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

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(Received July 31, 2018)

Summary We investigated the combined effects of ‘Benifuuki,’ a tea cultivar that contains O-methylated catechins like epigallocatechin-3-O-(3-O-methyl) gallocate, 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-lowering effects than that of ‘Benifuuki’ or quercetin alone. The liver TG-lowering 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) gallocate (EGCG3”Me) and epigallocatechin-3-O-(4-O-methyl) gallocate (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 Prosky 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).
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 Experiment Guidelines of Chiba University and 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 and triglyceride E-test Wako kit (both from FUJIFILM Wako Pure Chemical Corporation). 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′-TGGCTGTTTGGCTGTATGATC-3′, reverse: 5′-TGAGCGTAACAAGAACACGAG-3′); cat CYP7A1 (NM_012942.2, forward: 5′-CACCATTCTGCACACCTTTC-3′, reverse: 5′-GTACCCAGCTCATTACGT-3′); rat SREBP-2 (NM_001033694.1, forward: 5′-AGACTTTGGTCATGGGGACAG-3′, reverse: 5′-GGGGAGACTCATCAGAAGCAG-3′); rat CPT1 (NM_001276707.1, forward: 5′-CTTGAGGAGCCCGAGAGAC-3′, reverse: 5′-TGAGAGACCACATCATGAC-3′); rat ACC1 (NM_0131912.2, forward: 5′-GCTTCCAGATCTCCCTTACC-3′, reverse: 5′-CAAACCAACCCTCTCAGT-3′); rat ACAT1 (NM_022193.1, forward: 5′-TCATCGTGGAGTGGCAGG-3′, reverse: 5′-TACAAAGCTCAGC-3′); rat SCD1 (NM_017008.4, forward: 5′-GGCCCTGACCTTGACACC-3′, reverse: 5′-GATTTGCGCATCAAGAC-3′); rat PPARα (NM_013196.1, forward: 5′-TCACACACCATCATGGGTC-3′, reverse: 5′-TGAGCCCTGATGGTCAAGAT-3′); rat CPT1α (NM_031559.2, forward: 5′-GGCCCTGACCTTGACACC-3′, reverse: 5′-GATTTGCGCATCAAGAC-3′); rat ACC1 (NM_022193.1, forward: 5′-TCATCGTGGAGTGGCAGG-3′, reverse: 5′-TACAAAGCTCAGC-3′); rat ACAT1 (NM_0131912.2, forward: 5′-GCTTCCAGATCTCCCTTACC-3′, reverse: 5′-CAAACCAACCCTCTCAGT-3′); rat SCD1 (NM_017008.4, forward: 5′-GGCCCTGACCTTGACACC-3′, reverse: 5′-GATTTGCGCATCAAGAC-3′); rat PPARα (NM_013196.1, forward: 5′-TCACACACCATCATGGGTC-3′, reverse: 5′-TGAGCCCTGACCTTGACACC-3′, reverse: 5′-TGAGCCCTGACCTTGACACC-3′, reverse: 5′-TTCAGCTCCTGGGATGACCTT-3′) as an internal control.

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

| Ingredient | Standard (g/kg diet) | Control (g/kg diet) | ‘Benifuuki’ extract (B) | Quercetin (Q) | B+Q |
|------------|---------------------|---------------------|------------------------|---------------|-----|
| 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 |
| t-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                   | 0                   | 0                      | 0             | 0   |
| Cholic acid sodium salt | 2.5   | 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   |

Table 1. Composition of diets.
Table 2. Composition of 'Benifuuki' extract.

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

Unmethylated catechins significantly among the groups (Data not shown).

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   |
|----------------------------------|---------|---------|---------|---------|---------|---------|
| Food intake (g/15 d)             | 215±11  | 207±6   | 204±4   | 200±6   | 196±2   | 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      |
| Final body weight (g)            | 175.7   | 184±5   | 180±2   | 178±6   | 179±1   | 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      |
| Epididymal fat weight (%)        | 1.14±0.07| 0.95±0.05| 0.83±0.07| 0.90±0.06| 0.88±0.06| 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      |
| 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   |
| Brown fat weight (%)             | 0.16±0.02| 0.16±0.01| 0.19±0.02| 0.18±0.01| 0.14±0.01| NS      |
| 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   |

ANOVA: B: p<0.01, Q: p<0.01, B×Q: p<0.01

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-hoc Dunnett’s multiple comparison test was performed.

