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Green Tea, Black Tea, and Oolong Tea Polyphenols Reduce Visceral Fat and Inflammation in Mice Fed High-Fat, High-Sucrose Obesogenic Diets[1–3]

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Abstract

Green tea (GT) and caffeine in combination were shown to increase energy expenditure and fat oxidation, but less is known about the effects of black tea (BT) and oolong tea (OT). This study investigated whether decaffeinated polyphenol extracts from GT, BT, and OT decrease body fat and inflammation in male C57BL/6J mice fed high-fat/high-sucrose [HF/HS (32% energy from fat, 25% energy from sucrose)] diets. Mice were fed either an HF/HS diet with 0.25% of polyphenol from GT, OT, or BT or a low-fat/high-sucrose [LF/HS (10.6% energy from fat, 25% energy from sucrose)] diet for 20 wk. Monomeric tea polyphenols were found in the liver and adipose tissue of mice fed the HF/HS diet with GT polyphenols (GTPs) and OT polyphenols (OTPs) but not BT polyphenols (BTPs). Treatment with GTPs, OTPs, BTPs, and an LF/HS diet led to significantly lower body weight, total visceral fat volume by MRI, and liver lipid weight compared with mice in the HF/HS control group. Only GTPs reduced food intake significantly by ~10%. GTP, BTP, and LF/HS-diet treatments significantly reduced serum monocyte chemotactic protein-1 (MCP-1) compared with HF/HS controls. In mesenteric fat, monocyte chemotactic protein-1 (*Mcp1*) gene expression was significantly decreased by treatment with GTPs, BTPs, OTPs, and an LF/HS diet and in liver tissue by GTP and BTP treatments. *Mcp1* gene expression in epididymal fat was significantly decreased by the BTP and LF/HS diet interventions. In epididymal fat, consistent with an anti-inflammatory effect, adiponectin gene expression was significantly increased by GTPs and OTPs. Angiogenesis during adipose tissue expansion is anti-inflammatory by maintaining adipocyte perfusion. We observed significantly increased gene expression of vascular endothelial growth factor A by GTPs and vascular endothelial growth factor receptor 2 by BTPs and the LF/HS diet and a decrease in pigment epithelium-derived factor gene expression by OTPs and BTPs. In summary, all 3 tea polyphenol extracts induced weight loss and anti-inflammatory and angiogenic effects, although the tissue content of polyphenols differed significantly. J. Nutr. 144: 1385–1393, 2014.

Introduction

Energy-dense, nutrient-poor diets containing high amounts of fat and refined carbohydrates combined with sedentary lifestyles are believed to be the major drivers of the global obesity epidemic (1–3). The consumption of tea containing polyphenols and caffeine was shown in numerous clinical trials to affect body weight and fat metabolism in humans (4) and in rodents fed high-fat/high-sucrose (HF/HS)[4] diets (5). However, the effects of the tea polyphenols found in green tea (GT), black tea (BT), and oolong tea (OT) on fat deposition and inflammation, as well as potential mechanisms of action, were not adequately studied. All teas are derived from the leaves of *Camellia sinensis*, but different processing methods produce different types of tea. Fresh tea leaves are rich in polyphenols known as flavan-3-ols,

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[3] Supplemental Tables 1 and 2, and Supplemental Figures 1–3 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[4] Abbreviations used: *Adipoq*, adiponectin; BT, black tea; BTP, black tea polyphenol; *C/ebpα*, CCAAT/enhancer binding protein α; *Cpt1*, carnitine palmitoyltransferase I; ECG, (−)-epicatechin-3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin gallate; *FAS*, FA synthase; *Gpat*, glycerol-3-phosphate acyltransferase; GT, green tea; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; *Hmgcoa*, 3-hydroxy-3-methylglutaryl-CoA; *Klf7*, Kruppel-like factor 7; LF/HS, low-fat/high-sucrose; MCP-1, monocyte chemotactic protein-1; OT, oolong tea; OTP, oolong tea polyphenol; *Pedf*, pigment epithelium-derived factor; *SCD-1*, stearoyl-CoA desaturase; *SREBP1c*, sterol regulatory element binding protein-1c; *Vegfa*, vascular endothelial growth factor A; *Vegfr2*, vascular endothelial growth factor receptor 2; 4′′-MeEGCG, 4′′-*O*-methyl (–)-epigallocatechin gallate.

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including (−)-epicatechin, (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), and (−)-epicatechin-3-gallate (ECG) (6). The 3 major types of tea are GT, OT, and BT, with different degrees of fermentation during processing (6). During fermentation to manufacture BT, monomeric polyphenols are converted to polymeric polyphenols called theaflavins and thearubigins, which are not absorbed from the gastrointestinal tract like smaller catechins, such as EGCG (7). BT contains lower amounts of monomeric polyphenols (3–10% of solids) and higher concentrations of polymers (23–25% of solids) compared with GT (6). In addition, a higher concentration of gallic acid is found in BT (8). The chemical composition of thearubigins is under investigation (9). OTs are partially oxidized, leading to an intermediate tea with a lower concentration of polymeric polyphenols and higher concentrations of EGCG than BT.

Preparations of GT are used as aids in weight loss and weight maintenance. Catechins and caffeine, both contained in GT, are each believed to have a role in increasing energy metabolism and fat oxidation, which may lead to loss of body fat and weight loss. A number of randomized controlled trials evaluating the role of GT in weight loss were published previously (5). Dulloo et al. (10), using a whole-body energy expenditure chamber, found that a combination of GT extract and caffeine led to an increase in energy expenditure in the range of 2–4% of total daily energy expenditure in normal volunteers. Conversely, in a study by Hursel et al. (11), GT alone did not prevent weight regain after weight loss in obese individuals. Maki et al. (12) found that GT extract consumption in combination with exercise led to a decrease in abdominal visceral fat and TGs. A recent meta-analysis by Hursel et al. (13) concluded that a combination of GT and caffeine led to increased energy expenditure and fat oxidation in humans. To our knowledge, there are numerous studies of pure ECGC but no previous studies of the effects of tea polyphenols from the 3 major types of tea (GT, BT, and OT) on body fat in mice consuming an obesogenic HF/HS diet.

