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Note

The Combination of 'Benifuuki' with Quercetin Suppresses Hepatic Fat Accumulation in High-Fat High-Cholesterol Diet-Fed Rats

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Summary We investigated the combined effects of 'Benifuuki,' a tea cultivar that contains *O*-methylated catechins like epigallocatechin-3-*O*-(3-*O*-methyl) gallate, and quercetin on hepatic fat accumulation in male Sprague-Dawley rats fed a high-fat, high-cholesterol diet for 15 d. Rats given 'Benifuuki'+quercetin had synergistically lower liver triglyceride (TG) level compared with rats given 'Benifuuki' or quercetin alone. Compared with 'Benifuuki' or quercetin alone, supplementation with 'Benifuuki'+quercetin resulted in a low level of fatty acid synthase (*FAS*) and stearoyl-CoA desaturase1 (*SCD1*) gene expression levels. These results suggest that the combination of 'Benifuuki' and quercetin has greater liver lipid-lowing effects than that of 'Benifuuki' or quercetin alone. The liver TG-lowing effect of combination of 'Benifuuki' with quercetin may be partially mediated by the suppression of lipogenesis. The combination of 'Benifuuki' and quercetin suppresses hepatic fat accumulation in high fat high cholesterol diet fed rats, showing a new trend of 'Benifuuki' as synergist with quercetin.

Key Words 'Benifuuki,' quercetin, lipid metabolism, cholesterol, triglyceride, tea

Tea (*Camellia sinensis* L.) is a beverage widely consumed worldwide, and green tea consumption is particularly common in Asian countries. Green tea has been reported to have various health benefits, such as antioxidant (*1*), anti-carcinogenic (*2*), hypocholesterolemic (*3*), and hepatoprotective activities (*4*). Catechins, a group of polyphenolic compounds, have shown to be largely responsible for these activities. Methylated catechin derivatives (5), epigallocatechin-3-*O*-(3-*O*-methyl) gallate (EGCG3″Me) and epigallocatechin-3-*O*-(4-*O*-methyl) gallate (EGCG4″Me) were identified in some cultivars, such as 'Benifuuki,' Benihomare, and Tong ting oolong tea (*6*). These tea extracts and purified methylated catechins have anti-allergic effects in vivo and in vitro (*7*).

Quercetin is a flavonoid found in onions, apples, berries, and other fruits and vegetables (*8*). It has been shown to have antihypertensive effects in animal models of hypertension (*9*). Furthermore, it was reported that quercetin has hypolipidemic effects in hyperlipidemia and atherosclerosis rabbits (*10*), ameliorates metabolic syndrome (*11*), and improves the inflammatory status in obese Zucker diabetic rats (*12*).

While 'Benifuuki' and quercetin have some beneficial functions, there is little information on their synergistic effects in high-fat high-cholesterol induced fatty liver rats. In this study, we aimed to investigate the combined effects of 'Benifuuki' and quercetin on hepatic fat accumulation in rats fed a high-fat and high-cholesterol diet and its related mechanism. We used 'Benifuuki' extract because it is useful as an ingredient for food industrial applications.

Materials and Methods

Materials. 'Benifuuki' extract was obtained from Asahi Soft Drinks Co., Ltd. (Tokyo, Japan). 'Benifuuki' extract was extracted with hot water. The extract was concentrated by freezing, and dried using spray-dry. Quercetin was purchased from Sigma Chemicals (St. Louis, MO, USA). 'Benifuuki' extract catechins and anhydrous caffeine concentrations were simultaneously measured by high-performance liquid chromatography (HPLC) method as previously described (*13*). Protein was determined by Kjeldahl method. Fat concentration was determined by the acid decomposition method. Dietary fiber was determined by Proski method. Moisture, sugar and ash were measured using standard methods.

Animals and diets. Three-week-old male Sprague-Dawley rats were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The rats were individually housed in metal cages in a temperature-controlled room (22˚C)

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Table 1. Composition of diets.

