Supplemental material

Shivering threshold test

A detailed description of the STT protocol is available elsewhere (1). Upon arrival, subjects entered in a room (22.1 ± 1.6 °C) where they remained seated for 30 min. They were then conducted to a mild-cold room (19.8 ± 0.5°C) where they put on a cooling-vest (Polar Products Inc, Ohio, USA) covering the clavicular, chest, abdominal and back areas; this was connected to a temperature-controlled water circuit. The water temperature was reduced progressively every 10 min from the initial temperature (16.6°C) until shivering started or a temperature of 3.8°C was reached. The participants who did not shiver continued in the cold room for another 45 min when the test was terminated. Throughout the test, participants remained seated. Shivering was determined visually and by self-reporting. The temperature at which participants started to shiver was established as the individual shivering threshold.

Cold-induced thermogenesis

Cold-induced thermogenesis was assessed in a subgroup of 33 subjects. Before starting the assessment, the subjects laid on a bed for 20 min in a room at 23.2 ± 0.7ºC. Their resting metabolic rate (RMR) was then assessed over 30 min by indirect calorimetry, using a CCM Express or Ultima CardiO2 cart (Medgraphics Corp, Minnesota, USA) (2). The average value for the most stable 5 min period was taken as the RMR (3). Immediately after RMR assessment, subjects were accompanied to the cold room (19.7 ± 0.4ºC) where they put on the water perfused cooling vest once again, this time set at a temperature 4ºC above the shivering threshold, and laid on a bed for 65 min. CIT was assessed by indirect calorimetry over two periods of 30 min separated by 5 min, during which time the gas analysers were recalibrated. To obtain a single representative value for CIT, the recorded 60 min were divided into four 15 min periods, and the most stable 5 min period in every 15 min period selected for averaging. Finally, these four 5 min periods, together with the RMR, were used

to calculate the area under the curve (trapezoidal rule), expressing it as a percentage of RMR (3).

Table S1. Characteristics of participants included in the analyses of cold-induced thermogenesis.

	All n=33	Men n=12	Women n=21
Age (years)	21. (2.1)	22.2 (2.3)	21.7 (2.0)
Body composition parameters			
BMI (kg/m^2)	25.2 (4.5)	26.4 (4.8)	24.6 (4.3)
LMI (kg/m^2)	14.5 (2.2)	16.5 (1.7)	13.4 (1.7)
FMI (kg/m^2)	9.4 (3.2)	8.5 (3.5)	9.9 (3.0)
Fat mass (%)	37.5 (7.9)	31.9 (8.0)	40.4 (6.2)
BAT ^{18}F-FDG uptake parameters			
BAT volume (ml)	77.6 (55.2)	83.7 (57.4)	74.5 (54.8)
BAT mean radiodensity	-57.4 (11.2)	-52.6 (9.9)	-60.0 (11.2)
BAT SUVmean	4.2 (1.7)	3.7 (1.4)	4.4 (1.9)
BAT SUVpeak	13.2 (8.6)	11.8 (7.6)	13.9 (9.1)
Thyroid hormones			
TSH (µUI/ml)	1.8 (0.8)	1.8 (0.8)	1.8 (0.9)
FT4 (ng/dl)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
FT3 (pg/ml)	3.4 (0.3)	3.5 (0.4)	3.3 (0.3)
PTFQI	0.2 (0.3)	0.2 (0.3)	0.2 (0.2)

Data are presented as mean and standard deviation. Abbreviations: BMI, Body mass index; LMI, Lean mass index, FMI, Fat mass index; ^{18}F-FDG, ^{18}F-Fluorodeoxyglucose; BAT, Brown adipose tissue; SUV, Standardized uptake value; TSH, Thyroid-Stimulating Hormone; FT4, free thyroxine; FT3, free triiodothyronine; PTFQI, Parametric Thyroid Feedback Quantile based Index.

Figure S1.