Another potential benefit of weight loss is the reduction of obesity-associated inflammation, which was implicated as a major factor in age-related chronic diseases. Abdominal visceral fat, but not lower body fat, is implicated in obesity-associated inflammation (14). A decrease in microvessel density and blood circulation in visceral adipose tissue leads to hypoxia and adipocyte necrosis, leading to the release of proinflammatory cytokines into the circulation (15,16). Weight loss was shown to be associated with an increase in adiponectin (*Adipoq*) formation, which results in increased angiogenesis in visceral fat that counteracts the hypoxia due to decreased microvessel density, and can inhibit inflammation (14).

The present study was designed to compare the activities of the caffeine-free extracts of polyphenols from GT, BT, and OT in inhibiting fat deposition and systemic inflammation during weight gain in wild-type C57BL/6J mice fed an HF/HS diet.

Methods and Materials

Tea polyphenol extracts

Chemical reagents and plant materials. All solvents were HPLC grade and purchased from Fisher Scientific. Gallic acid (>98%), tea polyphenol, and caffeine standards were purchased from Sigma-Aldrich. All GT, OT, and BT leaves were collected and purchased in a selected location in Sichuan province, China. The samples were kept in sealed bags at room temperature before extraction.

Tea polyphenol extract preparation. A total of 500 g of tea leaves were extracted with 4 L of 75% ethanol in room temperature for 3 h. The leaves were separated and extracted with ethanol. The procedure was repeated twice. The ethanol was evaporated in a rotary evaporator under reduced pressure at 40°C. The dried extract was suspended in 500 mL of pure water and extracted with dichloromethane to remove caffeine. The decaffeinated water solution of tea extract was subjected to an XAD-16 resin column separation, rinsed with 5 bed volumes of water, and eluted with pure ethanol. The extract was dried using the rotary evaporator.

Gallic acid equivalent. The assays were performed as reported previously with some modification using Folin-Ciocalteau reagent (17). The absorbance was read at 755 nm in a ThermoMax microplate reader (Molecular Devices) at room temperature. The standard curves were used to convert the average absorbance of each sample into milligrams per gram gallic acid equivalent.

HPLC condition for analysis of catechins and caffeine. A Water Alliance 2695 HPLC system coupled with a PDA detector and Empower 2 software was used to analyze the catechins and caffeine. The separation of catechins and caffeine was conducted on an Agilent Zorbax SB C18 column with a gradient of acetonitrile and 0.4% phosphoric acid in water. The detection wavelength was 280 nm.

Experimental mouse and body composition studies

All mouse procedures were approved by the University of California, Los Angeles Animal Research Committee in compliance with the Association for Assessment and Accreditation of Laboratory Care International. Male C57BL/6J mice (strain *JAX 000664*) were received from The Jackson Laboratory at 6–7 wk of age (body weight: 16–18 g). After 1 wk of acclimation, 28-d-old male C57BL/6J mice were assigned to 5 groups with similar body weight distribution in each group (**Supplemental Fig. 1**) and were fed a low-fat/high-sucrose (LF/HS) diet (D12489B; Research Diets), an HF/HS diet (D12266B; Research Diets), or an HF/HS diet supplemented with GT polyphenols (GTPs), OT polyphenols (OTPs), or BT polyphenols (BTPs) (**Table 1**) at 0.5 g/100 g diet providing 0.25 g polyphenols/100 g diet. Tea extracts were mixed into the diet by Research Diets. Body weights were recorded weekly and food consumption 3 times per week. Groups of 3–5 mice from each group were killed after 4, 8, 12, 16, and 20 wk of dietary treatments (Supplemental Fig. 1). Tissues were collected, weighed, and stored at −80°C until analysis. Body fat

TABLE 1 Composition of LF/HS diet, HF/HS diet, and HF/HS diets containing different tea polyphenols fed to male C57BL/6J mice for 20 wk[1]

Ingredients	LF/HS diet	HF/HS diet	HF/HS-GTP diet	HF/HS-OTP diet	HF/HS-BTP diet
	g/kg	*g/kg*	*g/kg*	*g/kg*	*g/kg*
Casein	161.2	182.2	181.3	181.3	181.3
DL-Methionine	2.5	2.8	2.9	2.9	2.9
Corn starch	423.1	206.2	205.1	205.1	205.1
Maltodextrin 10	29.7	71.9	74.4	74.4	74.4
Sucrose	246.1	278.1	276.7	276.7	276.7
Cellulose	25.5	28.8	28.6	28.6	28.6
Butter fat	12.5	42.4	42.2	42.2	42.2
Corn oil	33.4	113.2	112.6	112.6	112.6
Mineral mix S10001	34.0	38.4	38.2	38.2	38.2
Calcium carbonate	4.7	5.3	5.2	5.2	5.2
Sodium chloride	4.7	5.3	5.2	5.2	5.2
Potassium citrate	11.5	12.9	12.4	12.4	12.4
Vitamin mix V10001	9.3	10.5	10.5	10.5	10.5
Choline bitartrate	1.6	1.9	1.9	1.9	1.9
GTPs			5		
OTPs				5	
BTPs					5

[1] A total of 0.25 g of GTPs, OTPs, and BTPs was added to 1 kg of diet based on gallic acid equivalents. BTP, black tea polyphenol; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; LF/HS, low-fat/high-sucrose; OTP, oolong tea polyphenol.

composition was measured at week 20. Five mice from each group were anesthetized by isoflurane inhalation and imaged using an Aspect imaging M2 MRI system. Total adipose tissue and abdominal adipose tissue were quantified using the T1-weighted spin-echo data (VivoQuant; inviCRO).

Measurement of liver total lipid content, TGs, and FA composition

Total lipid content. Total hepatic lipids were quantified by chloroform methanol extraction following a modification of the method by Bligh and Dyer (18).

