

## Gene Expression Analysis of the Anti-obesity Effect by Apple Polyphenols in Rats Fed a High Fat Diet or a Normal Diet

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**Abstract:** We previously reported that ingestion of apple polyphenols reduced the weight of visceral adipose tissue and the triglyceride content of blood and liver in rats fed a high-fat diet (1). To further elucidate the mechanism of the improvement of lipid metabolism by dietary polyphenols, the effects of feeding apple- and tea-derived catechins on hepatic gene expression profile was investigated using the GeneChip DNA microarray system. Male Sprague-Dawley rats were fed a normal diet, a high-fat diet, a high-fat diet supplemented with 1.0% apple polyphenols or a high-fat diet supplemented with 1.0% tea catechins for 9 weeks. Both polyphenols reduced the weight of visceral adipose tissue and the triglyceride content in blood and liver. Tea catechins increased the transcription of genes involved in cholesterol synthesis whereas apple polyphenols decreased the transcription of genes involved in fatty acid synthesis. Thus, each polyphenol exerts a different effect on hepatic gene expression. The reduction of the weight of visceral adipose tissue was also observed when apple polyphenols were fed with a normal-fat diet. The genes involved in fatty acid synthesis were down-regulated in both high fat and normal diets. These results suggest that apple polyphenols and tea catechins improve lipid metabolism through different manner of action. Apple polyphenols widely inhibit the expressions of genes involved in fatty acid synthesis.

**Key words:** nutrigenomics, apple, polyphenol, procyanidin, obesity, DNA microarray

### 1 Introduction

Obesity, which is due to the excessive intake of calories, is a worldwide health problem of modern times. Obesity increases the risk of hyperlipemia, hypertension and type2 diabetes, and contributes to the development of serious illnesses, such as coronary heart disease and brain diseases (2). Functional ingredients to

improve lipid metabolism and control obesity have been drawing increasing attention.

Tea catechins have been reported to reduce serum cholesterol levels (3) and visceral adipose tissue weight in rodents (4, 5), and these effects were confirmed in humans (6, 7). In addition, procyanidins, the main ingredient of apple polyphenols (8, 9), have recently been reported to reduce serum cholesterol levels in rats (10) and in humans (11). It is interesting to examine

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the differences of actions between both polyphenols because procyanidins consist of oligomers of catechins.

Novel technologies such as DNA microarrays allow the comprehensive gene expression analysis, thus nutrigenomics approaches are currently being applied to nutrition (12). Recently, polyphenols in ingested food have been reported to effect gene expression, and the mechanisms of action have been elucidated (13-15).

In order to study the mechanism of lipid metabolism improvement caused by apple polyphenols ingestion, we performed a nutrigenomics analysis using rats fed high-fat and normal diets.

## 2 Experimental

### 2.1 Animals and Diets

The high-fat diet experiment was performed as follows: three-week-old male Sprague-Dawley rats (CLEA Japan) were maintained on normal AIN-93G diets for one week to stabilize metabolic conditions before starting the experiments. Rats were housed individually in cages at room temperature (20-23°C) on a controlled lighting schedule (lights on between 08:00-20:00). After one week of acclimatization, rats were divided into four groups consisting of six animals fed the control diet. **Table 1** shows the compositions of the diets, determined on the basis of the AIN-93G formula. The polyphenol components mixed in the diets are shown in

**Table 2.** The normal diet group is referred to as “NC” (Normal Control), the high-fat diet group as “HC” (High-fat Control), the group fed the high-fat diet containing apple polyphenols as “AP”, and the group fed the high-fat diet containing tea catechins as “TP” (Tea Polyphenols). Rats were fed the controlled diets for nine weeks, during which precautions were taken to minimize the difference in food intake among individual rats.

The normal diet experiment was performed as follows: five-week-old male Wistar rats (Japan SLC) were maintained for one week as described above. After one week of acclimatization, rats were divided into two groups consisting of five animals. The normal diet group is referred to as “NC2”, and the group fed the normal diet with drinking water containing 0.1% of apple polyphenols *ad libitum* as to “AP2” for one week.

