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ARTICLE

Administration of Tomato and Paprika Beverages Modifies Hepatic Glucose and Lipid Metabolism in Mice: A DNA Microarray Analysis

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To examine whether the expression of hepatic genes, including biomarkers, is affected by the ingestion of tomato or paprika, mice were given tomato beverage (TB), paprika beverage (PB), or water (control) ad libitum for 6 weeks. The body weights in the TB and PB groups were significantly lower than those in the control group. Administration of PB significantly increased the plasma high-density lipoprotein-cholesterol level. Hepatic gene expression was investigated using DNA microarrays. The ingestion of TB or PB up-regulated the expression of 687 and 1045 genes and down-regulated the expression of 841 and 653 genes, respectively (false discovery rate < 0.05). These changes in gene expression suggest that TB ingestion promotes glycogen accumulation and stimulates some specific steps in fatty acid oxidation. PB ingestion promoted the entire glucose and fatty acid metabolic pathways to improve lipid profiles. These results provide useful genetic information about a variety of biochemical processes by which vegetables can contribute to good health.

KEYWORDS: DNA microarray; tomato; paprika; glucose; lipid; metabolism

INTRODUCTION

Fruits and vegetables contain many beneficial nutrients and phytochemicals that are thought to protect our bodies against chronic diseases, such as cardiovascular disease (CVD) (*1*, *2*) and diabetes (*3*, *4*). However, different types of fruits and vegetables may vary in their contents of vitamins, minerals, antioxidants, and other micronutrients. Some nutrients or phytochemicals may also show additive or interactive effects with each other. Although it is important to evaluate the effects of individual nutrients or other bioactive compounds on biological processes, it is equally important to examine the beneficial effects of whole fruits and vegetables to investigate the significance of fruit and vegetable consumption on health.

Different vegetables or their components may exert their distinct effects via different mechanisms. However, the biochemical pathways involved in these effects are largely unknown. Modification of gene expression by dietary vegetables has been investigated only in some pathological model animals for a limited number of genes, but many of the molecular targets at the genomic level remain unknown. DNA microarray technology has enabled comprehensive analysis of the expression of a large number of genes simultaneously (*5*).

To investigate the comprehensive effects of different vegetables on gene expression, we chose two vegetables, tomato (*Lycopersicon esculentum*) and paprika (*Capsicum annuum*). Many previous studies have shown that the dietary intake of tomatoes and tomato products is associated with a reduced risk of chronic diseases (*6*, *7*). Meanwhile, although only limited information is available on the biological activities of paprika (*8*), it is widely used as a vegetable and food additive and is a good source of carotenoids (*9*), lipids (*10*), and micronutrients (*11*−*13*). Therefore, these vegetables are expected to significantly modulate the expression of some genes, thus influencing physiological functions to maintain health and/or prevent some diseases.

In this study, we used DNA microarrays to investigate the effects of administration of tomato and paprika to normal mice for 6 weeks on gene expression in the liver.

MATERIALS AND METHODS

Preparation of Diluted Beverages. In this experiment, we prepared two types of diluted beverages from tomato and paprika. Commercially available salt-free tomato juice (Kagome Co., Ltd., Tokyo, Japan) was diluted 1:1 (v/v) with sterile water for drinking use. The resulting sample was used as the tomato beverage (TB). Paprika juice was made from a paprika concentrate (Invertec Food, Santiago, Chile), and its sugar content was adjusted to 6.0% with water and the pH to 4.3 with a lemon concentrate. After sterilization, it was diluted 1:1 (v/v) with sterile water and used as the paprika beverage (PB). The nutritional ingredients of the diluted beverages are shown in **Table 1**.

Animal Experiments. Eighteen specific-pathogen-free female Balb/c mice, aged 3 weeks, were purchased from Japan SLC Co. Ltd. (Hamamatsu, Japan). The mice were housed in an animal laboratory with

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Table 1. Nutrient and Carotenoid Compositions of Diluted Vegetable Beverages Used in This Study (per 100 g of Beverage)

	diluted tomato beverage	diluted paprika beverage
energy (kcal)	10	12
protein (g)	0.4	0.4
fat (g)	0.0	0.1
carbohydrate (g)	0.8	2.2
fiber (g)	0.3	0.0
ascorbic acid (mg)	3.0	30.9
α-tocopherol (mg)	1.0	0.5
carotenoid		
lycopene (mg)	5.0	0.0
β-carotene (mg)	0.2	0.2
capsanthin (mg)	0.0	2.3

a 12 h light/dark cycle (7:30 a.m.−7:30 p.m. and 7:30 p.m.−7:30 a.m.) under controlled ambient conditions: temperature, 20−24 °C; and humidity, 45−65%. Mice were divided into three groups with equal average body weight. The commercial normal chow, FR-2 (Funabashi Farms Co., Ltd., Chiba, Japan) and sterile water were given to the control group for 6 weeks, with ad libitum access. For the TB and PB groups, the sterile water was replaced with the diluted vegetable beverages. These diluted beverages and water were freshly prepared every other day. Beverage and water intakes were recorded every other day, and body weights were measured at regular intervals throughout the feeding study. All animals were treated in accordance with guidelines established by the Japanese Society of Nutrition and Food Science (Law 105 and Notification 6 of the Japanese government).