FA analysis by GC. The FA content of 6 FAs was determined as methyl esters by a GC–flame ionization detector method as published previously (19). The analysis was performed on an Agilent 7890 A GC as described previously (19). The FFAs were identified and quantified by comparing the retention times and area of peaks with those of commercial FFA methyl ester standards purchased from Sigma-Aldrich.

Liver Oil Red O staining. Liver tissue was fixed in 10% neutral-buffered formalin (VWR) and embedded in optimal cutting temperature compound. Sections were processed for Oil Red O staining as described previously (20). Liver specimens were evaluated by light microscopy, and the Oil Red–positive area was analyzed using VivoQuant data processing.

Liver and adipose tissue tea polyphenol analysis

Extraction and digestion (β-glucuronidase and sulfatase; Sigma-Aldrich) of liver and adipose tissue tea polyphenols was performed as described previously (21). Dried extracts were reconstituted in 50% methanol: water for detection by LC–MS. The LC–MS analysis was performed on a Zorbax SB C18 column (Agilent). Analyses were performed using the HPLC–electrospray ionization–MS system (Thermo Finnigan LCQ advantage) at negative mode. MS2 spectra were automatically performed with helium as the collision gas (EGC: m/z 305→219; EGCG: m/z 457→331; methyl-EGCG: m/z 471→287; and ECG: m/z 441→289). Concentrations were calculated by comparison of sample peak area with the commercial standard peak area (21).

Real-time qPCR

Liver and adipose tissues of mice after 16 wk of dietary treatment were dissected and immediately preserved in RNA*Later* Solution (Life Technologies). Total RNA was isolated using an RNeasy mini kit and an RNeasy lipid tissue mini kit (Qiagen). RNA treated with deoxyribonuclease I was quantified, and equal amounts of RNA were reverse transcripted into cDNA using a first-strand cDNA synthesis kit (Clontech). qRT-PCR was performed using a SYBR green PCR master mix (Clontech) and HT7900 Fast Real-Time PCR (Applied Biosystems). The mRNA levels of all genes were normalized using GAPDH as internal control. The primers were designed to evaluate the expressions of the pigment epithelium-derived factor (*Pedf*), vascular endothelial growth factor A (*Vegfa*), vascular endothelial growth factor receptor 2 (*Vegfr2*), *Gapdh*, monocyte chemotactic protein-1 (*Mcp1*), and *Adipoq* genes (**Supplemental Table 1**).

Analysis of serum MCP-1

The plasma concentration of mouse MCP-1 was measured with Quantikine M mouse MCP-1 ELISA kits (R&D Systems). Intra-assay and interassay precision indicated by percentage coefficient of variation are 4.6–7.3 and 5.1–8.3, respectively.

Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics version 21; mean values, SDs, and SEs were calculated using descriptive statistics. Energy intake, body weight, percentage visceral fat/body weight, percentage subcutaneous fat/body weight, percentage liver weight/body weight, and percentage lipid weight/liver weight were analyzed with 2-factor ANOVA, with the factors diet and time. The Tukey-Kramer multiple comparison procedure was used for post hoc comparisons of diet means. Total visceral fat volume (MRI), hepatic FAs, hepatic Oil Red–positive area, *Mcp1*, *Adipoq*, *Pedf*, *Vegfa*, and *Vegfr2* gene expression, and MCP-1 protein expression were analyzed with 1-factor ANOVA, with the factor diet. The Tukey-Kramer multiple comparison procedure was used for post hoc comparisons. P values <0.05 were considered statistically significant.

Results

Body weight and composition in mice fed the HF/HS diet supplemented with polyphenol-enriched tea extracts. During the 20-wk dietary intervention, the HF/HS-treated mice had significantly higher body weight and subcutaneous fat by weight (**Fig. 1***A*, *B*) and total visceral fat by weight compared with the LF/HS group (**Supplemental Fig. 2***B*). As shown in Figure 1*A*, *B* and Supplemental Figure 2*B*, body weight gain and visceral fat and subcutaneous fat gain normalized to body weight were decreased significantly in mice fed the HF/HS diets supplemented with all 3 types of tea polyphenols compared with HF/HS controls. We also assessed total visceral fat of mice by MRI at week 20. The HF/HS-induced gain in total visceral fat was significantly reduced by GTP, OTP, and BTP supplementation

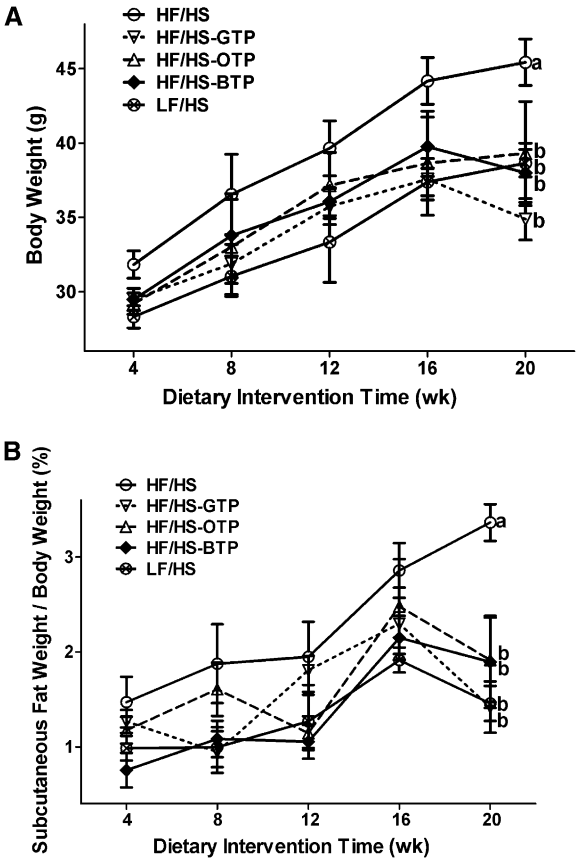

FIGURE 1 Effects of polyphenol-enriched tea extracts on body weight (*A*) and percentage subcutaneous fat normalized to body weight (*B*) in male C57BL/6J mice fed an HF/HS, LF/HS, HF/HS-GTP, HF/HS-OTP, or HF/HS-BTP diet for 20 wk. Data are means ± SEMs (n = 3–5). Statistical significance as evaluated by 2-factor ANOVA (diet × time), followed by the Tukey-Kramer multiple comparison procedure. Both diet and time affected body weight and percentage subcutaneous fat normalized to body weight, but there was no interaction between the effect of time and diet (**Supplemental Table 2**). Labeled means of dietary interventions without a common letter differ by diet throughout the intervention time (4–20 wk), $P < 0.05$. BTP, black tea polyphenol; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; LF/HS, low-fat/high-sucrose; OTP, oolong tea polyphenol.