Upon completion of the experiments, rats were fasted overnight, sacrificed by exsanguination of the aorta abdominalis under diethyl ether anesthesia, and samples were immediately dissected. Experiments were conducted in accordance with the guideline provided by the ethical committee on experimental animal care at Hirotsuki University.

### 2.2 Sample Collection and RNA Preparation

Immediately after collection, samples were fixed

**Table 1** Diet Compositions.

Component	Group			
	NC	HC	AP	TP
	weight %			
Corn starch	34.8	-	-	-
Casein	23.6	23.6	23.6	23.6
$\alpha$ -Cornstarch	13.2	8.4	7.4	7.4
Sucrose	10	28.8	28.8	28.8
Soybean oil	7	7	7	7
Lard	-	20.8	20.8	20.8
Cellulose	6.4	6.4	6.4	6.4
Mineral mix (AIN93)	3.5	3.5	3.5	3.5
Vitamin mix (AIN93)	1	1	1	1
L-Cystine	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2
Polyphenol	-	-	1	1

NC, normal control (normal diet); HC, high fat control (high fat diet); AP, high fat diet + apple polyphenols; TP, high fat diet + tea polyphenols.

overnight in RNeasy lysis buffer (Qiagen) at 4°C and frozen at -80°C after removal of supernatant fluids. Total RNA was isolated from liver samples using the RNeasy Mini Kit (Qiagen) according to manufacturer's instruction. After confirming no degradation using the Bioanalyzer 2100 (Agilent), the RNA from rats in each group were mixed together in equal amounts and used in Gene Chip analysis.

### 2.3 DNA Microarray Procedure

DNA microarray analysis was conducted using the Affymetrix RAE 230A (with 15,923 gene transcripts). RNA labeling, hybridization, washing, and staining were performed according to the Affymetrix protocols: 2 µg of total RNA was used for each array to generate cDNA, and biotinylated cRNA was then synthesized from the cDNA obtained using T7RNA polymerase and fractionated. The cRNA was hybridized on the Rat Expression Array 230A at 45°C for 16 hours. After hybridization, the arrays were washed, and labeling was performed using phycoerythrin. Fluorescence signals were scanned using the Gene Chip Scanner GC3000. Two arrays were used for each group.

### 2.4 DNA Microarray Data Analysis

Fluorescence signals obtained were converted into numeric using the Affymetrix GCOS (Gene Chip Operation System 1.0). The following quality control procedures were used to select appropriate probes for analysis: Selection by frag - the GCOS classifies the expression of each probe into "present," "marginal," or "absent." We selected "present" probes. Selection by

raw values - Probes that satisfied at least one of the two raw-value conditions (determined by scanning) were selected: a probe that has a raw value of 20 or over in all groups or a probe that has a raw value of 50 or over in at least in one group. Data from probes that met both of the frag and raw value criteria were used for analysis.

The data from selected probes were analyzed using gene expression analysis software GeneSpring 6.0 (Silicon Genetics). Signal corrections were made so that the 50% medians of the expression intensity of total probes became the same among groups. Probes whose expression intensity changed 1.5-fold or more, compared with the control group, were extracted as altered genes. To investigate the mechanism of the improvement of lipid metabolism, we analyzed the probes confined genes involved in lipid metabolism (Table 3).

### 2.5 Quantitative Real Time PCR

First strand cDNA solution obtained from 1 µg of total RNA was diluted 1:10, 1 µL of which was used as template for quantitative real-time PCR using the SYBR Green PCR Master Mix kit (Applied Biosystems) and the ABI 7700 Sequence Detector (Applied Biosystems). The following PCR cycle was performed: Step 1; 50°C for 2 min, Step 2; 95°C for 10 min, and Step 3; (95°C for 15 s and 60°C for 1 min) × 40 cycles. All data were normalized to the Glyceraldehyde-3-phosphate dehydrogenase gene (GAPD) signal. Table 4 shows a list of primers used in the quantitative real-time PCR. We confirmed the results of microarray analysis using quantitative PCR analysis for genes whose expression levels were changed by polyphenol

**Table 2** Dietary Polyphenol Compositions.

Apple polyphenols <sup>†</sup>		Tea catechins	
Component	Composition	Component	Composition
	weight %		weight %
Catechins	12.9	Epicatechin	9
Procyanidin 2mer	27.3	Epigallocatechin	11
Procyanidin 3mer	11.7	Epicatechin gallate	11
Procyanidin 4mer	3.5	Epigallocatechin gallate	57
Procyanidin 5mer	2.3	Other catechins	3
Procyanidin 6mer	1.3		
Procyanidin 7mer	0.9		
Procyanidin ≥ 8mer	5.4		
Phenol carboxylic acid	22.3		

<sup>†</sup> Procyanidins in apples consist of condensation polymers of catechin and epicatechin.

