Effects of intravenous thyrotropin-releasing hormone on ^{18}F -fluorodeoxyglucose uptake in human brown adipose tissue: a randomized controlled trial

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Abstract

Objective: Brown adipose tissue (BAT) activity in humans is stimulated by cold and by a limited number of pharmacological agents, including β 3-adrenergic agonists and bile acids. Although thyrotropin-releasing hormone (TRH) is known to activate BAT in several mammals, this has not been reported in humans.

Design: A randomized, placebo-controlled, double-blind, cross-over trial.

Methods: We investigated the effects of intravenous bolus administration of 400 μg TRH or 2 mL saline on BAT activity in healthy, lean men. BAT activity was measured as standardized ^{18}F -fluorodeoxyglucose (^{18}F -FDG) uptake and glucose metabolic rate (MRglu) using dynamic PET/CT imaging. The first six individuals were studied at room temperature, while subsequently nine were exposed to mild cold ($17^\circ\text{C} \pm 1^\circ\text{C}$) for 60 min before imaging. During the dynamic scan, blood was withdrawn for measurement of thyroid hormone and catecholamine concentrations. This trial is registered with The Netherlands National Trial Register (number NTR5512).

Results: Sixteen participants were recruited. Six men studied at room temperature showed no visible BAT activity during either session. After exposure to mild cold, four of nine men (44.4%) showed clear increase of ^{18}F -FDG uptake after TRH administration compared to placebo. Maximal standardized ^{18}F -FDG uptake showed a trend toward increase after TRH compared to placebo ($P=0.066$). MRglu showed a significant increase after TRH administration ($P=0.014$). The increase in ^{18}F -FDG uptake was not paralleled by changes in plasma thyroid hormone or catecholamine concentrations.

Conclusion: Systemic TRH administration can increase the activity of cold-stimulated BAT in adult men. These findings may assist developing pharmacological strategies for modulating BAT activity in the management of obesity.

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Introduction

Brown adipose tissue (BAT), a classic site of adaptive non-shivering thermogenesis in mammals, was only recently found to be present in adult humans (1, 2, 3).

Thermogenesis in BAT increases during cold exposure and during food ingestion in order to preserve thermal and caloric homeostasis respectively (4). Fatty acids derived

by lipolysis are a key substrate for BAT, providing energy to be converted to heat by uncoupling protein 1 (UCP1)-mediated mitochondrial uncoupling (5). It was estimated that continuous activation of both supraclavicular BAT depots may burn an amount of energy equivalent to several kilograms of adipose tissue in 1 year (2). In view of their promising metabolic potential, pharmacological BAT activators are a topic of high interest. In addition to cold exposure, to date, only β 3-adrenergic receptor agonists and bile acids have been shown to be able to increase BAT activity in humans (6, 7).

While BAT is primarily stimulated by the sympathetic nervous system (SNS) via release of norepinephrine (NE), for optimal and sustained activation, BAT requires a complex, intracellular interaction between NE and the biologically active thyroid hormone triiodothyronine (T3) (8). Sympathetic BAT innervation originates in the hypothalamus, and recent studies showed that intrahypothalamic T3 activates sympathetic outflow to BAT in rats, resulting in increased thermogenesis (9). Cold exposure activates cold-sensitive neurons in hypothalamic nuclei known to express thyrotropin-releasing hormone (TRH), which in turn activate neurons in the spinal cord that innervate BAT (10). Within the hypothalamic paraventricular nucleus (PVN), hypophysiotropic TRH neurons are involved in the regulation of the hypothalamus–pituitary–thyroid axis. Both hypophysiotropic and non-hypophysiotropic TRH neurons are activated by cold (10, 11). Thus, hypothalamic TRH may have a dual role in temperature regulation, via activation of the sympathetic outflow to BAT and via stimulation of plasma thyroid hormone concentrations which in turn modulate BAT activity. Indeed, TRH-knockout mice show cold intolerance (12). Interestingly, both central and peripheral administration of TRH was found to increase BAT thermogenesis in several mammals (13, 14). Whether peripheral administration of TRH has similar effects in humans is unknown. We aimed to examine the effects of peripheral TRH administration on the activity of BAT in male volunteers.