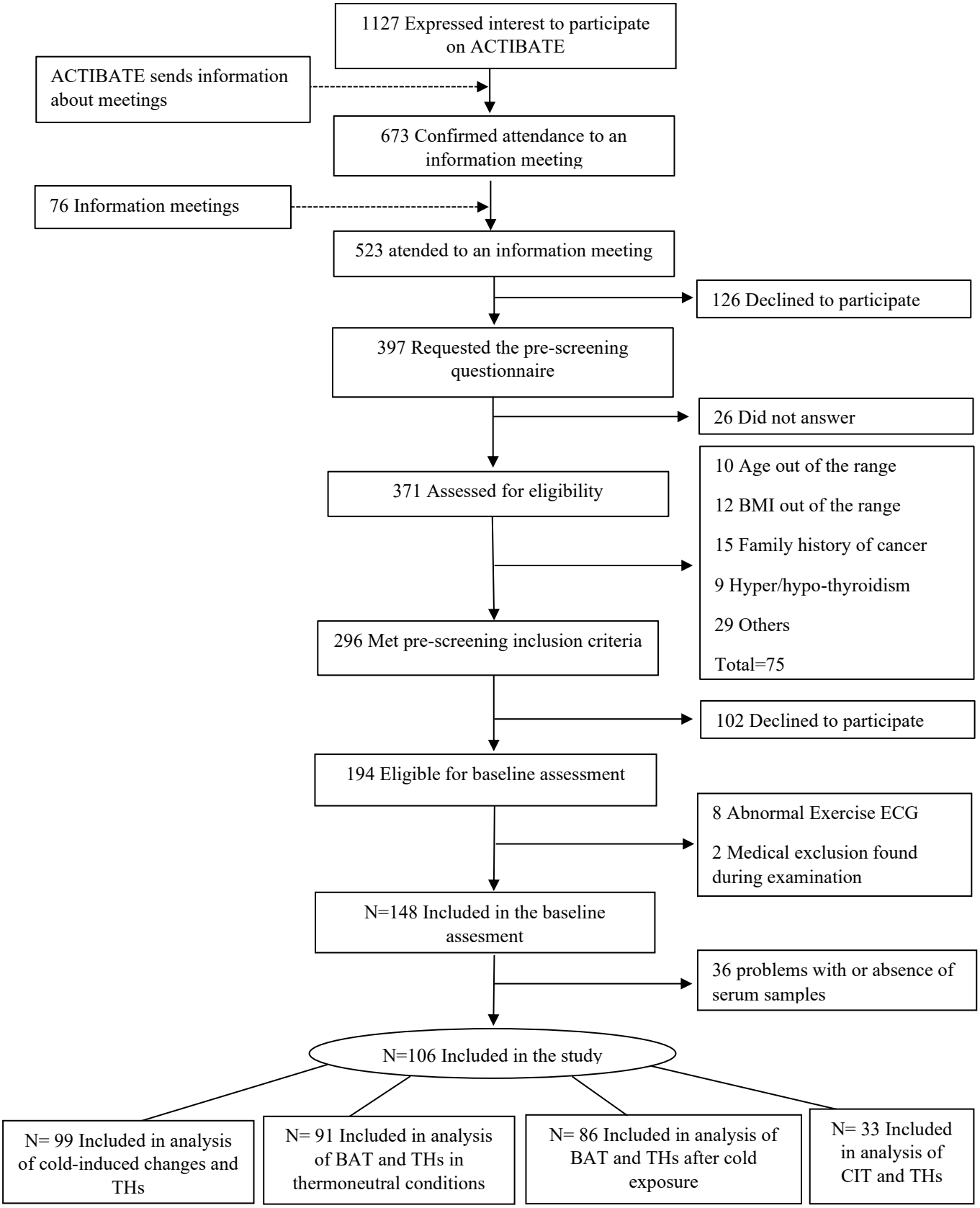

Figure S1. Study flow-chart. Abbreviations: BMI, Body mass index; BAT, Brown adipose tissue; THs, Thyroid hormones; CIT, cold induced thermogenesis.

