Theaflavin Synthesized in a Selective, Domino-Type, One-Pot Enzymatic Biotransformation Method with *Camellia sinensis* Cell Culture Inhibits Weight Gain and Fat Accumulation to High-Fat Diet-Induced Obese Mice

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The polyphenolic compound theaflavin, which is the main red pigment present in black tea, is reported to elicit various physiological effects. Because of the extremely low concentration of theaflavin present in black tea, its extraction from black tea leaves in quantities sufficient for use in medical studies has been difficult. We have developed a simple, inexpensive, selective, domino-type, one-pot enzymatic biotransformation method for the synthesis of theaflavin that is suitable for use in medical studies. Subsequent administration of this synthetic theaflavin to high-fat diet-induced obese mice inhibited both body weight gain and visceral fat accumulation, with no significant difference in the amount of faeces between the experimental and control mice.

Key words theaflavin; *Camellia sinensis* cell culture; peroxidase; hydrolase; selective domino-type one-pot enzymatic biotransformation

Theaflavins, which include theaflavin (TF) (1) and its galloyl esters TF 3-O-gallate (TF3G) (2), 3′-O-gallate (TF3′G) (3) and 3,3′-di-O-gallate (TFDG) (4), are the main red pigments in black tea (Fig. 1). These compounds are reported to elicit a variety of physiological effects, including anti-obesity,1 glucose-lowering,2,3) and anticancer effects.4) Therefore, TF (1) has become recognized as both a natural coloring agent and a bioactive substance. Because of the extremely low concentration of TF (1) present in black tea, its extraction from black tea leaves in quantities sufficient for use in medical studies has been difficult. Therefore, functional studies of TF (1) (particularly animal experiments with the long term dosage and testing in humans) have lagged seriously behind those of the catechins.

Recently, we reported the synthesis of TF (1) from epicatechin (EC) (5) and epigallocatechin (EGC) (7) in *Camellia (C.) sinensis* cell culture.5) The reaction proceeded in 70% yield and 100% conversion with a reaction time of 4 min. The effect of the synthetic TF (1) on increases in blood sugar was evaluated in mice. The oral administration of TF (1) (30.6 mg/kg) to 5-week-old C57BL6 mice (n = 6) significantly inhibited increases in blood glucose in a sucrose loading test (2 g/kg), relative to the effect of distilled water administration in the control mice (maximum blood glucose levels 20 min after administration of TF (1) 195.0±22.4 mg/dL, control 220.0±8.2).

Animal experiments with long term TF (1) dosing are very important for the elucidation of TF (1) function. However, our synthesis of TF (1) was expensive, because the price of raw materials [EC (5) and EGC (7)] was very expensive. Therefore, we could not use our synthesis of TF (1) for further medical studies.

Then, we developed a simple, inexpensive, and large-scale synthesis of TF (1) that is suitable for use in medical studies, and we examined whether this synthetic TF (1) inhibits weight gain and fat accumulation in the high-fat diet-induced obesity mouse model. To synthesize TF (1) inexpensively, we paid attention to the method by which black tea is manufactured. Black tea is manufactured through the fermentation of fresh green tea leaves. Fresh green tea leaves contain the four major *epi*-type catechins: EC (5), epicatechin 3-O-gallate (ECG) (6), EGC (7) and epigallocatechin 3-O-gallate (EGCG) (8). These chemical structures are shown in Fig. 2. During the fermentation process, these four major *epi*-type catechins (5–8) are converted into TF (1), TF3G (2), TF3′G (3) and TFDG (4) through the enzymatic activity of endogenous polyphenol oxidase (PPO) and/or peroxidase (POD).6,7) More specifically, TF (1) is biosynthesized from EC (5) and EGC (7); TF3G (2) is biosynthesized from EC (5) and EGCG (8); TF3′G (3) is biosynthesized from ECG (6) and EGC (7) and TFDG (4) is biosynthesized from ECG (6) and EGCG (8).

