Fermented green tea extract exhibits hypolipidaemic effects through the inhibition of pancreatic lipase and promotion of energy expenditure

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Abstract
Hyperlipidaemia is a major cause of atherosclerosis and related CVD and can be prevented with natural substances. Previously, we reported that a novel Bacillus-fermented green tea (FGT) exerts anti-obesity and hypolipidaemic effects. This study further investigated the hypotriglyceridaemic and anti-obesogenic effects of FGT and its underlying mechanisms. FGT effectively inhibited pancreatic lipase activity in vitro (IC50, 0.48 mg/ml) and ameliorated postprandial lipaemia in rats (26% reduction with 500 mg/kg FGT). In hypertriglyceridaemic hamsters, FGT administration significantly reduced plasma TAG levels. In mice, FGT administration (500 mg/kg) for 2 weeks augmented energy expenditure by 22% through the induction of plasma serotonin, a neurotransmitter that modulates energy expenditure and mRNA expressions of lipid metabolism genes in peripheral tissues. Analysis of the gut microbiota showed that FGT reduced the proportion of the phylum Firmicutes in hamsters, which could further contribute to its anti-obesity effects. Collectively, these data demonstrate that FGT decreases plasma TAG levels via multiple mechanisms including inhibition of pancreatic lipase, augmentation of energy expenditure, induction of serotonin secretion and alteration of gut microbiota. These results suggest that FGT may be a useful natural agent for preventing hypertriglyceridaemia and obesity.

Key words: Fermented green tea; Pancreatic lipase; Serotonin; Hyperlipidaemia; Energy expenditure; Gut microbiota

Energy homoeostasis is tightly regulated by the interaction of central and peripheral organs that control total energy intake and energy expenditure. Positive energy balance leads to excess storage of energy molecules, primarily in the form of TAG, in metabolic tissues including adipose, liver, muscle and pancreatic tissues, causing cellular dysfunction and lipotoxicity(1–22), which are the major causes for developing metabolic disorders such as type 2 diabetes and related CVD(3–7). Accordingly, treatment and prevention of hyperlipidaemia is critical for lowering the risk of CVD(8–10).

Several effective and potent hypolipidaemic drugs are available including statins, fibrates and metformin(9–12), however, these drugs may not be tolerated for long-term treatment and may cause significant side-effects. Thus, natural substances have been considered alternatives for the prevention of dyslipidaemia in humans – for example, resveratrol and berberine ameliorate hyperlipidaemia and related metabolic disorders by activating AMP-activated protein kinase (AMPK)(13–15).

On the other hand, numerous animal studies have demonstrated that green tea and its processed products (e.g. oolong tea and black tea) exhibit lipid-lowering effects(16–25). The hypotriglyceridaemic effects of green tea and its derivatives have also been well documented in clinical trials and have recently been intensively reviewed(26). For instance, consumption of green tea inhibits lipid digestion and absorption after a meal(27), and long-term supplementation with green tea improves plasma lipid profiles and increases the levels of antioxidants(28,29). Black tea also exerts hypotriglyceridaemic effects in humans(30,31). A meta-analysis of human studies revealed that black tea reduces serum cholesterol and LDL concentrations(32). Similar to other hypotriglyceridaemic agents (e.g. metformin and berberine), green and black teas activate

Abbreviations: AMPK, AMP-activated protein kinase; FF, fenofibrate; FGT, fermented green tea; HFD, high-fat diet; LPL, lipoprotein lipase.
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Evidently, a novel Bacillus-fermented green tea (FGT) exerts anti-obesity and hypolipidaemic effects. This study further investigated the hypotriglyceridaemic and anti-obesogenic effects of FGT and its underlying mechanisms. FGT effectively inhibited pancreatic lipase activity in vitro (IC50, 0.48 mg/ml) and ameliorated postprandial lipaemia in rats (26% reduction with 500 mg/kg FGT). In hypertriglyceridaemic hamsters, FGT administration significantly reduced plasma TAG levels. In mice, FGT administration (500 mg/kg) for 2 weeks augmented energy expenditure by 22% through the induction of plasma serotonin, a neurotransmitter that modulates energy expenditure and mRNA expressions of lipid metabolism genes in peripheral tissues. Analysis of the gut microbiota showed that FGT reduced the proportion of the phylum Firmicutes in hamsters, which could further contribute to its anti-obesity effects. Collectively, these data demonstrate that FGT decreases plasma TAG levels via multiple mechanisms including inhibition of pancreatic lipase, augmentation of energy expenditure, induction of serotonin secretion and alteration of gut microbiota. These results suggest that FGT may be a useful natural agent for preventing hypertriglyceridaemia and obesity.

