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Tea catechins enhance the mRNA expression of uncoupling protein 1 in rat brown adipose tissue

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Abstract

The aim of the present study was to determine whether the antiobesity effects of tea catechins (TCs) are associated with the expression of uncoupling protein 1 (UCP1) in brown adipose tissue (BAT). Male Sprague–Dawley rats were fed a high-fat (HF; 35% fat) diet for 5 weeks, then divided into four groups and fed an HF, HF with 0.5% TC (HFTC), normal-fat (NF; 5% fat) or NF with 0.5% TC (NFTC) diet for 8 weeks. At the end of the experimental period, perirenal and epididymal white adipose tissues (WATs) and interscapular BAT were isolated. The NFTC group had significantly lower perirenal WAT weights than the NF group (NF: 12.7±0.53 g; NFTC: 10.2±0.43 g; $P<.01$), but the HF and HFTC groups did not differ significantly. TC intake had no effects on epididymal WAT weights. The NFTC and HFTC groups had significantly lower BAT weights than the NF and HF groups, respectively. The NFTC group had significantly higher UCP1 mRNA levels in BAT than the NF group (NF: 0.35±0.02; NFTC: 0.60±0.11; $P<.05$), but the HF and HFTC groups did not differ significantly. Thus, TC intake in the context of the NF diet reduced perirenal WAT weight and up-regulated UCP1 mRNA expression in BAT. These results suggest that the suppressive effect of TC on body fat accumulation is associated with UCP1 expression in BAT.

Keywords: Tea catechins; Brown adipose tissue; Uncoupling protein 1; Normal-fat diet; High-fat diet

1. Introduction

Being overweight or obese is a significant public health threat in an increasing number of countries. Obesity is the outcome of a prolonged positive energy balance due to excess energy intake relative to energy expenditure. A negative energy balance is needed to produce weight loss and can be achieved by decreasing intake and/or by increasing expenditure. With regard to facilitating weight loss, increasing interest is being paid to therapies involving natural herbal supplements, one of which is green tea. Green tea contains high levels of polyphenols that are believed to help prevent several lifestyle-related diseases, including cardiovascular diseases and carcinogenesis [1]. The main tea polyphenols are tea catechins (TCs), which have been reported to have antioxidant, antihypertensive, anticarcinogenic and hypocholesterolemic activities [2–4]. TCs are also thought to be useful compounds for the treatment and prevention of obesity, since it has been shown that they reduce diet-induced adiposity in mice, rats and humans [5,6].

Although the mechanisms by which green tea helps treat and prevent obesity have not yet been fully determined, several studies suggest that it may act by decreasing fat absorption [7–9] and by accelerating fecal fat excretion [10–12]. In addition, green tea extracts have been found to increase energy expenditure in rats, possibly by activating brown adipose tissue (BAT) thermogenesis [13]. Supporting this is the fact that TC-enriched green tea extracts increase sympathetic-mediated thermogenesis in BAT in vitro [14]. Moreover, the intake of green tea extracts by humans has

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been shown to increase their 24-h energy expenditure [15]. These findings suggest that TCs may prevent or treat obesity by inducing thermogenesis. However, other studies have failed to observe this thermogenic effect of TCs on both rodents [10] and humans [16,17].

Green tea extracts also bear significant amounts of caffeine that could, at least in part, contribute to the thermogenic effect of green tea observed in the studies described above. However, Dulloo et al. [14] have shown that caffeine may potentiate the thermogenic effect of TCs in a synergistic fashion. Moreover, many studies have shown that pure TC supplements prevent diet-induced adiposity in mice and rats on their own [5,10,18,19]. Indeed, Klaus et al. [10] have demonstrated that a major compound of TCs, namely, epigallocatechin gallate (EGCG), blocks high-fat (HF)-diet-induced obesity in mice. However, it was suggested that this effect is not mediated by an EGCG-induced elevation in energy expenditure since BAT thermogenesis was unaltered, as shown by the unchanged expression of uncoupling protein 1 (UCP1), which plays a key role in thermogenesis. Interestingly, however, a closer analysis of the TC-supplemented animal studies described above [5,10,18,19] reveals that dietary TC intake reduces body fat accumulation more effectively when the fat content in the diet is relatively low. Thus, it remains possible that TC could enhance UCP1 expression and BAT thermogenesis when it is provided in the context of moderate dietary fat intake.