We previously reported the successful synthesis of TF (1), TF3G (2), TF3′G (3) and TFDG (4) 7) and we have also surveyed various oxidizing agents for potential use in TF (1) generation, including commercial PPO (Funakoshi Co., Ltd., Japan), commercial horseradish peroxidase (HRP) (Wako Pure Chemical Industries, Ltd., Japan), *C. sinensis* cell culture, *Nicotiana tabacum* cell culture and *Daucus carota* cell culture. Among these oxidizing agents, *C. sinensis* cell culture, a rich source of POD,7) is capable of synthesizing TF (1) from EC (5) and EGC (7). *C. sinensis* cell culture converted EC (5) and EGC (7) into TF (1) in 15 min with a 48% yield, with no residual starting materials. However, PPO was superior to *C. sinensis* cell culture with respect to the final yields of TF3G (2) from EC (5) and EGCG (8), TF3′G (3) from ECG (6) and EGC (7) and TFDG (4) from ECG (6) and EGCG (8). From these results, *C. sinensis* cell culture was superior for the production of TF (1).
leaves are used as a source of EC (C. sinensis) for TF, which is an efficient source of POD, is a superior system for selective synthesis of TF (from EC). Dry Japanese green tea leaves contain 0.7% EC, 0.2% ECG, 0.7% EGC (7) and 0.8% EGCG (8). However, enzyme-deactivated dry green tea leaves have a major problem: the ratio of EC (5), ECG (6), EGC (7) and EGCG (8) are present in the reaction mixture, preventing the selective synthesis of TF (1) when dried Japanese green tea leaves are used as a source of EC (5) and EGC (7).

We found the answer to this problem. C. sinensis cell culture, which is an efficient source of POD, is a superior system for TF (1) synthesis from EC (5) and EGC (7). However, C. sinensis cell culture did not effectively synthesize TF3G (2) from EC (5) and EGCG (8), TF3’G (3) from ECG (6) and EGC (7) or TFDG (4) from EGC (7) and EGCG (8). Furthermore, C. sinensis cell culture is a moderate source of hydrolyase. From these theories and established facts, we have developed a new one-pot method for domino-type, enzymatic, selective, large-scale and high-yield TF (1) production from enzyme-deactivated dry Japanese green tea leaves extracts containing EC (5), ECG (6), EGC (7), EGCG (8), caffeine, and gallic acid (9,10). Subsequently, the effects of TF (1) on lifestyle-related diseases were investigated in a high-fat diet-induced obesity mouse model. Administration of TF (1) (1.3 mg/d) in this model inhibited both body weight gain and visceral fat accumulation, with no significant difference observed in the amount of faeces between the experimental and control mice. These results and their implications are discussed.

MATERIALS AND METHODS

Spectral Data TF (1) structure was determined by comparing FAB-MS and 1H-NMR spectra with the spectra of verified samples. FAB-MS were measured using a JEOL JMS-SX102 mass spectrometer. 1H-NMR spectra were measured using a JEOL JNM-EX-270 FT NMR (270MHz). TF (1): FAB-MS m/z 564 (M+), 1H-NMR (d4-acetone) δ: 2.78–3.10 (4H, m, H(4), H(4’)), 4.41 (1H, brd, H(3’)), 4.50 (1H, brd, H(3’)), 5.02 (1H, s, H(2’)), 7.55 (1H, s, H(3’)), 7.99 (1H, s, H(5’)), 8.04 (1H, s, H(7’)), 8.26–8.40 (4H, m, H(6), H(8), H(6’), H(8’)), 5.04 (Tropolone OH).

HPLC Analysis HPLC analysis of TF (1), TF3G (2), TF3’G (3), TFDG (4), EC (5), ECG (6), EGC (7) and EGCG (8) was performed with a JASCO HPLC system (PU-980, UV-970) and a 4.6×250-mm ODS120A column (TOSOH, Japan) using with the following conditions: solvent, acetonitrile–ethyl acetate (AcOEt)–0.05% H3PO4 at 21:3:76; flow rate, 1.0 mL/min; temperature, 25°C and UV detection at 280 nm.

Materials B-5 medium, 2,4-dichlorophenoxyacetic acid (2,4-D) and pyrogallol were purchased from Wako Pure Chemical Industries Ltd., Purpurogallin was purchased from Kanto Chemical Co., Inc., Japan. Quick Fat was purchased from CLEA Japan, Inc., Japan.

Plant Material and Culture Condition Suspensions of C. sinensis cells were subcultured every 10 d by transferring a 1-week-old culture (10 mL) into B5 medium(9) (80 mL) containing 2,4-D (1.25 mg/L) and 5% sucrose (pH 5.8) and incubating on a rotary shaker (110 rpm) at 25°C in the dark.