(**Fig. 2**). Energy intake of the mice consuming the HF/HS-OTP and HF/HS-BTP diets was not significantly different from the HF/HS control mice, whereas HF/HS-GTP consumption was associated with a significant decrease in energy intake compared with HF/HS control mice (weeks 4–20) (Supplemental Fig. 2*A*).

Liver weight and lipid content. When normalized to body weight, no difference in liver weight was observed at the end of the 20-wk dietary intervention with the HF/HS diet group compared with the LF/HS diet group (**Fig. 3***A*). However, normalized liver weights of mice in the HF/HS-GTP and HF/HS-BTP groups, but not the HF/HS-OTP group, were significantly lower compared with HF/HS-fed mice (Fig. 3*A*). Weights of the spleen, heart, pancreas, and kidney were similar among all the experimental groups (data not shown). None of the tea polyphenol extracts caused liver toxicity. At 20 wk of intervention, serum alanine transaminase activity was below 33 U/L, which is in the normal range for mice (22). Hepatic total lipid and TG content was increased significantly in the HF/HS diet mice compared with the LF/HS diet mice. We observed that all 3 tea polyphenol extracts significantly decreased liver total lipid and TG content compared with HF/HS control mice (Fig. 3*B*, *C* and **Supplemental Fig. 3**).

The liver FA analysis at 20 wk demonstrated that the dietary tea polyphenol treatment was associated with changes in hepatic FA composition (**Table 2**). GTPs and BTPs significantly altered the percentage palmitic acid (16:0) and DHA (22:6n–3) of total FAs quantified compared with the HF/HS diet alone. All 3 tea polyphenol extracts significantly regulated the percentage stearic acid (18:0) and oleic acid (18:1n–9) compared with the HF/HS diet alone. The percentage linoleic acid (18:2n–6) and arachidonic acid (20:4n–6) were significantly increased by GTPs only compared with the HF/HS diet alone.

Effect of tea polyphenols on inflammatory responses in white adipose tissue and liver of mice with HF/HS-induced obesity. *Mcp1* gene expression was evaluated in white adipose tissue (epididymal and mesenteric fat) at the end of the 16-wk dietary tea treatment. In mesenteric fat, all 3 tea extracts

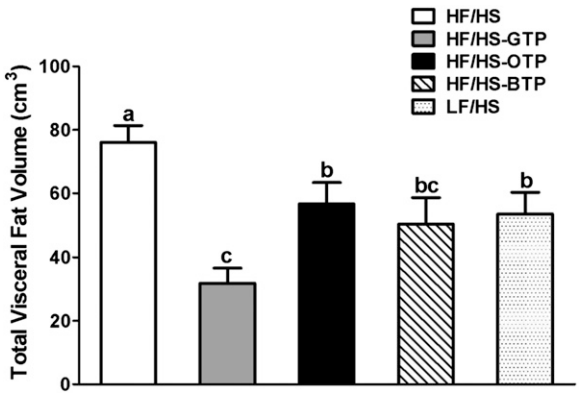

FIGURE 2 Coronal T1-weighted spin-echo MRIs were obtained from male C57BL/6J mice fed an HF/HS, LF/HS, HF/HS-GTP, HF/HS-OTP, or HF/HS-BTP diet for 20 wk. The images were processed with volume segmentation for total visceral fat. Quantification of MR images was performed using VivoQuant software. Data are means ± SEMs (n = 5). Data were analyzed by 1-factor ANOVA, followed by the Tukey-Kramer multiple comparison procedure. Labeled means without a common letter differ, $P < 0.05$. BTP, black tea polyphenol; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; LF/HS, low-fat/high-sucrose; OTP, oolong tea polyphenol.