**Table 3** Analyzed Lipid Metabolism-related Genes<sup>†</sup>.

Gene symbol	Description	Unigene	Gene symbol	Description	Unigene
HMGCL	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	Rn.12297	CYP51	Cytochrome P450, subfamily 51	Rn.107152
HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	Rn.9437	ELOVL5	ELOVL family member 5, elongation of long chain fatty acids (yeast)	Rn.4243
HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	Rn.5106	EHHADH	Enoyl-Coenzyme A hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	Rn.3671
HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Rn.29594	ECHS1	Enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	Rn.6847
DHCR7	7-dehydrocholesterol reductase	Rn.228	FDFT1	Farnesyl diphosphate farnesyl transferase 1	Rn.21071
ACAA1	Acetyl-Coenzyme A acyltransferase 1	Rn.8913	FAAH	Fatty acid amide hydrolase	Rn.89119
ACAA2	(peroxisomal 3-oxoacyl-Coenzyme A thiolase)				
ACADL	Acetyl-Coenzyme A acyltransferase 2	Rn.3786	FABP1	Fatty acid binding protein 1, liver	Rn.36412
ACADM	(mitochondrial 3-oxoacyl-Coenzyme A thiolase)				
ACADL	Acetyl-Coenzyme A dehydrogenase, long-chain	Rn.174	FABP2	Fatty acid binding protein 2, intestinal	Rn.91358
ACADL	Acetyl-coenzyme A dehydrogenase, medium chain	Rn.6302	FABP5	Fatty acid binding protein 5, epidermal	Rn.98269
ACSL1	Acyl-CoA synthetase long-chain family member 1	Rn.6215	FABP7	Fatty acid binding protein 7, brain	Rn.10014
ACSL4	Acyl-CoA synthetase long-chain family member 4	Rn.87821	FADS1	Fatty acid desaturase 1	Rn.28161
ACSL5	Acyl-CoA synthetase long-chain family member 5	Rn.105862	FADS2	Fatty acid desaturase 2	Rn.35189
ACADSB	Acyl-Coenzyme A dehydrogenase, short/branched chain	Rn.44423	FASN	Fatty acid synthase	Rn.9486
ACADS	Acyl-coenzyme A dehydrogenase, short chain	Rn.1167	G6PDX	Glucose-6-phosphate dehydrogenase	Rn.11040
ACADVL	Acyl-Coenzyme A dehydrogenase, very long chain	Rn.33319	GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	Rn.44456
ACOX1	Acyl-Coenzyme A oxidase 1, palmitoyl	Rn.31796	HADHA	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	Rn.3340
ACOX2	Acyl-Coenzyme A oxidase 2, branched chain	Rn.10622	HADHB	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	Rn.11253
ACOX3	Acyl-Coenzyme A oxidase 3, pristanoyl	Rn.10546	HADHSC	L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain	Rn.92789
ABCG5	ATP-binding cassette, sub-family G (WHITE), member 5	Rn.74258	LCAT	Lecithin cholesterol acyltransferase	Rn.10481
ACLY	ATP citrate lyase	Rn.29771	NCKAP1	NCK-associated protein 1	Rn.2611
CES3	Carboxylesterase 3	Rn.34885	SLC27A1	Solute carrier family 27 (fatty acid transporter), member 1	Rn.1047
CPT1A	Camitine palmitoyltransferase 1, liver	Rn.2856	SLC27A2	Solute carrier family 27 (fatty acid transporter), member 2	Rn.3608
CD36	CD36 antigen	Rn.102418	SCD1	Stearoyl-Coenzyme A desaturase 1	Rn.1023
CLTA	Clathrin, light polypeptide (Lca)	Rn.112599	SC4MOL	Sterol-C4-methyl oxidase-like	Rn.7167
CYP7A1	Cytochrome P450, family 7, subfamily a, polypeptide 1	Rn.10737	SC5D	Sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (S. cerevisiae)	Rn.18741
CYP8B1	Cytochrome P450, family 8, subfamily b, polypeptide 1	Rn.23013	SCP2	Sterol carrier protein 2	Rn.31887
			SREBP1	Sterol regulatory element binding protein1	Rn.95306

