.8 ± 0.6	10.7 ±0.4
Insulin (ng/mL)	3.5 ± 0.4	6.0 ± 0.8**	4.5 ± 0.5	5.6 ± 0.7*
Glucagon (pg/mL)	95.5 ± 2.8	93.3 ± 4.7	93.3 ± 4.1	100.1 ± 7.9
Corticosterone (ng/mL)	1005 ± 94	853 ± 85	843 ± 30	922 ± 45
Leptin (ng/mL)	49.3 ± 2.0	47.6 ± 2.5	47.4 ± 2.2	43.6 ± 1.0
Adiponectin (μg/mL)	11.9 ± 0.5	10.5 ± 0.5	12.9 ± 0.9	10.6 ± 0.6
Growth hormone (ng/mL)	2.1 ± 0.3	2.3 ± 0.2	1.8 ± 0.3	2.5 ± 0.3

Rats were fasted overnight before blood sample collection. Data are presented as mean ± SEM of each treated group (n = 8). nd indicates not determined.
* $P < .05$, topiramate vs vehicle.
** $P < .01$, topiramate vs vehicle.

prepared by using 5 μg of total RNA and hybridized to RG-U34A GeneChips (Affymetrix, Santa Clara, CA). Detailed protocols for the probe synthesis and hybridization reactions, as well as for the posthybridization washing and staining, have been described in the Affymetrix Genechip Expression Analysis Technical Manual (Affymetrix). The effects of TPM and pair feeding on the expression of selected genes were confirmed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR), which was performed with SYBR Green technology using a service order (GeneDirect, Princeton, NJ). Briefly, complementary DNA was prepared by reverse transcription of 1 to 1.5 μg of total RNA, using random hexamer as primer with a Taqman Reverse Transcription Kit (Applied Biosystems, Foster City, CA), and the resulting complementary DNA was amplified by using SYBR Green PCR Core Reagent (Applied Biosystems) on a DNA Engine Opticon-2 instrument (MJ Research, Waltham, MA) according to the manufacturer's protocol (Applied Biosystems). Target messenger RNA (mRNA) expression was normalized to 18S rRNA expression in the corresponding sample.

2.4. Oral glucose tolerance test

Rats were fasted overnight and a glucose challenge (2 g/kg body weight, via gavage) was administered the next morning. Tail blood samples were collected at 0, 30, 60, and 120 minutes to measure blood glucose levels with a glucometer (One Touch Ultra, Lifescan, Milpitas, CA), and plasma insulin (rat insulin ELISA kit, ALPCO, Windham, NH).

2.5. Energy expenditure

Indirect calorimetry was applied (CaloSys, TSE Technical & Scientific Equipment, Bad Homburg, Germany) to assess the effect of TPM on energy expenditure. Constant airflow (1.75 L/min) was drawn through each chamber and monitored by a mass-sensitive flowmeter. The concentrations of oxygen and carbon dioxide were monitored at the inlet and outlet of every chamber to calculate oxygen consumption and respiratory quotient (RQ).

2.6. Blood biochemistry analysis

Plasma levels of glucose, triglyceride, and high-density lipoprotein (HDL) were measured with a COBAS Mira Plus Blood Chemistry Analyzer (Roche Diagnostic Systems, Nutley, NJ). Commercial kits were used to measure plasma concentrations of free fatty acids (FFA; Waco Pure Chemical Industries, Osaka, Japan) and β-hydroxybutyrate

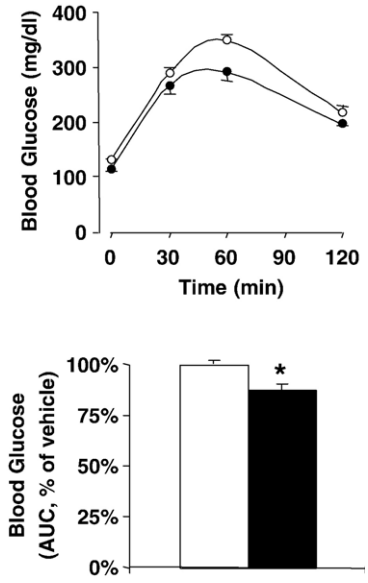


Fig. 2. TPM in OGTT in ZDF rats treated with TPM for 14 days. An OGTT was conducted in rats after 14 days of treatment with vehicle or TPM (100 mg/kg per day). Top, Blood glucose at every time point. Bottom, AUC_{glu} during 2-hour period of OGTT. (–○–) indicates vehicle; (–●–), TPM, 100 mg/kg per day. *$P < .05$, compared with that in vehicle-treated rats; Student t test.