Measurements of Plasma Biochemical Parameters. Six weeks after starting the test, the mice were euthanized under diethyl ether anesthesia, and their blood and livers were collected. Liver samples were frozen immediately after excision and kept at −80 °C. A small volume of each blood sample was used to measure the blood glucose level using a simplified glucose measurement kit (Glutest, Arkray Factory, Inc., Shiga, Japan). The rest of the blood was separated into plasma by centrifugation and frozen at −80 °C until analyzed. The concentrations of plasma total cholesterol, triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-C) were measured using commercial in vitro enzymatic test kits (Wako, Tokyo, Japan). Plasma insulin and adiponectin levels were measured using an Ultrasensitive Mouse Insulin ELISA Kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan) and a Mouse/Rat Adiponectin ELISA Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) according to the manufacturer's protocols. Statistical analyses were performed by ANOVA with Dunnett's multiple comparison of means test using SPSS 15.0 for Windows (SPSS Japan Inc., Tokyo, Japan). Differences were considered to be significant at $P < 0.05$.

DNA Microarray Assay. We selected four average mice from each group, as determined by body weights, relative liver weights, and plasma glucose levels. Total RNA was isolated from liver samples using TRIzol reagent (Invitrogen, Tokyo, Japan) according to the manufacturer's instructions. RNA was purified using the RNeasy Mini Kit (Qiagen, Tokyo, Japan). The quality and quantity of total RNA were checked by agarose gel electrophoresis and by spectrophotometry. DNA microarray analysis was performed using Affymetrix GeneChip mouse genome 430 2.0 array (Affymetrix, Santa Clara, CA), according to the manufacturer's instructions, for the detection of 43000 genes, as previously described (*14*). Briefly, 5 μg of purified total RNA was used to synthesize cDNA, and the resultant cDNA was used as a template for the synthesis of biotinylated cRNA by T7 polymerase. After fragmentation, the cRNA was hybridized to the GeneChip in accordance with the manufacturer's instructions. The fluorescence signals were scanned by the Affymetrix GeneChip System. Affymetrix GCOS software was used to reduce the array images to the intensity of each probe (CEL files).

DNA Microarray Data Analysis. The CEL files were quantified with the Factor Analysis for Robust Microarray Summarization (q.FARMS) algorithm (*15*) using the statistical language R (*16*) and Bioconductor (*17*). Hierarchical clustering was then performed by the pvclust() function (*18*) in R. To detect the differentially expressed genes between the control group and one of the diluted beverage groups, the Rank products (RP) method was used (*19*) as a nonparametric statistic derived from biological reasoning to detect items that are consistently highly ranked in a number of lists. It offers several advantages over linear modeling, including biologically intuitive fold-change criterion; the model contains fewer assumptions and increased performance with noisy data and/or low numbers of replicates (*20*). Moreover, a recent study revealed that the combination of the RP method and the q.FARMS preprocessing algorithm is one of the best combinations for accurately detecting differentially expressed genes (*21*), and these were applied to our microarray data. To detect the over-represented Gene Ontology (GO) categories in each group of differentially expressed genes, we used DAVID (*22*), a Web-accessible program, in accordance with the manuals available from the Web site (http://david.abcc.ncifcrf.gov/home.jsp).

Table 2. Metabolic Parameters of Mice Given the Experimental Beverages[a]

	group		
	control	TB	PB
beverage or water intake (g/day)	6.59 ± 0.53	6.45 ± 0.40	6.91 ± 0.49
initial BW[b] (g)	10.56 ± 0.28	10.21 ± 0.18	10.70 ± 0.38
halfway BW[c] (g)	18.11 ± 0.21	17.25 ± 0.26	18.11 ± 0.27
final BW[d] (g)	20.23 ± 0.11	17.00 ± 0.42**	18.80 ± 0.44*
relative liver weight (g/100 g of BW)	4.75 ± 0.05	4.25 ± 0.09**	5.01 ± 0.09
plasma insulin (ng/mL)	0.40 ± 0.22	0.39 ± 0.20	0.50 ± 0.11
blood glucose (mg/dL)	124.0 ± 9.1	93.8 ± 9.6	106.5 ± 11.9
plasma adiponectin (μg/mL)	33.9 ± 1.2	35.5 ± 2.7	33.9 ± 1.7
plasma lipids			
total cholesterol (mg/dL)	110.6 ± 4.0	119.9 ± 6.7	105.9 ± 6.7
TG (mg/dL)	163.7 ± 9.4	161.5 ± 10.4	133.3 ± 12.4
HDL-C (mg/dL)	58.5 ± 2.4	67.8 ± 2.1	69.0 ± 3.7*

[a] Values are means ± SEM, $n = 6$. * and ** indicate differences from the control group at $P < 0.05$ and $P < 0.01$ by Dunnett's multiple-comparison test. [b] Initial body weight was measured before starting dietary protocols. [c] Halfway body weight was measured after administering the experimental beverages for 3 weeks. [d] Final body weight was measured administering the experimental beverages for 6 weeks.

