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Effects of Ingested Turmeric Oleoresin on Glucose and Lipid Metabolisms in Obese Diabetic Mice: A DNA Microarray Study

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Turmeric, the rhizome of *Curcuma longa* L., has a wide range of effects on human health. Turmeric oleoresin, an extract of turmeric, is often used for flavoring and coloring. Curcuminoids and turmeric essential oil are both contained in turmeric oleoresin, and both of these fractions have hypoglycemic effects. In the present study, we comprehensively assessed the effect of turmeric oleoresin on hepatic gene expression in obese diabetic KK-Ay mice using DNA microarray analysis and quantitative real-time polymerase chain reaction (PCR). Female KK-Ay mice aged 6 weeks ($n = 6$/group) were fed a high-fat diet containing turmeric oleoresin, curcuminoids, and essential oil for 5 weeks. The same diet without any of these fractions was used as a control diet. Ingestion of turmeric oleoresin and essential oil inhibited the development of increased blood glucose and abdominal fat mass, while curcuminoids only inhibited the increase in blood glucose. DNA microarray analysis indicated that turmeric oleoresin ingestion up-regulated the expression of genes related to glycolysis, β-oxidation, and cholesterol metabolism in the liver of KK-Ay mice, while expression of gluconeogenesis-related genes was down-regulated. Real-time PCR analysis was conducted to assess the contribution of the curcuminoids and essential oil in turmeric oleoresin to the changes in expression of representative genes selected by DNA microarray analysis. This analysis suggested that curcuminoids regulated turmeric oleoresin ingestion-induced expression of glycolysis-related genes and also that curcuminoids and turmeric essential oil acted synergistically to regulate the peroxisomal β-oxidation-related gene expression induced by turmeric oleoresin ingestion. These changes in gene expression were considered to be the mechanism by which the turmeric oleoresin affected the control of both blood glucose levels and abdominal adipose tissue masses. All of these results suggest that the use of whole turmeric oleoresin is more effective than the use of either curcuminoids or the essential oil alone.

KEYWORDS: ***Curcuma longa* L.; DNA microarray; turmeric extract; turmeric oleoresin; type 2 diabetes; KK-Ay mice; blood glucose**

INTRODUCTION

The development of type 2 diabetes is mainly caused by insulin resistance, which is closely associated with abdominal obesity (*1*). This relationship between the clinical condition, insulin resistance, and obesity is also applicable in hypertension and dyslipidemia. Currently, diabetes, obesity/abdominal obesity, hypertension, dyslipidemia, and insulin resistance are recognized as being closely linked to each another. These clinical conditions are all risk factors for cardiovascular disease. The presence of a cluster of these risk factors in the same individual has been termed metabolic syndrome, which is a major public health problem (*2*, *3*).

Turmeric, the rhizome of *Curcuma longa* L., is widely used as a spice, coloring, and flavoring and in traditional medicines. Turmeric and its extracts have various beneficial effects on human health (*4*). Turmeric oleoresin, an extract prepared from turmeric using organic solvents such as acetone, ethanol, and hexane, is also widely used for flavoring or coloring. There are

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two major fractions in turmeric oleoresin: curcuminoids (CU) and turmeric essential oil (TE). CU, comprising curcumin, demethoxycurcumin, and bisdemethoxycurcumin, are yellowish turmeric pigments and have antioxidative (*5*, *6*), anticarcinogenic (*7*, *8*), antiinflammatory (*9*), antihepatotoxic (*10*), and hypocholesterolemic (*11*) activities. TE contains sesquiterpenoids, including ar-turmerone, α-turmerone, β-turmerone, and curlone, which have antioxidative (*12*) and mosquitocidal (*13*) activities, and the essential oil also inhibits the production of prostaglandin E2 and NO (*14*, *15*).

We previously reported that both the CU and the sesquiterpenoids present in turmeric suppress the increase in blood glucose levels in type 2 diabetic KK-Ay mice and that, in an in vitro study in these animals, the effect of turmeric extract or its components on adipocyte differentiation was found to be related to the antihyperglycemic effect (*16*).

In addition, turmeric and CU have both been suggested to have many effects on hepatic function, such as activation of cholesterol metabolism (*11*, *17*) and of fatty acid catabolism (*18*). Moreover, turmeric and CU are reported to suppress blood glucose levels by changing the activity of some of the enzymes related to gluconeogenesis and glycolysis in the liver of alloxan-induced diabetic rats (*19*, *20*). However, a comprehensive evaluation of the effects of turmeric on gene expression in vivo has not been conducted. In addition, there are no detailed studies on the effects of sesquiterpenoids, which we have reported have a hypoglycemic effect on glucose metabolism (*16*).

In the present study, we investigated the effects of three turmeric extracts, oleoresin, CU, and essential oil, on glucose and lipid metabolism in obese diabetic KK-Ay mice. Furthermore, to ascertain the mechanism by which turmeric oleoresin exerts an antihyperglycemic effect on the liver, we conducted DNA microarray analysis and screened genes that had changes in expression upon ingestion of turmeric. We also report on real-time polymerase chain reaction (PCR) analysis comparing the effects of the three turmeric extracts on the changes in expression of representative genes selected by DNA microarray analysis.