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AMPK and inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase – key molecules involved in the control of lipid metabolism. In addition, in humans, oolong tea enhances lipid excretion in faeces. Collectively, these data suggest that green tea and processed green teas may be effective agents for improving hyperlipidaemia and its related metabolic complications.

In a previous study, we proposed that fermented green tea (FGT) with Bacillus sp. had anti-obesogenic effects in diet-induced obese mice. We observed that FGT reduced plasma lipid levels as well as plasma glucose levels, implying that, similar to green tea and related products, FGT exerts hypotriglyceridaemic effects. To elucidate the effects and the underlying mechanism through which FGT influences lipid metabolism, we designed additional experiments in this study. Specifically, we examined the hypotriglyceridaemic effects of FGT in acute and diet-induced chronic hyperlipidaemic animal models. To determine the molecular mechanisms of FGT-mediated hypotriglyceridaemic effects, we evaluated the enzymatic activity of pancreatic lipase. We also measured energy expenditure and the expressions of lipid metabolism-related genes in FGT-administered animals. Finally, we analysed gut microbiota from faecal samples.

**Methods**

**Reagents and fermented green tea extract preparation**

Triton WR-1339 (Triton, a lipoprotein lipase (LPL) inhibitor) and fenofibrate (FF, PPARα agonist) were purchased from Sigma. FGT extracts were produced by Mizon Co., as described in the previous study with the de-cafﬁnation method. In brief, dried green tea leaves were mixed with 1% sucrose and B. subtilis (5 x 10⁷ colony-forming unit) and fermented at 50°C for 3 d, followed by further incubation at 90°C for 4 d to remove remaining B. subtilis. After fermentation, the FGT was dried and extracted with 50% ethanol at 70°C for 2 h. Analysis of catechin and caffeine composition was performed as described previously. The composition of catechins and caffeine in the FGT is shown in Table 1.

**Acute hypotriglyceridaemic effect of fermented green tea**

All animal experiments were approved by the Amorepacific Institutional Animal Care and Use Committee (PQ13-8007) and adhere to the Organisation for Economic Cooperation and Development (OECD) guidelines. Sprague–Dawley (SD) male rats, 6-week-old, were purchased from the Central Laboratory Animal Inc. and maintained in a 12 h dark–12 h light cycle chamber with controlled temperature of 22–25°C and 40–50% humidity. For adaptation, rats were fed normal chow *ad libitum* for 1 week. The average level of plasma TAG was not significantly different (online Supplementary Table S1). After adaptation, animals were divided into four groups (Saline, Triton, Triton + FGT and Triton + FF; n = 5/group). Triton was utilised to induce hyperlipidaemia, and FF was used as a positive control. Rats were orally injected with saline, 500 mg/kg of body weight of FGT or 65 mg/kg body weight of FF for 5 d. After 5 d, animals were fasted overnight, and the final administration of selected agents (saline, FGT and FF) was carried out 1 h before Triton treatment. Finally, Triton (200 mg/kg body weight) was delivered to all rats except those in the saline group through the tail vein. At 0, 3, 5, 18 and 20 h after Triton injection, blood samples were collected to measure plasma TAG levels. Plasma TAG levels were measured using an automated clinical chemistry analyzer (Cobas111; Roche).

