AJCN. First published ahead of print March 8, 2017 as doi: 10.3945/ajcn.116.144972.

Tea catechin and caffeine activate brown adipose tissue and increase cold-induced thermogenic capacity in humans[1,2]

Takeshi Yoneshiro,[3] Mami Matsushita,[5] Masanobu Hibi,[6] Hiroshi Tone,[6] Masao Takeshita,[6] Koichi Yasunaga,[6] Yoshihisa Katsuragi,[6] Toshimitsu Kameya,[7] Hiroki Sugie,[7] and Masayuki Saito[4]*

[3]Department of Biomedical Sciences, Graduate School of Veterinary Medicine, [4]Hokkaido University, Sapporo, Japan; [5]Department of Nutrition, School of Nursing and Nutrition, Tenshi College, Sapporo, Japan; [6]Health Care Food Research Laboratories, Kao Corporation, Tokyo, Japan; and [7]LSI Sapporo Clinic, Sapporo, Japan

ABSTRACT

Background: The thermogenic effects of green tea catechin have been repeatedly reported, but their mechanisms are poorly understood.

Objective: The aim of this study was to investigate the acute and chronic effects of catechin on brown adipose tissue (BAT), a site specialized for nonshivering thermogenesis, in humans.

Design: Fifteen healthy male volunteers underwent fluorodeoxyglucose-positron emission tomography to assess BAT activity. To examine the acute catechin effect, whole-body energy expenditure (EE) after a single oral ingestion of a beverage containing 615 mg catechin and 77 mg caffeine (catechin beverage) was measured. Next, to investigate the chronic catechin effects, 10 men with low BAT activity were enrolled. Before and after ingestion of the catechin beverage 2 times/d for 5 wk, cold-induced thermogenesis (CIT) after 2 h of cold exposure at 19°C, which is proportional to BAT activity, was examined. Both the acute and chronic trials were single-blinded, randomized, placebo-controlled, season-matched crossover studies.

Results: A single ingestion of the catechin beverage increased EE in 9 subjects who had metabolically active BAT (mean ± SEM: +15.24 ± 1.48 kcal, $P < 0.01$) but not in 6 subjects who had negligible activities (mean ± SEM: +3.42 ± 2.68 kcal). The ingestion of a placebo beverage containing 82 mg caffeine produced a smaller and comparative EE response in the 2 subject groups. Multivariate regression analysis revealed a significant interaction between BAT and catechin on EE ($\beta = 0.496$, $P = 0.003$). Daily ingestion of the catechin beverage elevated mean ± SEM CIT (from 92.0 ± 26.5 to 197.9 ± 27.7 kcal/d; $P = 0.009$), whereas the placebo beverage did not change it.

Conclusion: Orally ingested tea catechin with caffeine acutely increases EE associated with increased BAT activity and chronically elevates nonshivering CIT, probably because of the recruitment of BAT, in humans. These trials were registered at www.umin.ac.jp/ctr/ as UMIN000016361. *Am J Clin Nutr* doi: 10.3945/ajcn.116.144972.

Keywords: catechin, brown adipose tissue, energy expenditure, cold-induced thermogenesis, healthy humans

INTRODUCTION

Brown adipose tissue (BAT)[8] is a tissue specialized for nonshivering metabolic thermogenesis induced by cold exposure and food intake, thereby an intriguing target to combat obesity and related metabolic disorders (1). BAT thermogenesis is acutely activated by cold exposure; moreover, cold exposure generates long-term effects, such as increased BAT activity and thermogenic uncoupling protein 1 expression, which are associated with increasing energy expenditure (EE), decreasing adiposity, and improving insulin sensitivity (2–8). Although a β3-adrenergic receptor agonist and bile acids are capable of activating human BAT (9, 10), neither the cold regimen nor such pharmacologic agents would be easily applicable for sustained interventions because of unwanted side effects (9, 11).

The cold-induced activation of BAT through temperature-sensitive transient receptor potential (TRP) channels and the sympathetic nervous system can be mimicked by oral ingestion of natural food ingredients. For example, capsaicin analogs capsinoids, which have agonistic activities at TRPV1 and TRPA1, enhance efferent discharges of sympathetic nerves connecting to BAT (12), thereby triggering thermogenesis (13). In addition to capsinoids, there are thermogenic food ingredients consumed world-wide, one of which is green tea catechin, a class of low-molecular-weight polyphenols. Since the first report by Dulloo et al. (14), the thermogenic effect of green tea extract rich in catechin has repeatedly been shown in humans (15–18). A synergistic effect of caffeine, which is also rich in green tea, was also reported for the thermogenic effect of catechin (16, 19).

[1] Supported by Grant-in-Aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (24240092, 2610103, and 26860703).

[2] Supplemental Figures 1–4 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://ajcn.nutrition.org.

*To whom correspondence should be addressed. E-mail: yoneshiro@vetmed.hokudai.ac.jp.

[8] Abbreviations used: BAT, brown adipose tissue; CIT, cold-induced thermogenesis; COMT, catechol-*O*-methyltransferase; CT, computed tomography; EE, energy expenditure; EGCG, epigallocatechin gallate; FFM, fat-free mass; iAUC, incremental AUC; PET, positron emission tomography; SUV, standardized uptake value; TRP, transient receptor potential; VCO_2, carbon dioxide production; VO_2, oxygen consumption.

Received September 5, 2016. Accepted for publication February 3, 2017. doi: 10.3945/ajcn.116.144972.

Despite the abundance of evidence for the thermogenic effect of catechin, the action mechanisms of its active compound are not fully understood. Although catechin is believed to stimulate fatty acid degradation in the liver and adipose tissue (20, 21), controversy still exists as to how this food ingredient generates heat. Kurogi et al. (22, 23) reported agonistic activities of epigallocatechin gallate (EGCG), one of the major catechins found in green tea, at cold-sensitive TRPA1. Moreover, a combination of green tea and exercise induces brown-like (beige) adipocytes formation in mice (24). Given that BAT of adult humans mainly possesses beige adipocytes (25, 26), it seems conceivable that the thermogenic effect of catechin is mediated through the activation of BAT. To test this, in the present study, we investigated the acute and chronic effects of oral administration of catechin with caffeine on whole-body EE, with special references to BAT activity, in healthy humans.

METHODS

Study protocols

We performed 2 separate randomized, single-blinded, placebo-controlled, season-matched crossover trials; one was designed to investigate if a single oral ingestion of catechin acutely increases EE through the activation of BAT (acute trial), and another was designed to test whether BAT can be reactivated and recruited by daily ingestion of catechin even in subjects who had decreased BAT activity and mass (chronic trial). The protocols were approved by the Institutional Research Ethics Review Board of Tenshi College (Sapporo, Japan). The trials were registered at http://www.umin.ac.jp/ctr/ (UMIN000016361). All participants, who had been living in Sapporo, Japan, for ≥3 y, were carefully instructed regarding the study and provided written, informed consent.