MATERIALS AND METHODS

Reagents. CU, ca. 90% (w/w) pure, and TE, ca. 70% (w/w) pure, both derived from the plant *C. longa* L., were purchased from Maruzen Pharmaceuticals Co., Ltd. (Hiroshima, Japan). The CU and TE used in this study were food additive grade. Other chemicals, solvents, and reagents were reagent grade and were purchased from Nacalai Tesque (Kyoto, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Invitrogen Corp. (Carlsbad, CA).

Preparation of Oleoresin Powder. Turmeric oleoresin powder (TOP) is a fine powder containing turmeric oleoresin at 40% (w/w) and auxiliary substances such as zein, corn starch, and dextrin at 20% (w/w) each. To prepare TOP, turmeric oleoresin was extracted from the root of turmeric (*C. longa* L. harvested in India) using acetone and ethanol. After the solvents were removed, the oleoresin was mixed with starch and zein and spray-dried to produce a fine powder. This powder contained CU at about 14% (w/w) and essential oil at about 17% (w/w).

Animal Experiment. Genetically obese diabetic female KK-Ay/Ta mice were purchased from CLEA Japan, Inc. (Tokyo, Japan) and maintained on a normal laboratory diet CE-2 (CLEA) for 1 week before starting the experiment. The mice were housed in an animal laboratory under controlled ambient conditions: temperature, 20−24 °C; humidity, 45−65%; and a 12 h light/dark cycle (7:30−19:30 and 19:30−7:30). Mice aged 6 weeks were divided into six groups by body weight and blood glucose levels, and each group was reared in a separate cage. The control group was fed a basal high-fat diet (**Table 1**; Oriental Yeast, Japan), and test groups were fed a basal diet containing TOP (0.5, 1.0, or 2.0% w/w), CU (0.15% w/w), or TE (0.24% w/w). The diets containing CU or TE had equivalent amounts of CU or TE as that contained in the 1% TOP diet. The basal diet was adopted to exaggerate the status of diabetes. Mice received these diets and water ad libitum for 5 weeks. The total food and water intake in each individual test group was monitored every 3−4 days during the study. Blood samples for the measurement of blood glucose were obtained from the tail vein at a fixed time (8:30−9:00) 1, 2, 3, and 4 weeks after initiation of the test diets. At 5 weeks from initiation of the test diets, the mice were euthanized under diethylether anesthesia and their serum and liver, kidney, mesenteric, perirenal, and periuterine adipose tissues were sampled. The sampling was conducted at 9:00−10:30, during which time the mice were allowed continuous access to the feed. The plasma samples were stored at −80 °C until the time of glucose, insulin, leptin, and adiponectin analyses. The liver samples were stored in RNAlater (Qiagen, Valencia, CA) at −80 °C until DNA microarray and quantitative real-time PCR analyses. The study protocol was reviewed and approved by the animal ethics committee of Kaneka Corp.

Table 1. Composition of High-Fat Diet as Basal Experimental Diet[a]

ingredient	composition (g/kg)
casein	250
corn starch	148.69
sucrose	200
soybean oil	20
lard	140
beef tallow	140
cellulose powder	50
AIN-93 mineral mixture[b]	35
AIN-93 vitamin mixture[b]	10
choline bitartrate	2.5
tert-butylhydroquinone	0.06
L-cystine	3.75

[a] The high-fat diet as basal experimental diet was prepared at Oriental Yeast, Co., Ltd. The energy ratio was 53% fat, 27% carbohydrate, 20% protein, and 21 MJ/kg diet total energy. [b] AIN-93 mineral mixture and AIN-93 viamin mixture (*31*).

Measurements of Serum Glucose, Insulin, Leptin, and Adiponectin. Blood glucose levels were measured up to 4 weeks after the start of feeding using a blood glucose level monitor Glutest Ace kit (Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan). Serum insulin, leptin, and adiponectin levels were measured using an ELISA Insulin Kit, ELISA Mouse Leptin Kit (Morinaga Institute of Biological Science, Yokohama, Japan), and Mouse/Rat Adiponectin ELISA Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) according to the manufacturer's protocol.

DNA Microarray Analysis. To determine gene expression differences in the liver between the control and the 1% TOP groups, we conducted DNA microarray analysis using three mice selected from each group. The selection was based on the blood glucose levels at 4 weeks after initiation of the test diets. We selected three particular mice with blood glucose levels that were close to the median for each group. The total RNA was extracted from the liver using RNeasy Mini Kit (Qiagen) and checked for quality and quantity by agarose gel electrophoresis and spectrophotometry. DNA microarray analysis was conducted according to the manufacturer's protocol using the Affymetrix mouse genome 430 2.0 array (Affymetrix, Santa Clara, CA) with 43000 genes. Briefly, 10 μg of the total RNA was used to synthesize cDNA, and the resultant cDNA was used as a template for synthesis of biotinylated cRNA by T7 DNA polymerase. After fragmentation, the cRNA was added, hybridized, and stained to the Affymetrix mouse genome 430 2.0 array as per the manufacturer's protocol guides. The fluorescence signals were scanned by the Affymetrix GeneChip System.