**Prevention of diet-induced hyperlipidaemia by fermented green tea**

Golden Syrian male hamsters, 9-week-old male, purchased from the Central Laboratory Animal Inc., were maintained in a 12 h light–12 h dark cycle chamber with controlled temperature of 21–25°C and 50–60% humidity. After being fed a commercial chow diet (Central Laboratory Animal Inc.) for 1 week, hamsters were fed a high-fat diet (HFD) (Central Laboratory Animal Inc.) with 10% fructose in drinking water for 2 weeks, followed by a western diet (Central Laboratory Animal Inc.) with 10% fructose in drinking water for another 2 weeks. At first, the hamsters were randomly assigned to four groups: the control (water as a vehicle and the western diet), FF (positive control; western diet with 100 mg/kg body weight of FF) and two FGT groups (200 and 400 mg/kg body weight of FGT with the western diet); diets were orally administered for 4 weeks. During the experiment, plasma samples were collected every 2 weeks, and the concentrations of TAG were analysed by a Cobas C111 automated clinical chemistry analyzer following the manufacturer’s protocol. To examine the effect of long-term treatment with FGT on plasma TAG levels, 9-week-old male Golden Syrian hamsters were purchased from the Central Laboratory Animal Inc., adopted and fed a western diet as described above. Next, the hamsters were randomly assigned to five groups: control, FF (100 mg/kg body weight) and three FGT groups (200, 400 or 600 mg/kg body weight, respectively). Hamsters were administered water (as a vehicle), FGT or FF via oral gavage for 12 weeks, respectively. During administration of the reagent, a western diet with 10% fructose was still supplied to all hamsters. Plasma samples from hamsters were collected just before and after 12 weeks of treatment, and TAG concentrations were analysed as described above. All experiments involving mice and hamsters were performed according to a protocol approved by the Animal Experiment Committee of Korea University (Protocol No. KUIACUC-2013-139).

**Table 1. Composition of catechins (C) in green tea and fermented green tea (FGT)**

<table>
<thead>
<tr>
<th>Components</th>
<th>Green tea (% w/w)</th>
<th>FGT (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>26.56</td>
<td>8.27</td>
</tr>
<tr>
<td>GC</td>
<td>1.52</td>
<td>1.26</td>
</tr>
<tr>
<td>EGC</td>
<td>11.7</td>
<td>1.32</td>
</tr>
<tr>
<td>C</td>
<td>0.39</td>
<td>0.22</td>
</tr>
<tr>
<td>EC</td>
<td>2.16</td>
<td>0.26</td>
</tr>
<tr>
<td>EGC</td>
<td>8.74</td>
<td>2.63</td>
</tr>
<tr>
<td>GCG</td>
<td>0.82</td>
<td>2.02</td>
</tr>
<tr>
<td>ECG</td>
<td>1.23</td>
<td>0.56</td>
</tr>
<tr>
<td>Caffeine</td>
<td>8.13</td>
<td>3.70</td>
</tr>
<tr>
<td>Catechin + caffeine</td>
<td>34.69</td>
<td>11.97</td>
</tr>
</tbody>
</table>

GC, gallatechin; EGC, epigallocatechin; EC, epicatechin; EGC, epicatechin gallate; GCG, gallatechin gallate; EGC, epicatechin gallate.
Pancreatic lipase activity assay

Pancreatic lipase activity was measured as previously described (30). In brief, FGT was dissolved in distilled water (as a negative control) or in 50 μl of 4-methylumbelliferyl olate (4-MO; as a substrate; Sigma-Aldrich Co. LLC.) solution dissolved in an assay buffer (13 mM TRIS-HCl, 150 mM NaCl and 1·3 mM CaCl2 with pH 8.0). Subsequently, 25 μl of pancreatic lipase (50 U/ml; Sigma-Aldrich Co. LLC.) was added and incubated at 25°C for 50 min. To terminate the enzyme reaction, 100 μl of sodium citrate (100 mM) was added to the reaction mixture. The amount of 4-methylumbelliferone released from 4-MO by pancreatic lipase was measured using TECAN M200 PRO fluorometric plate reader (TECAN Trading AG; excitation 355 nm and emission 460 nm). The IC50 of FGT on pancreatic lipase was calculated from a regression line of the plots in the logarithm of FGT concentration vs. pancreatic lipase activity graph.

Measurement of energy expenditure and plasma neurotransmitter levels

C57BL/6J male mice, 6-week-old, were purchased from the Central Laboratory Animal Inc. and maintained in a 12h light–12h dark cycle chamber with controlled temperature of 21–25°C and 50–60% humidity. For adaptation, mice were fed an AIN-76A diet (Central Laboratory Animal Inc.) ad libitum for 1 week. After adaptation, mice were fed an AIN-76A-based HFD (45%) with orally administered 500 mg/kg body weight/d of FGT. The same volume of distilled water was given to the control group for 2 weeks. VO2 and carbon dioxide production (VCO2) were measured using the Oxylab Physiotoxic System (Panlab) and the software suite METABOLISM (version 2.2.01; Panlab). The respiratory exchange ratio used for estimating the RQ was calculated as VCO2/VO2, and energy expenditure was calculated according to the formula (kJ (kcal)/ (d kg-0.75)) = VO2·1.44+(3.815·+(1·232·resting energy requirement)).