Ingredient	Standard	Control	'Benifuuki' extract (B)	Quercetin (Q)	B+Q
	(g/kg diet)				
Corn starch	329.5	247	237	242	232
Sucrose	300	300	300	300	300
Soybean oil	40	40	40	40	40
Lard	30	100	100	100	100
Casein	200	200	200	200	200
L-Cystine	3	3	3	3	3
AIN 93G vitamin mix	10	10	10	10	10
AIN 93G mineral mix	35	35	35	35	35
Cellulose	50	50	50	50	50
Cholesterol	0	10	10	10	10
Cholic acid sodium salt	0	2.5	2.5	2.5	2.5
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
'Benifuuki' extract	0	0	10	0	10
Quercetin	0	0	0	5	5

under a 12-h light/dark cycle. After a 4-d adaptation period feeding with a commercial CE-2 pellet diet (CLEA Japan, Inc., Tokyo, Japan), rats were assigned equally by weight to five groups (standard group: $n=4$, high-fat high-cholesterol containing diet groups: $n=6$). Each group was fed with the diets shown in Table 1 with food and water ad libitum for 15 d.

At the end of the experiment, rats were fasted overnight and then sacrificed under anesthesia with sodium pentobarbital (65 mg/kg of body weight) and atropine sulfate (0.40 mg/kg of body weight). Blood was collected by cardiac puncture, and plasma was prepared and stored at −20°C until further analysis. The liver, abdominal subcutaneous fat, epididymal fat, mesenteric fat, retroperitoneal fat, and interscapular brown fat were dissected. Tissues were snap-frozen in liquid nitrogen and stored at −80°C until further analysis. All animal procedures were performed in accordance with the Animal Experiment Guidelines of Chiba University and complied with the "Guide for the Care and Use of Laboratory Animals" (NIH publication no. 85-23, revised 1985). The animal protocol was approved by the Animal Use Committee at Chiba University (Registration ID No. Dou-25-307).

Measurement of the plasma parameters. Plasma parameters were measured using assay kits. The levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride (TG), and glucose in the plasma were measured with a cholesterol E-test Wako kit, HDL-cholesterol E-test Wako kit, triglyceride E-test Wako kit, and glucose CII-test Wako kit (all from FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), respectively. The level of low-density lipoprotein (LDL) cholesterol in the plasma was calculated with the Friedewald formula (*14*), that is, LDL-cholesterol=total cholesterol−HDL-cholesterol−(TG/5).

Measurement of total cholesterol and TG in the liver. Liver lipids were extracted and purified as previously described (*15*). The concentrations of total cholesterol and TG were measured with a cholesterol E-test Wako kit and triglyceride E-test Wako kit (both from FUJIFILM Wako Pure Chemical Corporation).

Isolation of total RNA and quantitative real-time PCR analysis. Total RNA was isolated from rat liver with RNAiso Plus (Takara, Shiga, Japan) in accordance with the manufacturer's protocol. Real-time PCR was performed to quantify mRNA expression, using an ABI PRISM 7000 sequence detection system (Applied Bio Systems, Waltham, MA, USA) and SYBR Premix Ex Taq (Takara). Oligonucleotide primer pairs were designed with the Primer3 website (http://bioinfo.ut.ee/primer3-0.4.0/) as follows: rat *HMGCR* (accession no. NM_013134.2, forward: 5′-TGCTGCTTTGGCTGTATGTC-3′, reverse: 5′-TGAGCGTGAACAAGAACCAG-3′); rat *CYP7A1* (NM_012942.2, forward: 5′-CACCATTCCTGCAACCTTTT-3′, reverse: 5′-GTACCGGCAGGTCATTCAGT-3′); rat *SREBP-2* (NM_001033694.1, forward: 5′-AGACTTGGTCATGGGGACAG-3′, reverse: 5′-GGGGAGACATCAGAAGGACA-3′); rat *SREBP-1c* (NM_001276707.1, forward: 5′-CTTGACCGACATCGAAGACA-3′, reverse: 5′-AGAGAGGAGCCCAGAGAAGC-3′); rat *FAS* (X62888.1, forward: 5′-TCGAGACACATCGTTTGAGC-3′, reverse: 5′-TCAAAAAGTGCATCCAGCAG-3′); rat *SCD1* (NM_139192.2, forward: 5′-GCTTCCAGATCCTCCCTACC-3′, reverse: 5′-CAACAACCAACCCTCTCGTT-3′); rat *ACC1* (NM_022193.1, forward: 5′-TACAACGCAGGCATCAGAAG-3′, reverse: 5′-TGTGCTGCAGGAAGATTGAC-3′); rat *PPARα* (NM_013196.1, forward: 5′-TCACACAATGCAATCCGTTT-3′, reverse: 5′-GGCCTTGACCTTGTTCATGT-3′); rat *CPT1α* (NM_031559.2, forward: 5′-ATGACGGCTATGGTGTCTCC-3′, reverse: 5′-GTGAGGCCAAACAAGGTGAT-3′); and rat *GAPDH* (NM_017008.4, forward: 5′-CTCATGACCACAGTCCATGC-3′, reverse: 5′-TTCAGCTCTGGGATGACCTT-3′) as an internal control.