Cultivation of C. sinensis Cell Culture Suspensions of C. sinensis cells were subcultured every 14 d by transferring a 14-d-old culture (10 mL) into B5 medium (80 mL) containing 2,4-D (1.25 mg/L) and 5% sucrose. Cultures were incubated in a rotary shaker (110 rpm) and incubating at 25°C in the dark.

POD Activity of C. sinensis Cell Culture The POD activity of C. sinensis cell cultures was assayed using a purpurogallin assay. This assay combined 5% pyrogallol (2 mL), freshly-prepared 0.5% hydrogen peroxide (1 mL), water (14 mL), 0.1 mM pH 6.4 phosphate buffer (2 mL) and an enzyme sample (1 mL). Absorbance at 420 nm was recorded, and the number of enzymatic units present was calculated from a standard curve for purpurogallin. The POD activity of C. sinensis cell culture (cell) was 15.5±0.32 U/mL. The POD activity of C. sinensis cell culture (broth) was 12.4±0.50 U/mL. For reference, one unit (U) of peroxidase forms 1.0 mg of purpurogallin from pyrogallol in 20 s at pH 6.0 and 20°C.

Enzyme-Deactivated Dry Japanese Green Tea Leaves We used enzyme-deactivated dry Japanese green tea leaves harvested in September. Enzyme-deactivated dry Japanese green tea leaves were produced as follows. The tea leaves were either steamed or boiled to inactivate endogenous oxidase (PPO and POD) and then dried. Enzyme-deactivated dry Japanese green tea was purchased from Mizuuien Limited.

Water-Soluble Extract of Enzyme-Deactivated Dry Japanese Green Tea Leaves as a Raw Material for TF (1) Generation After soaking enzyme-deactivated dry Japanese green tea leaves harvested in September, enzyme-deactivated dry Japanese green tea leaves were produced as follows. The tea leaves were either steamed or boiled to inactivate endogenous oxidase (PPO and POD) and then dried. Enzyme-deactivated dry Japanese green tea was purchased from Mizuuien Limited.
green tea leaves (75 g) in water (1500 mL) for 12 h, the water
mixture was separated by filtration. By HPLC analysis, the
resulting tea-leaf filtrate (1840 mL) contained 488.5 mg of EC
(5), 122.4 mg of ECG (6), 556.5 mg of EGC (7), 610.5 mg of
EGCG (8), gallic acid (9) and 302.1 mg of caffeine.

**TF (1) Synthesis by C. sinensis Cell Culture from
Enzyme-Deactivated Dry Green Tea Leaf Filtrate** In total, C. sinensis cell culture (42 g of cells and 200 mL of
broth) and 14.5 mL of 3% H₂O₂ were added to the enzyme-
deactivated dry green tea leaf filtrate (1840 mL), and the
mixture was shaken at 110 rpm in air for 8 h. At the conclusion
of the reaction, the mixture was filtered, and the filtered
cells were washed with CHCl₃. The filtrate was then extracted
with CHCl₃. The aqueous layer was extracted with AcOEt. The
AcOEt layer was washed with brine and then dried over
MgSO₄ and concentrated *in vacuo*. The residue was subjected
to column chromatography using a Sephadex LH-20 column
and MeOH at 5°C to yield TF (1) (193 mg).

**Animals** Five-week-old male C57BL/6 mice were purchased
from Japan SLC, Inc. (Japan). Prior to the experiments, the
mice were acclimatized for 1 week in a 23±1°C environment
with 55±5% humidity and a light-dark cycle of 12 h. The
study was conducted in accordance with the guidelines for
animal care, handling and termination from the University of
Shizuoka, which are in line with both international and Japa-
nese guidelines on animal care and welfare.

**Feeding** After 1 week of acclimatization, healthy mice
were separated into a control water group (n = 7) and a TF (1)
water group (n = 7). Both groups were provided with a high-
fat diet (Quick Fat) *ad libitum* and either control water or TF
(1) water for 12 weeks. TF-water was produced by dissolving
50 mg of TF in 150 mL of water.

**Statistical Analyses** All data are presented as the
mean±standard deviation (S.D.). Group differences were sta-
tistically analysed for significance as follows: the Student’s
t-test was used after an F-test in experiments involving two
experimental groups. p-Values less than 0.05 were considered
significant.