Subjects and methods

Study design and participants

Subjects were studied according to a randomized, placebo-controlled, double-blind, cross-over design. Subjects were recruited through public advertisements. Inclusion criteria were age 30–50 years and BMI of 19–25 kg/m². Exclusion criteria were any thyroid, cardiovascular or renal disease,

use of medication affecting thyroid hormone metabolism or the autonomic nervous system, a recent stay in tropical countries and a desire to father a child within 1 month. Each subject underwent two dynamic ¹⁸F-FDG PET/CT scans, performed directly after intravenous administration of either 400 µg TRH (2 mL 200 µg/mL protiterin in 0.9% NaCl; Ferring, Kiel, Germany) (15) or placebo (2 mL 0.9% NaCl) (Fig. 1B). The interval between the scans was set between 1 and 3 weeks. All subjects were studied after a fast of at least 6 h, after which fasting plasma glucose was assessed. The subjects were weighed on a mechanical scale to the nearest 100 g, and their height was measured to the nearest 0.01 m. During the experiment, all subjects wore standardized light clothing (standard surgical shirts, trousers and socks provided by the Academic Medical Center). The study has been approved by the Ethics Committee of the Academic Medical Center of the University of Amsterdam, and all subjects signed an informed consent form.

Randomization and masking

Participants were randomly assigned to receive either TRH during the first scan and placebo during the second, or vice versa. The order of the two interventions was randomly determined by trial apothecaries with locally stratified, randomly permuted blocks of three and four. The apothecaries had no further involvement in the rest

Figure 1

Experiment #2 over time. (A) ¹⁸F-FDG uptake during 60 min after administration of TRH in responders. Data are expressed as median (interquartile range). (B) Study design. BAT, brown adipose tissue; BS, blood sampling; FDG, fluorodeoxyglucose; SUVmax, maximal standardized ¹⁸F-FDG uptake; TRH, thyrotropin-releasing hormone.

of the trial. Participants and all investigators (those giving the intervention, assessing the outcomes and analyzing the data) were masked to treatment allocation. As the placebo and the intervention had identical appearances (2 mL of colorless liquid), further concealment of the allocation was not necessary.

Procedures

Experiments

In experiment #1, six subjects were studied at room temperature (21°C). Because of negative findings in the first six subjects, the study was continued with an amended protocol in the following nine subjects, now exposing the men to mild cold prior to TRH or placebo administration. In experiment #2, nine subjects were exposed to mild cold ($17^{\circ}\text{C} \pm 1^{\circ}\text{C}$, controlled by a climate control ventilation system) for an hour prior to PET/CT imaging. During cold exposure, subjects wore the standardized light clothing provided by the Academic Medical Center. To prevent maximum BAT stimulation by cold, the subjects were not exposed to cold after this period. Scans for both groups were performed by the same PET/CT scanner in a room with an air temperature of 23°C. Before start of the experiment, an intravenous catheter was inserted in the subject's antecubital vein in each arm; one for bolus injection of ^{18}F -FDG and one for bolus injection of TRH or placebo according to randomization, and withdrawal of blood samples.

Scanning protocol

PET-CT images were acquired using a Gemini time-of-flight multidetector helical PET-CT scanner (Philips Medical Systems), in one bed position, starting from the bottom of the nasal aperture to the seventh thoracic vertebrae. A bolus of 80 MBq ^{18}F -FDG was administered intravenously in one arm, while a bolus of 400 µg TRH or placebo was concurrently administered intravenously in the other arm. Starting simultaneously with the administration of ^{18}F -FDG and TRH or placebo, a 60-min dynamic PET imaging session with variable frame lengths (8×30, 1×60, 3×300, 2×600, 1×1200 s) was performed. The first PET scan was combined with a low-dose CT scan (80 mAs) as anatomical reference. After completing the dynamic scanning, a single static PET scan was acquired (acquisition time 240 s). The total radiation dose from the low-dose CT scan and the ^{18}F -FDG was approximately 3.6 mSv.