<sup>†</sup> To analyze the mechanism of the improvement of lipid metabolism, we focused on these genes involved in lipid metabolism.

**Table 4** Primers Used to Generate Probes for Quantitative Real-time PCR.

Gene	Forward primer sequence	Reverse primer sequence
GAPD	5'-CCGAGGGCCCACTAAAGG-3'	5'-CATCCTGGGCTACACTGAGGACCAGG-3'
G6PDX	5'-TTGCTGCACAAGATTGATCGA-3'	5'-AGAAGCCCCAGCCCATCCCG-3'
FASN	5'-GGACATGGTCACAGACGATGAC-3'	5'-AAGGCTGGGCTCTATGGGTTGCCTAAG-3'
CYP51	5'-GGAGGCAACCTGCTTTCCA-3'	5'-CTCATCGCCTGCGCCTTCACG-3'
SC4MOL	5'-GGCTCGGCATCACGATTT-3'	5'-CACATGAACTTCATTGGGAATTACGCCTCC-3'
SCD1	5'-CACGCCGACCTCACAAAC-3'	5'-CCCGCCGTGGCTTTTTCTTCTCTC-3'
SREBP1	5'-CTGGCCAATGGACTACTAGTGTTG-3'	5'-CCTGCTTGGCTCTTCTTTTGTCTACGGG-3'
HMGCS1	5'-GTAGATGCTGGAAAGTATACCATTGG-3'	5'-TGGGCCAGGCCAGGATGGG-3'
GPAM	5'-CCAGGACGGAGAGGAACGT-3'	5'-CCTTAAACATTTTCACAGCATTCTTC-3'
ACADSB	5'-AAGCAAGTGCATCGAGTGGAT-3'	5'-TTCGCATCGCGGAAGAA-3'
CYP8B1	5'-TCTGCCATGCTCCCTGTAAGA-3'	5'-ACCACAGGTTTTCTCCTGAGA-3'
CYP4A12	5'-AGCATGCTGTCTGCCATTTG-3'	5'-AAGGTGAGAAGAAAGAATGAGATGTGA-3'
CD36	5'-CCTCTCCCTCTCTGGTGTCTT-3'	5'-GGCGAGGAACAGAACATATTAAGC-3'
FADS2	5'-GATGCCTACCTCCACAAATGAAG-3'	5'-CCTCTGGCTGTCACCCAAC-3'

GAPD, Glyceraldehyde-3-phosphate dehydrogenase; other abbreviations are the same as in **Table 3**.

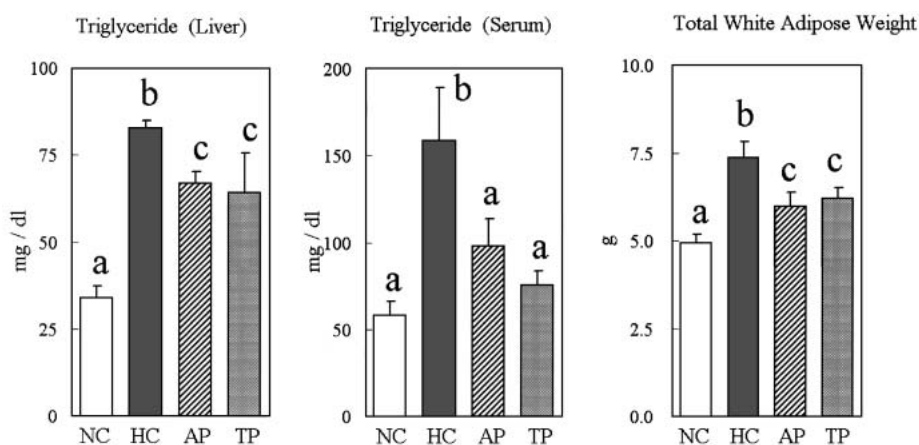
ingestion.