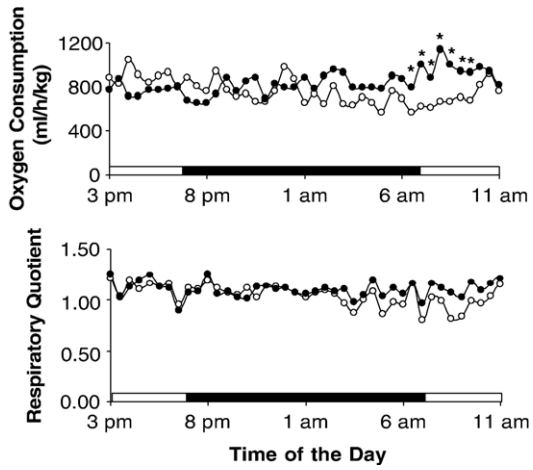


Fig. 3. TPM on energy expenditure in ZDF rats treated with TPM for 14 days. Indirect calorimetry was applied to measure oxygen consumption (top) and RQ (bottom) in ZDF rats after 14 days of treatment with TPM (●) or vehicle (○). Rats were housed in the calorimetry chamber for 24 hours with free access to water and food. *$P < .05$, compared with that in vehicle-treated rats at the same time point; Student t test.

(Stanbio Laboratory, Boerne, TX). A rat insulin ELISA kit (ALPCO) was used to measure plasma insulin concentrations. Plasma concentrations of glucagon, leptin, adiponectin, corticosterone, and growth hormone were measured by using a LINCO Diagnostic Services kit (St Louis, MO).

2.7. Statistical analysis

For gene expression study, the hybridization arrays were scanned, and raw data were extracted by Microarray Analysis Suite 5 (Affymetrix), log2-transformed, and normalized by quantile normalization [8]. Expression data from controls and TPM-treated rats from 2 separate times were analyzed by Student t test with a filter threshold of $P < .05$ and fold change of 1.5 or higher. The filtered genes were further tested in the general liner model of Partek Pro software (Partek, St Charles, MO) with drug (TPM vs vehicle) and food intake (pair feeding vs vehicle) as the 2 factors to test the factorial effects on gene expression changes.

Statistical analysis of other in vivo study data was performed by using the program Prism (Graphpad, Monrovia, CA) with a one-way analysis of variance (ANOVA) and Dunnett multiple comparison test, as well as Student t test (unpaired). All data are presented as mean ± SEM.

3. Results

3.1. Effects of TPM on diabetic syndromes and hormonal profiles in female ZDF rats

A 14-day TPM-treatment study was conducted to examine the effect of TPM on diabetic syndrome in female ZDF rats. The food consumption and body weight gain were monitored. Rats receiving TPM treatment showed no statistically significant reduction of accumulative food intake during 1 to 4, 5 to 8, and 9 to 12 days. Body weight gain was significantly reduced in rats treated with TPM (50 and 100 mg/kg per day, $P < .05$ compared with vehicle group) (Fig. 1). Blood biochemical analysis showed that compared with the vehicle-treated group, a 14-day TPM treatment decreased plasma glucose and triglyceride levels in a dose-dependent manner. TPM did not affect fasting plasma FFA and β-hydroxybutyrate concentration, but elevated plasma total cholesterol levels (Table 1). TPM treatment increased only the plasma insulin levels and had little effect on plasma levels of glucagon, corticosterone, adiponectin, leptin, and growth hormone.

Table 2
Differentially expressed genes in the liver of TPM- vs vehicle-treated rats after 7 days of treatment