Both image sets were reconstructed in axial, coronal and sagittal images with a slice thickness of 5 mm. Volumes

of interest (VOIs) were the cervical and supraclavicular depots. When ^{18}F -FDG uptake was registered by PET in areas in which fat was identified by CT, the maximal standardized ^{18}F -FDG uptake (SUV_{max}) defined as activity was quantified by auto contouring the areas (Hybrid Viewer; Hermes Medical Solutions, Stockholm, Sweden) in the static scans (settings: relative to max 80%, standardized ^{18}F -FDG uptake (SUV) 2.00). Additionally, the ^{18}F -FDG influx rate constant (K_i) was determined using the irreversible Patlak method (16). Subsequently, K_i was multiplied with the capillary glucose concentration divided by a lumped constant value of 1.14 for adipose tissue to obtain glucose metabolic rate (MR_{glu}) (17). BAT time-activity curves (TACs) and image-derived input functions (IDIFs) were generated from the dynamic PET images by placing VOIs in the BAT depots and carotid artery respectively (Syngo.via MM Oncology package; Siemens Healthineers, Erlangen, Germany). ^{18}F -FDG K_i values of the left and right BAT depots were derived from the TACs and IDIFs (30–60 min post injection) using in-house developed Matlab tools (Matlab R14 Mathworks, Nantick, Massachusetts). The left-right averaged BAT K_i was used for statistical analysis. ^{18}F -FDG K_i was regarded as indicator of activity. If active regions could not be quantified, VOIs were drawn in by hand in locations where CT identified possible BAT. All assessments were performed by a blinded investigator.

Vital signs and blood samples

After being positioned in the scanner, a Nexfin (BMEYE BV, Amsterdam, the Netherlands) finger cuff was placed on the third digit of the subject's left hand for continuous monitoring of heart rate, blood pressure and sympathovagal balance during 5 min. The finger cuff was then disconnected to allow the bed to move into the CT scanner and reconnected when the bed was in position.

Blood samples were collected from the intravenous catheter 10 min before start of the scan, directly before administration of TRH or placebo, and 10, 20, 30, 40 and 60 min after start of the scan. In all samples, plasma concentrations thyroid-stimulating hormone (TSH), thyroxine (T4), free thyroxine (FT4) and T3, and serum concentrations NE and normetanephrine (NM) were measured in batches. FT4 and TSH concentrations were measured by fluoroimmunoassay using the Delfia 1232 Fluorometer (PerkinElmer), T4 and T3 by an in-house RIA (18). NE was analyzed by liquid chromatography in combination with isotope dilution mass spectrometry (19). NM extracted from plasma using weak cation exchange solid-phase extraction and measured by

Hydrophilic Interaction Liquid Chromatography (HILIC) on an Acquity–Quattro Premier Liquid Chromatography (LC)–tandem Mass Spectrometry (MS) system (Waters, Millford, MA, USA). Capillary blood glucose was obtained with a Bayer Contour Glucometer (Bayer Healthcare, Mishawaka, IN, USA).