### RESULTS

**Enzyme-Deactivated Dry Japanese Green Tea Leaves as
a Raw Material for TF (1) Production** We selected fresh
green tea leaves harvested in September, as the best starting
material for TF (1) production. These fresh green tea leaves
contain the four major *epi*-type catechins: EC (5), ECG (6),
EGC (7) and EGCG (8), as well as endogenous enzymes
(PPO, POD and hydrolase etc.). The tea leaves were either
steamed or boiled to inactivate endogenous enzyme and then
dried.

**Domino-Type, Enzymatic Selective, One-Pot Biotrans-
formation of All *epi*-Type Catechins [EC (5), ECG (6),
EGC (7) and EGCG (8)] in Enzyme-Deactivated Dry
Japanese Green Tea Leaves to TF (1) Using C. sinensis
Cell Culture** After soaking enzyme-deactivated dry Japa-
nese green tea (75 g) in water for 12 h, the resulting tea-leaf
filtrate (1840 mL) contained EC (5) (488.5 mg), ECG (6)
(122.4 mg), EGC (7) (556.5 mg), EGCG (8) (610.5 mg) and
caffeine (302.1 mg). Subsequently, the *C. sinensis* cell culture
[42 g of cells (POD: 15.5±3.2 U/mL)] and 200 mL of broth
(POD: 12.4±0.50 U/mL) and 14.5 mL of 3% H₂O₂ were added
to the tea-leaf filtrate (1840 mL), and the mixture was shaken
at 110 rpm in air for 8 h. Figure 3 shows the HPLC chromato-
gram of the reaction mixture after 1 min of reaction time
[peaks: gallic acid (9) (3.1 min), EGCG (8) (5.1 min) and ECG
(6) (7.1 min)]. Figure 4 shows the HPLC chromatogram of the
reaction mixture at the reaction time of 5 h. The peak of TF
(1) production was at 12 min.

After 5 h (see Fig. 4), the levels of starting materials [ECG
(6) and EGCG (8)] were decreased and the peaks of TF (1)
and gallic acid (9) were increased, which is in contrast with the HPLC result from the starting reaction mixture (see Fig. 3). The HPLC chromatogram at a reaction of 5h shows that the reaction mixture contained a peak for TF (1), but no peaks corresponding to the galloyl esters [TF3G (2), TF3’G (3) and TFDG (4)] were observed (see Fig. 4). In addition, we observed an increase in the gallic acid (9) peak. At a reaction time of 8h, the peaks of ECG (6) and EGCG (8) were almost disappeared (see Fig. 5).

Figure 6 illustrates the time course of the reaction. Quantification of compounds [EC (5), EGC (6), EGC (7), EGCG (8), TF (1) and gallic acid (9)] was performed using calibration curves of corresponding authentic compounds. Yield [EC (5), EGC (6), EGC (7), and EGCG (8)] is expressed as a relative percentage. Relative percentage=[(abundance at the reaction time)/(starting abundance)]×100. Yield [TF (1) and gallic acid (9)] is expressed as relative percentage to the total amount. Relative percentage=[(abundance at the reaction time)/(total amount)]×100. TF (1) and gallic acid (9) levels increased with reaction times. On the other hand, EC (5), EGC (6), EGC (7) and EGCG (8) levels decreased (0–24.5%). After 8h, the mixture was filtered, and the filtrate was extracted using CHCl3 to remove caffeine from the filtrates that contained TF (1), caffeine, gallic acid (9) and epi-type catechins [EC (5), EGC (6), EGC (7) and EGCG (8)]. The CHCl3 layer contained caffeine. The aqueous layer which contained gallic acid (9), TF (1) and epi-type catechins [EC (5), EGC (6), EGC (7) and EGCG (8)] was extracted using AcOEt. The AcOEt extract TF (1) and epi-type catechins [EC (5), EGC (6), EGC (7) and EGCG (8)] were subjected to column chromatography on Sephadex LH-20 using MeOH to obtain TF (1) (193 mg). The chemical yield of TF (1) from epi-type catechins [EC (5), EGC (6), EGC (7) and EGCG (8)] was 20.3%. No TF3G (2), TF3’G (3) or TFDG (4) was obtained.

Effect of TF (1) on Fat Accumulation in a High-Fat Diet-Induced Obesity Mouse Model A high-fat diet-induced obesity mouse model was used to investigate the effects of TF (1) on human lifestyle-related diseases. In this study, 5-weeks-old healthy C57BL6 male mice were acclimatized for 1 week before being randomly assigned into the experimental groups. The groups were provided with either control water or TF (1) water and fed a high-fat diet (Quick Fat) ad libitum for 12 weeks. There was no significant difference in food consumption between the control and the TF (1) groups up to 18-weeks of age (Table 1), and liquid consumption was also approximately equal (4mL/d). Furthermore, there was no significance difference in faeces or urination production was observed between the control and TF (1) mice (Table 2).