### 3 Results

#### 3.1 Hepatic Gene Expression Analysis of Rats Fed a High-fat Diet

We previously reported that feeding a high-fat diet increases serum and liver triglyceride levels and white adipose tissue weight in rats, and that these parameters

are significantly improved by the intake of apple polyphenols or tea catechins (**Fig. 1**). Some reports suggested a high-fat diet reduces the expression of certain genes in the liver, such as fatty acid synthase (FASN), glucose-6-phosphate dehydrogenase (G6PDX) and ATP citrate lyase (ACLY) (16-18). We confirmed the expression of these genes in the HC group was reduced 1.5-fold or more compared with the NC group (**Table 5**).



**Fig. 1** Reducing Effect of Triglyceride and White Adipose Weight by Ingested Polyphenols (High-fat diet rats).

All values are presented as mean  $\pm$  standard error (n=6). Values without a common superscript in a row are significantly different at  $p < 0.05$ . Statistical comparisons between groups were analyzed by Duncan's new multiple-range test.

**Table 5** Lipid Metabolism-related Genes in the Liver Whose Expression Intensity Changed 1.5-fold or More in Rats Fed High-fat Diet.

Gene symbol	Fold change	Fold change
	(HC/NC) DNA microarray	(HC/NC) qPCR
CYP8B1	2.1	3.2
FABP2	2.2	-
RELO1	1.5	-
SREBP1	1.5	1.7
ACLY	-2.5	-
FABP5	-2.5	-
FASN	-2.5	-5.4
G6PDX	-1.7	-1.5
SCD1	-1.8	-5.2

Abbreviations are the same as those in **Table 3**.

**Table 6** lists lipid metabolism-related genes whose expression intensity was changed 1.5-fold or more by polyphenol ingestion. Common changes observed in the AP and TP groups, when compared against the HC group were: the fatty acid hydrolase gene, acyl-Coenzyme A dehydrogenase short / branched chain (ACADSB (19)) was up-regulated 2.2-fold (AP) and 1.9-fold (TP) by Gene Chip (According to the qPCR confirmation, they were 1.5-fold and 1.4-fold respectively). The fatty acid transport-related gene CD36 antigen (CD36 (20)) was 1.5-fold (AP) and 1.5-fold (TP) (qPCR: 2.0 and 2.8). And fatty acid synthase

gene, stearoyl-Coenzyme A desaturase 1 (SCD1) was down-regulated -1.8-fold (AP) and -4.5-fold (TP) (qPCR: -2.0 and -7.1). Changes in the expression of genes observed only in the AP group were: the fatty acid hydrolase gene, glycerol-3-phosphate acyltransferase, mitochondrial (GPAM (21)) was up-regulated 1.9-fold (qPCR: 1.4) and the fatty acid synthases genes, FASN and fatty acid desaturase 2 (FADS2) were down-regulated -1.6-fold and -1.5-fold respectively (qPCR: -1.7 and -1.5). Changes in the expression of genes observed only in the TP group were: the cholesterol synthase genes, 3-hydroxy-3-methylglutaryl Coenzyme A synthase 1 (HMGCS), CYP51 (22), the sterol synthase gene SC4MOL, and the bile acid biosynthesis-related gene CYP8B1 were up-regulated 1.5-fold, 2.6-fold, 2.9-fold and 1.5-fold respectively (qPCR: 2.3, 3.6, 4.6 and 1.4). And the transcription-factor gene sterol-regulatory element binding protein (SREBP1) was down-regulated -1.5-fold (qPCR: -1.9).

### 3.2 Effects of Apple Polyphenols Ingestion on Parameter of Rats Fed a Normal Diet

To examine whether the anti-obesity mechanism described above is also effective in a normal diet, we administered apple polyphenols to rats fed a normal diet (**Table 7**). We found the weight of visceral adipose tissue was reduced significantly, suggesting that apple polyphenols suppress adipose accumulation in a normal diet as well as a high-fat diet.