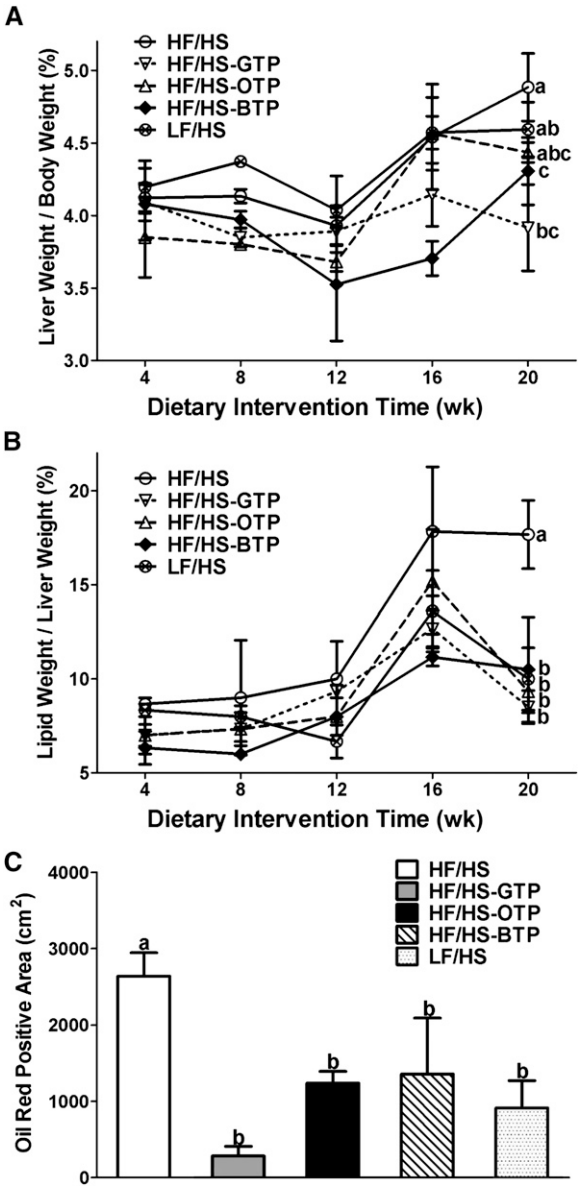

FIGURE 3 Effects of polyphenol-enriched tea extracts on HF/HS-induced fatty liver in male C57BL/6J mice fed an HF/HS, LF/HS, HF/HS-GTP, HF/HS-OTP, or HF/HS-BTP diet for 20 wk. Liver weight was normalized to body weight (*A*), and liver lipid weight was normalized to liver weight (*B*) over 20 wk. Data are means ± SEMs (n = 3–5). Statistical significance as revealed by 2-factor ANOVA (diet × time), followed by the Tukey-Kramer multiple comparison procedure. Both diet- and time-affected liver weight were normalized to body weight and liver lipid weight normalized to liver weight, but there was no interaction between the effect of time and diet (Supplemental Table 2). Labeled means of dietary interventions without a common letter differ by diet throughout the intervention time (4–20 wk), $P < 0.05$. Quantification of TG by Oil Red staining in liver sections (*C*). Data are means ± SEMs (n = 5). Data were analyzed by 1-factor ANOVA, followed by the Tukey-Kramer multiple comparison procedure. Labeled means without a common letter differ, $P < 0.05$. BTP, black tea polyphenol; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; LF/HS, low-fat/high-sucrose; OTP, oolong tea polyphenol.

significantly decreased HF/HS diet–induced *Mcp1* upregulation (**Fig. 4***A*), whereas BTP treatment significantly decreased HF/HS diet–induced *Mcp1* upregulation in epididymal fat (Fig. 4*B*). In liver, *Mcp1* gene expression was inhibited significantly by the addition of GTPs and BTPs but not OTPs (Fig. 4*C*). The serum

TABLE 2 Effects of polyphenol-enriched tea extracts on hepatic FA composition expressed as a percentage of the sum of all FAs analyzed in male C57BL/6J mice fed an HF/HS, LF/HS, HF/HS-GTP, HF/HS-OTP, or HF/HS-BTP diet for 20 wk[1]

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidonic acid	DHA
	Weight %	*Weight %*	*Weight %*	*Weight %*	*Weight %*	*Weight %*
LF/HS diet	25.3 ± 1.0^{a,b}	6.0 ± 1.8^{a,b}	30.9 ± 6.7^{a}	8.9 ± 1.6^{c}	5.6 ± 3.1^{a,b}	1.4 ± 0.7^{a,b}
HF/HS diet	26.3 ± 0.8^{a}	4.1 ± 1.1^{b}	30.3 ± 3.6^{a}	16.9 ± 1.9^{b}	4.1 ± 1.1^{b}	1.0 ± 0.2^{b}
HF/HS-GTP diet	20.8 ± 2.2^{c}	7.7 ± 2.6^{a}	19.2 ± 4.9^{b}	21.1 ± 4.4^{a}	7.6 ± 2.5^{a}	2.0 ± 0.8^{a}
HF/HS-OTP diet	25.6 ± 1.2^{a,b}	6.9 ± 1.4^{a}	22.5 ± 4.2^{b}	16.6 ± 2.0^{b}	6.2 ± 1.2^{a,b}	1.4 ± 0.3^{a,b}
HF/HS-BTP diet	23.5 ± 2.5^{b}	7.4 ± 2.9^{a}	22.4 ± 7.0^{b}	17.7 ± 1.9^{b}	6.9 ± 2.9^{a,b}	1.9 ± 0.9^{a}

[1] Values are means ± SDs (n = 5). Data were analyzed by 1-factor ANOVA, followed by the Tukey-Kramer multiple comparison procedure. Hepatic FA profiles of mice fed the indicated diets for 20 wk were determined by GC analysis. Data were expressed as percentage of the sum of all FAs. Means in a column without a common letter differ, $P < 0.05$. BTP, black tea polyphenol; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; LF/HS, low-fat/high-sucrose; OTP, oolong tea polyphenol.

concentration of MCP-1 in the HF/HS group was increased compared with the LF/HS group; BTPs and GTPs significantly inhibited HF/HS-induced serum MCP-1 upregulation (**Fig. 5**).

Gene expression of Adipoq, Pedf, Vegfa, *and* Vegfr2 *in white adipose tissue.* During initial adipose expansion (HF diet–induced obesity model), proangiogenic activities were shown to be associated with a reduction of inflammation and insulin resistance (15,16,23). Feeding the HF/HS-GTP and HF/HS-OTP diets significantly increased *Adipoq*, whereas HF/HS-OTP and HF/HS-BTP diets decreased *Pedf* gene expression in epididymal fat compared with HF/HS diet mice (**Fig. 6***A*, *B*). In addition, gene expression of 2 proangiogenic markers, *Vegfa* and *Vegfr2*, was determined. Compared with the HF/HS diet, the HF/HS-GTP diet significantly increased *Vegfa* gene expression in epididymal fat (Fig. 6*C*), and the HF/HS-BTP diet was associated with significantly increased *Vegfr2* gene expression in epididymal fat (Fig. 6*D*).

Concentration of tea polyphenols and metabolites in liver and adipose tissue. Polyphenol extracts of GT, OT, and BT contained 21%, 14%, and 3.6% of EGCG, respectively (**Table 3**). EGCG was found in liver and white adipose tissue of mice fed the GTP and OTP diets, with considerable individual variation (**Fig. 7***A*, *B*). 4′′-*O*-methyl EGCG (4′′-MeEGCG) was only found in liver of mice in the HF/HS-GTP and HF/HS-OTP groups (Fig. 7*A*). ECG was found in liver and white adipose tissue of mice in all 3 groups (Fig. 7*A*, *B*). In the liver, EGC was detected in 1 of 3 mice fed the HF/HS-GTP and HF/HS-OTP diets (Fig. 7*B*). Adipose EGC was detected in GTP-fed mice (Fig. 7*B*).