NS: not significant.

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   |
|--------------------------|--------|--------|--------|--------|--------|---------|
| 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      |
| 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      |

ANOVA: B: p<0.01, Q: p<0.01, B×Q: 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-hoc Dunnett’s multiple comparison test was performed.

NS: not significant.

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-methylate catechins (EGCG″3Me+GCG″3Me) and EGCG″3Me in hot water extract of 'Benifuuki' were found to be 24.8 and 21.0 mg/g, respectively (Table 2). Unmethylated cat-
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The expression of the other genes (diet group had lower expression of fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD1) compared with the control diet. The expression of 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 (PPARα), and carnitine palmitoyltransferase-1-α (CPT1α), sterol regulatory element-binding protein-2 (SREBP-2), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR), cholesterol 7α-hydroxylase (CYP7A1).

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 |
|-------------------|-----------------|-----------------|-----------------|-----------------|------------------|-------|
| SREBP-1           | 0.655±0.151     | 1.00±0.13       | 1.18±0.20       | 1.19±0.16       | 1.13±0.10        | 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|
| ACC1              | 0.608±0.157     | 1.00±0.17       | 1.27±0.19       | 1.54±0.21       | 1.51±0.15        | p<0.05|
| SCD1              | 0.288±0.102     | 1.00±0.20       | 0.550±0.116     | 0.670±0.181     | 0.376±0.074*     | p<0.05|
| PPARα             | 0.680±0.098     | 1.00±0.14       | 1.09±0.20       | 1.50±0.29       | 1.34±0.14        | NS    |
| CPT1α             | 1.43±0.14       | 1.00±0.05       | 1.41±0.26       | 1.70±0.42       | 1.74±0.31        | NS    |
| SREBP-2           | 0.772±0.024     | 1.00±0.11       | 1.11±0.13       | 1.01±0.18       | 1.08±0.19        | NS    |
| HMGCR             | 1.66±0.61       | 1.00±0.11       | 0.791±0.153     | 0.881±0.123     | 0.717±0.126      | NS    |
| CYP7A1            | 0.258±0.073     | 1.00±0.17       | 1.86±0.51       | 0.579±0.162     | 0.097±0.021*     | p<0.01|

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-hoc Dunnett’s multiple comparison test was performed.

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 ‘Benifuuki’ 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 (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 (PPARα), and carnitine palmitoyltransferase-1-α (CPT1α), sterol regulatory element-binding protein-2 (SREBP-2), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR), cholesterol 7α-hydroxylase (CYP7A1).
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, EAS, 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 down-regulation 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-lowering 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-lowering effects in vivo and in vitro requires further study.

Acknowledgments

This work was supported by a grant from the Research
REFERENCES

1) Osada K, Takahashi M, Hoshina S, Nakamura S, Sugano M. 2001. Tea catechins inhibit cholesterol oxidation accompanying oxidation of low density lipoprotein in vitro. *Comp Biochem Physiol C Toxicol Pharmacol* **128**: 153–164.

2) Lambert JD, Yang CS. 2003. Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. *Mutat Res* **523**: 201–208.

3) Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I. 2002. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. *Int J Obes Relat Metab Disord* **26**: 1459–1464.

4) Masterjohn C, Bruno RS. 2012. Therapeutic potential of green tea in nonalcoholic fatty liver disease. *Nutr Rev* **70**: 41–56.

5) Sano M, Tabata M, Suzuki M, Degawa M, Miyase T, Maeda-Yamamoto M. 2001. Simultaneous determination of twelve tea catechins by high-performance liquid chromatography with electrochemical detection. *Analyst* **126**: 816–820.

6) Suzuki M, Yoshino K, Maeda-Yamamoto M, Miyase T, Sano M. 2000. Inhibitory effects of tea catechins and O-methylated derivatives of (-)-epigallocatechin-3-O-gallate on mouse type IV allergy. *J Agric Food Chem* **48**: 5649–5653.