**Table 6** Lipid Metabolism-related Genes in the Liver Whose Expression Intensity Changed 1.5-fold or More in Rats Fed the Polyphenol Diet.

Gene symbol	AP		TP		
	Fold change	Fold change	Fold change	Fold change	
	(AP/HC) DNA microarray	(A/HC) qPCR	(TP/HC) DNA microarray	(TP/HC) qPCR	
ACADSB	2.2	1.5	ACADSB	1.9	1.4
CD36	1.5	2.0	CD36	1.5	2.8
SCD1	-1.8	-2.0	SCD1	-4.5	-7.1
GPAM	1.9	1.4	CYP51	2.6	3.6
FASN	-1.6	-1.7	CYP8B1	1.5	1.4
FADS2	-1.5	-1.5	HMGCS1	1.5	2.3
			SC4MOL	2.9	4.6
			SREBP1	-1.5	-1.9

Abbreviations are the same as in **Table 3**.

**Table 7** Reducing Effect of White Adipose Weight by Ingested Apple Polyphenols (Normal Diet Rats).

Group	NC2	AP2
Initial BW (g)	124.6 ± 1.67	122.6 ± 1.78
Weight gain (g)	42.6 ± 13.6	41.2 ± 21.2
Epididymal WAT (g/100 g of BW)	1.21 ± 0.07	1.03 ± 0.02*
Perirenal WAT (g/100 g of BW)	0.98 ± 0.06	0.84 ± 0.04
Mesenteric WAT (g/100 g of BW)	0.76 ± 0.03	0.67 ± 0.04
Total WAT (g/100 g of BW)	2.95 ± 0.14	2.54 ± 0.06*

NC2, normal control (normal diet); AP2, normal diet + apple polyphenols.

All values are presented as mean ± standard error.

Statistical comparisons between groups were analyzed by one-sided Student's T test.

\*Value is significantly different at  $p < 0.05$ .

### 3.3 Hepatic Gene Expression Analysis of Rats Fed a Normal Diet

Gene expression changes observed in the AP2 group, when compared against the NC2 group were: FASN and SCD1 were down-regulated -3.6-fold and -1.5-fold respectively (**Table 8**).

We also found that gene expression of G6PDX involved in the pentose phosphate pathway, ACLY involved in acetyl CoA synthesis, and SREBP1 involved in fatty acid metabolism in AP2 group were down-regulated compared with NC2 group. On the other hand, CD36, a gene involved in fatty acid transport was down regulated with apple polyphenols ingestion, unlike in the high-fat diet. Furthermore, genes involved in cholesterol metabolism, such as 7-dehydrocholesterol reductase (DHCR7), Farnesyl diphosphate farnesyl transferase 1 (FDFT1) and ATP-binding cassette, sub-family G, member 5 (ABCG5) were altered in the expression.

## 4 Discussion

The ingestion of apple polyphenols reduced the weight of visceral adipose tissue and the triglyceride content of blood and liver in rats fed a high-fat diet. To examine the effect of apple polyphenols on lipid-metabolism we analyzed hepatic gene expression using DNA microarrays.

SCD1, a gene found to be down-regulated in rats fed a high-fat diet was down-regulated by the intake of both

polyphenols. This result indicates that a high-fat feeding decreased fatty acid synthesis but polyphenols ingestion decreased it furthermore. Stearoyl-CoA desaturase is a microsomal enzyme for the biosynthesis of oleate and palmitoleate, which are the major monounsaturated fatty acids of triglycerides and cholesterol esters. Triglyceride synthesis in liver is highly dependent on the expression of the SCD1 gene. Previous reports showed that SCD1 activity correlates with blood triglyceride levels in rodents, and it is suggested that SCD1 activities in liver and adipose might be rate-limiting for triglyceride production (23-26). In this experiment, the low levels of serum and liver triglyceride observed in AP group and HP group might have resulted from down-regulating of SCD1 gene expression. Furthermore, it could be that adipose gene expression altered by both polyphenols ingestion. Some studies showed polyphenol ingestion improved lipid profiles and affected adipose gene expression such as FASN, SREBP1, Peroxisome Proliferative Activated Receptor, Gamma (PPARG) and Uncoupling Protein 2 (UCP2) (27-29). They hypothesized that polyphenol ingestions may cause the reduction of triglyceride accumulation and enhancement of thermogenesis in adipose tissues. We previously showed the intake of apple polyphenols reduce the PPAR  $\gamma$  protein levels in brown and white adipose tissue. PPAR  $\gamma$  is master regulator of the expression of genes that are associated with adipocyte differentiation. The reduction PPAR  $\gamma$  may lead to anti-obesity effect. In this study, we did not perform the analysis of adipose gene expression, so further studies are needed to help reveal the impacts on adipose tissues. Additionally, beta-oxidation may be facilitated by the administration of tea catechins (4, 5), and the increase in the expression of ACADSB by the intake of both polyphenols supports this. The genes involved in the cholesterol synthesis and cholesterol catabolism such as HMGCS, CYP51, SC4MOL and CYP8B1 were up-regulated by the tea catechins intake. Similar results were showed on other studies (30), and it is explained increased removal from circulation may link the expression of hepatic genes involved in cholesterol metabolism as a hypothesis.