For neurotransmitter measurements, 6-week-old male C57/BL6 mice were purchased from the Central Laboratory Animal Inc. and adapted for 1 week. After adaptation, mice were fed a 45% HFD. During the administration of a HFD, FGT (500 mg/kg body weight) or water (as a vehicle) was orally administered for 8 weeks. After FGT administration, mice were fasted overnight, and blood samples were collected and centrifuged (4°C, 3000 rpm, 5 min). Supernatants were transferred to new micro-centrifuge tubes. Plasma levels of dopamine, norepinephrine and serotonin were measured using a dopamine ELISA kit (Abnova), norepinephrine ELISA kit (LifeSpan Biosciences) and serotonin ELISA kit (Abcam), respectively, following each manufacturer’s instructions. White adipose tissue (WAT) and liver tissue were separated and stored at −80°C for further use. All animal experiments were approved by the Amorepacific Institutional Animal Care and Use Committee (AP11-FR008) and adhered to the OECD guidelines.

Pyrosequencing analysis of gut microbiota

For pyrosequencing analysis, faeces samples were collected for 3 consecutive days before the animals were euthanised with CO2.

The stool samples were stored at −80°C until analysis, and then genomic DNA was extracted from pooled faecal samples using the FastDNA™ SPIN kit for Faececs (MP Biomedical) according to the manufacturer’s protocol. For pyrosequencing, amplification of genomic DNA was performed using barcoded primers that target the V1–V3 region of the bacterial 16S rRNA gene. Amplification, sequencing and basic analysis were performed according to the methods described by Chun et al. (33) and were completed by ChunLab Inc. using the 454 GS FLX Titanium Sequencing Systems (Roche). Sequence reads were identified using EzTaxon-e database (http://eztaxon-e.ezbiocloud.net)(38) on the basis of 16S rRNA sequence data. We analysed the number of sequences, observed the diversity richness (operational taxonomic units (OTU)) and estimated the OTU richness (abundance-based coverage estimator and Chao1 indices). Bacterial community abundance and composition were generated using CLcommunity software (ChunLab Inc.).

RNA isolation, complementary DNA synthesis and quantitative RT-PCR

RNA from tissues was isolated using the RNeasy® Mini Kit (Qiagen) following the manufacturer’s protocol. Each RNA sample (2 μg) was subjected to complementary DNA (cDNA) synthesis using the RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Relative mRNA levels were determined by quantitative RT-PCR (qRT-PCR) using the appropriate primers (Bioneer) as described previously (33). Primer sequences used for qRT-PCR are provided in the online Supplementary Table S2.

Activity of lipoprotein lipase

HepG2 human hepatoma cells were obtained from the Korean Cell Line Bank and grown in Dulbeco’s modified Eagle’s medium (DMEM) supplemented 10% FBS and 1% penicillin and streptomycin (PEST) at 37°C in an atmosphere containing 5% CO2. HepG2 were cultured in six-well plates at a density of 104 cells/well for 24 h, and then cells were treated with various concentrations of FGT (0·03, 0·1, 0·3, 1, 3, 10, 30 and 100 μg/ml) in DMEM without FBS and PEST for another 24 h. After incubation, the supernatants were collected, and human LPL concentrations were analysed using an ELISA-based Human LPL Assay Kit (Immuno-Biological Laboratories Co., Ltd), according to the manufacturer’s instructions.

Statistical analysis

All data are shown as means with their standard errors. Student’s t test was performed for two-group comparison, and one-way ANOVA was performed for multiple-group comparison. P < 0·05 was considered as significant.

Results

Fermented green tea relieves acute hyperlipidaemia in rats

In the previous study, we showed that FGT decreased plasma lipid levels in proportion to the reduction in body weight in...
diet-induced obese mice\(^{(35)}\). To elucidate whether FGT exerts hypotriglyceridaemic effects, regardless of body weight control, we administered FGT to SD rats by oral gavage for 5 d. FGT administration did not significantly alter body weight (online Supplementary Table S3), implying that acute treatment of FGT is independent of body weight change with a normal diet. To provoke hyperlipidaemia acutely, we injected Triton, a LPL inhibitor, to SD rats. As shown in Fig. 1(a), Triton administration caused a robust increase in plasma TAG levels, whereas pretreatment with FF blunted the hyperlipidaemic effect induced by Triton. Similarly, FGT reduced hypertriglyceridaemia by 26% (Fig. 1(b)). Collectively, these results suggest that FGT exhibits hypotriglyceridaemic effects.