Figure S2

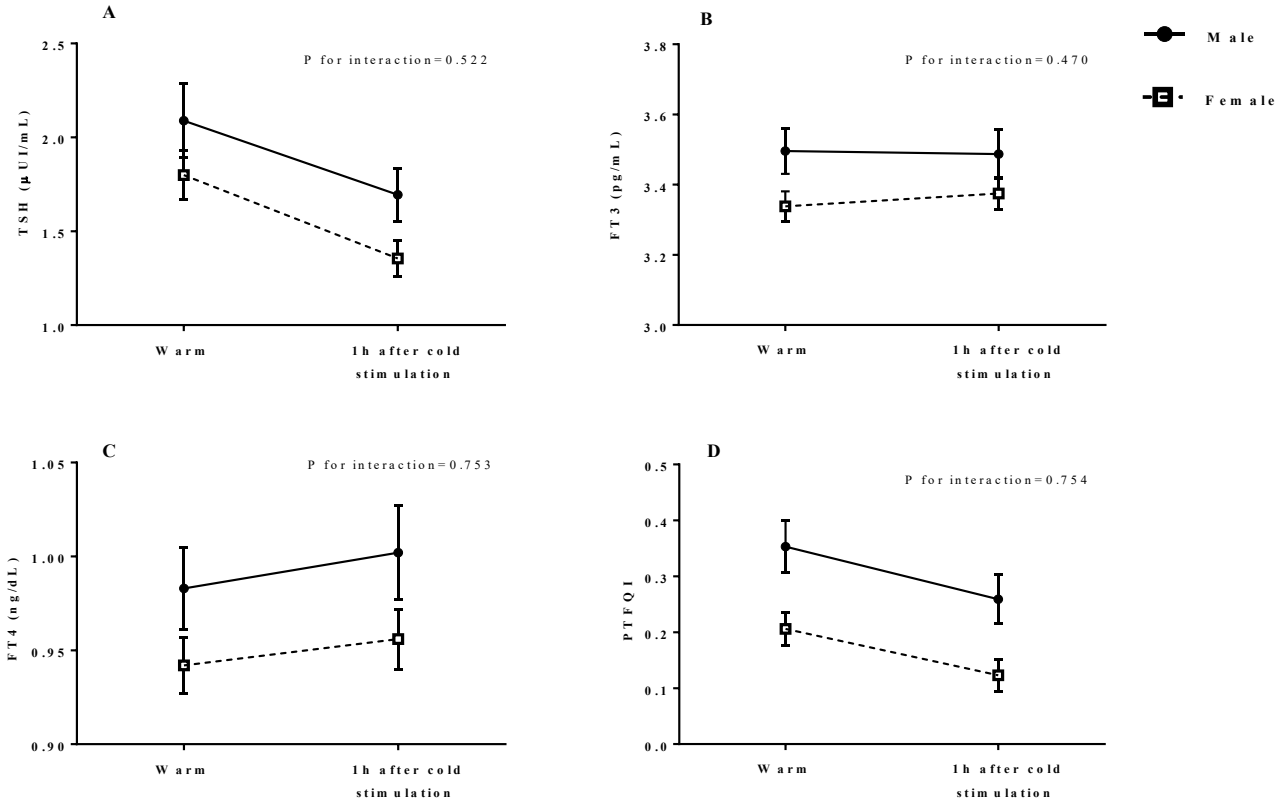

Figure S2. Cold-induced changes on Thyroid-Stimulating Hormone (TSH) (A), free Thyroxine (FT4) (B), free triiodothyronine (FT3) (C) circulating levels and Parametric Thyroid Feedback Quantile based Index (PTFQI) (D) by sex after one hour of cold stimulation in young euthyroid adults (n=102, 71 female). Analyses of variance (ANOVA) were performed.

REFERENCES

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3. Sanchez-Delgado G, Alcantara JMA, Acosta FM, Martinez-Tellez B, Amaro-Gahete FJ, Ortiz-Alvarez L, Löf M, Labayen I, & Ruiz JR. Estimation of non-shivering thermogenesis and cold-induced nutrient oxidation rates: Impact of method for data selection and analysis. *Clinical nutrition (Edinburgh, Scotland)* 2019 **38** 2168–2174. (doi:10.1016/j.clnu.2018.09.009)