However, the average weight of mice given the TF (1) drink was significant lower than that of the control mice. From the results of tissue dissection, we found that abdomen length, perirenal fat, periglu teal fat and total fat mass were significantly decreased. TF (1) also tended to decrease mesenteric fat, epididymal fat, and periscapular fat (Table 3).

### DISCUSSION

*C. sinensis* cell culture is a superior system for TF (1) synthesis from EC (5) and EGC (7). However, *C. sinensis* cell culture did not effectively synthesize TF3G (2) from EC (5) and EGCG (8), TF3’G (3) from EGC (6) and EGC (7) or TFDG (4) from EGC (6) and EGCG (8). From these facts, we developed a domino-type, enzymatic, selective one-pot synthesis of TF (1) from enzyme-deactivated dry green tea leaf extracts containing 0.7% EC (5), 0.7% EGC (7), 0.2% EGC (6) and 0.8% EGCG (8) using *C. sinensis* cell culture as an enzyme source. The key determinant in using these green tea leaves as a source for synthesis was establishing how ECG...
(6) and EGCG (8), which are not raw materials for TF (1) generation, would be transformed in the reaction. TF3G (2) and TF3G (3) might be formed when ECG (6) and EGCG (8) are present in the reaction mixture, preventing the selective synthesis of TF (1) when Japanese green tea leaves are used as a source of EC (5) and EGC (7).

A two-stage reaction method for producing TF (1) from green tea extract has been previously reported by William et al. (see Fig. 7). In the first stage of the reaction, the parent catechins EC (5), ECG (6), EGC (7) and EGCG (8) present in enzyme-deactivated green tea leaf extracts are converted to EC (5), EGC (7) and gallic acid (9) by exogenous tannase under argon or nitrogen. In other words, gallate groups of ECG (6) and EGCG (8) are cleaved by tannase to produce EC (5), EGC (7) and gallic acid (9) (Fig. 7, first stage reaction). In the second stage of the reaction, EC (5) and EGC (7) generated by the first reaction are converted to TF (1) by exogenous POD and H$_2$O$_2$. However, this reported method is not a one-pot reaction but instead has two reactions stages. Furthermore, it is both expensive and complicated because of the requirement for exogenous tannase, exogenous peroxidase and either argon or nitrogen.

When we use enzyme-deactivated dry green tea leaves, a key point is how to treat ECG (6) and EGCG (8), which are not starting materials for TF (1) generation. We investigated the biosynthetic pathways of EC (5), ECG (6), EGC (7), EGCG (8) and gallic acid (9) in green tea leaves. Previous reports show that the biosynthetic pathways of EC (5) and EGC (7) are similar to those of flavonoids. Furthermore, Saijo demonstrated gallic acid (9) biosynthesis and its esterification with EC (5) and EGC (7). At least two pathways have been proposed for gallic acid (9) biosynthesis in tea shoots. These include a) a pathway through phenylpropanoid and b) another through shikimic acid dehydrogenation. Gallic acid (9) is presumably esterified with EC (5) and EGC (7) to form ECG (6) and EGCG (8) in young tea shoots. Because gallic acid (9) levels are low and demand for gallic acid (9) is high in tea shoots, gallic acid (9) concentrations may be a rate limiting factor for ECG (6) and EGCG (8) formation. However, no previous study has reported the mechanisms of these esterification reactions in fresh green tea leaves. In addition, enzymatic esterification reactions, such as those of lipase, are generally reversible. Therefore, the esterification reaction in fresh green tea leaves is assumed to be reversible.

It is assumed that reactions A and B occur in fresh green tea leaves (see Fig. 8). These reactions are reversible reactions in which the forward reaction ($a_1, a_2$) is hydrolysis reaction and the reverse reaction ($b_1, b_2$) is esterification reaction. By Le Chatelier’s principle of the movement of equilibrium, the equilibrium moves in the direction where the concentration of material rises when the concentration of material participating in the reaction becomes lower. For example, the $a_1$ reaction occurs preferentially if the concentration of either EGC (7) or gallic acid (9) decreases. It is assumed that an enzyme exists in fresh green tea leaves that catalyses these forward ($a_1, a_2$) and reverse ($b_1, b_2$) reaction. We hypothesized that the C. sinensis cell culture might possess these enzymes (see Fig. 8). Furthermore, in our previous study of C. sinensis cell cultures, we reported that the TF (1) generation speed was much faster than the generation speed of TF3G (2), TF3G (3) and TFDG (4).