**Fermented green tea reduces plasma TAG levels in diet-induced hyperlipidaemic hamsters**

FGT partially improved acute hyperlipidaemia induced by Triton treatment (Fig. 1). To assess whether FGT inhibited diet-induced elevations in plasma lipid levels, we administered FGT (200 or 500 mg/kg body weight) to western diet-induced hyperlipidaemic hamsters for 4 weeks. Although low-dose FGT (200 mg/kg) failed to lower plasma TAG levels, FF (100 mg/kg) and high-dose FGT (500 mg/kg) treatment blunted further elevations in plasma TAG (Fig. 2(A)). These data imply that high doses of FGT are required to acutely lower plasma TAG levels. To further elucidate long-term and dose-responsive effects of FGT on Western diet-fed hyperlipidaemic animals, Western diet-induced hyperlipidaemic hamsters were administered FF (100 mg/kg, as positive control) or FGT (200/400/600 mg/kg) for 12 weeks. Interestingly, FGT lowered plasma TAG levels in a dose-dependent manner (Fig. 2(B)). Thus, low-dose FGT likely requires a long time to exert its hypotriglyceridaemic effect, whereas a high-dose of FGT rapidly reduces plasma TAG levels.

**Fermented green tea inhibits pancreatic lipase activity**

We found that FGT blunted plasma TAG levels in hyperlipidaemic animal models (Fig. 1 and 2). However, it is unclear how FGT reduces plasma lipid levels. In order to identify potential hypolipidaemic mechanisms, we examined the promoter activity of PPAR\(\alpha\), liver X receptor and forkhead box O, protein levels of LPL and activity of diacylglyceride acyltransferase; however, none of them was affected by FGT (online Supplementary Fig. S1). Dietary lipids are digested by pancreatic lipases and absorbed in the gut. Therefore, inhibition of pancreatic lipase would be a mechanism for the treatment of acquired hyperlipidaemia. To elucidate whether FGT-mediated hypotriglyceridaemic effect requires modulation of pancreatic lipase activity, we assessed pancreatic lipase inhibition assay using FGT. As shown in Fig. 3, FGT effectively and dose-dependently suppressed enzymatic activity of pancreatic lipase. Calculated from the experimental data, the IC\(_{50}\) of FGT on pancreatic lipase is 0.49 mg/ml.

![Fig. 1. Fermented green tea (FGT) alleviates acute hyperlipidaemia. Sprague-Dawley rats were administered saline ( ), FGT (Triton + FGT ( ); 500 mg/kg body weight) or fenofibrate (FF) (Triton + FF ( ); 65 mg/kg body weight) for 5 d. After overnight fasting, Triton (200 mg/kg body weight) was injected via the tail vein, and blood samples were collected at 0, 3, 5, 18 and 20 h after Triton administration. (a) Changes in plasma TAG levels are shown as line graphs, and (b) the AUC was calculated and presented as bar graphs (n 5/group). * P < 0.05 v. Triton, ** P < 0.01 v. Triton, *** P < 0.001 v. Triton. To convert TAG from mg/dl to mmol/l, multiply by 0.0113.](https://doi.org/10.1017/S0007114516004621)

![Fig. 2. Fermented green tea (FGT) exerts hypotriglyceridaemic effects. Hamsters fed a Western diet were administered vehicle, fenofibrate (FF , ) or FGT for 4 weeks (A) or 12 weeks (B). Plasma TAG levels are presented as line graphs (n 7/group). Control, Triton, FGT 200, FGT 500, FGT 400; FGT 600. Mean values with unlike letters were significantly different (P < 0.05). To convert TAG from mg/dl to mmol/l, multiply by 0.0113.](https://doi.org/10.1017/S0007114516004621)
Fermented green tea augments energy expenditure, modulates the expressions of lipid metabolism-related genes and increases plasma serotonin levels

FGT inhibited pancreatic lipase activity and increased faecal lipid content (Fig. 3 and online Supplementary Fig. S2).

However, dietary lipids and pancreatic lipase are not the causal factors in Triton-induced acute hyperlipidaemia. This suggests that the hypotriglyceridaemic effect of FGT could be mediated by multiple mechanisms. Interestingly, we observed that FGT-administered animals were more active, compared with the vehicle group (data not shown), suggesting that FGT might affect energy expenditure. To determine FGT-mediated changes in energy expenditure, C57BL/6J mice were fed a HFD with oral administration of FGT (500 mg/kg) for 2 weeks in an animal metabolic monitoring system. VO₂ (Fig. 4(a)) and energy expenditure (Fig. 4(b)) were significantly elevated during the light and dark cycles in the FGT-fed mice, whereas the RQ was unchanged between FGT and vehicle groups (Fig. 4(c)). Thus, FGT appears to encourage energy expenditure without affecting energy source.