Function	Probe set	Genes	Fold change	Description
Signaling proteins	AA892251_at	*Avpr1a*	−3.4	Arginine vasopressin receptor 1A
	AI012183_at	*Nr2f2*	−2.1	Nuclear receptor subfamily 2, group F, member 2
	D00698_s_at	*Igf1*	−1.7	Insulinlike growth factor 1
	X12752_at	*Cebpa*	−1.6	CCAAT/enhancer binding protein, α
	U42413_at	*Prkag1*	−1.5	Protein kinase, AMP-activated, γ 1 noncatalytic subunit
	L16995_at	*Srebp-1c*	−1.5	Sterol regulatory element binding protein-1c
	U02553cds_s_at	*Ptpn16*	1.8	Protein tyrosine phosphatase, nonreceptor type 16
Energy metabolism	M96601_at	*Slc6a6*	4.3	Solute carrier family 6, member 6
	AI236284_s_at	*Slc21a5*	2.1	Solute carrier family 21, member 5
	AI175764_s_at	*Facl4*	−2.1	Fatty acid CoA ligase, long chain 4
	D17370_at	*Scd1*	−1.8	Stearoyl-CoA desaturase-1
	L01793_at	*chk*	−1.7	Choline kinase
	D13667cds_s_at	*Gyg*	−1.6	Glycogenin
	U53706_at	*Agxt*	−1.6	Alanine-glyoxylate aminotransferase
	U88036_at	*Mpd*	1.8	Mevalonate pyrophosphate decarboxylase
	AF003835_at	*Idi1*	2.4	Isopentenyl-diphosphate δ isomerase
	D37920_at	*Sqle*	2.4	Squalene epoxidase

The experiment was done twice with 3 rats in each group each time. Expression data were analyzed by using t test ($P < .05$, n = 6) with batch effect from the two repeated experiments removed.

Table 3
Differentially expressed genes in the hypothalamus of TPM- vs vehicle-treated rats after 7 days of treatment

Function	Probe set	Genes	Fold change	Description
Signaling proteins	L08595_at	*Nr4a2*	−1.8	Nuclear receptor subfamily 4, group A, member 2
	AI012534_at	*Gtf2a2*	−2.1	General transcription factor IIa
	U38653_s_at	*Itpr1*	1.9	Inositol 1,4,5-triphosphate receptor 1
	U88986_s_at	*Pld1*	2.2	Phospholipase D1
	S53987_at	*α7*	1.9	Nicotinic receptor alpha 7 subunit
Energy metabolism	AA957917_s_at	*Slc7a1*	−1.6	Solute carrier family 7, member 1
	X12459_at	*Ass*	−1.6	Arginosuccinate synthetase

The experiment was done twice with 3 rats in each group each time. Expression data were analyzed by using *t* test ($P < .05$, n = 6) with batch effect from the 2 repeated experiments removed.

When OGTT was conducted, TPM-treated rats showed an improved glucose-handling capacity, as shown by a decrease in blood glucose area under the curve (AUC) during 120 minutes (Fig. 2). The plasma insulin levels during OGTT did not show significant difference between the 2 groups, with AUC during 2 hours of 1837 ± 95 ng/mL in vehicle-treated rats compared with 1897 ± 168 ng/mL in TPM-treated rats.

The energy expenditure was measured on day 12 of treatment. Rats were housed in the indirect calorimetry chamber for 24 hours with free access to water and food. The oxygen consumption and carbon dioxide production were constantly measured at 30-minute intervals. Fig. 3 shows the average values of oxygen consumption and RQ values at every time point. The 24-hour average RQ values were similar between compound- and vehicle-treated groups. The oxygen consumption values at most time points were not markedly different between vehicle- and TPM-treated groups; however, TPM-treated rats showed significantly increased oxygen consumption values during 6 to 10 AM compared with rats in the vehicle group ($P < .05$).

3.2. Effects of TPM on gene expression profile in liver, hypothalamus, white adipose tissue, and skeletal muscle

Two groups of rats (n = 3 of each group) were treated with either vehicle or TPM (100 mg/kg per day). Food consumption was monitored daily in this study. TPM-treated rats showed a 33% reduction of food intake on the first day of study ($P < .01$, compared with that in vehicle-treated rats). However, these TPM-treated rats consumed a similar amount of food in 2 to 7 days compared with vehicle-treated rats. Rats from each group were killed after 7 days of dosing. Samples from liver, white adipose tissue (periovarian), skeletal muscle (soleus), and whole hypothalamus were collected for microarray analysis.

3.2.1. Effect of TPM on hepatic gene expression profile

TPM treatment altered the gene expression of metabolic enzymes and signaling regulatory proteins involved in energy metabolism in the liver as shown in Table 2. After 7 days' treatment, TPM decreased mRNA amounts for sterol regulatory element binding protein-1c (SREBP-1c), stearoyl-coenzyme A (CoA) desaturase (SCD)-1, choline kinase, which catalyzes the synthesis of phosphatidylcholine [9], and fatty acid CoA ligase, long chain 4 (FACL4). Three cholesterol synthesis genes, MPD, IDI1, and SQLE were up-regulated. TPM treatment also decreased the expression of arginine vasopressin receptor 1A (AVPR1a, V1A), a possible blood pressure control gene [10], and AMPKγ1, an AMP-binding subunit of universal cellular energy sensor AMPK [11]. In addition, TPM diminished mRNA of the glycogen metabolism enzyme glycogenin.