RESULTS

Growth and Biochemical Data. The metabolic parameters of mice in each group are shown in **Table 2**. All mice consumed similar amounts of food together with water or beverages throughout the experimental period. At 3 weeks, there was no significant difference in body weight (BW) between each group, but the body weights at 6 weeks in the TB and PB groups were significantly lower than those in the control group. In addition, the relative liver weight was significantly lower in the TB group than in the control group. Administration of PB significantly increased HDL-C. There were no significant differences in the plasma insulin or adiponectin concentrations.

DNA Microarray Analysis. To characterize the mechanism underlying the significant effects observed in the TB and PB groups, hepatic gene expression levels were analyzed using a DNA microarray. Four mice from each group, whose final body weights, relative liver weights, and plasma glucose levels approximated the mean values for the six mice in each group, were selected for further DNA microarray analysis. In this selection, the biochemical parameters of the selected mice, including plasma HDL-C, reflected the changes observed in the full study groups. Hierarchical clustering analysis was performed for all genes, revealing that the mice in the control and beverage-treated groups separately formed apparent clusters (Supporting Information). Therefore, gene expression profiles were formed to assess the beverage-induced transcriptional changes. In this study, the genes showing a false discovery rate (FDR) of < 0.05 between the control group and one of the beverage groups were defined as genes showing biologically significant changes in expression levels. On the basis of this estimation, we found that the ingestion

Table 3. Significantly Enriched GO Terms Found in 687 Up-Regulated Genes by Tomato Beverage Ingestion ($P < 0.05$)

GO-ID	GO term	No. of genes	FDR-corrected P-value
0006457	protein folding	27	7.02E-06
0006091	generation of precursor metabolites and energy	41	2.16E-04
0006629	lipid metabolic process	43	1.11E-03
0044255	cellular lipid metabolic process	38	2.43E-03
0006638	neutral lipid metabolic process	9	1.15E-03
0046486	glycerolipid metabolic process	9	1.16E-03
0006639	acylglycerol metabolic process	9	1.15E-03
0006641	triacylglycerol metabolic process	8	1.12E-03
0006662	glycerol ether metabolic process	9	1.15E-03
0006082	organic acid metabolic process	38	1.73E-03
0019752	carboxylic acid metabolic process	37	1.20E-03
0032787	monocarboxylic acid metabolic process	22	2.70E-03
0051789	response to protein stimulus	13	1.39E-03
0006986	response to unfolded protein	13	1.39E-03
0006066	cellular alcohol metabolic process	23	2.11E-02

Table 4. Significantly Enriched GO Terms Found in 841 Down-Regulated Genes by Tomato Beverage Ingestion ($P < 0.05$)

GO-ID	GO term	No. of genes	FDR-corrected P-value
0006629	lipid metabolic process	72	5.84E-11
0008610	lipid biosynthetic process	38	1.76E-08
0006694	steroid biosynthetic process	24	4.54E-11
0016126	sterol biosynthetic process	15	8.58E-09
0006695	cholesterol biosynthetic process	12	8.36E-07
0008203	cholesterol metabolic process	19	2.61E-07
0016125	sterol metabolic process	23	5.01E-10
0008202	steroid metabolic process	33	4.86E-11
0044255	cellular lipid metabolic process	67	2.64E-11
0006066	cellular alcohol metabolic process	40	3.11E-08
0006082	organic acid metabolic process	52	2.31E-06
0019752	carboxylic acid metabolic process	52	2.35E-06
0032787	monocarboxylic acid metabolic process	32	2.49E-06
0006631	fatty acid metabolic process	23	3.59E-04
0051186	cofactor metabolic process	25	4.35E-03
0006732	coenzyme metabolic process	22	4.51E-03