To further elucidate the effect of FGT on energy metabolism, we measured mRNA expressions of lipid metabolism-related genes (e.g. sterol regulatory element-binding protein-1c (SREBP1c), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl CoA desaturase-1 (SCD1), acyl-CoA oxidase

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**Fig. 3.** Fermented green tea (FGT) inhibits pancreatic lipase activity. Enzymatic activity of pancreatic lipase is shown as line graph (n 5). **P < 0.01 v. 0, ***P < 0.001 v. 0.

**Fig. 4.** Fermented green tea (FGT) increases energy expenditure. VO₂ (a), energy expenditure (EE) (b) and RQ (c) of FGT-treated mice were measured as described in the Methods and shown as bar and line graphs (n 5). *P < 0.05 v. control, **P < 0.01 v. control (—, control; —, FGT). To convert kcal to kJ, multiply by 4-184.
(ACO), carnitine palmitate transferase-1 (CPT1), medium-chain acyl CoA dehydrogenase (mCAD) and PPARα) in WAT and the liver. Notably, expressions of lipogenic genes (SREBP1c, ACC, FAS and SCD1) were down-regulated (Fig. 5(a)), whereas expressions of fatty acid oxidation-related genes (ACO, CPT, mCAD and PPARα) remained up-regulated (Fig. 5(b)) in both tissues. These data imply that FGT might control the expressions of lipid metabolism-related genes to modulate circulating lipid levels. Surprisingly, plasma concentrations of serotonin, a neurotransmitter associated with energy expenditure and behaviour\(^{40,41}\), were significantly increased in FGT-administered mice (Fig. 6(a)), and the expressions of fatty acid oxidation genes were up-regulated by FGT as well as serotonin treatments in cultured adipocytes and myocytes, respectively (Fig. 6(b) and (c)). These results suggest that FGT stimulates lipid metabolism to increase energy expenditure by inducing serotonin.

**Fig. 5. Fermented green tea (FGT) modulates mRNA expressions of lipid metabolism-related genes in peripheral tissues.** Gene expressions of sterol regulatory element-binding protein-1c (SREBP1c), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), acyl-CoA oxidase (ACO) and carnitine palmitate transferase-1 (CPT1) in adipose tissue (a) and liver (b) are shown as bar graphs (n 5/group). WAT, white adipose tissue; SCD1, stearyl CoA desaturase-1; CPT1, carnitine palmitate transferase-1; mCAD, medium-chain acyl CoA dehydrogenase; Control; ■, high-fat diet (HFD); ○, HFD + FGT. * P < 0.05 v. control; ** P < 0.01 v. control; *** P < 0.001 v. control; † P < 0.05 v. HFD, †† P < 0.01 v. HFD, ††† P < 0.001 v. HFD.

**Fig. 6. Fermented green tea (FGT) increases plasma serotonin levels and fatty acid oxidation-related genes.** (a) Plasma levels of neurotransmitters (dopamine (DOP), norepinephrine (NEP) and serotonin (SER); Control, ■, high-fat diet (HFD) group; □, HFD with FGT. * P < 0.05 v. control; *** P < 0.001 v. control, †† P < 0.01 v. HFD. Expressions of fatty acid oxidation genes in (b) 3T3-L1 adipocytes and (c) C2C12 myocytes. FGT (500 μg/ml) or serotonin (SER; 4 ng/ml) treatment was performed for 24 h. mRNA was isolated using Trizol™ Reagent (Life Technologies). Each RNA sample (2 μg) was subjected to complementary DNA (cDNA) synthesis using the RevertAid™ First Strand cDNA Synthesis Kit, and relative mRNA levels were determined by quantitative RT-PCR using the appropriate primers (n 4/group). ACO, acyl-CoA oxidase; CPT1, carnitine palmitate transferase-1; Control, ■, not treated (negative control); □, FGT; ○, SER. * P < 0.05 v. negative control, ** P < 0.01 v. negative control, *** P < 0.001 v. negative control.
microbiota in Western diet-induced hyperlipidaemic hamsters, gut microbiota was analysed by pyrosequencing. Similar to previous results(35), FGT slightly reduced the abundance of the Firmicutes phylum and enhanced the Bacteroidetes phylum, when compared with the vehicle group (Fig. 7(a)). The ratio of Firmicutes:Bacteroidetes was also reduced by FGT treatment (Fig. 7(b)). Collectively, it appears that FGT modulates gut microbiota by suppressing the prevalence of the Firmicutes phylum and facilitating the growth of Bacteroidetes, supporting a role for FGT in the development of metabolic disorders such as obesity and type 2 diabetes.