3.2.2. Effects of TPM on gene expression in hypothalamus, white adipose tissue, and skeletal muscle

In contrast to changes in hepatic gene expression by TPM treatment, fewer genes in the hypothalamus, white adipose tissue, and skeletal muscle were affected by TPM

Table 4
Differentially expressed genes in the white adipose tissue of TPM- vs vehicle-treated rats after 7 days of treatment

Function	Probe set	Genes	Fold change	Description
Signaling proteins	AI235758_s_at	*Prkar2b*	−1.8	Protein kinase, cAMP-dependent regulatory, type II β
	X77237_at	*Ppp5c*	−1.8	Protein phosphatase 5, catalytic subunit
	AA900476_g_at	*MGR1*	2.0	Cbp/p300-interacting transactivator
	AA799729_g_at	*Pde4b*	−1.9	Phosphodiesterase 4B
Energy metabolism	J02773_at	*Fabp3*	−2.1	Fatty acid binding protein 3
	AI236284_s_at	*Facl4*	−1.5	Fatty acid CoA ligase, long chain 4
	M26594_at	*Me1*	−1.5	Malic enzyme 1
	X52625_at	*Hmgcs1*	−1.5	3-Hydroxy-3-methylglutaryl-CoA synthase 1
	U64451_at	*Acadsb*	1.5	Acyl-CoA dehydrogenase, short/branched chain
	U36771_at	*Gpat*	1.8	Glycerol-3-phosphate acyltransferase, mitochondrial

The experiment was done twice with 3 rats in each group each time. Expression data were analyzed by using *t* test ($P < .05$, n = 6) with batch effect from the 2 repeated experiments removed.

Table 5
Differentially expressed genes in skeletal muscle of TPM vs vehicle-treated rats after 7 days of treatment

Function	Probe set	Genes	Fold change	Description
Signaling proteins	AF030163_s_at	*Ucp3*	−2.3	Uncoupling protein 3
	U16655_at	*Plcd4*	−1.7	Phospholipase C, δ 4
	AI171796_at	*Capn6*	−1.7	Calpain 6
	AA875225_at	*Gnai2*	1.9	GTP-binding protein (G-α-i2)
Energy metabolism	AF034577_at	*Pdk4*	−1.7	Pyruvate dehydrogenate kinase 4
	AF080468_at	*G6pt1*	−1.7	Glucose-6-phosphatase, transport protein 1
	X78593_g_at	*Gpd2*	−1.7	Glycerol-3-phosphate dehydrogenase 2
	D63834_at	*Slc16a1*	1.6	Solute carrier family 16, member 1
	M18467_at	*Got2*	1.7	Glutamate oxaloacetate transaminase 2

The experiment was done twice with 3 rats in each group each time. Expression data were analyzed by using *t* test ($P < .05$, n = 6) with batch effect from the 2 repeated experiments removed.

7-day treatment as shown in Tables 3-5. In the hypothalamus, TPM treatment affected the expression of several signaling genes, such as nicotinic receptor. In white adipose tissue, TPM treatment affected the expression of some genes involved in lipid metabolism (fatty acid oxidation, cholesterol synthesis, triglyceride synthesis) as well as a few signaling genes. In skeletal muscle, TPM down-regulated gene expression of UCP3, a key gene mediating energy expenditure.