Analysis of DNA Microarray Data. The global normalization method was used to correct for minor differences in the amount of each cRNA added to the microarrays. In each microarray, gene expression values, calculated from fluorescence signals by the Affymetrix GeneChip Operating Software, were classified with detection calls indicating whether or not the transcript of the gene was reliably detected

Table 2. Body Weight Gain and Adipose Tissue Weights of KK-Ay Mice Fed High-Fat Diets Containing TOP, CU, or TE[a–c]

item	control diet	TOP diet 0.5%	TOP diet 1%	TOP diet 2%	CU diet	TE diet
body weight gain (g/5 weeks)	24.1 ± 1.0	21.4 ± 0.5	19.4% ± 1.6*	19.1% ± 0.8**	22.0 ± 1.4	21.7 ± 0.5
			adipose tissue weight (g)			
periuterine	5.96 ± 0.21	5.93 ± 0.18	5.34 ± 0.16	5.20 ± 0.22	6.09 ± 0.29	6.00 ± 0.23
perirenal	2.43 ± 0.07	2.09 ± 0.10	2.09 ± 0.15	1.78 ± 0.07**	2.22 ± 0.09	1.94 ± 0.16*
mesenteric	1.90 ± 0.05	1.81 ± 0.07	1.69 ± 0.08*	1.56 ± 0.04**	1.71 ± 0.03	1.63 ± 0.02*

[a] Values are means ± SEM, $n = 6$. [b] * and ** indicate differences from the control at $P < 0.05$ and $P < 0.01$ by Dunnett's multiple comparison test. [c] The diet containing CU or TE has the equivalent amounts of CU or TE included in 1% TOP diet, and the control diet does not contain any of them.

(Present), marginally detected (Marginal), or not detected (Absent), using Affymetrix Statistical Algorithms. In our study, we selected genes for use in the evaluation of the effects of TOP administration using the following criteria: genes detected as "present" in all microarrays in one test group, which has a higher mean expression value for that gene than seen in the other test group; genes with mean expression values >20 in one test group, which has a higher mean expression value for that gene than seen in the other test group; genes with changes in expression of more than 1.25-fold in the TOP group, as compared with the mean expression value in the control group; and genes with significantly different expression values ($P < 0.05$) between the control and the TOP groups on the Student *t*-test.

Real-Time PCR. Quantitative real-time PCR was performed according to the TaqMan probe method. Appropriate primers and FAM (carboxyfluorescein)-labeled TaqMan probes for mouse G6pc (glucose-6-phosphatase, catalytic), Gck (glucokinase), Acox1 (acyl-CoA oxidase 1, palmitoyl), Ehhadh (enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase), Hmgcs (3-hydroxy-3-methylglutaryl-coenzyme A synthase 1), and Gapdh (glyceraldehyde-3-phosphate dehydrogenase) were commercially available (TaqMan Gene Expression Assays; Applied Biosystems, Japan). The products were designed so that they did not amplify genomic DNA. The respective TaqMan Gene Expression Assay identities for mouse G6pc, Gck, Acox1, Ehhadh, Hmgcs, and Gapdh were Mm00839363_m1, Mm00439129_m1, Mm00443579_m1, Mm01343141_m1, Mm01304569_m1, and Mm99999915_g1. Total RNA from the liver was extracted as mentioned above. Reverse transcription (RT) was carried out with 1 μg of the total RNA in a 50 μL reaction mixture using a High Capacity cDNA Archive kit (Applied Biosystems). The universal master mix (Applied Biosystems) containing the PCR buffer, MgCl2, dNTPs, and thermal stable AmpliTaq Gold DNA polymerase were used in the PCR reactions. Then, the PCR mixture containing primers, TaqMan probes, RNase/Dnase free water, and 1 μL of the RT product was added to the universal master mix to obtain a final volume of 50 μL per reaction. The reaction mixture was incubated at 95 °C for 10 min to activate the AmpliTaq Gold DNA polymerase and then run for 40 cycles at 95 °C for 15 s and 60 °C for 60 s in the ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The PCR results were analyzed with ABI SDS software (Applied Biosystems). The relative expression levels of each sample were calibrated by the standard curve method, and the expression of Gapdh was used for normalization.

Statistical Analysis. Data were analyzed using the SAS/STAT software computerized statistical analysis program (SAS Institute, Cary, NC). When significant differences were detected by one-way analysis of variance, Dunnett's multiple comparison test was applied. Differences were considered significant at $P < 0.05$. Values are given as means ± standard errors of means (SEM).

RESULTS

Animal Experiment. When female KK-Ay control mice aged 6 weeks were fed a high-fat diet, their body weight increased from 28.9 ± 0.5 to 52.9 ± 1.5 g in 5 weeks, with a body weight gain of 24.1 ± 1.1 g/5 weeks (**Table 2**). Body weight gain was significantly lower in the 1 and 2% TOP groups than in the control group (**Table 2**). Food intake was nearly the same in all groups. On the basis of food intake and body weight, the doses of TOP in the 0.5, 1, and 2% TOP groups were set at 478, 987, and 1894 mg/kg body weight/day, and those of CU or TE in the CU and TE groups were set at 156 and 230 mg/kg body weight/day, respectively. The amounts of CU in the CU group and of TE in the TE group were calculated to be approximately the same as the amount of CU and TE given to the 1% TOP group.

Mesenteric adipose tissue weight was significantly lower in the 1 and 2% TOP groups and in the TE group than in the control group (**Table 2**). In the CU group, mesenteric adipose tissue weight tended to be lower, although not significantly ($P < 0.07$), than in the control group. Perirenal adipose tissue weight was significantly lower in the 2% TOP group and in the TE group than in the control group (**Table 2**). In contrast, no significant differences were observed in the weights of liver and kidney samples (data not shown).