Table 5. DNA Microarray Data on Glucose Metabolism-Related Genes Induced by Tomato Beverage Ingestion in Liver of Balb/c Mice

gene name	symbol	gene expression	false discovery rate[a]	accession no.[b]
glycogenesis				
glucokinase	*Gck*	up	<0.0001	NM_010292
glycogen synthase 2	*Gys2*	up	0.00015	NM_145572
glycolysis				
dihydrolipoamide *s*-acetyltransferase (e2 component of pyruvate dehydrogenase complex)	*Dlat*	down	0.00074	AV336908
pyruvate kinase liver and red blood cell	*Pklr*	down	0.00133	NM_001099779, NM_013631
gluconeogenesis				
phosphoenolpyruvate carboxykinase 1, cytosolic	*Pck1*	up	0.00017	NM_011044
fructose bisphosphatase 1	*Fbp1*	up	0.03393	NM_019395

[a] False discovery rate (FDR) between the control group and the tomato beverage group. In this experiment, genes at FDR < 0.05 were defined as showing biologically significant changes in expression levels. [b] GenBank ID.

of the TB or PB up-regulated the expression of 687 and 1045 genes, respectively, and down-regulated the expression of 841 and 653 genes, respectively.

Using DAVID, the differentially expressed genes by ingestion of each beverage were classified into functional categories according to GO. The significantly enriched categories of genes that were up- or down-regulated by the administration of the TB are summarized in **Tables 3** and **4**. The most specific over-represented categories of up-regulated genes were "protein folding", "generation of precursor metabolites and energy", "lipid metabolic process", "organic acid metabolic process", "response to protein stimulus", and "cellular alcohol metabolic process" (**Table 3**). Similarly, the categories of down-regulated genes were "lipid metabolic process", "cellular alcohol metabolic process", "organic acid metabolic process", and "cofactor metabolic process" (**Table 4**). Accordingly, the categories of up-regulated and down-regulated genes were predominantly related to lipid and glucose metabolism in mice given the TB. Furthermore, the changes in lipid and glucose metabolism may be responsible for the decreases in body and liver weights in these mice. Therefore, we subsequently selected and categorized the genes related to glucose and lipid metabolism. The selection was based on metabolic function in the GO and metabolic pathway map.

The genes that were notably affected by the ingestion of TB and those associated with glucose metabolism are listed in **Table 5**. The ingestion of the TB altered the expression of six genes related to glucose metabolism, of which two were related to glycogenesis and were up-regulated. Two genes related to glycolysis were down-regulated, and the two genes related to gluconeogenesis were up-regulated. These changes in gene expression suggest that the ingestion of the TB enhances glycogen accumulation.

Table 6. DNA Microarray Data on Lipid Metabolism-Related Genes Induced by Tomato Beverage Ingestion in the Liver of Balb/c Mice

gene name	symbol	gene expression	false discovery rate[a]	accession no.[b]
fatty acid synthesis				
elovl family member 5, elongation of long-chain fatty acid	*Elovl5*	down	<0.0001	NM_134255
elovl family member 6, elongation of long-chain fatty acid	*Elovl6*	down	<0.0001	NM_130450
fatty acid synthase	*Fasn*	down	<0.0001	NM_007988
stearoyl-coenzyme A desaturase 1	*Scd1*	down	<0.0001	NM_009127
malic enzyme, supernatant	*Me1*	down	0.00135	NM_008615
sterol regulatory element binding factor 1	*Srebf1*	down	0.00276	NM_011480
atp citrate lyase	*Acly*	down	0.00342	NM_134037
acetyl-coenzyme A carboxylase α	*Acaca*	down	0.00888	NM_133360
fatty acid degradation				
cytochrome P450, family 4, subfamily a, polypeptide 14	*Cyp4a14*	up	0.00037	NM_007822
carnitine palmitoyltransferase 1a, liver	*Cpt1a*	up	0.00053	NM_013495
acyl-coA synthetase long-chain family member 4	*Acsl4*	up	0.00512	NM_001033600, NM_019477, NM_207625
cytochrome P450, family 4, subfamily a, polypeptide 10	*Cyp4a10*	up	0.00512	NM_010011
acyl-coenzyme A dehydrogenase family, member 9	*Acad9*	down	0.00043	NM_172678
enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase	*Ehhadh*	down	0.00218	NM_023737
acyl-coenzyme A oxidase 2, branched chain	*Acox2*	down	0.00328	NM_053115
cholesterol synthesis				
3-hydroxy-3-methylglutaryl-coenzyme A reductase	*Hmgcr*	up	0.00418	NM_008255,
7-dehydrocholesterol reductase	*Dhcr7*	down	<0.0001	NM_007856
3-hydroxy-3-methylglutaryl-coenzyme A synthase 1	*Hmgcs1*	down	<0.0001	NM_145942
isopentenyl-diphosphate δ isomerase	*Idi1*	down	<0.0001	NM_145360, NM_177960
nad(p) dependent steroid dehydrogenase-like	*Nsdhl*	down	<0.0001	NM_010941
sterol-c5-desaturase (fungal erg3, δ-5-desaturase) homologue (*S. cerevisae*)	*Sc5d*	down	<0.0001	NM_172769
sterol-c4-methyl oxidase-like	*Sc4 mol*	down	<0.0001	AK005441
phosphomevalonate kinase	*Pmvk*	down	0.00016	NM_026784
cytochrome p450, family 51	*Cyp51*	down	0.00071	NM_020010
cholesterol catabolism (bile acid biosynthesis)				
hydroxy-δ-5-steroid dehydrogenase, 3β- and steroid δ isomerase 7	*Hsd3b7*	down	<0.0001	NM_001040684, NM_133943
lysosomal acid lipase 1	*Lipa*	down	0.00021	AI596237
retinol dehydrogenase 11	*Rdh11*	down	0.00058	NM_021557
sterol *o*-acyltransferase 2	*Soat2*	down	0.00325	NM_146064
hydroxysteroid (17-β) dehydrogenase 12	*Hsd17b12*	down	0.00348	NM_019657
cytochrome P450, family 7, subfamily b, polypeptide 1	*Cyp7b1*	down	0.00153	NM_007825