To test this notion, we examined the effect of dietary TC intake on body fat accumulation in rats and UCP family (UCP1, UCP2 and UCP3) gene expression in BAT. For this, we employed a unique feeding protocol (Fig. 1) where 5-week-old rats were fed an HF diet for 5 weeks and then divided into four groups and fed an HF or normal-fat (NF) diet with or without TC supplementation for 8 weeks.

2. Materials and methods

2.1. TCs

TCs were prepared from the leaves of green tea (*Camellia sinensis*) and analyzed as described previously [20]. The TC preparation used in the present study consisted of 81.3% catechins. Of this, EGCG, epigallocatechin, epicatechin gallate, epicatechin, gallocatechin, gallocatechin gallate, catechin and catechin gallate comprised 40.6%, 23.1%, 12.4%, 9.2%, 6.8%, 3.9%, 2.6% and 1.3%, respectively. The caffeine content was 0.1%.

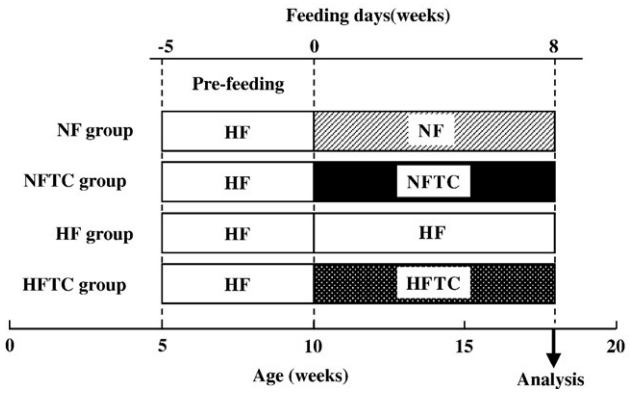

Fig. 1. Schematic depiction of the experimental protocol.

Table 1
Composition of the test diets

Ingredients (g/100 g)	Group			
	NF	NFTC	HF	HFTC
Casein	18.0	18.0	18.0	18.0
D,L-Methionine	0.3	0.3	0.3	0.3
Sucrose	33.5	33.5	19.0	19.0
Wheat starch	33.5	33.0	18.0	17.5
Olive oil	5.0	5.0	5.0	5.0
Coconut oil	–	–	30.0	30.0
Cellulose	5.0	5.0	5.0	5.0
Mineral mixture [a]	3.5	3.5	3.5	3.5
Vitamin mixture [b]	1.0	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2	0.2
TCs	–	0.5	–	0.5
Dietary energy (kJ/100 g)	1621	1612	2248	2240
% Energy from fat	11.6	11.6	58.6	58.6

NF, normal fat; NFTC, NF+TCs; HF, high fat; HFTC, HF+TCs.

[a] AIN 76 mineral mixture (g/kg): calcium phosphate dibasic, 500; sodium chloride, 74; potassium citrate, 220; potassium sulfate, 52; magnesium oxide, 24; manganous carbonate, 3.5; ferric citrate, 6; zinc carbonate, 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01; chrominium potassium sulfate, 0.55.

[b] AIN 76 vitamin mixture (g/kg): dry vitamin A palmitate (500,000 U/g), 0.8; vitamin D_3 trituration (500,000 U/g), 0.2; dry vitamin E acetate (50%), 10; thiamin, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; vitamin B_{12} (0.1% trituration in mannitol), 1; niacin, 3; calcium pantothenate, 1.6; folic acid, 0.2; biotin, 0.02; vitamin K_3, 0.005.

2.2. Diets

Table 1 details the composition of the experimental diets used in the present study. These diets were prepared as described previously [21] in accordance with the recommendations of the American Institute of Nutrition. The HF diet contained 30 g coconut oil/100 g chow as a fat source, in addition to 5 g olive oil/100 g chow. The NF diet was formulated by replacing the coconut oil energy of the HF diet with carbohydrate energy (sucrose and wheat starch). The NF and HF diets provide 1621 and 2248 kJ/100 g chow, respectively, and ~12% and ~60% of their dietary energy come from fat, respectively. The NFTC and HFTC diets contained 500 mg TC/100 g chow. When the diets were supplemented with TC, this was done so at the expense of wheat starch. All of the diets were supplied in powder form. Aliquots of the diets were sealed with nitrogen gas and stored at 4°C until use.