After feeding, the blood glucose level gradually increased in the control group (**Figure 1**), indicating the development of hyperglycemia. On the other hand, blood glucose levels were significantly lower in the 1 and 2% TOP groups than in the control group at 2, 3, and 4 weeks (**Figure 1**). However, no dose-dependent blood glucose level responses were observed in the 1 and 2% TOP groups. Blood glucose levels were significantly lower in the CU group than in the control group at 2, 3, and 4 weeks group and were significantly lower in the TE group than in the control group at 2 and 4 weeks (**Figure 1**). With respect to serum leptin, adiponectin, and insulin levels, no significant differences were observed among any of the test groups (data not shown).

DNA Microarray Analysis. To characterize the mechanism of the effects of TOP in KK-Ay mice fed the high-fat diet, gene expression levels in the livers of three mice in the control group and in the 1% TOP group were analyzed using a DNA microarray with 43000 genes. In this study, genes with significant changes in expression levels ($P < 0.05$) and where there was a >1.25-fold difference in mean expression levels between the control group and the 1% TOP group were defined as the genes with biologically significant changes in expression levels. According to our estimate, TOP ingestion up-regulated the expression of 548 genes and down-regulated the expression of 621 genes. We subsequently categorized and screened the resulting genes for their relation to glucose and lipid metabolism, since TOP acted to suppress the increase in blood glucose and abdominal fat accumulation. Screening was based on metabolic function in gene ontology and the mouse metabolic pathway map. The genes that we categorized are shown in **Table 3** for glucose metabolism and in **Table 4** for lipid metabolism.

TOP ingestion changed the expression of nine genes related to glucose metabolism (**Table 3**). Among these nine genes, five related to glycolysis, including glucokinase (Gck) and enolase 1, α non-neuron (Eno1), were up-regulated, and the remaining four, related to gluconeogenesis, including glucose-6-phos-

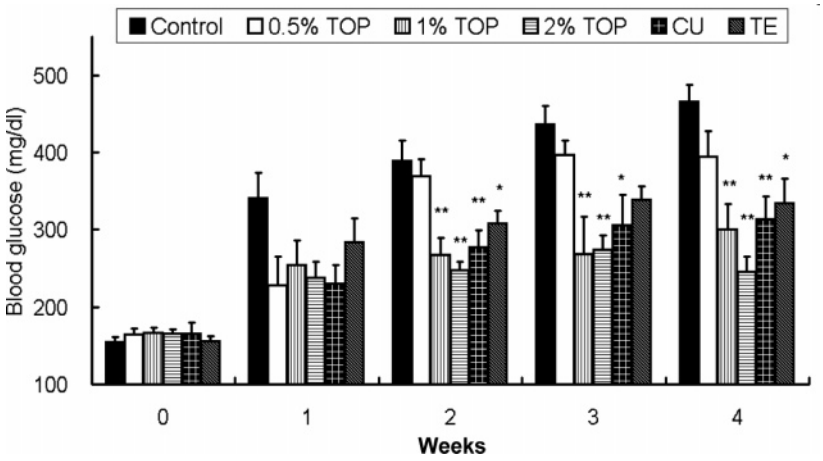

Figure 1. Blood glucose levels in KK-Ay mice fed a diet containing various amounts of TOP (turmeric oleoresin powder), CU (curcuminoids), or TE (turmeric essential oil), where the control diet does not contain any of them. Values are means ± SEM ($n = 6$). Significant differences were observed at $P < 0.05$* and $P < 0.01$** in Dunnett's multiple comparison test.

Table 3. DNA Array Data on Glucose Metabolism-Related Genes Induced by TOP Ingestion in Liver of KK-Ay Mice

gene name	symbol	fold change[a]	accession no.[b]
glycolysis-related enzyme			
glucokinase	Gck	1.78	BC011139
enolase 1, α non-neuron	Enol	1.34	NM_023119
aldolase 3, C isoform	Aldo3	2.09	BC008184
2,3-bisphosphoglycerate mutase	Bpgm	1.58	NM_007563
pyruvate dehydrogenase complex, component X	Pdhx	1.31	BB667859
gluconeogenesis-related enzyme			
glucose-6-phosphatase, catalytic	G6pc	−1.48	NM_008061
phosphoenolpyruvate carboxykinase 1, cytosolic	Pck1	−1.35	AW106963
serine dehydratase	Sds	−1.93	BC021950
glutamate oxaloacetate transaminase 1, soluble	Got1	−1.46	AA792094

[a] Relative gene expression levels between control and 1% TOP groups; a positive value means that an up-regulated gene was induced by TOP ingestion, and a negative value means that a down-regulated gene was induced by TOP ingestion. [b] GenBank ID.