Test beverages

Test beverages used in the present study were provided by Kao Corporation and were prepared from a green tea extract as described previously (27). A bottle of the active beverage contained 615 mg tea catechin and 77 mg caffeine in 350 mL, and the control beverage contained 0 mg catechin and 81 mg caffeine, hereinafter referred to as the catechin beverage and the placebo beverage, respectively (**Table 1**).

TABLE 1
Composition of the test beverages

	Catechin beverage	Placebo beverage
Water, g	350.0	350.0
Energy, kcal	14.0	0.0
Carbohydrate, g	3.5	0.0
Fat, g	0.0	0.0
Protein, g	0.0	0.0
Ash, g	0.0	0.0
Sodium, mg	35.0	6.3
Catechin, mg	33.2	0.0
Epicatechin, mg	34.8	0.0
Gallocatechin, mg	135.2	0.0
Epigallocatechin, mg	114.5	0.0
Catechin gallate, mg	22.9	0.0
Epicatechin gallate, mg	39.9	0.0
Gallocatechin gallate, mg	108.4	0.0
Epigallocatechin gallate, mg	125.9	0.0
Total catechins, mg	614.9	0.0
Caffeine, mg	77.0	81.2

Participants

Fifteen healthy young male volunteers participated in the acute catechin trial (**Table 2**). The BAT activity was evaluated by using fluorodeoxyglucose-positron emission tomography (PET) combined with computed tomography (CT) in the winter season (December or January). They were randomly assigned to 2 groups based on age, body composition, and BAT activity. Then their whole-body EE and its response to the catechin or placebo ingestion were measured by indirect calorimetry between January and March (winter) in a randomized crossover design with a washout period of ∼2 wk (**Figure 1**A). Anthropometric variables were also measured on the day of the measurements.

For the chronic catechin trial, healthy young men who participated in our previous study (28) and showed low or no BAT activity assessed by fluorodeoxyglucose-PET/CT after 2 h of cold exposure were enrolled until a population of 10 subjects was achieved. They were randomly assigned to 2 groups based on age, body composition, and BAT activity and were treated with either the catechin beverage or the control beverage for 5 wk in the crossover design. Five men were first treated with the catechin beverage and second with the placebo beverage, whereas the other 5 men were treated with the test beverages in the reverse order (Figure 1B). To minimize the possible effects of season on BAT and cold-induced thermogenesis (CIT) (2, 29), both treatments were conducted in the winter season from January to March of either 2012 or 2013, thus an 11-mo washout period was applied. Before and after the treatments, CIT on 2 h of cold exposure was measured by indirect calorimetry as a noninvasive predictive index of BAT activity (6, 30) instead of the repeated 4-time fluorodeoxyglucose-PET/CT examination. Likewise, anthropometric variables were also monitored.

Fluorodeoxyglucose-PET/CT

After overnight fasting for 6–12 h, subjects were exposed to cold by being kept in an air-conditioned room at 19°C with light clothing, and they intermittently placed their feet on an ice block wrapped in cloth. After 1 h under these cold conditions, they were given an intravenous injection of ^{18}F-fluorodeoxyglucose (1.66–5.18 MBq/kg body weight) and subsequently kept under the same cold conditions. One hour after the fluorodeoxyglucose injection, PET/CT scans were performed by using a PET/CT system (Aquiduo; Toshiba Medical Systems). According to the detection of cold-activated BAT with a standardized uptake value (SUV) of fluorodeoxyglucose ≥2.0 and Hounsfield Units from −300 to −10 in the supraclavicular region, subjects for the acute trial were divided into the high- and low-BAT groups (2, 5, 6, 13, 28–32). Their BAT activity was quantitatively assessed from an SUV in the supraclavicular fat deposits. Similarly, BAT activity of 10 subjects selected for the chronic trial was also quantified as an SUV, and their activity was undetectably or faintly detectable as low by visual inspection of the PET/CT images and an SUV of 1.9 ± 0.3.

TABLE 2
Profiles of the participants for the acute trial[1]

	All (N = 15)	High BAT (n = 9)	Low BAT (n = 6)
Age, y	23.1 ± 0.6	22.7 ± 0.6	23.8 ± 1.1
Height, cm	170.1 ± 1.6	169.7 ± 2.6	170.7 ± 0.8
Weight, kg	62.1 ± 2.3	60.4 ± 2.3	64.7 ± 4.6
BMI, kg/m^2	21.4 ± 0.7	20.9 ± 0.5	22.2 ± 1.6
Body fat content, %	15.4 ± 1.3	15.6 ± 1.0	15.2 ± 2.9
Fat mass, kg	9.8 ± 1.1	9.5 ± 0.8	10.3 ± 2.6
Fat-free mass, kg	52.3 ± 1.5	50.9 ± 1.8	54.3 ± 2.6
Waist circumference, cm	75.7 ± 1.9	74.6 ± 1.6	77.3 ± 4.4

[1] Values are means ± SEMs. Statistical analysis was performed by using the Student's t test or Mann-Whitney U test, as appropriate. There were no significant differences between the high- and low-BAT groups ($P > 0.05$). BAT, brown adipose tissue.

Anthropometric parameters

Body weight and body fat mass were estimated by using the multifrequency bioelectric impedance method (HBF-361; Omron Health Care). The BMI (in kg/m^2) and fat-free mass (FFM) were calculated as body weight in kilograms divided by the square of height in meters and as the difference between the body weight and fat mass, respectively. Waist circumference was measured at the level of the umbilicus by using a flexible plastic tape. Systolic and diastolic blood pressure and fasting blood glucose were measured with the subjects in a quietly resting state by using a standard electronic sphygmomanometer (W500; Terumo) and a blood glucose meter (GT-1641; Sanwa Kagaku Kenkyusho), respectively.

EE response to a single oral ingestion of the test beverages (acute trial)

After overnight fasting for 6–12 h, the subjects relaxed on a bed in an air-conditioned room while wearing light clothing for >30 min under a thermoneutral condition at 27°C. Then oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were recorded for ~30 min by using a respiratory gas analyzer connected to a ventilated hood (AR-1; Arco System). Subsequently, the subjects ingested a bottle of either the catechin or placebo beverage in 2 min, and VO_2 and VCO_2 were again recorded for 3 h with 4–5 short breaks to avoid restraint stress. At 0, 15, 30, 60, 90, and 120 min after ingestion, EE was calculated from the stable values of a 10-min period. Overall thermogenic effects of the catechin and placebo beverages were calculated as the incremental AUC (iAUC) of EE.