Outcomes

The primary outcome was the presence of SUVmax above 2.00 in cervical and supraclavicular BAT depots on the single static PET scan acquired after completion of dynamic scanning. Secondary outcomes were vital signs (heart rate, diastolic and systolic blood pressure and sympathovagal balance), thyroid hormone concentrations (TSH, T4, FT4 and T3) and catecholamine concentrations (NE and NM) before and during 60 min of imaging after administration of TRH or placebo. Although skin temperature during this same time period was initially specified as secondary outcome, it will not be reported in this study. Skin temperature was initially measured continuously with a resolution of 0.0625 using iButtons (Thermochron iButton, DS1921H, Dallas, Maxim). However, the measurements fluctuated broadly both during cold exposure ($17^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and during imaging (21°C). As we felt the changes in skin temperature reflected the changes in environmental temperature rather than the effects of the intervention, this secondary outcome will not be reported. Because TRH is a well-known and often-used substance with only minor side effects, no adverse events were expected or assessed.

Statistical analysis

Our hypothesis was that BAT activity significantly increased after administration of TRH. BAT activity was considered a continuous variable and considered to be present when ^{18}F -FDG uptake reached a SUVmax of 2.0 (20). According to previous research, a standard deviation of differences of 1.5 was chosen. Assuming $\alpha=5\%$ in a two-sided outcome, ten subjects needed to be included to obtain a power of 90% to detect a 2.0 difference between two groups. Assuming that 80% of the normal population has detectable BAT (20) and to allow for unexpected findings, we included 15 subjects.

SPSS, version 22 for Windows was used for the statistical analysis. Subjects were categorized as responders ($\geq 25\%$ SUVmax percentage change between the two scans) and non-responders ($< 25\%$ SUVmax percentage change between the two scans). Endpoints were assessed

by the Wilcoxon signed-ranks (paired data) and Mann-Whitney U (independent data) tests. Measurements over time were compared using a two-way repeated-measures ANOVA, with Bonferroni confidence interval adjustment. For parameters differing at baseline, the deltas were used in the ANOVA. Cases with missing values were excluded from analysis. P values < 0.05 were considered to be significant.

The study was not overseen by a data monitoring committee. This clinical trial was registered with The Netherlands National Trial Register (NTR5512).

Results

Between 3 October 2014 and 18 April 2016, we included 16 healthy lean men (Table 1). In experiment #2, one subject who was randomized to receive TRH before the first scan, and placebo before the second scan, withdrew from the study after completion of the first scan as he found lying in the PET/CT scan too uncomfortable. He was replaced by a newly randomized subject and was not included in the analysis. In experiment #1, the outside temperature was significantly higher during the TRH scan compared to the placebo scan ($P=0.028$), while in experiment #2, there were no differences in outside temperature between the two scans (Supplementary Table 1, see section on supplementary data given at the end of this article).

In experiment #1, none of the subjects showed any visible activation of BAT during either scan, nor were there significant differences in ^{18}F -FDG K_i ($P=0.610$).

In experiment #2, BAT glucose uptake was visibly higher in four of the nine subjects (44.4%) after administration of TRH compared to placebo (Fig. 2A). SUVmax after placebo was set at 100%, and the relative change after TRH was calculated (ΔSUVmax ; Fig. 2B). ΔSUVmax showed a trend toward increase after TRH compared to placebo ($P=0.066$, Fig. 2B and Table 2). Using predefined criteria, four subjects were TRH responders ($\geq 25\%$ increase SUVmax), while five subjects were non-responders. ^{18}F -FDG K_i showed a significant increase after administration of TRH compared to placebo ($P=0.014$,

Table 1 Clinical characteristics of subjects in experiment #1 (room temperature) and experiment #2 (cold exposure).

Characteristics (units)	Room temperature group (n=6)	Cold-exposed group (n=9)
Age (years)	37.3 (35.2–41.4)	33.1 (30.8–34.3)
Weight (kg)	78.9 (70.0–85.3)	83.9 (70.5–85.8)
Height (cm)	184.0 (178.0–189.5)	183.8 (174.5–191.0)
BMI (kg/m ²)	23.2 (21.8–23.2)	23.7 (22.9–24.2)

Values expressed as median (interquartile range).