phatase, catalytic (G6pc), phosphoenolpyruvate carboxykinase 1, cytosolic (Pck1), glutamate oxaloacetate transaminase 1, soluble (Got1), and serine dehydratase (Sds), were down-regulated. These results suggest that TOP suppressed glucose production and enhanced glucose catabolism in the liver (**Table 3**). With regard to lipid metabolism (**Table 4**), TOP ingestion up-regulated the expression of 32 genes, two of which were related to fatty acid synthesis, including malic enzyme, supernatant (Mod1), and seven of which were related to β-oxidation, including acyl-coenzyme A oxidase 1, palmitoyl (Acox1), hydroxyacyl-coenzyme A dehydrogenase/3-ketoacyl-coenzyme A thiolase/enoyl-coenzyme A hydratase (trifunctional protein), β-subunit (Hadhb), and enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase (Ehhadh), and the subtypes of fatty acid-coenzyme A ligase, long chain (Facl2, Facl4, and Facl5). The 32 up-regulated genes included 11 genes involved in cholesterol synthesis by 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (Hmgcs1) and four in cholesterol catabolism (bile acid biosynthesis) by acetyl-coenzyme A acyltransferase 1 (Acaa1). On the other hand, the expressions of only two genes related to β-oxidation and cholesterol metabolism were down-regulated by TOP ingestion (**Table 4**). The two genes were carnitine palmitoyltransferase 1a, liver (Cpt1a), which was related to β-oxidation, and cytochrome P450, family 39, subfamily a, polypeptide 1 (Cyp39a1), related to cholesterol catabolism (**Table 4**). These results suggested that TOP ingestion up-regulated β-oxidation and cholesterol metabolism. With respect to other genes related to fatty acid metabolism, the expression of several genes, including the acyl-CoA thioesterase family (Cte1, Pte1, Mte1, Pte2a, and Acot12), was up-regulated by TOP ingestion.

Quantitative Real-Time PCR. To validate the DNA microarray analysis and compare the effects of each turmeric compound, we measured the representative genes changed by TOP ingestion in DNA microarray analysis using quantitative real-time PCR in the liver of the control, 1% TOP, CU, and TE groups. The representative genes were selected according to metabolic functions in terms of gluconeogenesis (G6pc), glycolysis (Gck), β-oxidation (Acox1 and Ehhadh), and cholesterol synthesis (Hmgcs). In the 1% TOP group, the expression of Gck, Acox1, Ehhadh, and Hmgcs was significantly higher than in the control group on real-time PCR evaluation. The expression of G6pc in the 1% TOP group was slightly, but not significantly, lower than in the control group. The values for changes in gene expression between the control and the 1% TOP groups that were obtained on quantitative real-time PCR analysis were consistent with those seen on DNA microarray analysis. These results indicate that the changes in expression of these genes in our DNA microarray analysis were clearly correlated with the quantitative real-time PCR analysis on all samples in each group. Furthermore, the expression of Gck and Acox1 in the CU group and of Acox1 in the TE group was significantly higher than in the control group. Although not significant, there was a tendency toward up-regulation of Ehhadh ($P < 0.08$) and down-regulation of G6pc ($P < 0.08$) in the TE group (**Figure 2**). In addition, the expression of Hmgcs in the CU group tended to be higher, although not significantly ($P < 0.06$), than in the control group (**Figure 2**).

DISCUSSION

TOP is a fine powder containing turmeric oleoresin at 40% (w/w). It is easier to handle and ingest than oleoresin itself, which has a higher viscosity. CU and TE are the main constituents of turmeric oleoresin, amounting to approximately 80% (w/w) of turmeric oleoresin. The most abundant component in TE is considered to be ar-turmerone, a sesquiterpenoid (*21*, *22*). Our previous study demonstrated that CU and sesquiterpenoids suppress the increase in blood glucose levels in obese

Table 4. DNA Array Data on Lipid Metabolism-Related Genes Induced by TOP Ingestion in Livers of KK-Ay Mice

gene name	symbol	fold change[a]	accession no.[b]
	fatty acid synthesis-related enzymes		
NADH dehydrogenase (ubizuinone) 1, α/β subcomplex, 1	Ndufab 1	1.44	AK101307
malic enzyme, supernatant	Mod1	2.82	BC011081
	β-oxidation-related enzymes		
acyl-coenzyme A oxidase 1, palmitoyl	Acox1	1.27	AB034914
enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase	Ehhadh	1.40	NM_023737
carnitine acetyltransferase	Crat	1.71	BC006668
fatty acid coenzyme A ligase, long chain 2	Facl2	1.37	BI413218
fatty acid-coenzyme A ligase, long chain 4	Facl4	1.70	AB033886
fatty acid coenzyme A ligase, long chain 5	Facl5	1.39	AK006541
hydroxyacyl-coenzyme A dehydrogenase/ 3-ketoacyl-Coenzyme A thiolase/ enoyl-coenzyme A hydratase (trifunctional protein), β-subunit	Hadhb	1.28	BG866501
carnitine palmitoyltransferase 1a, liver	Cpt1a	–1.78	BB119196
	other fatty acid metabolism-related enzymes		
cytosolic acyl-CoA thioesterase 1	Cte1	1.36	NM_012006
lipase, endothelial	Lipg	1.45	BC020991
peroxisomal acyl-CoA thioesterase 1	Pte1	1.25	NM_133240
mitochondrial acyl-CoA thioesterase 1	Mte1	1.81	NM_134188
peroxisomal acyl-CoA thioesterase 2A	Pte2a	2.25	NM_134246
acyl-CoA thioesterase 12	Acot12	1.33	AB078618
glycerol-3-phosphate acyltransferase, mitochondrial	Gpam	1.72	NM_008149
ATP-binding cassette, subfamily D (ALD), member 2	Abcd2	2.60	NM_011994
L-3-hydroxyacyl-coenzyme A dehydrogenase, short chain	Hadhsc	–2.12	BB114220
adiponectin receptor 1	Adipor1	–1.29	BC014875
lysophospholipase 1	Lypla1	–1.32	BC013536
	cholesterol synthesis-related enzymes		
phosphomevalonate kinase	Pmvk	2.76	BI713896
3-hydroxy-3-methylglutaryl-coenzyme A synthase 1	Hmgcs1	2.64	BB705380
squalene epoxidase	Sqle	4.80	NM_009270
cytochrome P450, 51	Cyp51	2.88	NM_020010
sterol-C4-methyl oxidase-like	Sc4mol	2.14	AK005441
isopentenyl-diphosphate δ isomerase	Idi1	3.13	BC004801
mevalonate kinase	Mvk	1.63	BC005606
farnesyl diphosphate farnesyl transferase 1	Fdft1	2.57	NM_010191
mevalonate (diphospho) decarboxylase	Mvd	2.88	NM_38656
NAD(P)-dependent steroid dehydrogenase-like	Nsdhl	2.66	BC019945
jydroxysteroid (17-β) dehydrogenase 7	Hsd17b7	1.77	NM_010476
	cholesterol catabolism (bile acid biosynthesis)-related enzymes		
acetyl-coenzyme A acyltransferase 1	Acaa1	1.30	NM_130864
NAD(P)-dependent steroid dehydrogenase-like	Nsdhl	2.66	BC019945
hydroxysteroid (17-β) dehydrogenase 12	Hsd17b12	1.50	AK012103
aldehyde dehydrogenase family 3, subfamily A2	Aldh3a2	1.56	NM_007437
cytochrome P450, family 39, subfamily a, polypeptide 1	Cyp39a1	–1.56	NM_018887