EE and CIT before and after the daily ingestion of the test beverages (chronic trial)

The subjects ingested a bottle of either the catechin or placebo beverage with breakfast and another bottle with dinner every day for 5 wk. Total daily dose of catechins was 1230 or 0 mg/d, and that of caffeine was ~160 mg/d. Participants were instructed to maintain their daily lifestyle, including dietary intake and physical activity during the 5-wk treatment period. Consumption

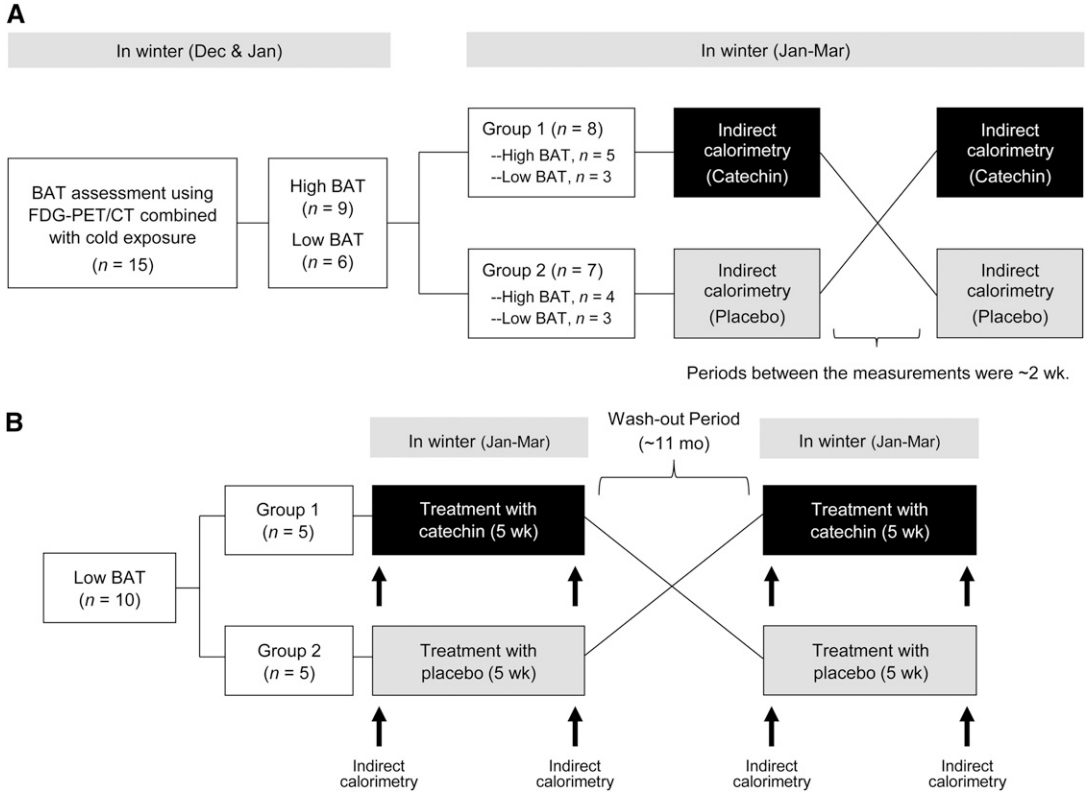

FIGURE 1 Study protocols for the acute and chronic trials. (A) Study protocol of the acute trial. (B) Study protocol of the chronic trial. Both of the trials were single-blinded, randomized, placebo-controlled, season-matched crossover studies. BAT, brown adipose tissue; Dec, December; FDG-PET/CT, fluorodeoxyglucose-positron emission tomography and computed tomography; Jan, January; Mar, March.

of caffeine-rich drinks and foods such as tea, coffee, cocoa, other commercial drinks containing caffeine, and cacao-rich chocolate were limited throughout the treatment period. Alternatively, decaffeinated coffee and caffeine-free barley tea were supplied to them as they needed.

Because frequent, repeated fluorodeoxyglucose-PET/CT for the same subjects may result in an unacceptable radiation exposure, in this intervention trial we assessed the change in BAT activity by measuring BAT-dependent CIT as the difference between the whole-body EE at 27°C and at 19°C. Before and after the 5-wk treatment, respiratory parameters were measured at 27°C and after 2 h of cold exposure at 19°C (Figure 1B). Subjects fasted overnight for 6–12 h and were seated in a comfortable chair in an air-conditioned room at thermoneutral 27°C while wearing light clothing and relaxing for >30 min. Then VO_2 and VCO_2 were recorded for ~20 min as described above. Subsequently, the subjects moved to a cold room kept at 19°C and intermittently placed their feet on an ice block wrapped in cloth while they were in the sitting position. After 100 min under these cold conditions, VO_2 and VCO_2 were recorded for 20 min. The EE and respiratory exchange ratio were calculated from stable values of the last 10-min period. Because protein oxidation remains unaltered by mild cold exposure (33), VO_2 and VCO_2 were adjusted for those by estimated protein oxidation (21.4% of resting EE at 27°C) and used for the calculation of fat and carbohydrate oxidation, as described previously (29). CIT and cold-induced fat and carbohydrate oxidation were calculated from the difference between the values at 27°C and 19°C.

Statistical analyses

Data were expressed as the means ± SEMs and analyzed by using statistical software (SPSS 18.0; IBM Japan). Whole-body EE was adjusted for FFM by means of the linear regression equation (29). Comparisons between the 2 groups were analyzed by using the paired 2-tailed *t* test or Wilcoxon's Signed Rank test and Student's *t* test or the Mann-Whitney *U* test, as appropriate. Changes in EE over 3 h after single ingestion of the catechin or placebo beverage were analyzed by using 2- or 3-factor ANOVA for repeated measures on 2 within-subject factors (time and treatment) and/or one between-subject factor (BAT) with post hoc multiple comparisons by Tukey's post hoc test, as appropriate. Simple correlations were assessed by using the Pearson correlation, the Spearman rank correlation coefficient, or Kendall's τ rank correlation coefficient, as appropriate. Independent associations of age, body compositions, and BAT activity with the iAUC of EE were estimated by using stepwise multiple regressions. CIT and cold-induced fat and carbohydrate oxidations before and after daily ingestion of either the catechin or placebo beverages were analyzed by means of 1-factor repeated-measures ANOVA. A *P* value was considered statistically significant if ≤0.05.

RESULTS

Acute thermogenic effects of a single ingestion of tea catechin and caffeine

To examine the acute thermogenic effects of catechin in our settings, first we measured the response of whole-body EE to a single ingestion of either a beverage containing 615 mg catechin and 77 mg caffeine or a placebo beverage containing 0 mg catechin and 81 mg caffeine in the crossover design with a 2-wk washout period (Figure 1A). Whole-body EE before the ingestion of the test beverages was positively correlated with FFM ($r = 0.691$, $P < 0.001$; **Figure 2**A). Moreover, a close positive correlation between whole-body EE measured before the catechin and placebo ingestions (y = 1.012x, $r = 0.869$, $P < 0.001$; Figure 2B) was observed, indicating a minimal intraindividual variation of resting EE and validating our reproducible measurements.