2.3. Animals

Male Sprague–Dawley rats at 3 weeks of age (body weight, 30–50 g) were purchased from CLEA Japan (Tokyo, Japan). Before experimentation, all rats were acclimated to

their new environment for 2 weeks, during which time they were allowed free access to a standard powdered diet (CE-2; CLEA Japan) composed of 25% crude protein, 4.7% crude fat and 50.6% nitrogen-free extract. The gross energy of the standard diet was calculated to be 1446 kJ/100 g chow. The present study was approved by the Animal Care and Use Committee of Waseda University School of Human Sciences (no. 06J0018) and was performed in accordance with the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences" of the Physiological Society of Japan.

2.4. Experimental setup

Fig. 1 shows the experimental procedure for the present study. In total, 32 rats aged 5 weeks were put on an HF diet for 5 weeks to induce adiposity, after which they were given an NF, NFTC, HF or HFTC diet (n=8 per group) for 8 weeks. The rats were housed individually under standard conditions at a temperature of 23–25°C and a 12-h:12-h dark–light cycle. Food intake and body weight were monitored daily throughout the experiment. At the end of the experimental period, all animals were fasted overnight, with free access to water. Blood samples were collected from the tails, after which the rats were sacrificed by decapitation. Plasma samples were prepared and stored at −80°C. The perirenal and epididymal white adipose tissues (WATs) and interscapular BAT were removed and weighed. For RNA extraction, 50–100 mg of interscapular BAT was permeated with the tissue storage reagent RNAlater (Ambion, Austin, TX) in accordance with the manufacturer's instructions. After overnight incubation at 4°C to allow thorough penetration of the tissues, the samples were transferred to −80°C.

2.5. Blood analyses

Plasma glucose and triglyceride levels were determined using Glucose C II and Triglyceride E kits (Wako Pure Chemical Industries, Osaka, Japan), respectively. Plasma concentrations of hormones were measured with enzyme-linked immunosorbent assays using a Rat Insulin Kit (Shibayagi Co., Gunma, Japan) and a Rat Leptin Kit (Morinaga Institute of Biological Science, Yokohama, Japan).

2.6. Real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR)

Real-time RT-PCR was used to quantify UCP gene expression levels. Total RNA was extracted from the interscapular BAT using the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). Briefly, frozen tissue samples were thawed at room temperature and homogenized in lysis reagent (a monophasic solution of phenol and guanidine thiocyanate). After the addition of chloroform, the homogenate was separated into aqueous and organic phases by centrifugation. The RNA-containing aqueous phase was extracted, and ethanol was added to provide appropriate binding conditions. The sample was then applied onto a silica membrane. After several washes, RNA was eluted in RNase-free water. The purity and concentration of total RNA were determined by measuring absorbance at 260 and 280 nm (U-3310 Spectrophotometer; HITACHI, Tokyo, Japan). The extracted RNA was then subjected to first-strand cDNA synthesis using the ExScript RT reagent Kit (Takara Bio Inc., Shiga, Japan). The final volume was adjusted to 50 μl with EASY Dilution (Takara Bio Inc.) in accordance with the manufacturer's instructions. In quantitative RT-PCR, diluted cDNA was added to a SYBR Premix Ex Taq reaction mixture (Takara Bio Inc.) containing 200 nM PCR (forward and reverse) primers. The reference gene used was glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The oligonucleotide sequences for the primers were as follows:

1. UCP1: 5′-TACCCAGCTGTGCAATGACCA (forward), 5′-GCACACAAACATGATGACGTTCC (reverse);
2. UCP2: 5′-CAGAGCACTGTCGAAGCCTACAAG (forward), 5′-CAATGGCATTTCGGGCAAC (reverse);
3. UCP3: 5′-AGCTGCTGGACTCTCACCTGTCC (forward), 5′-CGTTCATGTATCGGGTCTTTACCAC (reverse);
4. GAPDH: 5′-GACAACTTTGGCATCGTGGA (forward), 5′-ATGCAGGGATGATGTTCTGG (reverse).