[a] Relative gene expression levels between control and 1% TOP group; a positive value means that an up-regulated gene was induced by TOP ingestion, and a negative value means that a down-regulated gene was induced by TOP ingestion. [b] GenBank ID.

diabetic KK-Ay mice (*16*). However, the objective of the present study was to analyze the effects of TOP on gene expression in the liver of KK-Ay mice by DNA microarray analysis and to assess whether CU or TE containing sesquiterpenoids contributed to the transcriptional changes induced by TOP.

The active component of turmeric in terms of the effect on blood glucose levels has been reported to be curcumin (*20*). In addition, we have previously reported that curcumin, demethoxycurcumin, and bisdemethoxycurcumin, belonging to CU, and ar-turmerone, belonging to essential oil, are related to the blood glucose lowering effect, induced via activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) (*16*). The representative components of the CU and the essential oil were measured using the high-performance liquid chromatography (HPLC) method reported previously (*16*). The concentrations of curcumin, demethoxycurcumin, bisdemethoxycurcumin, and ar-turmerone were 6.5, 2.3, 1.9, and 3.9%, respectively, in TOP; 74.7, 17.6, 3.6, and <1.0%, respectively, in CU; and <1.0, <1.0, <1.0, and 12.8%, respectively, in TE.

We found that TOP, CU, and TE ingestion significantly suppressed the increase in blood glucose levels (**Figure 1**). These results are highly consistent with our previous report (*16*). The relation between the blood glucose lowering effect and the dosage of individual components such as curcumin, demethoxy-

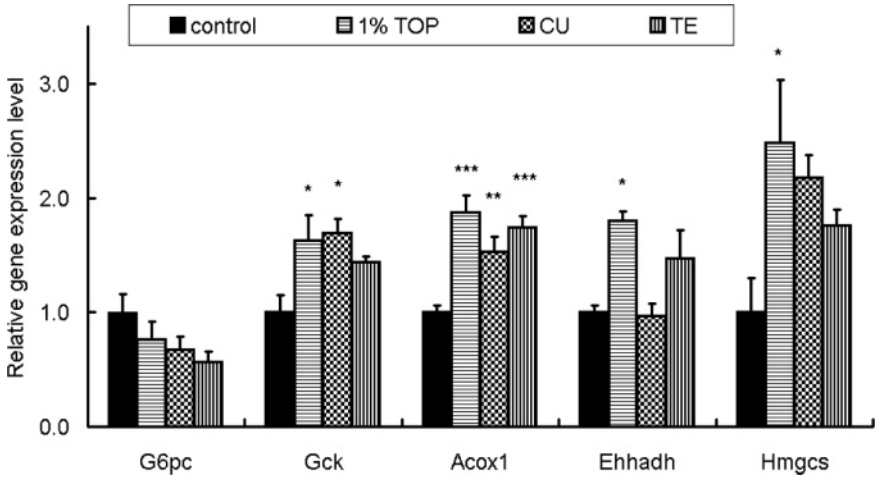

Figure 2. Validation of the changed gene induced by TOP-consuming KK-Ay mice in DNA microarray analysis by using quantitative real-time PCR and the effect of individual TOP components (CU and TE) to the expression of these changed genes. Values are means ± SEM ($n = 6$). Significant differences were observed at $P < 0.05$*, $P < 0.01$**, and $P < 0.001$*** in Dunnett's multiple comparison test. G6pc, glucose-6-phosphatase, catalytic; Gck, glucokinase; Acox1, acyl-coenzyme A oxidase 1, palmitoyl; Ehhadh, enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase; and Hmgcs, 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1.