From these theories and established facts, we have developed a novel, simple, inexpensive and scalable one-pot method of TF (1) production from enzyme-deactivated Japanese green tea leaves extracts containing EC (5), ECG (6), EGC (7), EGCG (8), caffeine, and gallic acid (9).

Figure 6 shows a decrease in the peak of EGCG (8) and ECG (6) and an increase in the peak of gallic acid (9) as the reaction progressed. The presence of gallic acid (9), which is produced when EGCG (8) and ECG (6) are hydrolysed to EGC (7) and EC (5), respectively, indicated that EGCG (8) and ECG (6) were hydrolysed during the reaction. Conversely, the peak of TF (1) increased as the reaction progressed. However, no TF3G (2), TF3G (3) or TFDG (4) peaks appeared. These results demonstrated that EC (5), ECG (6), EGC (7) and EGCG (8) were selectively converted to TF (1) and gallic acid (9) during the reaction. Both generation [TF (1) and gallic acid (9)] and degradation [EC (5), ECG (6), EGC (7) and EGCG (8)] occurred simultaneously in the one-pot reaction (Fig. 6). From these results, we surmised that the following mechanism was responsible (Fig. 9). C. sinensis cell culture is a rich source of POD and a moderate source of hydrolase. In the one-pot reaction, EC (5) and EGC (7) are immediately converted to TF (1) by endogenous POD. When the amount of EC (5) and EGC (7) in the reaction mixture has sufficiently decreased, the C. sinensis cell culture hydrolase initiates hydrolysis of ECG (6) and EGCG (8) to produce EC (5), EGC (7) and gallic acid (9). Importantly, a hydrolysis reaction is an equilibrium reaction (Fig. 8). It is thought that a hydrolysis reaction of EGCG (8) and ECG (6) progressed to make up for EC (5) and EGC (7) in the reaction mixture immediately, after TF (1) generation from EC (5) and EGC (7) progressed. The EC (5) and EGC (7) generated by the hydrolysis reaction in turn is converted to TF (1) by POD. Thus TF (1) synthesis and hydrolysis of ECG (6) and EGCG (8) are repeated to yield TF (1) and gallic acid (9) from EC (5), EGC (7), ECG (6) and EGCG (8) in the one-pot reaction. This new TF (1) production method does not require the use of exogenous tannase, exogenous POD, nitrogen or argon, making it completely different from the method described by William et al. Furthermore, all of the necessary enzymes are present in the C. sinensis cell.
In this one-pot reaction, the major *epi*-type catechins EC (5), EGC (7), ECG (6) and EGCG (8) were selectively converted to TF (1) and gallic acid (9) through a domino-type enzymatic reaction involving endogenous POD and hydrolase present in *C. sinensis* cell culture. TF3G (2), TF3’G (3) and TFDG (4) were not generated. The advantages of this new method are that it provides a simple, inexpensive, and large-scale TF (1) production method that is suitable for use in medical studies.

Subsequently, the effects of this synthetic TF (1) on lifestyle-related diseases were investigated in a high-fat diet-induced obesity mouse model. This is the first study showing the long-term anti-obesity effect of TF (1) in mice given a high fat diet. The TF (1) (1.3 mg/d) that was generated using the new synthetic method inhibited both body weight increase and visceral fat accumulation. Kojima *et al.* reported that adzuki polyphenol inhibited weight gain in mice on high-fat diets by blocking the digestion and absorption of dietary fat and promoting fat elimination in faeces.\(^1\) Furthermore, Murase *et al.* reported that the stimulation of lipid metabolism in the liver may possibly be a factor for the anti-obesity effect of tea catechins in mice.\(^1\) TF (1) also inhibited both body weight gain and visceral fat accumulation, with no significant difference observed in the amount of food consumption, urination and faeces between the experimental and control mice. Further studies are warranted to elucidate the underlying mechanisms of TF (1).

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**Conflict of Interest** The authors declare no conflict of interest.

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