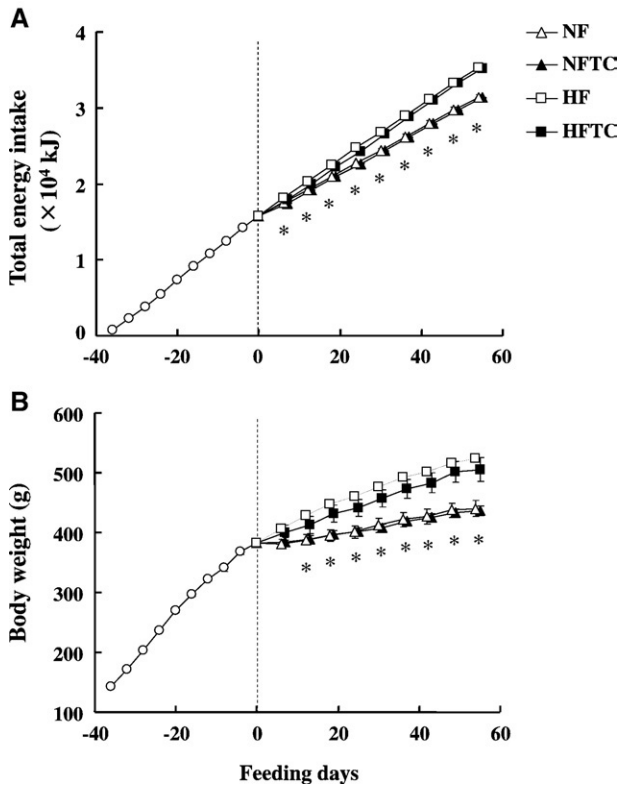

Fig. 2. Cumulative energy intake (A) and body weight (B) during the experimental period. Values shown are expressed as mean±S.E. Asterisks indicate significant differences between the animals fed the NF diet and the animals fed the HF diet without TCs (P<.05).

Table 2
Effect of TC intake on plasma glucose, triglyceride and hormone levels

	Group			
	NF	NFTC	HF	HFTC
Glucose (mg/dl)	117±5	124±4	121±6	115±6
Triglyceride (mg/dl)	203±32	184±28	131±18	111±15
Insulin (ng/ml)	2.3±0.4	1.3±0.2 *	1.8±0.4	1.4±0.3
Leptin (ng/ml)	6.3±1.0	4.8±0.6	12.1±3.9	9.3±1.3

Values shown are expressed as mean±S.E. Asterisks indicate significant differences between the NF and NFTC groups or between the HF and HFTC groups (P<.05).

* P<.05 NF versus NFTC.

Amplification was performed using an ABI-Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). The PCR amplification program consisted of an initial denaturation step for 10 s at 95°C, followed by the Shuttle PCR standard protocol and a dissociation protocol. UCP1 mRNA levels were determined using the Threshold Cycle (C_t) method in accordance with the manufacturer's protocol and were expressed as ratios relative to GAPDH mRNA levels.

2.7. Statistical analysis

All data were expressed as mean±S.E. F test was used as a prelude to unpaired Student's t test to determine whether the assumption of homogeneity of variances was supported (STAT View-J 5.0 statistical software; SAS Institute, Cary, NC). When the assumption of homogeneity of variances was not supported, nonparametric test was employed to determine the effect of NF diet feeding or TC supplementation. Differences with P<.05 were considered statistically significant.