[a] False discovery rate (FDR) between the control group and the tomato beverage group. In this experiment, genes at FDR < 0.05 were defined as showing biologically significant changes in expression levels. [b] GenBank ID.

With respect to lipid metabolism, 5 genes were up-regulated and 25 genes were down-regulated by the ingestion of the TB (**Table 6**). Eight genes in the fatty acid synthesis pathway were down-regulated. Four genes related to fatty acid degradation were up-regulated, whereas three other genes were down-regulated. With respect to cholesterol synthesis, the expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Hmgcr) was up-regulated. Furthermore, down-regulation of eight genes related to cholesterol synthesis was observed, of which six genes were related to cholesterol catabolism (bile acid biosynthesis). These results indicate that the ingestion of the TB would decrease the biosynthesis of fatty acids and stimulate specific steps in the fatty acid oxidation pathway (**Figure 1**).

The significantly enriched categories of genes that were up- or down-regulated by the ingestion of PB are summarized in **Tables 7** and **8**, respectively. The most specific over-represented categories in the up-regulated genes were "transport", "establishment of cellular localization", "protein localization", "protein folding", "response to protein stimulus", "cellular carbohydrate metabolic process", and "alcohol catabolic process" (**Table 7**). The categories of down-regulated genes were "lipid metabolic process", "alcohol catabolic process", "organic acid metabolic acid", "cofactor metabolic process", and "nitrogen compound catabolic process" (**Table 8**). Similar to that with TB ingestion, the categories related to "glucose metabolic process" (up-regulated) and "lipid metabolic process" (down-regulated) were significantly affected by PB ingestion.

The genes showing notable changes in expression due to the ingestion of PB are listed in **Tables 9** and **10**. Ingestion

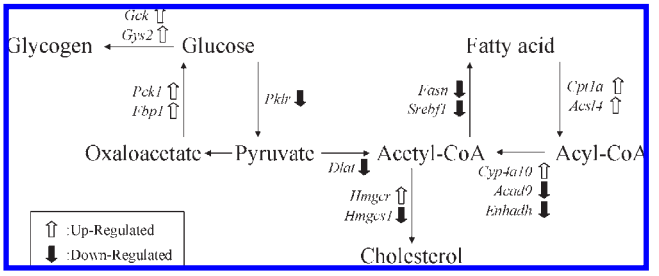

Figure 1. Summarized pathways of probable glucose and lipid metabolism in mouse liver affected by the ingestion of tomato beverage.

of PB up-regulated the expression of nine genes related to glucose metabolism, two of which were related to glycogenesis, three were related to glycolysis, and four were related to gluconeogenesis (**Table 9**). With respect to lipid metabolism, seven genes were up-regulated and eight genes were down-regulated. Four genes in the fatty acid synthesis pathway and two genes related to fatty acid degradation were up-regulated. With respect to cholesterol synthesis, the expression of one gene was up-regulated and four genes were down-regulated. Four genes involved in cholesterol catabolism were also down-regulated. In addition, PB up-regulated the expression of the low-density lipoprotein receptor (Ldlr), a gene related to lipid circulation (**Table 10**; **Figure 2**). These results indicate that PB ingestion promotes glucose and fatty acid metabolism and stabilizes lipid circulation.