Figure 2

Effects of TRH on BAT activity. (A) PET images of a 30-year-old man after administration of placebo (left) and 400 µg TRH (right). (B) BAT activity 60 min after administration of placebo and TRH in nine cold-exposed men, reflected by maximal standardized ^{18}F -FDG uptake (SUVmax; left), SUVmax relative change (ΔSUVmax ; middle) and glucose metabolic rate (MRglu; right). BAT, brown adipose tissue; FDG, fluorodeoxyglucose; MRglu, glucose metabolic rate; SUVmax, maximal standardized ^{18}F -FDG uptake; TRH, thyrotropin-releasing hormone.

Table 2). As PET scans were acquired in a single bed position for dynamic scanning, all BAT was detected in the cervical-supraclavicular adipose tissue depots. The extent of BAT activity after TRH administration was comparable to that reported for cold-activated BAT (20). The dynamic scans indicated that SUVmax increased immediately after TRH administration (Fig. 1A).

In experiment #1, baseline plasma TSH, FT4, T4 and T3 were within the reference range in all subjects. In experiment #2, while baseline plasma TSH, FT4 and T3 were within the

reference range in all subjects, plasma T4 was decreased during both study days in two subjects (55 and 65 nmol/L, normal range (NR): 70–150 nmol/L). In these subjects, thyroxine-binding globulin (TBG) appeared to be below reference range (170 and 180 nmol/L, NR: 200–650 nmol/L). Partial TBG deficiency is a common cause of decreased plasma T4 in combination with normal FT4 and does not point to hypothyroidism (21). In both experiments, baseline hormone measurements were not different between the two scans (Supplementary Fig. 1A and B).

In both experiments, TSH concentrations were significantly affected by both time (experiment #1: $P < 0.001$, experiment #2: $P < 0.001$) and TRH treatment from 10 min after administration compared to the other time points (experiment #1: $P < 0.001$, experiment #2: $P = 0.001$). All subjects showed normal timing and peak concentration of TSH (22) confirming euthyroidism, and a normal responsiveness of the pituitary gland to TRH. In both experiments, FT4 concentrations remained unaltered (Supplementary Table 2). There was no significant change in T4 concentrations in experiment #1. T4 concentrations in experiment #2 significantly fluctuated over time ($P = 0.003$), but were unaffected by treatment. In both experiments, T3 concentrations significantly decreased over time (experiment #1: $P = 0.040$, experiment #2: $P = 0.001$), but increased 60 min after administration of TRH compared to the other time points (experiment #1: $P = 0.005$, experiment #2: $P = 0.003$). Although the rise in T3 seemed slightly higher in subjects with activated BAT compared to subjects without BAT activation, this difference did not reach statistical significance ($P = 0.127$). In both experiments, there was no difference in baseline capillary glucose between the two scans (Supplementary Table 1). In both experiments, both serum NM and NE concentrations significantly decreased over time during the experiment (Supplementary Table 4). Both NM and NE concentrations were unaffected by treatment. There were no differences between responders and non-responders.

In experiment #1, heart rate significantly fluctuated over time ($P < 0.001$), but was unaffected by treatment.

Table 2 BAT activity 60 min after TRH and placebo in experiment #2: cold-exposed subjects ($n = 9$). Values expressed as median (interquartile range).

	TRH	Placebo	Z	P
BAT activity				
SUVmax	3.03 (0.91–4.62)	1.01 (0.87–1.51)	–1.84	0.066
ΔSUVmax	113.3 (94.0–361.4)	100.0 (100.0–100.0)	–1.84	0.066
MRglu	0.047683 (0.013258–0.074988)	0.000067 (0.000000–0.006077)	–2.45	0.014

Statistics: Mann-Whitney test (MRglu), Wilcoxon signed-rank test (SUVmax).

BAT, brown adipose tissue; MRglu, glucose metabolic rate; SUVmax, maximal standardized ^{18}F -FDG uptake; ΔSUVmax , SUVmax relative change.