curcumin, bisdemethoxycurcumin, and ar-turmerone were also highly consistent with our previous report (*16*). This suggested that the auxiliary substances used in TOP were less likely to have any significant effect on blood glucose levels. The weight of the mesenteric and perirenal adipose tissues in the abdomen was decreased by TOP ingestion. These effects were also observed with TE ingestion. On the other hand, a tendency toward a decrease in weight was observed for the mesenteric adipose tissue in the CU group, but the effect was weaker than that evoked by either TOP or TE ingestion (**Table 2**). Asai et al. (*18*) reported that CU suppressed the high-fat diet-induced increase in abdominal adipose tissue. The discrepancy between this study and our study was considered to be due to a difference in the dose of CU used. In our study, the 1% TOP group was designed to ingest the equivalent amount of CU in the CU group and essential in the TE group. The effect of the three samples on decreasing abdominal adipose tissue can be summarized as follows: 1% TOP = TE ≫ CU. Thus, we concluded that the contribution of TE to the effect of TOP on decreasing abdominal adipose tissue was stronger than that of CU. Also, body weight gain was suppressed by TOP ingestion (**Table 2**) without a concomitant decrease in the weights of other tissues, such as the liver and kidney (data not shown). Therefore, the suppressing effect of TOP on body weight gain was considered to at least partially relate to the decreases in the weight of adipose tissue induced by an additive effect of the CU and TE contained in TOP.

In our previous study, we showed that both the CU and the sesquiterpenoids in turmeric activate PPAR-γ in vitro and considered that this effect was related to the hypoglycemic effect of these compounds in KK-Ay mice. Thiazolidinediones (TZDs), well-known compounds that are PPAR-γ activators, are known to reduce insulin resistance via improvement in adipose cell functions, such as glucose uptake and adipocytokine secretion. Treatment with TZDs has been reported to increase body weight (*23*−*25*) and mesenteric adipose tissue (*24*) and to change levels of serum adipocytokines, including adiponectin and leptin (*25*), in KK-Ay mice. In this study, the effects of TOP, CU, or TE ingestion were not similar to reported effects of TZDs ingestion in KK-Ay mice (*23*−*25*), because TOP and TE ingestion decreased body weight or abdominal adipose tissue weight (**Table 2**) and TOP, CU, and TE ingestion did not changed serum adiponectin and leptin levels (data not shown). On the basis of this comparison, we hypothesized that there was another mechanism associated with the suppressant effect seen with turmeric compounds on the increase in blood glucose and abdominal adipose tissue. On the other hand, ingestion of either turmeric extract or curcumin has been reported to increase the activity of hepatic enzymes related to glucose metabolism (*20*) and lipid oxidation (*18*). Therefore, we analyzed the expression of various genes to characterize the effect of TOP, CU, and TE in the liver.

We assessed the effect of TOP containing both CU and TE on gene expression profiles in the liver using DNA microarray analysis. To the best of our knowledge, this study provides the first DNA microarray analysis of the effect of turmeric. TOP ingestion up-regulated the expression of five genes related to glycolysis and down-regulated the expression of four genes related to gluconeogenesis (**Table 3**). Gck, one of the up-regulated glycolysis-related genes, is a member of the hexokinase family (hexokinase type IV) that catalyzes the first committed step in glycolysis. This indicates that TOP ingestion activates glycolysis. Pck1, one of the down-regulated gluconeogenesis-related genes, is a rate-determining enzyme catalyzing the first committed step in gluconeogenesis. Another down-regulated gluconeogenesis-related gene, G6pc, is a key enzyme in gluconeogenesis, catalyzing the hydrolysis of D-glucose 6-phosphate to D-glucose. In addition, Got1 species are key enzymes in the malate-aspartate shuttle, having an important role in gluconeogenesis. Therefore, down-regulation of the expression of these enzymes indicated that TOP ingestion was associated with inactivation of gluconeogenesis. Moreover, real-time PCR analysis of the liver in all groups showed that ingestion of CU and TOP, but not of TE, was associated with up-regulation of Gck expression (**Figure 2**). These results indicate that the CU contained in TOP plays a major role in the up-regulation of Gck expression upon TOP ingestion. The evaluation of mean expression values showed that TOP, CU, and TE ingestion tended to down-regulate the expression of G6pc, although not significantly (**Figure 2**).

Glucose homeostasis depends largely on the balance between the formation of sugar in the liver and its utilization in liver, muscle, and adipose tissue. Expression of the Gck gene is known to be low in type 2 diabetes (*26*, *27*). Either up-regulation of Gck gene expression or Gck enzyme activity has been reported to be able to suppress an increase in blood glucose (*26*−*28*).

Thus, we concluded that the TOP or CU ingestion-induced suppression of the increase in blood glucose levels was attributable mainly to the activation of glycolysis and inactivation of gluconeogenesis in liver. Arun et al. (*20*) suggested that ingestion of either turmeric or curcumin was associated with suppression of increased blood glucose in the alloxan-induced diabetic rat via elevation of the activity of hexokinase and reduction in the activity of G6pc in the liver. Although the change in expression of G6pc was not found to significant on real-time PCR analysis, our gene expression results somewhat support this aforementioned report.