Statistical analysis. All values are expressed as mean±standard error (SE). All data except for standard

Table 2. Composition of 'Benifuuki' extract.

Ingredient	'Benifuuki' extract
	(mg/g)
Epigallocatechin gallate (EGCG)	111.8
Gallocatechin gallate (GCG)	17.7
Epicatechin gallate (ECG)	29.8
Catechin gallate (CG)	2.8
Epigallocatechin (EGC)	81.5
Gallocatechin (GC)	22.1
Epicatechin (EC)	28.6
Catechin (C)	6.7
Epigallocatechin-3-*O*-(3-*O*-methyl) gallate (EGCG″3Me)	21.0
Gallocatechin-3-*O*-(3-*O*-methyl) gallate (GCG″4Me)	3.8

group were analyzed using two-way ANOVA with Dunnett's multiple comparison post hoc test using BellCurve for Excel ver. 2.10 (Social Survey Research Information Co., Ltd., Tokyo, Japan). Differences were considered significant at $p<0.05$.

Results

The plasma parameters of rats in each group were measured. The levels of total cholesterol, HDL cholesterol, LDL cholesterol, TG, and glucose did not differ significantly among the groups (Data not shown).

The 'Benifuuki' sample was extracted using hot water. 'Benifuuki'-specific *O*-methylated catechins (EGCG″3Me+GCG″3Me) and EGCG″3Me in hot water extract of 'Beifuuki' were found to be 24.8 and 21.0 mg/g, respectively (Table 2). Unmethylated cat-

Table 3. Effect of 'Benifuuki' extract and quercetin on food intake, body weight and organ weights in rats fed high-fat high-cholesterol diet.

Parameter	S	C	B	Q	B+Q	ANOVA		
						B	Q	B×Q
Food intake (g/15 d)	215±11	207±6	204±4	200±6	196±2	NS	NS	NS
Initial body weight (g)	72.6±1.6	73.9±1.3	73.8±1.4	73.8±1.6	73.9±1.4	NS	NS	NS
Final body weight (g)	175±7	184±5	180±2	178±6	179±1	NS	NS	NS
Subcutaneous fat weight (%)	2.44±0.31	1.90±0.15	1.80±0.24	1.68±0.14	1.74±0.17	NS	NS	NS
Epididymal fat weight (%)	1.14±0.07	0.95±0.05	0.83±0.07	0.90±0.06	0.88±0.06	NS	NS	NS
Mesenteric fat weight (%)	1.27±0.08	1.40±0.13	1.08±0.11	1.11±0.10	1.13±0.10	NS	NS	NS
Retroperitoneal fat weight (%)	1.59±0.17	1.34±0.12	0.81±0.08	0.99±0.15	0.69±0.05*	$p<0.01$	$p<0.05$	NS
Brown fat weight (%)	0.16±0.02	0.16±0.01	0.19±0.02	0.18±0.01	0.14±0.01	NS	NS	$p<0.05$
Liver weight (%)	5.19±0.16	8.27±0.20	7.15±0.17*	7.54±0.23	6.38±0.16*	$p<0.01$	$p<0.01$	NS

Values are expressed as mean±standard error (S group: $n=4$, C, B, Q, B+Q groups: $n=6$). Statistical analysis was performed by two-way ANOVA using 'Benifuuki' and quercetin as factors. Post-hok Dunnett's multiple comparison test was performed.
*$p<0.05$ represents significant difference from the control group (C group).
NS: not significant.
(%)=organ weights (g)/carcass weight (g)×100.
S, low-fat diet standard group; C, high-fat and high-cholesterol diet control group; B, high-fat and high-cholesterol diet treated with 'Benifuuki' extract group; Q, high-fat and high-cholesterol diet treated with quercetin group; B+Q, high-fat and high-cholesterol diet treated with 'Benifuuki' extract and quercetin group.

Table 4. Effect of 'Benifuuki' extract and quercetin on liver triglyceride and cholesterol levels in rats fed high-fat high-cholesterol diet.