On the other hand, down-regulation of the fatty acid synthases genes, FASN and FADS2 observed only in the AP group. It was suggested that apple polyphenols suppress the expression of multiple kinds of fatty acid synthesis genes. In our study, AP group and TP group

showed similar improvement effect on lipid profiles, in spite of tea polyphenols had stronger inhibition of SCD1 gene expression than apple polyphenols (Table 6). It may be possible that the down-regulation of FASN contributed to the reducing of triglycerides and adipose tissue weight. We found that the ingestion of apple polyphenols suppresses adipose accumulation in a normal diet as well as a high-fat diet (Table 7). The down-regulation of genes involved in fatty acid synthesis was observed in this lean condition (Table 8), however the alternation of genes involved in beta-oxidation was not observed. This suggests the ingestion of apple polyphenols correlate with adipose accumulation by suppression of fatty acid synthesis in the liver. Moreover, SCD1 and FASN were down-regulated in common with a high-fat diet experiment. These genes may play a major role in suppression of adipose accumulation. Taken together, it is suggested that apple polyphenols decrease transcription of genes involved in fatty acid synthesis, and it may contribute to suppression of visceral adipose tissue accumulation.

As stated above, these polyphenols had similar effects on lipid metabolism-related phenotypes, such as triglyceride levels and white adipose tissue weight, however they showed different effects on the expression intensity of hepatic lipid metabolism-related genes. It is considered that the inhibition effect of polyphenols on lipid absorption plays a great role in improvements

of lipid profiles (3, 31, 32). In addition to this mechanism, we postulate that the ingestion of apple polyphenols prevents triglyceride accumulation by suppression of fatty acid synthesis in the liver. Previous studies confirmed intestinal absorption of catechins and procyanidins (33, 34), suggesting that components of these polyphenols reach the liver after intestinal absorption and exert specific effects there. This indicates the usefulness of ingesting multiple types of polyphenols, rather than a single type, for maintaining our health. In addition to the functions of single polyphenol types, further investigation into the synergistic effects of multiple polyphenol types is very important and are underway in our laboratory.

Obesity due to excessive calorie intake causes various diseases; further studies on apple polyphenols as potential substances that reduce the risk of metabolic syndromes are necessary.

In conclusion, apple polyphenols improve lipid metabolism and have anti-obesity effect as well as tea catechins. However, this study showed that the mechanisms of action of these polyphenols are not necessarily the same and that apple polyphenols have widely inhibition effects of fatty acid synthesis in the liver. Since polyphenols are believed to have different mechanisms of action, it would be useful, for maintaining our health, to ingest polyphenols derived from multiple food products.

**Table 8** Lipid Metabolism-related Genes in the Liver Whose Expression Intensity Changed 1.5-fold or More Due to Apple Polyphenols Ingestion in Rats Fed Normal Diet.

Gene symbol	Fold change (AP2 / NC2) DNA microarray
ABCG5	3.4
ACYL	-2.0
CD36	-1.7
DHCR7	-1.8
FABP5	-2.4
FASN	-3.6
FDFT1	-1.5
G6PDX	-2.7
SCD1	-1.5
SREBF1	-1.6

Abbreviations are the same as those in Table 3.

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