Discussion

In the present study, the addition of standardized GTP-, BTP-, and OTP-enriched extracts to an HF/HS diet significantly decreased body weight, abdominal visceral fat volume, and biomarkers of inflammation compared with an HF/HS obesogenic diet alone.

In addition, gene expression of angiogenesis markers and *Adipoq* were modulated by GTP-, BTP-, and OTP-enriched extracts. The final body weight in mice fed tea extracts with an HF/HS diet was similar to mice fed an LF/HS diet, except that liver fat in the GTP-fed group was greater than in the LF/HS diet. However, energy intake was decreased by ~10% with GTPs but unchanged with BTPs or OTPs added to the HF/HS diet. Although all 3 tea polyphenol extracts were standardized to similar phenolic contents, the GTP extract had the highest

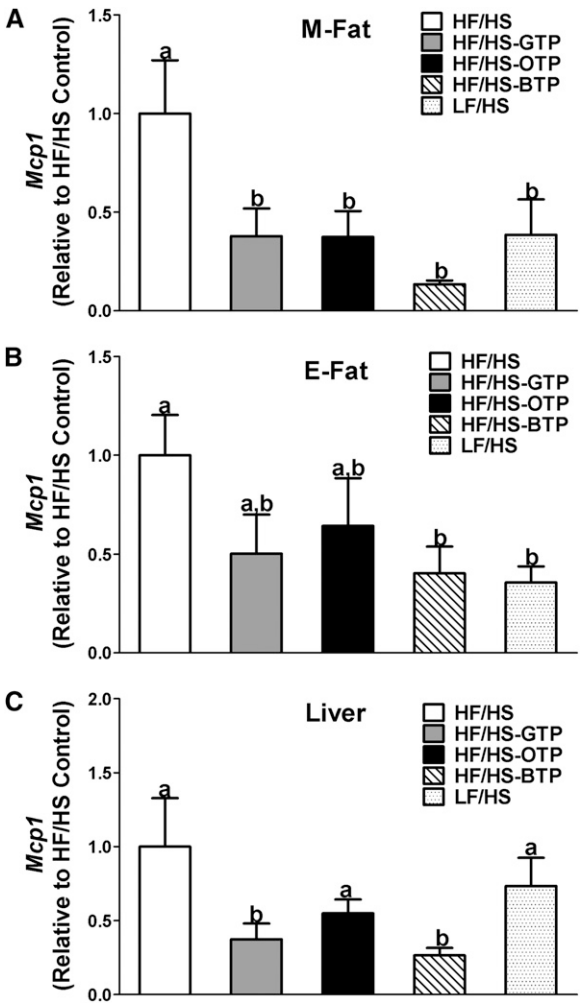

FIGURE 4 Effects of polyphenol-enriched tea extracts on *Mcp1* gene expression in white adipose tissue and liver in male C57BL/6J mice fed an HF/HS, LF/HS, HF/HS-GTP, HF/HS-OTP, or HF/HS-BTP diet for 16 wk. Mesenteric fat (*A*), epididymal fat (*B*), and liver (*C*). Data are means ± SEMs (n = 5). Data were analyzed by 1-factor ANOVA, followed by the Tukey-Kramer multiple comparison procedure. Labeled means without a common letter differ, $P < 0.05$. BTP, black tea polyphenol; E-Fat, epididymal fat; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; LF/HS, low-fat/high-sucrose; M-Fat, mesenteric fat; *Mcp1*, monocyte chemotactic protein-1; OTP, oolong tea polyphenol.

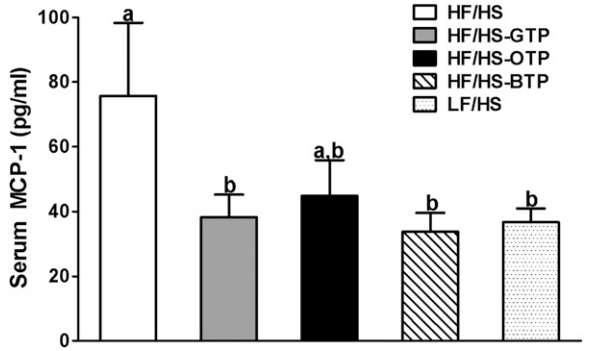

FIGURE 5 Effects of polyphenol-enriched tea extracts on serum concentrations of MCP-1 in male C57BL/6J mice fed an HF/HS, LF/HS, HF/HS-GTP, HF/HS-OTP, or HF/HS-BTP diet for 16 wk. Data are means ± SEMs (n = 5). Data were analyzed by 1-factor ANOVA, followed by the Tukey-Kramer multiple comparison procedure. Labeled means without a common letter differ, $P < 0.05$. BTP, black tea polyphenol; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; LF/HS, low-fat/high-sucrose; MCP-1, monocyte chemotactic protein-1; OTP, oolong tea polyphenol.

content of EGCG, which could explain the decreased energy intake through its known effects of increasing hepatic FA oxidation (4). Monomeric GTPs are absorbed in the small intestine, whereas the larger polymeric polyphenols in BT and OT are only marginally absorbed even after the administration of large amounts equivalent to 6 L (30 cups) of BT (24). GT or EGCG administration with HF diets can cause weight loss independent of effects on energy intake (25,26) through increased thermogenesis, increased lipid oxidation, and/or reduced lipogenesis (4,5). In addition i.p. injection of EGCG (85 mg/kg body weight) in rats is associated with a decrease in energy intake of ~15% along with decreases in serum leptin and luteinizing hormone concentrations (27).