Parameter	S	C	B	Q	B+Q	ANOVA		
						B	Q	B×Q
Triglyceride (mg/g liver)	19.4±6.1	39.9±3.5	46.7±2.3	40.3±3.8	27.2±2.1*	NS	$p<0.01$	$p<0.01$
Cholesterol (mg/g liver)	5.96±0.14	28.3±1.9	24.7±1.5	20.6±2.2*	18.7±0.9*	NS	$p<0.01$	NS

Values are expressed as mean±standard error (S group: $n=4$, C, B, Q, B+Q groups: $n=6$). Statistical analysis was performed by two-way ANOVA using 'Benifuuki' and quercetin as factors. Post-hok Dunnett's multiple comparison test was performed.
*$p<0.05$ represents significant difference from the control group (C group).
NS: not significant.
S, low-fat diet standard group; C, high-fat and high-cholesterol diet control group; B, high-fat and high-cholesterol diet treated with 'Benifuuki' extract group; Q, high-fat and high-cholesterol diet treated with quercetin group; B+Q, high-fat and high-cholesterol diet treated with 'Benifuuki' extract and quercetin group.

Table 5. Effect of 'Benifuuki' extract and quercetin on liver gene expression in rats fed high-fat high-cholesterol diet.

Parameter	S	C	B	Q	B+Q	ANOVA		
						B	Q	B×Q
SREBP-1	0.655±0.151	1.00±0.13	1.18±0.20	1.19±0.16	1.13±0.10	NS	NS	NS
FAS	0.891±0.275	1.00±0.24	0.400±0.049*	0.417±0.058*	0.299±0.053*	$p<0.05$	$p<0.05$	NS
ACC1	0.608±0.157	1.00±0.17	1.27± 0.19	1.54± 0.21	1.51±0.15	NS	$p<0.05$	NS
SCD1	0.288±0.102	1.00±0.20	0.550±0.116	0.670±0.181	0.376±0.074*	$p<0.05$	NS	NS
PPARa	0.680±0.098	1.00±0.14	1.09± 0.20	1.50±0.29	1.34± 0.14	NS	NS	NS
CPT1a	1.43±0.14	1.00± 0.05	1.41± 0.26	1.70±0.42	1.74±0.31	NS	NS	NS
SREBP-2	0.772±0.024	1.00±0.11	1.11±0.13	1.01±0.18	1.08± 0.19	NS	NS	NS
HMGCR	1.66± 0.61	1.00±0.11	0.791±0.153	0.881±0.123	0.717±0.126	NS	NS	NS
CYP7A1	0.258±0.073	1.00±0.17	1.86± 0.51	0.579±0.162	0.097±0.021*	NS	$p<0.01$	$p<0.05$

Values are expressed as mean±standard error (S group: $n=4$, C, B, Q, B+Q groups: $n=6$). Statistical analysis was performed by two-way ANOVA using 'Benifuuki' and quercetin as factors. Post-hok Dunnett's multiple comparison test was performed.
* $p<0.05$ represents significant difference from the control group (C group).
NS: not significant.
S, low-fat diet standard group; C, high-fat and high-cholesterol diet control group; B, high-fat and high-cholesterol diet treated with 'Benifuuki' extract group; Q, high-fat and high-cholesterol diet treated with quercetin group; B+Q, high-fat and high-cholesterol diet treated with 'Benifuuki' extract and quercetin group.
Sterol regulatory element-binding protein-1 (*SREBP-1*), fatty acid synthase (*FAS*), acetyl-CoA carboxylase 1 (*ACC1*), stearoyl-CoA desaturase 1 (*SCD1*), peroxisome proliferator-activated receptor-α (*PPAR*α), carnitine palmitoyltransferase-I-α (*CPT1*α), sterol regulatory element-binding protein-2 (*SREBP-2*), 3-hydroxy-3-methyl-glutaryl-CoA reductase (*HMGCR*), cholesterol 7α-hydroxylase (*CYP7A1*).

echins in 'Benifuuki' extract were more than 300 mg/g, and epigallocatechin gallate was 111.8 mg/g (Table 2). As other ingredients, moisture, protein, sugar, fat, ash, dietary fiber and anhydrous caffeine were 3.0%, 5.4%, 34.3%, 0.5%, 9.3%, 4.1% and 6.6%, respectively.

Food intake, body weight, and organ weights of rats are shown in Table 3. The food intake, body weight, epididymal fat weight, mesenteric fat weight, and brown fat weight did not differ among the groups. The 'Benifuuki'+quercetin diet rats had significantly lower liver weight and retroperitoneal fat weight compared with these in the control diet group.