After ingestion of either the catechin or placebo beverage, EE increased significantly and peaked at 15 min (time effect $P < 0.001$; Figure 2C, **Supplemental Figure 1**). EE after ingestion of the catechin beverage remained high at 30, 60, and 90 min, whereas EE after ingestion of the placebo beverage declined to a nadir at 60 and 90 min (Figure 2C). Two-factor ANOVA revealed a significant treatment effect on EE ($P < 0.001$), although there was no significant interaction between time and treatment ($P = 0.157$). The thermogenic effect of the catechin beverage expressed as iAUC was notably higher than that of

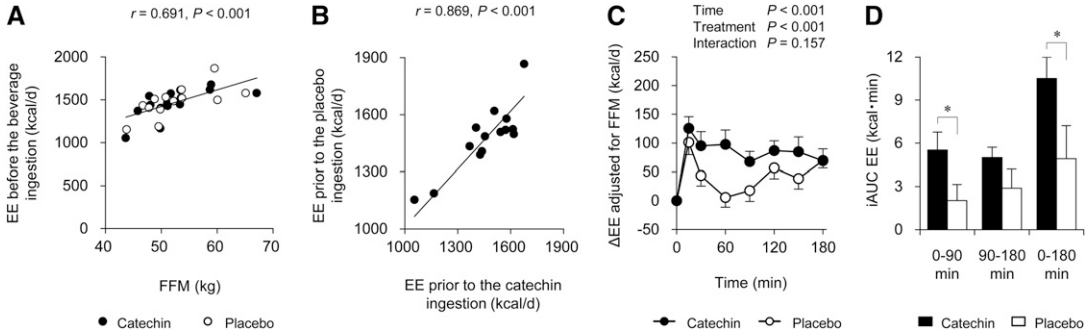

FIGURE 2 Acute thermogenic effects of catechin and caffeine ($n = 15$). (A) Relation of FFM with resting EE measured before ingestion of either the catechin beverage containing 615 mg catechin and 77 mg caffeine or the placebo beverage containing 0 mg catechin and 81 mg caffeine. (B) Correlation between resting EE measured before oral ingestion of the catechin beverage and EE measured before the placebo ingestion. The Pearson correlation coefficient was used to determine the relation between the variables. (C) ΔEE after the ingestion of the test beverages. EE was adjusted for FFM by means of the linear regression equation. Two-factor ANOVA for repeated measures was used to analyze the ΔEE adjusted for FFM before and after the ingestion of either catechin or the placebo. (D) Thermogenic effects of the catechin or placebo beverage expressed as iAUC of EE. A paired 2-tailed *t* test or Wilcoxon's Signed Rank test was used to compare the iAUC between the catechin and placebo beverages. Data are expressed as means ± SEMs. *$P < 0.05$. EE, energy expenditure; FFM, fat-free mass; iAUC, incremental AUC; ΔEE, change in energy expenditure.

the placebo beverage at 0–90 and 0–180 min ($P < 0.05$; Figure 2D) although the difference disappeared at 90–180 min ($P = 0.13$). These results suggest that a single ingestion of catechin elicits a slight but significant increase in EE in healthy, young men.

Involvement of BAT in the acute thermogenic effects of tea catechin and caffeine

To examine a possible role of BAT in the thermogenic effect of catechin, we assessed the BAT activity of 15 subjects by using fluorodeoxyglucose-PET/CT combined with 2 h of cold exposure, which is a standard method for the evaluation of human BAT. The fluorodeoxyglucose-PET/CT examination revealed detectable cold-activated BAT with an SUV ≥2.0 in 9 subjects (high-BAT group, mean SUV = 9.6 ± 1.7) and undetectable BAT with an SUV <2.0 in 6 subjects (low-BAT group, mean SUV = 1.1 ± 0.2) (**Figure 3**A, B). The age and anthropometric variables, including FFM, were not significantly different between the subject groups (Table 1). After the single ingestion of the test beverages, EE in the high-BAT group significantly increased; it was greater after ingestion of the catechin beverage than after ingestion of the placebo beverage (Figure 3C, **Supplemental Figure 2**). In contrast, in the low-BAT group, the EE responses to ingestion of the catechin beverage were not significantly different from responses to ingestion of the placebo beverage (Figure 2C, **Supplemental Figure 3**). Three-factor ANOVA revealed a significant time effect ($P < 0.001$), treatment effect ($P = 0.002$), BAT effect ($P < 0.001$), and interaction effect (time × treatment × BAT, $P = 0.004$). Thus, the iAUC of EE after ingestion of the catechin beverage was notably higher in the high-BAT group than in the low-BAT group (Figure 3D). Moreover, iAUC of EE for the catechin beverage, but not for the placebo beverage, was positively correlated with the BAT activity assessed by the SUV (**Figure 4**A–D). Multivariate regression analysis confirmed a significant interaction effect between BAT and treatment during 0–90 min ($P = 0.008$), 90–180 min ($P = 0.006$), and 0–180 min ($P = 0.003$) independent of age, fat mass, and FFM (**Table 3**). A significant beverage effect on iAUC of EE ($P = 0.017$ for 0–90 min, $P = 0.031$ for 0–180 min; Table 3) was also observed independent of BAT.

Increased cold-induced thermogenic capacity after daily ingestion of tea catechin and caffeine

To test whether tea catechin has the potential to recruit BAT, we next examined the effects of daily ingestion of the catechin beverage in 10 young, male subjects with low BAT activity (**Figure 5**A) in a season-matched crossover design (Figure 1B). The mean BAT activity, the SUV, of the 10 subjects was 1.9 ± 0.3, which was significantly lower than that of the high-BAT subjects ($P < 0.001$) but not significantly different from that of the low-BAT subjects shown in Figure 2B. There was no significant change in body weight, BMI, fat mass, FFM, waist circumference, fasting glucose, or blood pressure before and after the 5-wk treatment with either the catechin or placebo beverage (**Table 4**). Whole-body EE at thermoneutral 27°C depended partly on FFM ($r = 0.65$, $P < 0.001$; Figure 5B), and then EE was adjusted for FFM. Although whole-body EE and that adjusted for FFM at 27°C did not change by the 5-wk treatment, they increased in response to 2 h of cold exposure at