Fewer studies examined the effect of BT on weight loss in humans and animals (28–30). The large-molecular-weight tea polyphenols in BT suggest that the antiobesity effects of BTPs may be through inhibition of pancreatic lipase, leading to decreased lipid absorption (31–33). OTPs also cause weight loss in mice fed an HF diet (34), most likely due to effects similar to those of BT because OT also contains less catechin and EGCG than GT.

Rapid weight gain in humans and C57BL/6J mice leads to accumulation of intra-abdominal or visceral fat (35), which is accompanied by apoptosis, immune system activation, and recruitment of macrophages, leading to an increase in blood concentrations of circulating chemokines, MCP-1, IL-6, TNF-α, and other cytokines (36). MCP-1 is a small cytokine that recruits monocytes, memory T cells, and dendritic cells to the site of injury. Plasma concentrations of MCP-1 are increased with obesity (37). In the present study, all 3 tea polyphenol extracts at the same total polyphenol content led to a decrease in MCP-1 concentrations in serum. Similar results were observed in mice fed 60% energy from fat supplemented with 1% and 2% of GT extract or 0.37% EGCG (35,36,38). Treatment with low concentrations of gallic acid (0.1 and 1 μmol/L) inhibited proinflammatory cytokine gene expression in vitro, which may explain the anti-inflammatory effect of BTPs (39).

Tissue FA composition reflects the FA composition of the diet (40). Corn oil is rich in linoleic acid and oleic acid. A 2-fold relative increase of linoleic acid was seen in mice consuming the HF/HS diet compared with the LF/HS diet. Decreases in palmitic acid and oleic acid were observed in the hepatic fat of mice consuming the HF/HS diet with tea polyphenols compared with

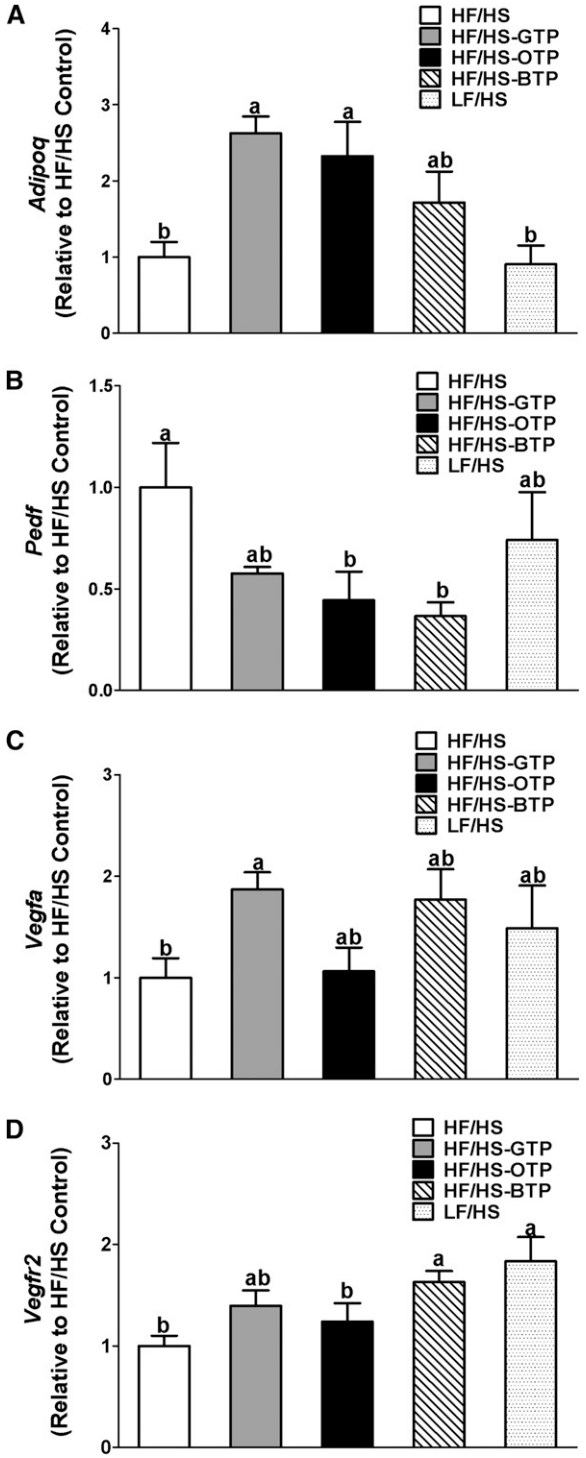

FIGURE 6 Effects of polyphenol-enriched tea extracts on *Adipoq* and angiogenesis-related gene expression in epididymal fat from male C57BL/6J mice fed an HF/HS, LF/HS, HF/HS-GTP, HF/HS-OTP, or HF/HS-BTP diet for 16 wk: *Adipoq* (*A*), *Pedf* (*B*), *Vegfa* (*C*), and *Vegfr2* (*D*) gene expression. Data are means ± SEMs (n = 5). Data were analyzed by 1-factor ANOVA, followed by the Tukey-Kramer multiple comparison procedure. Labeled means without a common letter differ, $P < 0.05$. *Adipoq*, adiponectin; BTP, black tea polyphenol; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; LF/HS, low-fat/high-sucrose; OTP, oolong tea polyphenol; *Pedf*, pigment epithelium-derived factor; *Vegfa*, vascular endothelial growth factor A; *Vegfr2*, vascular endothelial growth factor receptor 2.