3.2.3. Short-term effect of TPM on gene expressions in liver

Next, we examined the short-term effect of TPM on gene expression focused on liver tissue. Considering that changes in food intake observed in the first day of treatment could affect gene expression, we conducted a 16-hour pair-feeding study to eliminate the effect of food and to identify the effect of TPM on genes in liver that is independent of changes in food intake. In this study, in addition to vehicle- and TPM-treated rats, which had free access to food, we added 2 pair-feeding groups (Veh-PF and TPM-PF), which were fed with a limited amount of food similar to that consumed by TPM-treated rats. The gene expression data from these 4 groups were analyzed by using the general linear model of Partek Pro software to identify the treatment effect and the food effect. As shown in Table 6, the DNA microarray data revealed that 16 hours after a single administration, TPM markedly reduced hepatic gene expression related with fatty acid synthesis, eg, SCD1 (−2.3-fold vs vehicle group, $P < .01$), SCD2 (−5.3-fold vs vehicle group, $P < .01$), and acetyl-CoA carboxylase 2 (ACC2; −1.5-fold vs vehicle group, $P < .01$). Compared with vehicle-treated rats, the hepatic genes related with fatty acid β-oxidation, eg, 3-2-*trans*-enoyl-CoA isomerase and mitochondrial acyl-CoA thioesterase, were also increased by 1.8- and 1.6-fold, respectively ($P < .01$). However, TPM also decreased fatty acid CoA ligase (long chain 2 and long chain 5) by 1.5-fold compared with vehicle-treated rats. The TPM effect (treatment factor) on expression of the above-mentioned genes in liver was significant, whereas the pair-feeding effect (food factor) and the interaction between TPM and pair feeding were statistically insignificant, suggesting the expression of these genes involved in lipid metabolism was affected by TPM treatment but not by pair feeding.

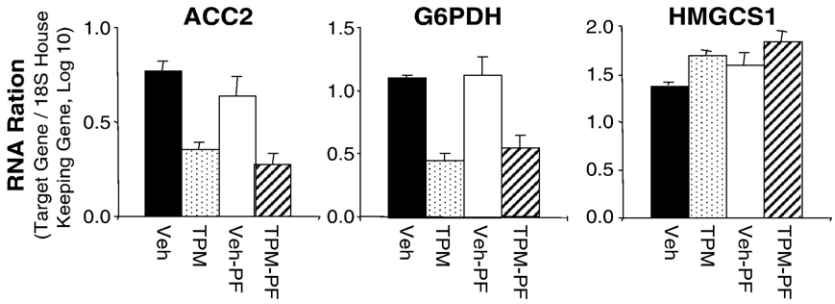
We further verified some of these genes by quantifying mRNA using qRT-PCR (Fig. 4). Compared with the vehicle group, the amounts of ACC2 and glucose-6-phosphate dehydrogenase (G6PDH) mRNA were decreased by approximately 60% and 70% in TPM and TPM-PF groups ($P < .01$ compared with Veh group, 2 × 2 factorial design), respectively, suggesting ACC2 and G6PDH expression was affected by TPM only. The 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) expression in TPM-PF rats

Table 6
Lipid metabolic genes in liver regulated by TPM treatment independently of reduced food intake[a]

Description	Fold change (TPM vs Veh)	*P*				Function
		TPM	Veh-PF[a]	TPM-PF[a]	Model	
Fatty acid CoA ligase, long chain 2	−1.5	.009	.942	.153	.033	Fatty acid oxidation
Fatty acid CoA ligase, long chain 5	−1.5	.004	.622	.969	.023	Fatty acid oxidation
3-2-*trans*-Enoyl-CoA isomerase	1.8	.001	.109	.567	.003	Fatty acid oxidation
Mitochondrial acyl-CoA thioesterase 1	1.6	.007	.538	.269	.031	Fatty acid oxidation
Isopentenyl-diphosphate δ isomerase	2.3	.003	.335	.866	.015	Cholesterol synthesis
Squalene epoxidase	2.2	.001	.301	.887	.005	Cholesterol synthesis
3-Hydroxy-3-methylglutaryl-CoA synthase 1	2.4	.009	.055	.275	.027	Cholesterol synthesis
Stearoyl-CoA desaturase 2	−5.3	.004	.314	.630	.023	Fatty acid synthesis
Acetyl-CoA carboxylase 2	−1.5	.001	.295	.389	.008	Fatty acid synthesis
Acetyl-CoA carboxylase 1	−2.3	.006	.230	.419	.027	Fatty acid synthesis
Glucose-6-phosphate dehydrogenase	−2.6	.000	.189	.241	.002	NADPH production

NADPH indicates nicotinamide adenine dinucleotide phosphate, reduced form.

[a] The experiment was done as a 2 × 2 factorial design (2 factors, TPM and pair feeding or PF, n = 3).