Table 7. Significantly Enriched GO Terms Found in 1045 Up-Regulated Genes by Paprika Beverage Ingestion ($P < 0.05$)

GO-ID	GO term	No. of genes	FDR-corrected P-value
0006810	transport	178	3.79E-06
0046903	secretion	35	4.01E-03
0032940	secretion by cell	33	5.59E-04
0016192	vesicle-mediated transport	40	2.76E-02
0048193	Golgi vesicle transport	24	9.91E-09
0006888	ER to Golgi vesicle-mediated transport	21	1.60E-09
0046907	intracellular transport	83	5.73E-11
0051649	establishment of cellular localization	86	5.95E-08
0008104	protein localization	81	9.59E-08
0045184	establishment of protein localization	80	6.89E-09
0015031	protein transport	79	1.79E-09
0006886	intracellular protein transport	62	1.27E-10
0006605	protein targeting	31	2.87E-04
0006612	protein targeting to membrane	7	3.80E-02
0006457	protein folding	39	1.40E-09
0051789	response to protein stimulus	19	1.25E-06
0006986	response to unfolded protein	19	1.25E-06
0044262	cellular carbohydrate metabolic process	37	2.37E-05
0005996	monosaccharide metabolic process	26	1.75E-05
0046364	monosaccharide biosynthetic process	8	3.26E-02
0019318	hexose metabolic process	26	1.66E-05
0006096	gluconeogenesis	7	2.37E-02
0006006	glucose metabolic process	24	1.14E-06
0006096	glycolysis	15	4.85E-05
0006007	glucose catabolic process	16	4.87E-05
0019320	hexose catabolic process	16	5.61E-05
0046365	monosaccharide catabolic process	16	5.61E-05
0044275	cellular carbohydrate catabolic process	37	2.37E-05
0016052	carbohydrate catabolic process	17	2.69E-04
0046164	alcohol catabolic process	17	1.76E-05

Table 8. Significantly Enriched GO Terms Found in 653 Down-Regulated Genes by Paprika Beverage Ingestion ($P < 0.05$)

GO-ID	GO term	No. of genes	FDR-corrected P-value
0006629	lipid metabolic process	57	5.83E-09
0008610	lipid biosynthetic process	27	8.00E-05
0044255	cellular lipid metabolic process	53	2.76E-09
0008202	steroid metabolic process	28	1.28E-09
0006694	steroid biosynthetic process	21	8.70E-10
0016126	sterol biosynthetic process	11	2.57E-05
0006695	cholesterol biosynthetic process	9	2.97E-04
0008203	cholesterol metabolic process	14	1.55E-04
0016125	sterol metabolic process	17	2.62E-06
0006066	alcohol metabolic process	25	4.99E-03
0006082	organic acid metabolic process	42	2.73E-05
0019752	carboxylic acid metabolic process	42	2.90E-05
0032787	monocarboxylic acid metabolic process	27	1.60E-05
0066631	fatty acid metabolic process	17	1.90E-02
0051186	cofactor metabolic process	21	7.50E-03
0006732	coenzyme metabolic process	20	1.45E-03
0044270	nitrogen compound catabolic process	10	3.70E-02
0009310	amine catabolic process	10	3.70E-02

Table 9. DNA Microarray Data on Glucose Metabolism-Related Genes Induced by Paprika Beverage Ingestion in the Liver of Balb/c Mice

gene name	symbol	gene expression	false discovery rate[a]	accession no.[b]
glycogenesis				
glucokinase	*Gck*	up	0.00016	NM_010292
glycogen synthase 2	*Gys2*	up	0.00259	NM_145572
glycolysis				
enolase 1, α non-neuron	*Eno1*	up	0.00090	NM_001025388, NM_023119
pyruvate dehydrogenase (lipoamide) β	*Pdhb*	up	0.00189	BC002188
pyruvate kinase liver and red blood cell	*Pklr*	up	0.00419	NM_001099779, NM_013631
gluconeogenesis				
fumarate hydratase 1	*Fh1*	up	0.00012	NM_010209
phosphoenolpyruvate carboxykinase 1, cytosolic	*Pck1*	up	0.00085	NM_011044
glucose-6-phosphatase, catalytic	*G6pc*	up	0.00087	NM_008061
fructose bisphosphatase 1	*Fbp1*	up	0.00830	NM_019395

[a] False discovery rate (FDR) between the control group and the tomato beverage group. In this experiment, genes at FDR < 0.05 were defined as showing biologically significant changes in expression levels. [b] GenBank ID.

Table 10. DNA Microarray Data on Lipid Metabolism-Related Genes Induced by Paprika Beverage Ingestion in Liver of Balb/c Mice