TOP ingestion up-regulated the expression of many genes related to β-oxidation (**Table 4**). First, Facl2, Facl4, and Facl5, known as the acyl-CoA synthetase family, play a key role in the early stage of β-oxidation. In particular, Facl4 and Facl5 (acyl-CoA synthetase 4 and 5) are known to be located in the peroxisome and in the mitochondria, respectively, and both play important roles in β-oxidation (*29*). Second, Acox1 is the first and rate-determining enzyme in the peroxisomal β-oxidation pathway. Ingestion of CU was suggested to reduce liver and plasma triacylglycerol levels in obese rats fed a high-fat diet, the mechanism of this reduction being the activation of β-oxidation in the liver (*18*). In this case, an increase in activity of the Acox1 enzyme, induced by ingestion of CU, is attributable to activation of β-oxidation in the liver (*18*). This agrees in part with our DNA microarray results obtained in mice that ingested TOP. Finally, Ehhadh and Hadhb are the enzymes involved in the late stages of the β-oxidation pathway in peroxisomes (for Ehhadh) or in the mitochondrial matrix (for Hadhb). On the other hand, expression of Cpt1a, which is known to be a rate-determining enzyme in mitochondrial β-oxidation, was down-regulated by TOP ingestion. Therefore, our microarray analysis at least suggests that up-regulation of the genes involved in peroxisomal β-oxidation might be one of the mechanisms for the reduction in abdominal adipose tissue mass in the TOP group, since expression of genes involved in peroxisomal β-oxidation, such as Acox1 and Ehhadh, was up-regulated by TOP ingestion. Moreover, real-time PCR analysis of the liver in all groups showed that ingestion of CU, TE, and TOP was associated with up-regulation of Acox1 expression (**Figure 2**). These results indicate that both the CU and the TE contained in TOP play roles in the up-regulation of Acox1 expression upon TOP ingestion. With respect to the real-time PCR analysis of Ehhadh, TE ingestion tended to up-regulate the expression of the gene, although not significantly ($P < 0.08$), while TOP ingestion significantly up-regulated the expression of the gene. On the other hand, CU ingestion did not up-regulate the expression of Ehhadh (**Figure 2**). These results show that the TE contained in TOP is responsible for the up-regulation of Ehhadh expression upon TOP ingestion. Therefore, the greater reduction in abdominal adipose tissue seen with TOP ingestion as compared with CU ingestion might be explained by the activation of Ehhadh expression induced by the ingestion of the TE contained in TOP. We thus conclude that the ingestion of TOP is more effective for triglyceride utilization in the liver than ingestion of CU alone, because the TE contained in the TOP appears to act synergistically to support the triglyceride utilization, not only by the additive activation of Acox1 expression and Facls expression with the CU but also by activation of Ehhadh expression.

TOP ingestion up-regulated numerous genes related to cholesterol synthesis and catabolism (bile acid biosynthesis). It was suggested that the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr), a key enzyme in the cholesterol synthesis pathway, was low, while the activity of cholesterol 7α hydroxylase (cytochrome P450, family 7, subfamily a, polypeptide 1: Cyp7a1), a key enzyme in cholesterol catabolism (bile acid synthesis), was high in the diabetic or high cholesterol-fed rat (*17*). In those rat models, ingestion of curcumin or turmeric increased the activity of both Hmgcr and Cyp7a1, which resulted in lowering of plasma and hepatic cholesterol levels (*17*). The effect of curcumin or turmeric on lowering plasma and hepatic cholesterol levels was reported in other papers (*11*, *17*) as well. Although expression of Hmgcr and Cyp7a1 was unchanged in our gene expression analysis, our results showing up-regulation of genes related to cholesterol synthesis and catabolism are thought to be important for the future study of curcumin or turmeric.

It should be noted that TOP ingestion up-regulated the expression of many genes belonging to the acyl-CoA thioesterase family (5 genes). Acyl-CoA thioesterases catalyze the hydrolysis of fatty acyl-CoAs to free fatty acids and CoASH and are assumed to control intracellular levels of Acyl-CoA, free fatty acids, and CoASH (*28*). Although the physiological functions of the acyl-CoA thioesterase family are not yet fully understood, it should be noted that an increase in expression of this gene family has also been observed in a study on the enhancement of hepatic β-oxidation by sesamin, a major lignan in sesame seeds (*30*).

In conclusion, our results show that TOP ingestion is associated with reductions in blood glucose and abdominal adipose tissue in KK-Ay mice. Both CU and TE contained in TOP contributed to the blood glucose lowering, and TE mainly contributed to the reduction in abdominal adipose tissue. DNA microarray analysis indicated that TOP ingestion up-regulated the expression of genes related to glycolysis, β-oxidation, and cholesterol metabolism and down-regulated the expression of gluconeogenesis-related genes in the liver of KK-Ay mice. Furthermore, real-time PCR analysis on some representative genes suggested that CU regulated glycolysis-related gene expression and that CU and TE acted synergistically to regulate β-oxidation-related gene expression. These gene expression changes were thought to contribute to the effects of TOP observed in this study. Therefore, these results indicate that TOP ingestion is more effective than ingestion of either CU or TE alone.

ABBREVIATIONS USED

Turmeric, rhizome of *Curcuma longa* L.; TOP, turmeric oleoresin powder; CU, curcuminoids; TE, turmeric essential oil; PCR, polymerase chain reaction.

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