	P value			
	TPM	Veh*PF	TPM*PF	Model
Acetyl-CoA carboxylase 2	0.001	0.295	0.389	0.008
Glucose-6-phosphate dehydrogenase	0.000	0.189	0.241	0.002
HMG-Co A synthase 1	0.009	0.055	0.275	0.027

Fig. 4. qRT-PCR–determined relative hepatic mRNA expression of ACC2, G6PDH, and HMGCS1 in vehicle (Veh), topiramate (TPM), vehicle pair-feeding (Veh-PF), and TPM pair-feeding (TPM-PF) groups. Rats (n = 3) were treated with a single, orally administered dose of TPM (100 mg/kg), and total RNA was isolated from liver samples collected after 16 hours of treatment. Data are presented as the RNA ration of target gene/18s housekeeping gene with mathematical transformation using log (*Y*). The *P* values are results of 2 × 2 factorial ANOVA analysis.

showed an even higher expression compared with that in the TPM group. This phenomenon has been reconfirmed, although the explanation of this increase is still unknown.

4. Discussion

In our present study, female ZDF rats were used. Different from male rats, female ZDF rats do not become severely hyperglycemic unless they are fed a diabetogenic diet (Gmi/RD 13004) for more than 3 weeks [5]. However, when these rats were fed Purina 5008, they displayed mild hyperglycemia, insulin resistance, and glucose intolerance at the age of 8 to 10 weeks [6]. Our previous study showed that in female ZDF rats fed Purina 5008, TPM treatment for 7 to 9 days significantly improved insulin sensitivity during a hyperinsulinemic-euglycemic clamp study [6]. To explore the potential mechanisms underlying the TPM effect, we used this female ZDF model and applied DNA microarray technology in the present study to explore the effect of TPM on genes related with energy metabolism and signaling transduction.

Our present results showed that long-term TPM treatment significantly decreased the plasma glucose and triglyceride levels in female ZDF rats. These results are consistent with those of previous studies and published reports from other researchers [5,6]. TPM-treated rats also showed a significant reduction in AUC compared with vehicle-treated rats during OGTT, although the plasma insulin levels were similar between these 2 groups, suggesting an improved insulin sensitivity in TPM-treated rats. Furthermore, TPM seems not to affect the plasma levels of glucagon, corticosterone, leptin, adiponectin, and growth hormone, indicating that it is unlikely that these hormones are involved in the action of TPM on glucose and lipid metabolism.

We observed that there was a significant reduction of body weight gain in TPM-treated rats, although their food consumption was similar to that of vehicle-treated rats. Our energy expenditure data showed that TPM significantly increased oxygen consumption in the morning. This limited increase in oxygen consumption could partially contribute to the mechanisms by which TPM reduces body weight gain. TPM treatment did not induce a change in the RQ value, suggesting the increased energy expenditure might not be due to an increase of fatty acid utilization. Our gene expression data showed that TPM increased 2 genes involved in β-oxidation (3-2-*trans*-enoyl-CoA isomerase and mitochondrial acyl-CoA thioesterase). However, TPM decreased another 2 genes (fatty acid CoA ligase, long chain 2 and long chain 5) in the fatty acid oxidation process. The impact of the changes in these genes on lipid metabolism requires further evaluation. We also observed that TPM reduced UCP3 expression in skeletal muscle. Whether this down-regulation of UCP3 gene has any impact on energy expenditure in skeletal muscle needs to be examined. Thus, more detailed energy expenditure studies both in vivo and extra vivo are required to further analyze the effect of TPM on body weight control.

In the present study, we applied DNA microarray and quantitative RT-PCR methods to search potential genes involved in glucose and lipid metabolism that are affected by TPM treatment. Our microarray data showed that (1) the most marked effect of TPM on gene expression occurred in the liver, (2) most genes affected by TPM are genes associated with lipid metabolism, and (3) in some instances, the effect of TPM on gene expression is independent of food intake.

The results of diminished expression of ACC2 and SCD1 by TPM would partially mimic the phenotypes of knockout mice of ACC2 and SCD1, namely, lowered hepatic and plasma triglyceride concentrations, decreased hepatic very low-density liproprotein secretion, as well as altered glycerolipid fatty acid composition [12-14]. It has also been reported that in the liver of *ob/ob* and *aP2-SREBP-1c* transgenic lipodystrophy mice, fatty acid composition was markedly altered, with a large increase in C18:1 and decreases in C18:0 and C18:2, whereas elevated SCD1 in these mice could contribute to these abnormalities [15]. Therefore, a decrease in SCD1 and SCD2 mRNA expression and up-regulation of genes involved in β-oxidation of unsaturated fatty acids by TPM treatment would attenuate the perturbation of hepatic fatty acid composition. It is worth emphasizing that further studies (eg, using Tryton-treated animal models) are needed to explore the mechanisms of TPM on liver triglyceride secretion and lipid metabolism. The elevated plasma concentrations of total and HDL cholesterol and hepatic expression of cholesterol synthesis genes and down-regulation of bile synthesis genes suggest that TPM may elevate hepatic cholesterol synthesis and decrease bile synthesis.