Discussion

In the present study, we show that FGT effectively rescued postprandial hypertriglyceridaemia and Western diet-induced hyperlipidaemia. We conducted in vivo experiments in different rodent models to confirm the effects of FGT in vivo. First, acute hypolipidaemic effects were examined in rats induced with postprandial lipaemia. Hamsters but not mice readily develop hypertriglyceridaemia on diets; thus, the long-term hypotriglyceridaemic effects of FGT were studied in hamsters. In addition, the metabolic rate and energy expenditure were measured in mice. Each animal model used in this study has been widely used for these experiments. The use of different animal models also shows that the hypolipidaemic effects of FGT are repeatedly found in different animal models, which confirms the validity of the hypolipidaemic effects of FGT.

Plasma TAG concentrations may be reduced by several biological mechanisms. Common therapeutics for hypertriglyceridaemia include the use of fibrates or niacin(45). Fibrates are ligand activators for the nuclear receptor PPARα. Activation of PPARα re-programmes gene expression in lipid metabolism, especially in the liver, thereby increasing fatty acid uptake and oxidation while suppressing VLDL secretion to lower plasma and hepatic TAG levels. Niacin binds and activates GPR109A, a niacin receptor, and suppresses the protein kinase A signalling pathway to lower adipocyte lipolysis, mobilisation of fatty acids to the liver and secretion of VLDL(46). These effects result in the reduction of plasma TAG concentrations as well. In addition, activation of liver X receptor(47) and inhibition of forkhead box O transcription factor(48), LPL(49) and diacylglycerol acyltransferase(50) are associated with the reduction in plasma TAG levels; however, none of those processes was affected by FGT in our activity screening experiments (online Supplementary Fig. S1).

Acute hypertriglyceridaemia in the postprandial state was ameliorated by inhibition of pancreatic lipase activity. Pancreatic lipase suppresses digestion and absorption of dietary lipids from meals; thus, inhibition of pancreatic lipase ameliorates postprandial lipaemia by FGT. Pancreatic lipase, a key-step enzyme in lipid digestion, catalyses the hydrolysis of dietary TAG into monoglyceride and fatty acids, so that dietary lipids are readily absorbed in the digestive tract(51). Therefore, inhibition of pancreatic lipase activity serves as a primary target in the treatment of hyperlipidaemia. Indeed, orlistat, a pancreatic lipase inhibitor, is used to treat obesity by reducing excess energy intake. Interestingly, orlistat also has other biological effects including the reduction of blood pressure and reduction of the incidence of diabetes in human clinical trials with obese patients(52). Whether these additional effects of orlistat are mediated through the suppression of lipid metabolism needs further evaluation. As orlistat, a pancreatic lipase inhibitor, controls hyperlipidaemia, it is feasible that FGT can potentially control hyperlipidaemia and related complications such as hypertension, type 2 diabetes and related CVD, as well as obesity.

In long-term feeding studies, plasma TAG levels were reduced in hamsters. FGT may reduce plasma TAG levels by increasing energy expenditure and serotonin secretion. We suggest that serotonin stimulates the consumption of stored lipid to lower plasma TAG levels, which could be due to induction of fatty acid oxidation gene expressions. Serotonin is a well-known neurotransmitter that is considered to be a ‘happy hormone’ because it is associated with feeding behaviour and mood(53). Mood control has been identified as an important factor in reducing the progression of CHD and its associated mortality(58). The FGT-associated increase in serotonin levels...
may be beneficial in alleviating this risk factor for CVD, which are often accompanied by hyperlipidaemia. Recently, serotonin has been associated with energy expenditure\(^{(40,41)}\). In this study, we demonstrated that FGT augments plasma serotonin levels (Fig. 6). Interestingly, we did not observe marked changes in the expression of tryptophan hydroxylase 1 (tph1) (online Supplementary Fig. S3), an enzyme that is involved in serotonin biosynthesis in the gut of FGT-treated mice. This observation suggests that the effects of FGT on serotonin metabolism involve a different pathway of regulation of serotonin metabolism by FGT, which requires further studies.