3. Results

3.1. Body weight and food intake

Fig. 2A shows the total energy intake of the four rat groups (designated as NF, NFTC, HF and HFTC). Immediately after starting the test diets, the NF group had a significantly lower total energy intake than the HF group (NF: 16,055±21.478 kJ; HF: 16,152±27.880 kJ; P<.05). As a result, the NF group had a significantly lower body weight than the HF group from Day 8 onwards (NF: 381.9±11.2 g; HF: 413.8±8.36 g; P<.05), as shown in Fig. 2B. By the final day of the experiment, the energy intake of the NF rats had been reduced by 11% (NF: 31,358±0.205 kJ; HF: 35,365±0.421 kJ; P<.0001), and their body weight was 16% (NF: 440.3±11.4 g; HF: 523.7±13.8 g; P<.0005) lower than that of the HF rats. However, there were no significant differences in total energy intake (NFTC: 31,338±0.077 kJ; HFTC: 35,219±0.490 kJ) or body weight (NFTC: 436.5±8.2 g; HFTC: 505.4±19.2 g) between the NF and NFTC groups or between the HF and HFTC groups.

3.2. Blood analyses

The fasting-state plasma glucose levels of the rats were tested at the end of the experiment (Table 2), and significant differences between the NF and HF groups were not observed. The glucose levels of the NF and HF groups were also similar to those of the NFTC and HFTC groups, respectively. However, the plasma triglyceride levels of the NF group were 1.5-fold higher than those of the HF group (NF: 203±32 mg/dl; HF: 131±18 mg/dl), although this did not reach statistical significance. The fasting-state insulin levels of the NF group were 1.3-fold higher than those of the HF group (NF: 2.3±0.4 ng/ml; HF: 1.8±0.4 ng/ml), although this did not reach statistical significance either. However, the plasma insulin levels of animals fed the NFTC diet were significantly lower (NFTC: 1.3±0.2 ng/ml; P<.05) than those of animals fed the NF diet, while there were no significant differences between the HF and HFTC groups (HFTC: 1.4±0.3 ng/ml). The NF group also had approximately 50% lower fasting-state leptin levels than the HF group (NF: 6.3±1.0 ng/ml; HF: 12.1±3.9 ng/ml), and the plasma leptin levels of the animals fed the NFTC or HFTC diet were 23% lower than

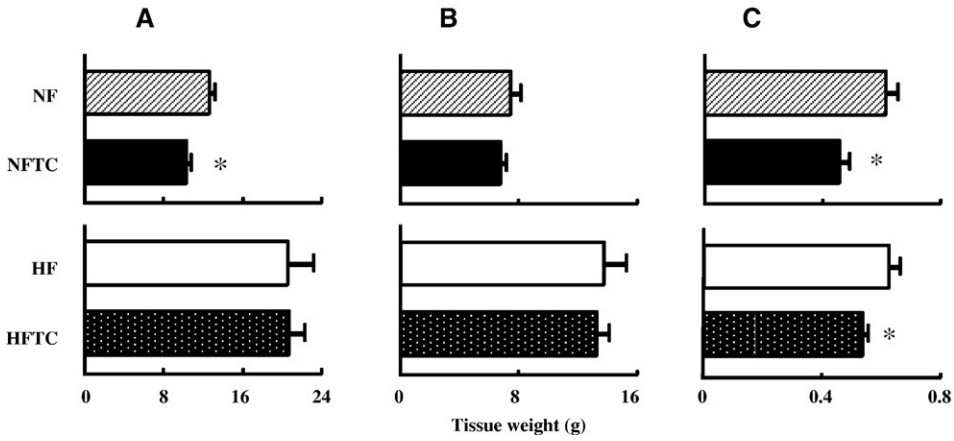

Fig. 3. Effect of TC intake on the weight of perirenal WATs (A), epididymal WATs (B) and interscapular BATs (C). Values shown are expressed as mean±S.E. Asterisks indicate significant differences between the NF and NFTC groups or between the HF and HFTC groups (P<.05).