gene name	symbol	gene expression	false discovery rate[a]	accession no.[b]
fatty acid synthesis				
malic enzyme, supernatant	*Me1*	up	0.00140	NM_008615
fatty acid synthase	*Fasn*	up	0.00066	NM_007988
atp citrate lyase	*Acly*	up	0.00090	NM_134037
acetyl-coenzyme A carboxylase α	*Acaca*	up	0.00481	NM_133360
fatty acid degradation				
acyl-coA synthetase long-chain family member 4	*Acsl4*	up	0.00130	NM_001033600, NM_019477, NM_207625
carnitine palmitoyltransferase 1a, liver	*Cpt1a*	up	0.00247	NM_013495
cholesterol synthesis				
acetoacetyl-coA synthetase	*Aacs*	up	0.00017	NM_030210
3-hydroxy-3-methylglutaryl-coenzyme A synthase 1	*Hmgcs1*	down	<0.0001	NM_145942
phosphomevalonate kinase	*Pmvk*	down	0.00016	NM_026784
sterol-c5-desaturase (fungal erg3, δ-5-desaturase) homologue (*S. cerevisae*)	*Sc5d*	down	0.00177	NM_172769
isopentenyl-diphosphate δ isomerase	*Idi1*	down	0.00693	NM_145360, NM_177960
cholesterol catabolism (bile acid biosynthesis)				
hydroxy-δ-5-steroid dehydrogenase, 3β- and steroid δ isomerase 7	*Hsd3b7*	down	<0.0001	NM_001040684, NM_133943
lysosomal acid lipase 1	*Lipa*	down	0.00159	AI596237
sterol *o*-acyltransferase 2	*Soat2*	down	0.00235	NM_146064
cytochrome P450, family 7, subfamily b, polypeptide 1	*Cyp7b1*	down	0.00490	NM_007825
lipid circulation				
low-density lipoprotein receptor	*Ldlr*	up	0.00290	NM_010700

[a] False discovery rate (FDR) between the control group and the tomato beverage group. In this experiment, genes at FDR < 0.05 were defined as showing biologically significant changes in expression levels. [b] GenBank ID.

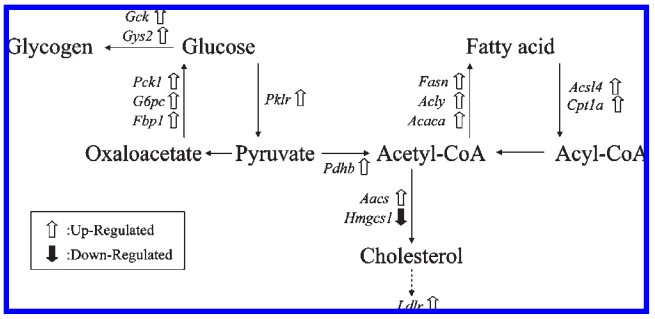

Figure 2. Summarized pathways of probable glucose and lipid metabolism in mouse liver affected by the ingestion of paprika beverage.

DISCUSSION

Microarray analysis is widely used as a tool to study genes potentially involved in metabolic pathways and homeostatic control (*23*). Here, we examined the effects of continuous ingestion of TB and PB on comprehensive gene expression in normal mice and found that the expression of many genes is regulated differently by the consumption of various vegetables.

With respect to TB ingestion, the DNA microarray analysis revealed down-regulation of sterol regulatory element-binding proteins (SREBPs) and up-regulation of peroxisome proliferator-activated receptors (PPARs). In this study, we found that Srebf1 and most SREBP-1c-responsive genes (Elovl5, Elovl6, Fasn, Scd1, Me1, Acly, and Acaca) were consistently down-regulated by TB ingestion (**Tables 5** and **6**). These changes in gene expression strongly suggest the suppressed expression of SREBP-1c. We also found a tendency for up-regulation of the PPARα gene (FDR = 0.01444), and significant up-regulation of the Cpt1a gene was observed, indicating that TB ingestion activated PPARα in the livers of mice. Previous papers have indicated that the overexpression of SREBP-1c in the livers of transgenic mice resulted in the development of a TG-enriched fatty liver (*24*) and that the absence of SREBP-1 significantly reduced the hepatic expression of lipogenic genes and prevented the development of fatty liver in leptin-deficient mice (*25*). In addition, reductions of fatty acid levels were reported to be affected by the activation of PPARα, which reduces hepatic de novo fatty acid synthesis (*26*, *27*). Therefore, it is possible that the decreases in body weight or liver weight loss in the TB group (**Table 2**) could be attributed to the down-regulation of SREBP-1c and the up-regulation of PPARα.

Compared with the findings with TB, changes in the expression of SREBP-1c and its responsive genes were not observed following PB ingestion. Meanwhile, PB ingestion increased the expression of Cpt1a genes (Tables**9** and **10**), which suggests that PPARα is activated by PB. These results indicate that paprika ingestion promotes the oxidation of fatty acids, without depleting fatty acids in the liver by down-regulating SREBP-1c. These changes in gene expression are likely to be involved in the moderate suppression of body weight gain by PB ingestion (**Table 2**).

Interestingly, there were no significant differences in terms of food/feed and water/beverage intake between the three experimental groups. The intake of commercial normal chow (FR-2) was generally between 4 and 6 g/day/mice, and the intake of experimental beverage or water was between 6 and 7 g/day/mice in this study (**Table 2**). The diluted TB and PB have caloric values of 10 and 12 kcal/100 g of beverage (**Table 1**); therefore, the ingestion of TB or PB increased the daily caloric intakes by 3.6 and 4.6%, respectively. However, TB and PB were associated with less weight gain despite the slight increase in calorie intake compared with the control group, which would suggest that tomato and paprika contained nutritional components that ultimately influence weight gain.