The 'Benifuuki'+quercetin diet group had synergistically lower liver TG level compared with that in the control diet group (Table 4). The 'Benifuuki'+quercetin diet group had lower liver cholesterol level compared with that in the control diet group due to the additive effect of 'Benifuuki' and quercetin.

To study the mechanism, we used real-time PCR to find the expression of genes involved in cholesterol metabolism and fatty acid metabolism in the liver. The 'Benifuuki'+quercetin diet group had lower expression of cholesterol 7α-hydroxylase (*CYP7A1*) compared with the control diet group due to the synergistic effect of each test sample. The expression of other genes associated with cholesterol metabolism such as 3-hydroxy-3methyl-glutaryl-CoA reductase (*HMGCR*) and sterol regulatory element-binding protein-2 (*SREBP-2*) did not differ among the groups. The 'Benifuuki'+quercetin diet group had lower expression of fatty acid synthase (*FAS*) and stearoyl-CoA desaturase 1 (*SCD1*) compared with the control diet. The expression of the other genes associated with fatty acid metabolism such as sterol regulatory element binding protein-1c (*SREBP-1c*), acetyl-CoA carboxylase 1 (*ACC1*), peroxisome proliferator-activated receptor-alpha (*PPAR*α), and carnitine palmitoyltransferase-1-α (*CPT1*α) did not differ among the groups (Table 5).

Discussion

In this study, we examined the combined effects of 'Benifuuki' and quercetin on hepatic fat accumulation in rats fed a high-fat and high-cholesterol diet. The concentrations of the test samples in the diets were determined for the following reasons. We previously reported that the increase of plasma TG after the administration of corn oil in rats was suppressed by 'Benifuuki' extract (100 mg per head) (*13*). Moreover, when 1% 'Benifuuki' extract in high-fat high-cholesterol diet was given to rats for 60 d, 'Benifuuki' extract had no obvious toxicity (*16*). On the other hands, 0.5% quercetin reduces serum homocysteine level and malondialdehyde (MDA) level in rats fed a methionine-enriched diet (*17*). Based on these information, we determined the 1% 'Benifuuki' extract and 0.5% quercetin-supplemented diets.

The 'Benifuuki' extract was extracted using hot water. 'Benifuuki'-specific *O*-methylated catechins (EGCG″3Me+GCG″3Me) and EGCG″3Me in hot water extract of 'Beifuuki' were 24.8 and 21.0 mg/g, respectively. Unmethylated catechins in 'Benifuuki' extract were more than 300 mg/g, and epigallocatechin gallate was 111.8 mg/g. The previous study had reported the similar results (*13*). Fat and dietary fiber contents are low. High level of catechins may affect *FAS* and *SCD1* gene expression. On the other hands, caffeine in 'Benifuuki' extract was 6.6%. Helal et al. reported that caf-

feine affected high-fat diet-induced hepatic steatosis in rats and the hepatic mRNA expression of *FAS* and *ACC* was decreased by 20 or 30 mg/kg bw/d caffeine orally feeding (*18*). In this study, caffeine also may affect the expression of some genes related to energy metabolism.

We found that the combination of 'Benifuuki' and quercetin in food helps to suppress liver fat accumulation synergistically. 'Benifuuki'+quercetin-fed rats had lower liver cholesterol compared with control rats due to the additive effect of 'Benifuuki' and quercetin (Table 4). Furthermore, rats given 'Benifuuki'+quercetin had lower liver TG compared with rats given 'Benifuuki' or quercetin alone due to the synergistic effect of 'Benifuuki' and quercetin. The liver weights of the 'Benifuuki'+quercetin-fed rats were significantly lower than those of the control rats due to the additive effect of 'Benifuuki' and quercetin, while no significant differences in body weight or food intake were found among the other groups. These results suggested that the 'Benifuuki'+quercetin diet inhibited fat accumulation in the liver.