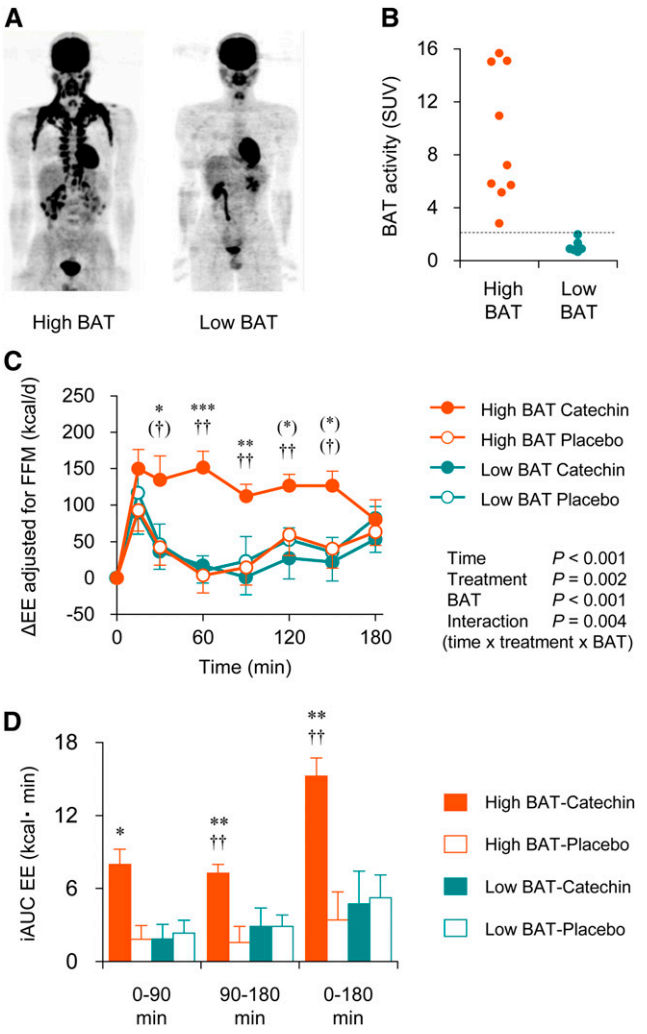

FIGURE 3 Catechin-induced thermogenesis and BAT activity assessed by FDG-PET/CT. (A) Representative FDG-PET/CT images of the high- and low-BAT subjects. (B) Quantitative BAT activity as the SUV of the high- ($n = 9$) and low-BAT ($n = 6$) subjects. (C) ΔEE of the high- and low-BAT subjects. Three-factor ANOVA for repeated measures on 2 within-subject factors (time and treatment) and 1 between-subject factor (BAT) with Tukey's post hoc test was used to analyze the ΔEE adjusted for fat-free mass. (D) iAUC of EE for the high- and low-BAT subjects. Two-factor ANOVA with post hoc multiple comparisons by Tukey's post hoc test was used to analyze the iAUC of the high- and low-BAT groups. Interaction effects between BAT and treatment were $P = 0.014$ for iAUC 0–90 min, $P = 0.026$ for iAUC 90–180 min, and $P = 0.010$ for iAUC 0–180 min. Data are expressed as means ± SEMs. $^{(*)}$,*,**,***Compared with placebo: $^{(*)}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $^{(\dagger)}$,††Compared with low BAT: $^{(\dagger)}P < 0.1$, $^{\dagger\dagger}P < 0.01$. BAT, brown adipose tissue; EE, energy expenditure; FDG-PET/CT, fluorodeoxyglucose-positron emission tomography and computed tomography; FFM, fat-free mass; iAUC, incremental AUC; SUV, standardized uptake value; ΔEE, change in energy expenditure.

19°C (Figure 5C, **Supplemental Figure 4**). Cold exposure for 2 h reduced the respiratory exchange ratio (Figure 5D), reflecting an increased fat oxidation. The calculated CIT was significantly increased after the catechin beverage treatment from 92.0 ± 26.5 to 197.9 ± 27.7 kcal/d ($P = 0.009$; Figure 5E), whereas it did not change after the placebo beverage treatment (70.2 ± 46.5 and 99.2 ± 37.0 kcal/d, $P = 0.507$). Cold-induced fat oxidation was notably higher after the treatment with the catechin beverage (33.7 ± 4.8 mg/min; Figure 5F) than before

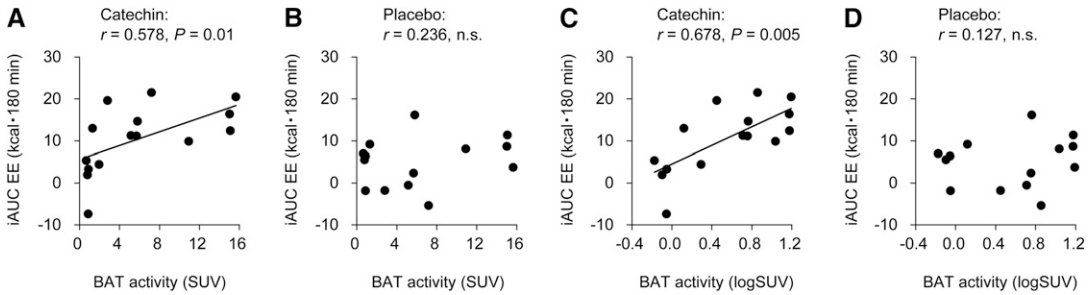

FIGURE 4 Linear regression analysis between catechin-induced thermogenesis and BAT activity (n = 15). Correlation of BAT activity expressed as the SUV (A and B) and log SUV (C and D) with iAUC of EE for catechin (A and C) and placebo ingestions (B and D). The Pearson correlation coefficient (C and D) or Spearman's correlation coefficient (A and B) was used to determine the relations between BAT activity and iAUC of EE, as appropriate. BAT, brown adipose tissue; EE, energy expenditure; iAUC, incremental AUC; n.s., not significant; SUV, standardized uptake value.

the treatment (22.8 ± 3.8 mg/min, P = 0.042) and after the treatment with the placebo beverage (15.2 ± 5.8 mg/min, P = 0.019). In contrast, there was no difference in cold-induced changes in carbohydrate oxidation between before and after the treatments (Figure 5G). No subjects reported any harmful effect of the catechin or placebo beverage treatment.

DISCUSSION

Although BAT may be a novel target to modulate EE, acceptable human interventions aiming at activating BAT have been limited. In the current study, we examined the stimulatory effects of tea catechin with caffeine on BAT thermogenesis in humans. In the acute trial of the present study, we observed a rapid increase in EE after an oral ingestion of a beverage containing catechin and caffeine (the catechin beverage) in subjects with active BAT assessed by fluorodeoxyglucose-PET/CT but not in subjects with undetectable activity. Multivariate regression analysis revealed that BAT is significantly involved in the catechin beverage–induced thermogenesis independent of age and body compositions. These results suggest that the acute thermogenic effect of the catechin beverage is attributable to the activation of BAT in humans. This may explain the inconsistent results in the earlier studies examining the thermogenic effects of similar tea beverages, in which the interindividual and seasonal variations of BAT activity were not taken into account. In addition, the effect of the catechin beverage on EE was still statistically significant after the adjustment for BAT, suggesting that some other mechanisms may also be involved in the observed effects of the catechin beverage. It is to be noted that the placebo ingestion slightly elevated EE in the early phase (15 min). As the placebo beverage used was free of catechin but contained 81 mg caffeine in 350 mL, the observed responses after the placebo ingestion may reflect the thermogenic action of caffeine- (19, 20) and/or water-induced thermogenesis (34). Collectively, it is likely that the thermogenic response to the catechin beverage is largely attributable to catechin itself; in other words, human BAT is activated by oral ingestion of catechin. This does not exclude, however, a possible synergistic action of catechin and caffeine; that is, catechin action is potentiated by caffeine or vice versa, as proposed previously (20, 21).

