Supplemental Methods

FDG-PET/CT

All subjects underwent FDG-PET/CT after acute cold exposure because BAT activity was substantially underestimated by FDG-PET/CT without cold. After fasting for 6-12 h, they were kept in an air-conditioned room at 19˚C with light clothing and intermittently placed their feet on an ice block wrapped in cloth for 2 h. FDG-PET/CT scans were performed by using a PET/CT system (Aquiduo, Toshiba Medical Systems, Otawara, Japan). BAT activity in the supraclavicular region was quantified by calculating a standardized uptake value of FDG (SUV), defined as the radioactivity per milliliter within the region of interest divided by the injected dose in megabecquerels per gram of body weight. The presence (BAT-positive) or absence (BAT-negative) of radioactivity greater than that of the background (SUV < 2) in the supraclavicular and paravertebral adipose tissue was assessed by two experienced, blinded observers. All examinations were performed during the winters (January-March) of 2007-2013.

Indirect calorimetry

After fasting for 6-12 h, whole-body EE was estimated by using a respiratory gas analyzer (O-Jiro, Alko System, Tokyo, Japan) at 27˚C and after 2-h cold exposure at

19˚C with light clothing and intermittent cooling of feet on an ice block wrapped in cloth while in a sitting position. Cold-induced thermogenesis (CIT) was calculated from the difference between the values before (27˚C) and after 2-h cold exposure (19˚C).

Anthropometric and body fat measurement

Body weight and the body fat mass were estimated by employing the multifrequency bioelectric impedance method (InBody 320 Body Composition Analyzer; Biospace, Seoul, Korea). The fat-free mass was calculated as the difference between the body weight and body fat mass.

Repeated cold exposure

Within 4 wk after the FDG-PET/CT, 22 subjects showing undetectable or low BAT activities were selected and randomly divided into two groups: one was exposed to cold at 17˚C with light clothing for 2 h every day for 6 wk (cold group, N = 12) while the other maintained their usual lifestyles without cold exposure (control group, N = 10) during the same period from January to March (winter). BAT activity of the cold and control groups at Wk 0 was almost the same (SUV = 2.5 ± 0.4 and 2.7 ± 0.5). As described above, body fat content and EE at 27˚C and after 2-h cold exposure at 19˚C were measured before and after the 6-wk period by employing the multifrequency bioelectric impedance method and indirect calorimetry, respectively. Eight out of 12 subjects who agreed repeated FDG-PET/CT underwent the examination again after a 6-wk cold exposure, the scan being restricted only from neck to chest regions to minimize radiation exposure.

Daily ingestion of capsinoids

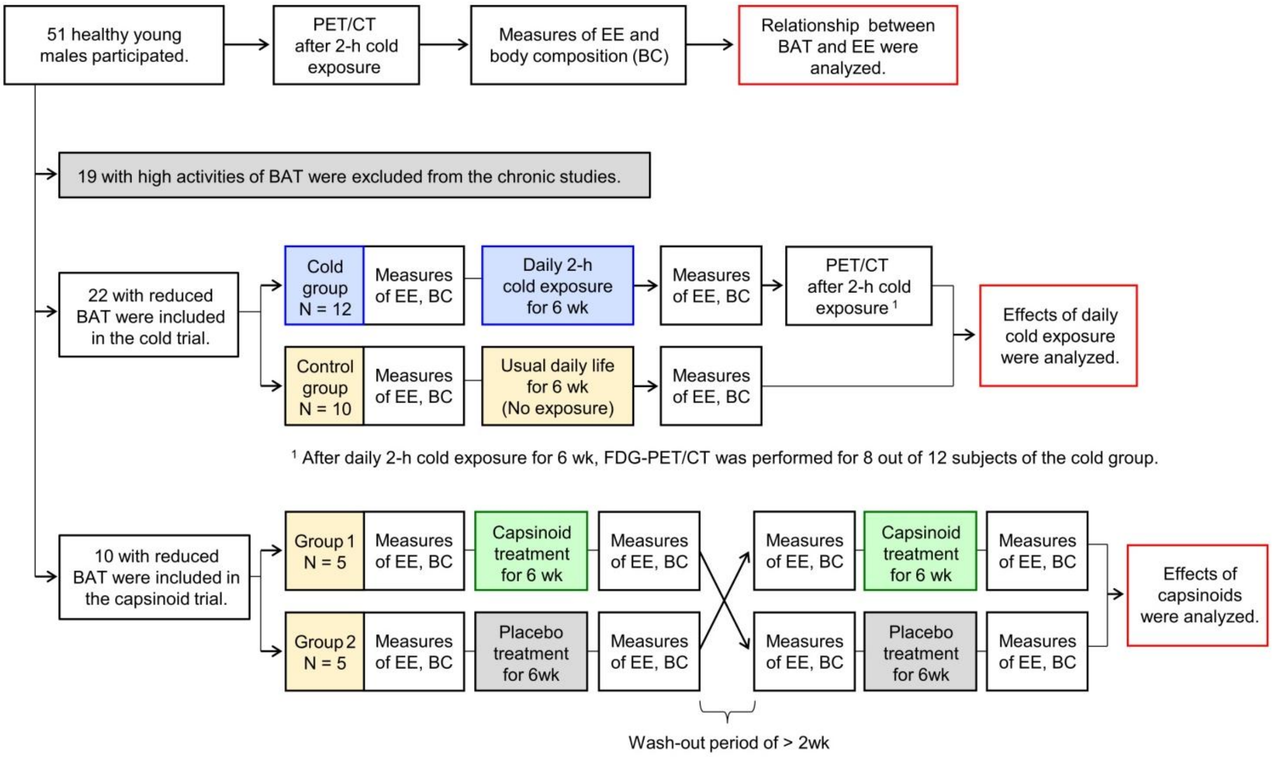
Ten subjects with undetectable or low activities of BAT (mean BAT activity: 2.3 ± 0.4) participated in this single-blinded, randomized, placebo-controlled, crossover trial with washout period of more than 2 wk (Supplemental Figure 1). Capsinoids were extracted from CH-19 Sweet (Capsicum anuum L.), purified, and encapsulated. Each capsule contained no (placebo) or 1.5 mg capsinoids (capsiate: dihidrocapsiate: nordihydrocapsiate = 7:2:1), and 199 mg of a mixture of rapeseed oil and medium-chain triglycerides. Three capsules containing 4.5 mg or 0 mg (placebo) of capsinoids were ingested orally at 7-9 a.m. and again at 8-11 p.m. every day for 6 wk. The total dose of capsinoids was 9 or 0 mg/day. Before and after the 6-wk period, body fat content and EE at 27˚C and after 2-h cold exposure at 19˚C were measured.