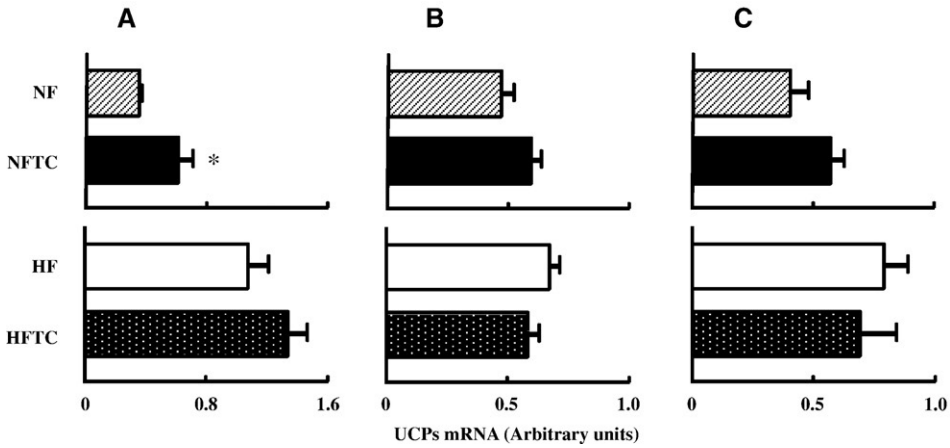

Fig. 4. Effect of TC intake on UCP1 (A), UCP2 (B) and UCP3 (C) mRNA expression in interscapular BAT. Values shown are expressed as mean±S.E. Asterisks indicate significant differences between the NF and NFTC groups or between the HF and HFTC groups ($P<.05$).

those of the animals fed the NF or HF diet (NFTC: 4.8±0.6 ng/ml; HFTC: 9.3±1.3 ng/ml). However, none of the differences attained statistical significance.

3.3. Weights of adipose tissues

To examine the effect of TC intake on visceral fat accumulation, we measured the perirenal and epididymal WATs of the rats at the end of the experiment (Fig. 3A and B). The perirenal WATs of the NF group were 38% lighter than those of the HF group (NF: 12.7±0.53 g; HF: 20.5±2.62 g; $P<.05$), while the epididymal WATs of the NF group were 45% lighter than those of the HF group (NF: 7.5±0.54 g; HF: 13.6±1.42 g; $P<.005$). The animals fed the NFTC diet also had significantly lighter perirenal WATs than the animals fed the NF diet (NFTC: 10.1±0.43 g; $P<.005$; Fig. 3A), although their epididymal WAT weights did not differ significantly (Fig. 3B). In contrast, the perirenal and epididymal WAT weights of the animals fed an HFTC diet were almost identical to those of the animals fed an HF diet (Fig. 3A and B). Thus, with regard to perirenal WAT weight, dietary TC intake further promotes the weight-reducing effect of the NF diet.

The interscapular BATs of the animals were also weighed at the end of the experiment (Fig. 3C). The BATs of the NF-diet- and HF-diet-fed animals were equally heavy, but those of the NFTC-diet- and HFTC-diet-fed animals were 26% and 15% lighter than those of the NF-diet-fed (NF: 0.612 ±0.042 g; NFTC: 0.453±0.031 g; $P<.01$) and HF-diet-fed (HF: 0.623±0.034 g; HFTC: 0.532±0.020 g; $P<.05$) animals, respectively.

3.4. UCP mRNA levels in BAT

We analyzed the UCP mRNA levels in the BATs by real-time quantitative RT-PCR. As shown in Fig. 4, the NF-diet-fed animals had significantly lower BAT UCP1 mRNA levels than the HF-diet-fed animals (NF: 0.346±0.016; HF: 1.078±0.130; $P<.0005$; Fig. 4A). The NF-diet-fed animals also had significantly lower mRNA levels of UCP2 (NF: 0.468±0.051; HF: 0.675±0.038; $P<.01$; Fig. 4B) and UCP3 (NF: 0.404±0.075; HF: 0.790±0.096; $P<.01$; Fig. 4C) than the HF-diet-fed animals. The NFTC-diet-fed animals had 70% higher UCP1 mRNA expression than the NF-diet-fed animals (NFTC: 0.600±0.105; $P<.05$) but did not differ significantly in UCP2 (NFTC: 0.586±0.043) and UCP3 (NFTC: 0.563±0.059) mRNA expressions. In contrast, the HF-diet- and HFTC-diet-fed animals had similar mRNA expressions of the three UCPs.