7) Maeda-Yamamoto M, Ema K, Shibuchi I. 2007. In vitro and in vivo anti-allergic effects of ‘benifuuki’ green tea containing O-methylated catechin and ginger extract enhancement. *Cytotechnology* **55**: 135–142.

8) Erlund I. 2004. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. *Nutr Res* **24**: 851–874.

9) Perez-Vizcaino F, Duarte J, Jimenez R, Santos-Buelga C, Osuna A. 2009. Antihypertensive effects of the flavonoid quercetin. *Pharmacol Rep* **57**: 604–609.

10) Panchal SK, Poudyal H, Brown L. 2012. Quercetin ameliorates diabetic cardiovascular, hepatic, and metabolic changes in diet-induced metabolic syndrome in rats. *J Nutr* **142**: 1026–1032.

11) Rivera L, Morón R, Sánchez M, Zarruezo A, Galisteo M. 2008. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. *Obesity (Silver Spring)* **16**: 2081–2087.

12) Egashira Y, Kamohara T, Yamaguchi W, Irie H, Toyoda Y, Hanamura M, Hirai S, Shinoda Y, Maeda-Yamamoto M. 2013. Suppression of postprandial hypertriglyceridaemia in rats by benifuuki tea extract. *Nippon Shokuhin Kagaku Kaishi* **60**: 407–411 (in Japanese).

13) Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18**: 499–502.

14) Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**: 497–509.

15) Hanamura M, Irie H, Shinoda Y, Yamaguchi W, Maeda-Yamamoto M, Hirai S, Egashira Y. 2012. Effects of Benifuuki green tea and its extract on growth and plasma components in high-cholesterol diet-fed rats. *Hort Research* **66**: 37–42.

16) Meng B, Gao W, Wei J, Yang J, Wu J, Pu L, Guo C. 2013. Quercetin reduces serum homocysteine level in rats fed a methionine-enriched diet. *Nutrition* **29**(4): 661–666.

17) Helal MG, Ayoub SE, Elkashefand WF, Ibrahim TM. 2018. Caffeine affects HDL-induced hepatic steatosis by multifactorial intervention. *Hum Exp Toxicol* **37**(9): 983–990.

18) Ai ZL, Zhu CH, Min M, Wang J, Lan CH, Fan LL, Sun WJ, Chen DE. 2011. The role of hepatic liver X receptor α and sterol regulatory element binding protein-1c-mediated lipid disorder in the pathogenesis of non-alcoholic steatohepatitis in rats. *J Int Med Res* **39**: 1219–1229.

19) Dobrzyn A, Ntambi JM. 2004. The role of stearoyl-CoA desaturase in body weight regulation. *Trends Cardiovasc Med* **14**: 77–81.

20) Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendzierski CM, Yandell BS, Song Y, Cohen P, Friedman JM, Attie AD. 2002. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci USA* **99**: 11482–11486.

21) Jiang G, Li Z, Liu F, Ellsworth K, Dallas-Yang Q, Wu M, Ronan J, Esau C, Murphy C, Szallowski D, Bergeron R, Doebber T, Zhang BB. 2005. Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. *J Clin Invest* **115**: 1030–1038.

22) Ferré P, Foulé E. 2007. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. *Horm Res* **68**: 72–82.

23) Shimano H. 2009. SREBP’s: physiology and pathophysiology of the SREBP family. *FEBS J* **276**: 616–621.

24) Murase T, Misawa K, Haramizu S, Hase T. 2009. Catechin-induced activation of the LKB1/AMP-activated protein kinase pathway. *Biochem Pharmacol* **78**(1): 78–84.

25) Wang P, Heber D, Henning SM. 2012. Quercetin increased the antiproliferative activity of green tea polyphenol (-)-epigallocatechin gallate in prostate cancer cells. *Nutr Cancer* **64**: 580–587.

26) Wang P, Heber D, Henning SM. 2012. Quercetin increased bioavailability and decreased methylation of green tea polyphenols in vitro and in vivo. *Food Funct* **3**: 635–642.