To investigate the molecular mechanism by which the combination of 'Benifuuki' and quercetin regulates hepatosteatosis, we investigated hepatic gene expression using real-time PCR (Table 5). Rats given the 'Benifuuki'+quercetin diet had lower *CYP7A1*, *FAS* and *SCD1* expression than control rats due to the additive effect of 'Benifuuki' and quercetin. FAS catalyzes the last step in the biosynthetic pathway of fatty acids. Therefore, it is believed to be a determinant of the maximal capacity of the liver tissue to synthesize fatty acids by de novo lipogenesis. Increases in the activity of FAS are attributed to elevations in serum and liver TG levels (*19*). Therefore, low level of *FAS* expression might explain the reduction in lipid levels in the livers of 'Benifuuki'+quercetin-fed rats. SCD1, which catalyzes the biosynthesis of monounsaturated fatty acids from saturated fatty acids, also has an important role in energy metabolism and regulation of fat accumulation (*20*). The 'Benifuuki'+quercetin group had lower *SCD1* expression than that in the control group. SCD1 plays an essential role in monounsaturated fatty acid synthesis for hepatic lipogenesis of triacylglyceride (*21*). Treatment of mice with *SCD1* antisense oligonucleotides has been shown to result in a higher metabolic rate, prevention of diet-induced obesity, and steatosis (*22*). Therefore, decreased *SCD1* expression could explain the lower liver TG observed in 'Benifuuki'+quercetin-fed rats. The expression of lipogenic genes such as *ACC1*, *FAS*, and *SCD1* is regulated by *SREBP-1c* at the transcriptional level (*23*, *24*). However, we found no effect from 'Benifuuki' and quercetin on *SREBP-1c* or *ACC1* levels. Our data suggested that 'Benifuuki'+quercetin suppresses the expression of *FAS* and *SCD1* without the expression of *SREBP-1c*. In addition, 'Benifuuki' and quercetin did not influence genes related to the oxidation of fatty acids (e.g. *PPARα* and *CPT1α*). Taken together, a plausible mechanism for the hypolipidemic activity of the combination of 'Benifuuki' and quercetin may be their downregulation of genes associated with lipid synthesis (*FAS* in particular). In this study, fatty acid oxidation was not enhanced at mRNA levels in the 'Benifuuki'+quercetin group. However, the suppression of fatty acid synthesis seems to be not enough to decrease lipid accumulation in the liver. There is a possibility of the increase in fatty acid oxidation at enzymatic activity levels. Murase et al. reported that oral administration of EGCG (200 mg/kg bw) to BALB/c mice induced an increase in AMP-activated protein kinase (AMPK) activity in the liver concomitant with a significant increase in AMPKα and ACC phosphorylation (*25*). EGCG administration also increased oxygen consumption and fat oxidation in mice (*25*). So, it is necessary to examine the effect of the combination of 'Benifuuki'+quercetin on fatty acid oxidation at protein levels in the future.

CYP7A1 is a liver-specific enzyme that catalyzes the rate-limiting step in the biosynthesis of bile acid from cholesterol. We showed that the 'Benifuuki'+quercetin group had synergistically lower *CYP7A1* expression compared with the control group. This result indicated that the biosynthesis of bile acid from cholesterol was inhibited in 'Benifuuki'+quercetin-fed rats. 'Benifuuki'+quercetin fed rats had lower liver cholesterol contents, so *CYP7A1* mRNA expression may be lower to maintain cholesterol metabolism homeostasis.

The results from the present study demonstrate that quercetin is able to enhance the hypolipidemic effect of 'Benifuuki' by modulating the expression of genes involved in fatty acid metabolism due to the additive effect of 'Benifuuki' and quercetin. In the presence of quercetin, 'Benifuuki' decreased the mRNA expression levels of *FAS*, leading to a decrease in the biosynthesis of fatty acids, and a decrease in liver TG levels. It was previously demonstrated that the combination of quercetin and green tea polyphenols was able to increase the bioavailability of green tea polyphenols in cancer cells and in severe combined immunodeficiency mice (*26*, *27*). While supplementation with 'Benifuuki' alone had no significant hypolipidemic effect, supplementation with 'Benifuuki'+quercetin had a marked hypolipidemic effect via downregulation of the gene expression levels of *FAS* in the liver. This suggests that the enhanced TG-lowering ability of 'Benifuuki'+quercetin compared with 'Benifuuki' alone is because of increased bioavailability of 'Benifuuki.'

In conclusion, combination of 'Benifuuki' and quercetin was found to decrease liver weight, and decrease liver TG levels compared with 'Benifuuki' or quercetin alone. In addition, compared with supplementation 'Benifuuki' alone, supplementation with 'Benifuuki'+quercetin resulted in a downregulation of the gene expression levels of *FAS*. Based on the data and information available, the TG-lowing effect of 'Benifuuki'+quercetin in the liver may be mediated partially via the suppression of lipogenesis. However, whether the combination of 'Benifuuki' and quercetin has lipid-lowing effects in vivo and in vitro requires further study.

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