Statistical analyses

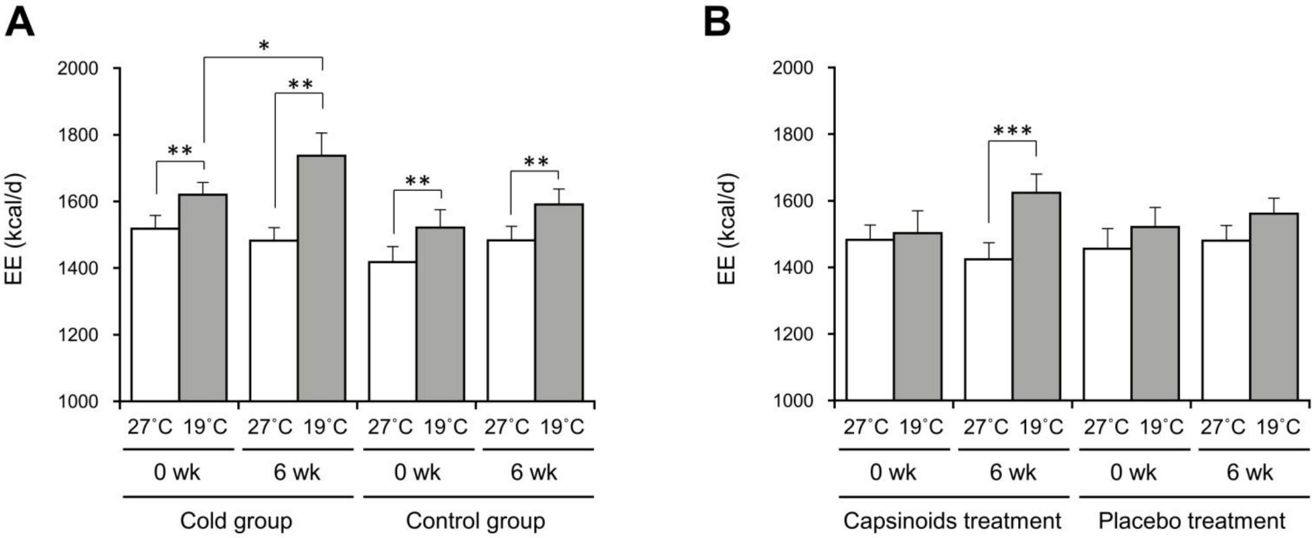
Data was expressed as the means ± SE and analyzed by using a statistical software (SPSS 18.0, IBM Japan, Tokyo, Japan). Comparisons between groups were analyzed by using the paired t-test or Wilcoxon signed rank test and the Student's t-test or Mann-Whitney test (two-side *P* values were given), as appropriate. Correlations were assessed by using the Pearson or Spearman correlation coefficient (one- or two-side *P* values were given), as appropriate. Independent associations of age, fat mass, fat-free mass, and BAT activity with EE were estimated by using stepwise multiple regressions. A *P* value was considered statistically significant if < 0.05.

Supplemental Table 1. Simple and multiple regression analysis for whole-body EE.

	Univariate regression		Multivariate regression		
	R	*P*	β	Standardized β	*P*
Energy expenditure at 27˚C (kcal/d)					
Age (yr old)	−0.22	0.12	-	-	-
Fat-free mass (kg)	0.57	< 0.0001	16.62	0.57	0.004
Fat mass (kg)	0.17	0.24	-	-	-
BAT activity (SUV)	−0.27	0.06	-	-	-
Energy expenditure after 2-h cold exposure at 19˚C (kcal/d)					
Age (yr old)	−0.30	0.03	-	-	-
Fat-free mass (kg)	0.33	0.02	19.67	0.44	< 0.001
Fat mass (kg)	0.17	0.24	-	-	-
BAT activity (SUV)	0.30	0.04	33.61	0.49	< 0.001
Cold-induced thermogenesis (kcal/d)					
Age (yr old)	−0.11	0.43	-	-	-
Fat-free mass (kg)	−0.06	0.69	-	-	-
Fat mass (kg)	0.02	0.89	-	-	-
BAT activity (SUV)	0.52	< 0.0001	36.73	0.60	< 0.0001



Supplemental Figure 1. Study protocols.



Supplemental Figure 2. Whole-body EE before and after chronic stimulation by cold and capsinoids. (A) Effects of repeated cold exposure for 6 wk. (B) Effects of daily ingestion of capsinoids for 6 wk. Calculated CIT values are shown in Figures 3D and 3E. Data represent mean ± SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.