We and others have shown that BAT is chronically recruited by cold exposure and some TRPV1 agonists in humans (2, 4). To test that this is also true for catechin, we randomly selected low-BAT subjects for the chronic catechin trial and found that BAT-dependent thermogenic capacity, CIT, was significantly increased after the catechin, but not the placebo, treatment. This implies that daily ingestion of the catechin beverage successfully recruits BAT even

TABLE 3
Univariate and multiple regression analysis for iAUC EE[1]

	Univariate regression		Multivariate regression		
	R	P	β	Standardized β	P
iAUC EE,[2] kcal/0–180 min					
Age,[3] y	0.030	0.877	—	—	—
Fat mass,[3] kg	0.009	0.962	—	—	—
FFM,[3] kg	−0.322	0.089	—	—	—
BAT[4,5]	0.268	0.088	—	—	—
Treatment[4,6]	0.349	0.026	3.304	0.396	0.017
BAT × treatment[4]	0.421	0.004	1.204	0.448	0.008
iAUC EE,[7] kcal/0–180 min					
Age,[3] y	−0.038	0.845	—	—	—
Fat mass,[3] kg	−0.180	0.351	—	—	—
FFM,[3] kg	−0.200	0.297	—	—	—
BAT[4,5]	0.346	0.028	—	—	—
Treatment[4,6]	0.226	0.150	—	—	—
BAT × treatment[4]	0.432	0.003	1.209	0.500	0.006
iAUC EE,[8] kcal/0–180 min					
Age,[3] y	−0.072	0.711	—	—	—
Fat mass,[3] kg	−0.067	0.732	—	—	—
FFM,[3] kg	−0.304	0.109	—	—	—
BAT[4,5]	0.332	0.035	—	—	—
Treatment[4,6]	0.322	0.040	5.205	0.350	0.031
BAT × treatment[4]	0.483	<0.001	2.373	0.496	0.003

[1] Independent associations of various parameters with iAUC of EE were assessed by using univariate and multivariate stepwise regression analysis. N = 15 subjects. BAT, brown adipose tissue; EE, energy expenditure; FFM, fat-free mass; iAUC, incremental AUC.

[2] ANOVA R = 0.616, P = 0.002.

[3] Univariate regression analysis was performed by using Pearson's product-moment correlation coefficient.

[4] Univariate regression analysis was performed by using Kendall's τ rank correlation coefficient.

[5] Subjects were divided into 2 groups based on the BAT activity assessed by fluorodeoxyglucose-positron emission tomography and computed tomography and coded as 0 (the low-BAT group) and 1 (the high-BAT group).

[6] The catechin ingestion and the placebo ingestion were coded as 2 and 1, respectively.

[7] ANOVA R = 0.500, P = 0.006.

[8] ANOVA R = 0.625, P = 0.002.

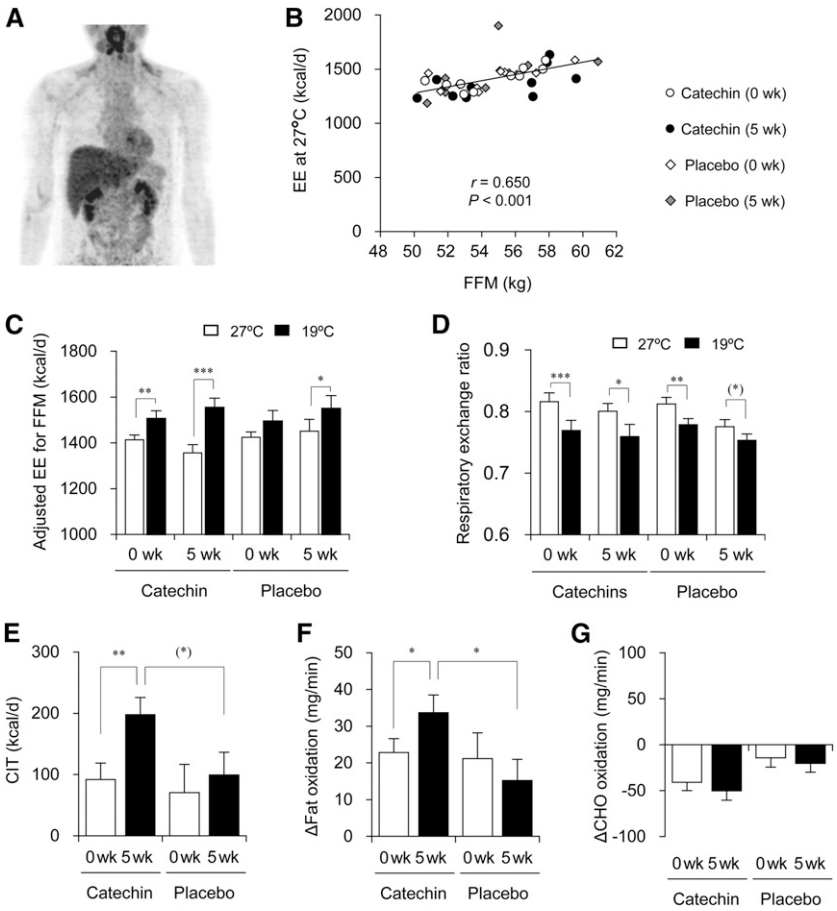

FIGURE 5 Chronic effects of catechin and caffeine on cold-induced thermogenic capacity. (A) Representative FDG-PET/CT image of the subjects for the chronic trial. (B) Relation of FFM with resting EE at 27°C. Resting EE at 27°C before and after daily ingestion of either the catechin or placebo beverage was plotted against FFM, thus $n = 40$. Spearman's correlation coefficient was used to determine the relations between EE and FFM. (C) EE adjusted for FFM by means of the linear regression equation before and after 2 h of cold exposure at 19°C. (D) Respiratory exchange ratio. (E) CIT. (F) Cold-induced fat oxidation. (G) Cold-induced CHO oxidation. Data are expressed as means ± SEMs. N for each data point for panels C–G is 10. $^{(*)}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. One-factor repeated-measures ANOVA revealed significant main effects for CIT (E, $P = 0.03$) and cold-induced fat oxidation (F, $P = 0.05$) but not for cold-induced CHO oxidation (G, $P = 0.20$). A paired 2-tailed t test or Wilcoxon's Signed Rank test was used to analyze the changes in EE, respiratory exchange ratio, CIT, cold-induced fat oxidation, and cold-induced CHO oxidation between the 2 groups, as appropriate. CHO, carbohydrate; CIT, cold-induced thermogenesis; EE, energy expenditure; FDG-PET/CT, fluorodeoxyglucose-positron emission tomography and computed tomography; FFM, fat-free mass.

in the low-BAT individuals. This seems compatible with reports that catechin administration induces browning of white fat in small rodents (24, 35) and that prolonged ingestion of a catechin-rich beverage increases the BAT density assessed by near-infrared time-resolved spectroscopy in humans (36). Moreover, a significant increase in cold-induced fat oxidation was observed after daily

TABLE 4
Profiles of the participants in the chronic trial[1]

	Catechin (n = 10)		Placebo (n = 10)	
	0 wk	5 wk	0 wk	5 wk
Age, y	22.6 ± 0.6	—	22.8 ± 0.8	—
Height, cm	176.0 ± 1.4	—	176.0 ± 1.4	—
Weight, cm	64.2 ± 1.4	64.8 ± 1.5	63.5 ± 1.3	63.7 ± 1.2
BMI, kg/m^2	20.8 ± 0.5	20.9 ± 0.5	20.5 ± 0.5	20.6 ± 0.5
Body fat content, %	14.8 ± 0.9	15.1 ± 0.7	14.2 ± 0.8	14.3 ± 0.7
Body fat mass, kg	9.6 ± 0.8	9.8 ± 0.6	9.1 ± 0.7	9.2 ± 0.5
Fat-free mass, kg	54.6 ± 0.8	55.0 ± 1.0	54.4 ± 0.8	54.6 ± 1.0
Waist circumference, cm	73.7 ± 2.0	74.1 ± 1.9	72.5 ± 1.5	73.4 ± 1.5
Fasting glucose, mg/dL	86.1 ± 2.2	86.7 ± 3.1	85.7 ± 2.9	83.1 ± 1.7
Systolic blood pressure, mm Hg	106 ± 2	107 ± 1	106 ± 2	108 ± 1
Diastolic blood pressure, mm Hg	71 ± 2	71 ± 1	70 ± 2	73 ± 2

[1] Values are means ± SEMs. There were no significant differences between before and after the treatments and between catechin and placebo treatments (2-factor repeated-measures ANOVA).

ingestion of the catechin beverage. These results, together with a report demonstrating a large amount of fatty acid uptake into cold-activated BAT (37), imply that the BAT recruited by catechin preferentially utilizes fatty acids as an energy substrate for heat production.