TABLE 3 GAE, caffeine, and EGCG concentrations of GTPs, OTPs, and BTPs[1]

	GAE	Caffeine	EGCG
	mg/g	*mg/g*	*mg/g*
GTPs	565 ± 24	0.5 ± 0.1	214 ± 4.5
OTPs	588 ± 29	0.6 ± 0.1	142 ± 0.6
BTPs	532 ± 25	1.4 ± 0.1	36 ± 0.3

[1] Values are means ± SDs ($n = 6$). Total phenolic content was expressed as GAE. BTP, black tea polyphenol; EGCG, (−)-epigallocatechin gallate; GAE, gallic acid equivalent; GTP, green tea polyphenol; OTP, oolong tea polyphenol.

controls. These observations are consistent with the effect of tea polyphenols inhibiting de novo fat synthesis from sucrose through acyl-CoA synthase reaction and the conversion of stearic acid to oleic acid by the stearoyl-CoA desaturase (*SCD-1*) (41). In addition, GT, BT, and pu-erh (completely fermented) tea were shown to inhibit FA synthase (*FAS*) through AMP-activated protein kinase phosphorylation in rats fed a high-fructose diet (42). GT extract also decreased the expression of hepatic lipogenic genes, including sterol element binding protein-1c (*SREBP1c*) and its downstream regulatory target genes *FAS* and *SCD-1*, in fructose-fed ovariectomized rats (43).

In adipose tissue, we focused on the gene expression of the following: *1*) the adipokines *Adipoq*, leptin, and *Pedf*; *2*) the angiogenesis-related genes *Vegfa* and *Vegfr2*; *3*) the adipogenic factors Kruppel-like factor 7 (*Klf7*), CCAAT/enhancer binding protein α (*C/ebpα*), *Pparγ*, and *Srebp1*; and *4*) the energy expenditure genes carnitine palmitoyltransferase I (*Cpt1*) and glycerol-3-phosphate acyltransferase (*Gpat*). The adipocyte secreted hormone ADIPOQ has multiple functions in lipid and glucose metabolism, inflammation, and vascular remodeling (14), including increased angiogenesis (14). PEDF, an adipose secreted factor, inhibits angiogenesis and is elevated in response to obesity (44–47). In this study, a 2-fold increase of *Adipoq* gene expression by GTPs and 63% decrease of *Pedf* gene expression by BTPs were associated with increased angiogenic factor gene expression by *Vegfa* in the GTP group and significantly enhanced *Vegfr2* expression in the BTP group. Our observations are consistent with the notion that tea polyphenols increase blood vessel formation in adipose tissue, which contributes to their anti-inflammatory effects. This finding is in contrast to the traditional concept that inhibition of angiogenesis results in weight loss (48) but is consistent with recent observations of proangiogenic activity during adipose tissue expansion mediating protective effects on metabolism and inflammation (23). Expression of genes associated with adipogenesis, energy expenditure, and lipid metabolism, including *Klf7*, *C/ebpα*, *Pparγ*, *Cpt1*, *Gpat*, *Srebp*, and 3-hydroxy-3-methylglutaryl-CoA (*Hmgcoa*), were not changed by tea polyphenols (data not shown).

The bioavailability and biotransformation of tea polyphenols are the limiting factor in mediating the biologic activity of tea polyphenols in target tissues (49). 4′′-MeEGCG, the major biotransformation product of EGCG, is formed by catechol-*O*-methyltransferase in the liver and in the current study was not found in adipose tissue (50). In the current study, EGCG, 4′′-MeEGCG, EGC, and ECG were found in higher concentrations in the livers of mice consuming GTPs compared with OTPs and not after BTPs, which reflects the tea polyphenol content of GT, OT, and BT due to the difference of fermentation during the processing and manufacturing of these different teas.

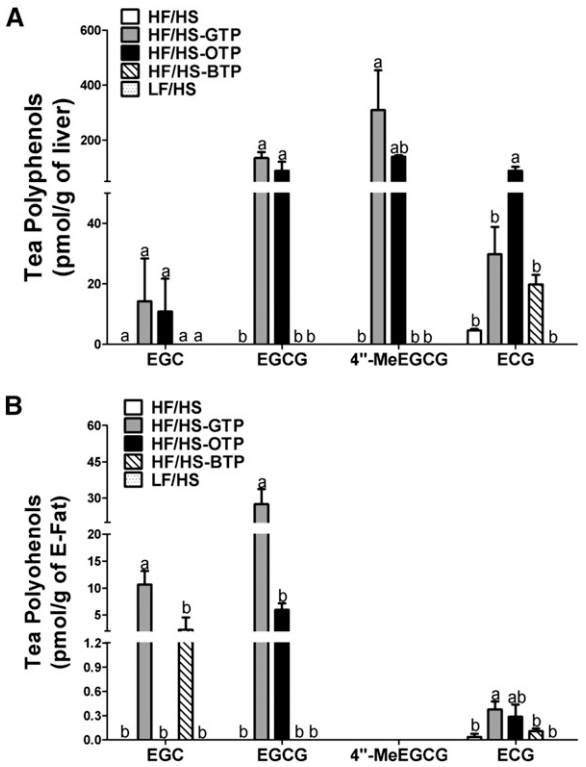

FIGURE 7 Effects of polyphenol-enriched tea extracts on tissue concentrations of tea polyphenols and their metabolites in liver (*A*) and E-fat (*B*) in male mice fed an HF/HS, LF/HS, HF/HS-GTP, HF/HS-OTP, or HF/HS-BTP diet for 16–20 wk. Data are means ± SEMs ($n = 3$). Data were analyzed by 1-factor ANOVA, followed by the Tukey-Kramer multiple comparison procedure. Labeled means without a common letter differ, $P < 0.05$. No comparison was performed for E-fat 4′′-MeEGCG because it was undetectable in all E-fat samples. BTP, black tea polyphenol; ECG, (−)-epicatechin-3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin gallate; E-Fat, epididymal fat; GTP, green tea polyphenol; HF/HS, high-fat/high-sucrose; LF/HS, low-fat/high-sucrose; OTP, oolong tea polyphenol; 4′′-MeEGCG, 4′′-*O*-methyl (–)-epigallocatechin gallate.

Our observations of the effects of caffeine-free extracts of GT, BT, and OT demonstrate that the polyphenol fractions of teas are biologically active in inhibiting weight gain on an HF/HS diet. However, the induction of weight loss by the 3 types of tea extracts was induced through different mechanisms. The combination of GTPs and BTPs, by working through different mechanisms as suggested by the results of the present study, may provide an interesting combination approach for future human studies of the effects of tea polyphenols in obesity treatment and weight maintenance.

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