It is possible that FGT boosts whole-body energy expenditure to reduce circulating lipid levels by regulating serotonin, at least in part. By augmenting serotonin metabolism, FGT is also expected to modulate happiness and reduce the development of CVD, both of which are thought to be influenced by lipid metabolism and mood. Enhanced energy expenditure reflects a huge consumption of energy, which accompanies a robust increase in lipid catabolism to supply ATP demand. We observed that FGT administration suppressed lipogenic gene expression while enhancing catalytic gene expression in peripheral tissues (Fig. 5), implying that the pattern of mRNA expression of lipid metabolism-related genes shifted favourably from lipogenic to lipolytic following FGT treatment. By combining two mechanisms, inhibition of pancreatic lipase and induction of serotonin secretion, FGT may effectively reduce plasma TAG levels.

In addition, it is possible that FGT compounds may modulate key metabolic regulators including AMPK, silent mating type information regulation 2 homolog 1 (Sirt1) and PGC1\(\alpha\)\(^{(54–56)}\). In a previous study, the amount of gallic acid robustly increased during green tea fermentation\(^{(57)}\). Recently, gallic acid has been reported to exhibit anti-obesity and anti-diabetic properties through the activation of AMPK, Sirt1 and PGC1\(\alpha\)\(^{(58)}\). EGCG, a major component of green tea, also modulates energy metabolism through AMPK activation\(^{(59–61)}\). Although the content of EGCG in FGT is much lower than that of green tea, the catechins and increased gallates (possibly due to metabolism of catechin gallates) are able to mediate the hypotriglyceridaemic effects of FGT. Furthermore, there are more active compounds that are effective in modulating lipid metabolism in processed green teas. For instance, theaflavins from black tea reduce cholesterol incorporation into micelles\(^{(62)}\), thereby reducing cholesterol uptake. Although we have not yet identified active compounds for key metabolic regulators, we are presently attempting to identify the major polyphenolic compounds in FGT by utilising various biochemical analytical methods. Further research is required to identify the active components and to evaluate the detailed mechanism underlying FGT-mediated hypotriglyceridaemic effects.

Changes in the gut microbiota is closely correlated with the development and treatment of lipid metabolism-related disorders including obesity and type 2 diabetes\(^{(63,64)}\). In the analysis of microbiota changes, the ratio of Firmicutes:Bacteroidetes ratio in mouse gut microbiota, and the present study confirms the previous findings in hamster microbiota\(^{(55)}\). In the present study, changes in microbiota were associated with complex metabolic alterations including reduced TAG levels and body weight; thus, it is not possible to characterise microbiota changes specific to hypotriglyceridaemic effects. However the Firmicutes:Bacteroidetes ratio in hamsters was significantly reduced, which confirmed our previous findings. It has been suggested that the phylum Firmicutes predominates the gut microbiota of obese mice\(^{(52)}\); thus, the host likely receives more energy content with increasing Firmicutes levels in the gut. Therefore, FGT-induced alteration of the composition of the gut microbiota (reduced Firmicutes) contributed to the reduction in energy intake in the absence of a change in food intake, thereby reducing body weight gain and fat mass increase, at least in part.

In the analysis of gut microbiota, the most abundant genera was Allobaculum, which was increased in the FGT group (45-7 and 53-6% in control and FGT, respectively). Allobaculum was shown to be enriched after exercise in rats\(^{(66)}\), augmented when supplemented with grain sorghum lipid extract in hamsters\(^{(67)}\) and increased with improved metabolic parameters in obese and insulin-resistant rats after berberine feeding\(^{(68)}\). In addition, Ruminococcus was reduced in hamsters fed FGT (8-3 and 5-7% in control and FGT, respectively). Ruminococcus has been found to be more abundant in obese subjects than in non-obese subjects\(^{(69)}\). These changes may be associated with the hypotriglyceridaemic effects of FGT, and further studies will be performed on this issue in the future.

In conclusion, FGT inhibits pancreatic lipase activity and induces serotonin secretion to modulate lipid metabolism and reduces hyperlipidaemia in animal models. We propose that FGT may be a novel hypotriglyceridaemic agent for the treatment of lipid dysregulation and related complications.

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Supplementary material
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References