With regard to glucose metabolism, the microarray analysis revealed distinct gene expression profiles with the two vegetables. Although whole glucose metabolism was promoted by PB (**Table 9**; **Figure 2**), glycogenesis was facilitated and glycolysis repressed by TB ingestion (**Table 5**; **Figure 1**). In terms of the biochemical data, TB ingestion tended to decrease blood glucose levels (75.6% compared with the control group, $P = 0.06$), whereas PB ingestion did not significantly affect blood glucose levels (85.9%, $P = 0.12$). In this experiment, blood samples were obtained from nonfasted mice, because food deprivation before killing is known to significantly down-regulate genes involved in

fatty acid synthesis and cholesterol metabolism (*28*, *29*). Although it is seems likely that blood glucose levels are affect by food/feed intake status shortly before the collection of blood samples, the changes in blood glucose levels can be interpreted by profiling gene expression by microarray analysis.

Interestingly, a significant increase in the plasma HDL-C level and up-regulation of Ldlr were observed only in the PB group. A recent clinical intervention study supports the idea that increased levels of HDL-C may protect against clinical CVD (*30*) and that HDL has antiatherogenic roles by promoting reverse cholesterol transport (*31*) and by acting as an antioxidant (*32*). Furthermore, elevated activity of the LDL receptor increases the efficiency of cholesterol clearance (*33*). These results would suggest that paprika ingestion may constitute a favorable environment for lipid circulation. With regard to the elevated of plasma HDL-C level, our group has previously demonstrated that purified capsanthin, the main carotenoid in paprika, increases the plasma HDL-C level in normal rats (*34*). Accordingly, the active compound responsible for the increase in HDL-C is capsanthin, which is present in the experimental PB at a level of 2.3 mg/100 g. In addition, the same paper reported that capsanthin administration up-regulated the mRNA expression of apo A5, without a significant difference in the mRNA levels of genes related to HDL-C metabolism such as the ATP-binding cassette transporter A1, apo A1, apo C3, hepatic lipase, lipoprotein lipase, endothelial lipase, cholesterol ester transfer protein, phospholipids transfer protein, and scavenger receptor class B type 1 using RT-PCR techniques (*34*). In the present study, the expression of genes related to HDL metabolism was not notably affected, but only the apo A5 gene was up-regulated (FDR = 0.00521). This microarray analysis has revealed consistent findings regarding the effects of paprika on gene expression and resulting HDL-C levels.

The main objective of this paper is to statistically examine the comprehensive changes in hepatic gene expression caused by the ingestion of TB and PB, to determine the significance of consuming these vegetables on health. Therefore, we did not explore the identification of any particular active compounds contained in these vegetables that affected gene expression. It was reported that tomato contains valuable components such as lycopene (*9*) and numerous nutrients and phytochemicals (*35*). Paprika is also known to be a good source of carotenoids (*9*), lipids (*10*), and micronutrients (*11*−*13*). Therefore, the changes in gene expression observed in this study may be due to additive or interactive effects between each nutrient and/or phytochemical. Some papers have reported that dietary lycopene, the main carotenoid in tomato, affects the expression of genes related to lipid metabolism (*36*, *37*). Similarly, our group has previously revealed that capsanthin, the main active carotenoid in paprika, increases the plasma HDL-C level in normal mice (*34*). Therefore, it seems likely that these carotenoids are the principal components that modify gene expression. Further studies on the administration of purified carotenoids and the accumulation of carotenoids in tissues would clarify the active compounds present in these vegetables.

Taken together, it seems likely that the intake of these two vegetables modifies genetic and physiological profiles; tomato ingestion improves lipid metabolism, whereas paprika ingestion provides a favorable environment for lipid circulation. Although many papers have revealed the benefits of high-vegetable diets on health, the genetic pathways through which vegetables exert their effects are still mostly unknown. The modulation of gene expression by dietary vegetables has only been investigated in pathological animal models and with a limited number of genes. To our knowledge, the present study is the first to define the effects of vegetables such as tomato and paprika on the expression of a large number of genes corresponding to biomarkers in normal mice with microarray technology. This study by such bioinformatics enhances our understanding that different vegetables contribute to our health in different ways. The results in this study also demonstrate the importance of consuming vegetables daily to maintain healthy body conditions and/or reduce the risk of chronic diseases.

Supporting Information Available: Hierarchical cluster dendrogram showing the effects of tomato or paprika beverage on the overall gene expression. This material is available free of charge via the Internet at http://pubs.acs.org.

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