Because catechins are known to inhibit the catecholamine-degrading enzyme catechol-*O*-methyltransferase (COMT) in vitro, this enzyme has been argued as a primary target of the thermogenic effect of catechin. However, the circulating levels of catechins after a single ingestion of catechin-tea were reported to be 50–100 nM (27), which are much lower than the half-maximal inhibitory concentration of catechins for the COMT activity (12–14 μmol EGCG/L) in rat hepatocytes (38). Consistently, Lorenz et al. (39) showed that COMT activity is not impaired by high doses of EGCG in humans. It is thus obvious that further studies are needed to specify COMT as a significant target for the thermogenic effect of catechin. Meanwhile, EGCG and its auto-oxidation products were reported to activate TRPA1 and TRPV1, with a concentration of 100–200 mmol/L (22, 23). Because the EGCG concentration in the test beverage we used was $\sim$700 mmol/L, it is possible that the ingested catechin activates and recruits BAT via the direct action on TRPA1/TRPV1 located in the sensory neuron of the gastrointestinal tract similarly to capsinoids (12). Further studies in vitro and in vivo are needed to clarify the role of TRP in the catechin-induced activation of BAT.

A limitation of this work is that the BAT activity was assessed by fluorodeoxyglucose-PET/CT after acute cold exposure. Although fluorodeoxyglucose uptake is accepted as an index of BAT thermogenesis under cold conditions, it may not always be proportional to total fuel utilization and absolute caloric consumption in this thermogenic tissue. It would be more appropriate in future studies to evaluate the absolute thermogenic activity of BAT by measuring the rates of oxygen consumption and blood flow in the tissue itself (40) in addition to PET by using fluorodeoxyglucose and fatty acid derivatives including ^{11}C-acetate (37). Moreover, in the present study, the chronic effect of the catechin beverage on BAT was assessed by measuring CIT but not by fluorodeoxyglucose-PET/CT. However, frequent PET/CT scanning in the crossover study design, in which measurements are necessary 4 times, is difficult because of unacceptable radiation exposure. Although CIT could be the sum of BAT thermogenesis, skeletal muscle shivering, and nonshivering thermogenesis in some organs including skeletal muscle (41), neither apparent signs of shivering nor response of fluorodeoxyglucose uptake into the skeletal muscle is detected under our experimental condition of mild cold exposure (29, 30). Earlier studies confirmed that, during mild cold exposure causing no muscle shivering, CIT is fairly proportional to cold-activated BAT activity (5–7, 42, 43). Consistently, our previous finding demonstrated that intraindividual and interindividual variations of CIT are significantly related to BAT activity (6, 29); thus CIT is a predictive index of BAT activity (6, 30).

Another limitation of this study is that, despite the increased thermogenesis, there was no significant change in adiposity variables, such as body fat content (+1.5%, $P = 0.66$ for catechin; +0.9%, $P = 0.80$ for placebo) and waist circumstance (+0.5%, $P = 0.62$ for catechin; +1.3%, $P = 0.32$ for placebo) after the 5-wk treatment period. This seems conflicting with previous reports showing a slight but significant reduction of body fatness after a catechin treatment of 8–12 wk in obese subjects (18). This may be because of the difference in the adiposity of participants and the treatment period. Further studies on chronic effects of catechin on BAT and their effect on adiposity in obese human subjects are required.

In conclusion, our results indicate that oral ingestion of tea catechin with caffeine acutely increases EE by triggering BAT thermogenesis and chronically elevates nonshivering thermogenic capacity during mild cold exposure, suggesting the recruitment of BAT, in healthy adults. These effects on BAT are quite similar to those of cold exposure and thermogenic capsinoids (7). Unlike cold exposure or pharmacologic agents, the tea catechin regimen would be applicable to the sustained interventions aimed at activating BAT.

TY, MH, HT, and MS: designed the research; TY and MM: enrolled the subjects and conducted the research; TK and HS: conducted PET/CT examination; TY: analyzed the data and wrote the manuscript; MH, HT, MT, KY, and YK: contributed the materials; TY and MS: had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

REFERENCES

1. Kajimura S, Saito M. A new era in brown adipose tissue biology: molecular control of brown fat development and energy homeostasis. Annu Rev Physiol 2014;76:225–49.
2. Saito M, Okamatsu–Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio–Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 2009;58:1526–31.
3. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S, et al. Functional brown adipose tissue in healthy adults. N Engl J Med 2009;360:1518–25.
4. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold–activated brown adipose tissue in healthy men. N Engl J Med 2009;360:1500–8.
5. Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y, Saito M. Brown adipose tissue, whole–body energy expenditure, and thermogenesis in healthy adult men. Obesity (Silver Spring) 2011;19:13–6.
6. Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, Iwanaga T, Saito M. Recruited brown adipose tissue as an antiobesity agent in humans. J Clin Invest 2013;123:3404–8.
7. van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ, Hansen J, Jörgensen JA, Wu J, Mottaghy FM, et al. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J Clin Invest 2013;123:3395–403.
8. Lee P, Smith S, Linderman J, Courville AB, Brychta RJ, Dieckmann W, Werner CD, Chen KY, Celi FS. Temperature–acclimated brown adipose tissue modulates insulin sensitivity in humans. Diabetes 2014;63:3686–98.
9. Cypess AM, Weiner LS, Roberts-Toler C, Franquet Elía E, Kessler SH, Kahn PA, English J, Chatman K, Trauger SA, Doria A, et al. Activation of human brown adipose tissue by a β3–adrenergic receptor agonist. Cell Metab 2015;21:33–8.
10. Broeders EP, Nascimento EB, Havekes B, Brans B, Roumans KH, Tailleux A, Schaart G, Kouach M, Charton J, Deprez B, et al. The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. Cell Metab 2015;22:418–26.
11. Islam KB, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, Ogura Y, Hayashi T, Yokota A. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology 2011;141:1773–81.
12. Yoneshiro T, Saito M. Transient receptor potential activated brown fat thermogenesis as a target of food ingredients for obesity management. Curr Opin Clin Nutr Metab Care 2013;16:625–31.