4. Discussion

The purpose of the present study was to elucidate the effect of dietary TC supplementation after fat restriction on body fat accumulation and BAT UCP gene expression in the rat. Our main findings are as follows: (a) while dietary TC supplementation significantly reduced the perirenal WAT weight in the NF-diet-fed animals, it had no effect when the animals were fed the HF diet (Fig. 3A); (b) dietary TC intake had no effect on epididymal WAT weights, regardless of whether the rats were fed the NF or the HF diet (Fig. 3B); (c) dietary TC supplementation significantly reduced BAT weights in both the NF-diet- and the HF-diet-fed animals (Fig. 3C); (d) dietary TC intake significantly elevated the BAT UCP1 mRNA levels in the NF-diet-fed rats but not in the HF-diet-fed animals (Fig. 4A); and (e) dietary TC intake had no effect on the mRNA levels of UCP2 and UCP3 in BAT, regardless of whether the rats were fed the NF or the HF diet (Fig. 4B and C).

One of the most frequently used animal models of obesity involves feeding the animals with an HF diet. We found that total energy intake, body weight and WAT weights decreased after the HF-diet-fed rats were placed on the NF diet (Figs. 2 and 3). Similar observations were made by Portillo et al. [21]. Our blood analyses also revealed that the NF-diet-fed rats had higher plasma triglyceride levels than the HF-diet-fed animals (Table 2). This probably reflects the higher sucrose levels in the NF diet, since the coconut oil energy of

the HF diet was replaced with carbohydrate energy in the NF diet. It is well known that sucrose-rich diets induce hyperlipidemia [22,23] and hyperinsulinemia [24] in rats. We also observed that the NF-diet-fed rats had slightly higher fasting-state insulin levels than the HF-diet-fed animals, while the NFTC-diet-fed rats had significantly lower insulin levels than the NF-diet-fed rats (Table 2). This supports a previous study by Wu et al. [25], who showed that green tea intake reduced the plasma insulin levels in rats. They also showed that green tea increased insulin sensitivity in adipocytes and that green tea polyphenol was one of the active components [25]. Thus, it is likely that the NFTC-diet-fed rats developed increased insulin sensitivity and that this was responsible for their lower fasting-state plasma insulin levels.

We found that TC supplementation had no effect on body weight or energy intake, regardless of the diet used (Fig. 2). Similarly, Wolfram et al. [26] showed that the body weight and energy intake of animals fed an HF diet with 20% fat did not change significantly when the diet was supplemented with EGCG. Moreover, Ikeda et al. [18] found that dietary gallate esters of TCs did not decrease the body weight and food intake of rats fed a diet containing 10% fat. We also discovered that the perirenal WAT weights of NF-diet-fed animals, but not HF-diet-fed animals, were reduced by TC intake (Fig. 3A). Again, this is confirmed by the observations of Ikeda et al. [18] and Wolfram et al. [26]: Ikeda et al. found that catechin intake lowered the visceral WAT weights of rats fed a 10% fat-containing diet, while Wolfram et al. found that EGCG supplementation did not alter the WAT weights of HF-diet-fed rats. These studies together show that dietary TC intake is more effective in reducing body fat accumulation when the fat content in the diet is relatively low. This is entirely consistent with our observation that dietary TC intake only significantly reduced WAT weight when the rats changed from the HF diet to the NF diet; this reduction was not observed when the rats kept consuming the HF diet.

In the present study, we found that while TC intake significantly reduced the perirenal WAT weight in the NF-diet-fed animals, it had no effect on the epididymal WAT weights of these animals. A previous study has shown that EGCG supplementation reduced visceral WAT weights more than subcutaneous WAT weights [26], while another study found that EGCG reduced the weights of both WATs equally well [19]. However, the disparate effects of TC on the weights of visceral WATs from different regions of the body that we detected have not been observed previously in various animal studies [5,10,18,19]. It is unclear why TC supplementation affected perirenal and epididymal WAT weights differently, but it may reflect possible gene expression differences in these adipose tissues that alter their lipid metabolism [27].