SERBP-1c up-regulates fatty acid and triglyceride synthesis genes and thus plays a key role in lipid metabolism. In ZDF rats, there is an elevated hepatic SREBP-1c expression and triglyceride synthesis in liver [16,17]. TPM treatment decreased SREBP-1c expression that would, in turn, decrease expression of its target genes in fatty acid/triglyceride synthesis, thereby lowering plasma triglyceride concentration.

Factors that regulate SREBP-1c expression and nuclear translocation include insulin, dietary carbohydrates, fatty acids, and cholesterol [18]. Fasting could down-regulate SREBP-1c gene expression [19]. In our present study, we observed a 1.5-fold decrease in SREBP-1c mRNA level and down-regulation of its target gene, SCD1, after TPM treatment for 7 days when food intake was unaltered. This result suggested that TPM down-regulates SREBP-1c gene expression independently of food intake. Further studies need to be conducted to explore whether SREBP-1c is one of the initial targets of TPM to lower plasma triglyceride levels.

TPM collectively up-regulated cholesterol synthesis genes, suggesting that TMP may have a direct effect in the regulation of cholesterologenic genes. Gene expression of the entire cholesterol synthesis pathway appears to be almost exclusively regulated by SREBP-2 through its proteolytic processing and mRNA expression [18]. Although it remains to be determined whether and how TPM affects SREBP processing, TPM apparently differentially regulated the expression of SREBP-2 and SREBP-1c and their target genes. We found that TPM did not statistically increase SREBP-2 mRNA amounts, but up-regulated SREBP-2 target genes of cholesterol synthesis. Differential regulation of proteolytic cleavage of SREBPs was shown in stain and bile acid sequestrant–treated hamsters in which SREBP-2 expression and processing was increased, whereas SREBP-1c processing was decreased [20]. Stain and bile acid sequestrant decreased plasma cholesterol concentration, whereas TPM elevated plasma cholesterol concentration. The mechanisms whereby TPM differentially regulates SREBPs warrant further study.

Although we observed the expression of lipid metabolic–related genes changed after 16 hours of a single dose of TPM, we did not see a decrease in plasma triglyceride and glucose concentrations at the same time (data not shown), and these changes occurred after 14 days of treatment. This finding suggested that although TPM has a short-term effect on gene expression (minutes to hours), altering protein expression and subsequent physiology would take hours to days. The timing phenomenon about gene expression and physiologic responses has also been reported in a study with bile acid–treated mice [21]. Furthermore, the impacts of these genes changed by TPM need to be explored by using molecular techniques to understand the molecular mechanisms of TPM in lipid and glucose metabolism. In addition to changing gene expression, TPM could also regulate lipid and glucose metabolism at posttranslation levels, as Wilkes et al [22] have reported that TPM administration resulted in a 3- to 4-fold increase in circulating levels of total and high-molecular-weight adiponectin (Acrp30) and a 2-fold increase in phospho-AMPK in skeletal muscle in TPM-treated rats.

Although our present study suggested that improving hepatic lipid metabolism might be part of the mechanisms underlying the effect of TPM on obese-associated diabetic rodent models, several important questions remain to be addressed: (1) Whether these changes in hepatic gene expression are correlated with TPM effect on blood glucose control and lipid metabolism in ZDF rats requires further assessment. (2) Does TPM directly target these lipid metabolic genes, or indirectly via other mechanisms, such as an insulin/leptin signaling system? (3) Considering that TPM-induced changes in gene expression mostly occurred in the liver and not in the hypothalamus, skeletal muscle, and white adipose tissue, what is the relationship of changes in hepatic lipid metabolism with increased energy expenditure and improved insulin sensitivity in TPM-treated ZDF rats? Further studies focused on these issues will help us to better understand the mechanisms of the antidiabetes and antiobesity effects of TPM and to find novel targets to treat these metabolic disorders.

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