13. Yoneshiro T, Aita S, Kawai Y, Iwanaga T, Saito M. Nonpungent capsaicin analogs (capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. Am J Clin Nutr 2012;95: 845–50.
14. Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, Chantre P, Vandermander J. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24–h energy expenditure and fat oxidation in humans. Am J Clin Nutr 1999;70:1040–5.
15. Gosselin C, Haman F. Effects of green tea extracts on non–shivering thermogenesis during mild cold exposure in young men. Br J Nutr 2013;110:282–8.
16. Hursel R, Westerterp-Plantenga MS. Catechin- and caffeine-rich teas for control of body weight in humans. Am J Clin Nutr 2013;98:1682S–93S.
17. Nagao T, Komine Y, Soga S, Meguro S, Hase T, Tanaka Y, Tokimitsu I. Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde–modified LDL in men. Am J Clin Nutr 2005;81:122–9.
18. Farrar MD, Nicolaou A, Clarke KA, Mason S, Massey KA, Dew TP, Watson RE, Williamson G, Rhodes LE. A randomized controlled trial of green tea catechins in protection against ultraviolet radiation–induced cutaneous inflammation. Am J Clin Nutr 2015;102: 608–15.
19. Dulloo AG, Seydoux J, Girardier L, Chantre P, Vandermander J. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. Int J Obes Relat Metab Disord 2000;24:252–8.
20. Westerterp-Plantenga MS. Green tea catechins, caffeine and body–weight regulation. Physiol Behav 2010;100:42–6.
21. Ferreira MA, Silva DM, de Morais AC Jr., Mota JF, Botelho PB. Therapeutic potential of green tea on risk factors for type 2 diabetes in obese adults - a review. Obes Rev 2016;17:1316–28.
22. Kurogi M, Miyashita M, Emoto Y, Kubo Y, Saitoh O. Green tea polyphenol epigallocatechin gallate activates TRPA1 in an intestinal enteroendocrine cell line, STC–1. Chem Senses 2012;37: 167–77.
23. Kurogi M, Kawai Y, Nagatomo K, Tateyama M, Kubo Y, Saitoh O. Auto–oxidation products of epigallocatechin gallate activate TRPA1 and TRPV1 in sensory neurons. Chem Senses 2015;40:27–46.
24. Sae-Tan S, Rogers CJ, Lambert JD. Decaffeinated green tea and voluntary exercise induce gene changes related to beige adipocyte formation in high fat-fed obese mice. J Funct Foods 2015;14:210–4.
25. Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 2012;150:366–76.
26. Sharp LZ, Shinoda K, Ohno H, Scheel DW, Tomoda E, Ruiz L, Hu H, Wang L, Pavlova Z, Gilsanz V, et al. Human BAT possesses molecular signatures that resemble beige/brite cells. PLoS One 2012;7: e49452.
27. Takahashi M, Miyashita M, Suzuki K, Bae SR, Kim HK, Wakisaka T, Matsui Y, Takeshita M, Yasunaga K. Acute ingestion of catechin–rich green tea improves postprandial glucose status and increases serum thioredoxin concentrations in postmenopausal women. Br J Nutr 2014; 112:1542–50.
28. Matsushita M, Yoneshiro T, Aita S, Kameya T, Sugie H, Saito M. Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. Int J Obes (Lond) 2014;38:812–7.
29. Yoneshiro T, Matsushita M, Nakae S, Kameya T, Sugie H, Tanaka S, Saito M. Brown adipose tissue is involved in the seasonal variation of cold–induced thermogenesis in humans. Am J Physiol Regul Integr Comp Physiol 2016;310:R999–1009.
30. Yoneshiro T, Saito M. Activation and recruitment of brown adipose tissue as anti–obesity regimens in humans. Ann Med 2015;47:133–41.
31. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, et al. Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009;360:1509–17.
32. Yoneshiro T, Aita S, Matsushita M, Okamatsu–Ogura Y, Kameya T, Kawai Y, Miyagawa M, Tsujisaki M, Saito M. Age–related decrease in cold–activated brown adipose tissue and accumulation of body fat in healthy humans. Obesity (Silver Spring) 2011;19:1755–60.
33. Haman F, Péronnet F, Kenny GP, Massicotte D, Lavoie C, Weber JM. Partitioning oxidative fuels during cold exposure in humans: muscle glycogen becomes dominant as shivering intensifies. J Physiol 2005; 566:247–56.
34. Boschmann M, Steiniger J, Franke G, Birkenfeld AL, Luft FC, Jordan J. Water drinking induces thermogenesis through osmosensitive mechanisms. J Clin Endocrinol Metab 2007;92:3334–7.
35. Nomura S, Ichinose T, Jinde M, Kawashima Y, Tachiyashiki K, Imaizumi K. Tea catechins enhance the mRNA expression of uncoupling protein 1 in rat brown adipose tissue. J Nutr Biochem 2008; 19:840–7.
36. Nirengi S, Amagasa S, Homma T, Yoneshiro T, Matsumiya S, Kurosawa Y, Sakane N, Ebi K, Saito M, Hamaoka T. Daily ingestion of catechin-rich beverage increases brown adipose tissue density and decreases extramyocellular lipids in healthy young women. Springerplus 2016;5:1363.
37. Ouellet V, Labbé SM, Blondin DP, Phoenix S, Guérin B, Haman F, Turcotte EE, Richard D, Carpentier AC. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J Clin Invest 2012;122:545–52.
38. Kadowaki M, Ootani E, Sugihara N, Furuno K. Inhibitory effects of catechin gallates on O-methyltranslation of protocatechuic acid in rat liver cytosolic preparations and cultured hepatocytes. Biol Pharm Bull 2005;28:1509–13.
39. Lorenz M, Paul F, Moobed M, Baumann G, Zimmermann BF, Stangl K, Stangl V. The activity of catechol–O–methyltransferase (COMT) is not impaired by high doses of epigallocatechin–3–gallate (EGCG) in vivo. Eur J Pharmacol 2014;740:645–51.
40. Muzik O, Mangner TJ, Leonard WR, Kumar A, Janisse J, Granneman JG. 15O PET measurement of blood flow and oxygen consumption in cold-activated human brown fat. J Nucl Med 2013;54: 523–31.
41. Blondin DP, Labbé SM, Phoenix S, Guérin B, Turcotte ÉE, Richard D, Carpentier AC, Haman F. Contributions of white and brown adipose tissues and skeletal muscles to acute cold-induced metabolic responses in healthy men. J Physiol 2015;593:701–14.
42. Orava J, Nuutila P, Lidell ME, Oikonen V, Noponen T, Viljanen T, Scheinin M, Taittonen M, Niemi T, Enerbäck S, et al. Different metabolic responses of human brown adipose tissue to activation by cold and insulin. Cell Metab 2011;14:272–9.
43. Chen KY, Brychta RJ, Linderman JD, Smith S, Courville A, Dieckmann W, Herscovitch P, Millo CM, Remaley A, Lee P, et al. Brown fat activation mediates cold–induced thermogenesis in adult humans in response to a mild decrease in ambient temperature. J Clin Endocrinol Metab 2013;98:E1218–23.