While it remains unclear how TCs attenuate body fat accumulation, an in vitro study has indicated that TCs inhibit catechol-*O*-methyltransferase (COMT), the enzyme that degrades noradrenalin (NA) [28]. This prolongs the effect of NA on adrenergic receptors at the postsynaptic cleft of the nerve terminal [14,29,30]. It has been shown that NA-mediated β-adrenergic stimulation increases BAT thermogenic capacity [31] in a process that involves the β-adrenergic signaling cascade, which is mediated by the transcription factor cAMP response element-binding protein, which has been reported to activate the expression of a variety of genes, including the UCP1 gene [32]. Notably, Choo [13] reported that the simultaneous administration of the β-adrenoceptor antagonist propranolol inhibited the increasing effects of green tea extract on the total protein levels in BAT. They concluded that green tea extract increases BAT protein levels via β-adrenoceptor activation.

As shown in Fig. 4, NF-diet-fed animals had significantly lower BAT UCP1, UCP2 and UCP3 mRNA levels than HF-diet-fed animals ($P<.0005$, $P<.01$ and $P<.01$, respectively). These results are consistent with those of previous studies showing that high-energy-fed animals have greater BAT UCP mRNA levels than control-diet-fed animals [33–35]. We also found that NFTC-diet-fed animals had significantly higher UCP1 mRNA expression than NF-diet-fed animals ($P<.05$), while there was no significant difference between the HF-diet- and the HFTC-diet-fed animals. Thus, dietary TC intake appears to counter the attenuating effect of NF diet feeding on UCP1 mRNA expression. Figs. 3C and 4 show that in both NF-diet- and HF-diet-fed animals, dietary TC intake significantly reduced the BAT weights and increased BAT UCP1 mRNA levels, although the elevation in BAT UCP1 levels in HF-diet-fed animals did not attain statistical significance. These results suggest that TC elevated BAT lipolysis and thermogenesis in both the NF-diet- and the HF-diet-fed rats. However, this enhancement of thermogenic capacity only became significant when the TCs were delivered in the context of an NF diet. Supporting this is the fact that TC intake reduced the perirenal WAT weights of the NF-diet-fed animals, but not the HF-diet-fed animals (Fig. 3A). These results together suggest that UCP1 mRNA expression is already high and cannot be enhanced by TC in HF-diet-fed animals, which is why TC intake does not significantly reduce body fat when incorporated into the HF diet. It was notable that the NF-diet- and NFTC-diet-fed animals and the HF-diet- and HFTC-diet-fed animals did not differ significant in terms of UCP2 and UCP3 mRNA expressions. Therefore, the enhancing effect of TCs in the context of the NF diet may be specifically due to UCP1 mRNA expression.

Evidence from various animal studies clearly indicates that the effect of green tea extract on obesity is probably associated with decreasing fat absorption and accelerating fecal fat excretion. For example, rats fed green TCs showed marked increases in fecal total lipids and cholesterol relative to the control group [7,10–12], although it should be noted that one study yielded an inconsistent result [5]. In the

present study, TC intake reduced the perirenal WAT weights of the NF-diet-fed animals, but not the HF-diet-fed animals. It is possible that this was due to TC-induced changes in fat absorption in the NF-diet-fed animals, but not the HF-diet-fed animals. Whether TC affected the fecal fat excretion of the rats in our study is unclear, as indices of this parameter were not tested. However, the enhanced UCP1 mRNA expression in the NFTC group is unlikely to reflect the fat-absorption-reducing effects of TCs since the inhibition of fat absorption induces low energy intake, which in turn would decrease, rather than increase, UCP1 mRNA expression [34]. Thus, it seems that TCs have at least two distinct effects on energy intake and expenditure.

With regard to the potential beneficial effects of TCs on human health, we can conclude that the ability of TC intake to suppress body fat accumulation is enhanced by lowering the fat and/or energy intake. Food restriction decreases sympathetic stimulation and thereby inactivates BAT thermogenesis [35], in part by reducing UCP1 levels [36]. TC may reverse this effect, perhaps by inhibiting COMT, thereby overturning the attenuation of sympathetically released NA that is caused by food restriction. Further studies are necessary to evaluate the effects of TC intake on energy expenditure and substrate oxidation in both NF-diet- and HF-diet-fed animals.

In conclusion, TC intake reduced perirenal WAT weight and enhanced BAT UCP1 mRNA expression in NF-diet-fed animals. Thus, the suppressive effects of TC on body fat accumulation appear to be associated with UCP1 expression in BAT.

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