In both experiments, diastolic blood pressure and sympathovagal balance remained unaltered throughout the scans (Supplementary Fig. 2 and Supplementary Table 3). Systolic blood pressure was significantly higher after TRH administration compared to placebo ($P=0.015$), but was unaffected by time. In experiment #2, heart rate remained unaltered throughout the scan, while systolic blood pressure significantly increased over time ($P<0.001$), but was unaffected by treatment. This was also true in the subset of responders.

Anticipated adverse effects of TRH (23) included nausea, flushing and urinary urgency and were reported as well tolerable. There were no adverse events.

Discussion

In this randomized, placebo-controlled, double-blind, cross-over study, we found that activity of cold-exposed BAT increases in a subset of healthy, lean men by intravenous administration of TRH. Of the nine subjects exposed to mild cold during 1 h prior to scanning, four showed a rapid increase of BAT ^{18}F -FDG uptake after administration of TRH, but not after placebo. One subject showed comparable BAT activation in both scans, while four had no BAT activation in either scan. As expected, TRH significantly increased TSH concentrations in both experiments. During the hour of PET/CT imaging, TRH administration had no significant effect on plasma FT4 or T4 concentrations. T3 concentrations were significantly higher after TRH compared to placebo, however, only 60min after TRH administration. Moreover, the T3 concentration 60min after TRH administration was not significantly different between responders and non-responders. It is thus unlikely that the effect of TRH on BAT activity can be explained by changes in plasma T4 or T3. Despite the key role for the SNS in BAT activation, we found no consistent changes in cardiac parameters, including the sympathovagal balance, suggesting that activation of the SNS may be either limited to BAT innervation or not involved in the response of BAT to TRH. So far, two pharmacological agents have been shown to increase BAT activation in humans: β_3 -adrenergic receptor agonist mirabegron (6) and bile acid chenodeoxycholic acid (7). The current study now adds TRH to the list.

During cold exposure, the SNS is stimulated by the hypothalamus to increase noradrenergic input to BAT, where the β -adrenergic pathway increases fatty acid uptake and lipolysis (5). This stimulates UCP1 to maximize the proton leak across the inner mitochondrial membrane, thus increasing heat production (24). For optimal UCP1

activation, high intracellular concentrations of T3 are also required (8). The increase of NE in BAT stimulates conversion of T4 to T3 by the enzyme deiodinase type 2 (D2), causing a BAT-specific T3 increase within hours after cold exposure (25). Intracellular T3 both directly stimulates UCP1 gene transcription and maintains long-term BAT activation under minimal sympathetic stimulation (26). Direct stimulation of β_3 receptors with mirabegron is sufficient for a marked increase in BAT activity in healthy men (6). Furthermore, bile acids activate D2 and thus increase T3 in BAT. Two doses of chenodeoxycholic acid in healthy women resulted in a modest increase in BAT activity (7). The proportion of subjects with active BAT after TRH administration in our study is lower than that in these two studies. Cypess *et al.* screened eligible subjects using cold exposure prior to inclusion, after which only those with detectable BAT were included (6). Broeders *et al.* did not select subjects on detectable BAT, but subjects underwent a third cold-exposed PET/CT scan to assess their maximum BAT activation (7). Of these exclusively female subjects, all had active BAT after cold exposure. The fact that women are more likely to display active BAT than men may have contributed to the high proportion of subjects with detectable BAT (27). We did not obtain permission from our ethical committee to perform a third PET/CT scan after cold exposure. We therefore may have included subjects without cold-inducible BAT. Additionally, the mean age of subjects in the previous studies were 22.2 ± 0.6 years and 22 ± 3 years respectively, while the subjects in the present study had a median age of 34.3 years (IQR 31.3–39.0). Due to the strong inverse relation between age and BAT responsiveness (28), this may have reduced the number of subjects with activated BAT.

While previous studies have investigated the effects of circulating thyroid hormones on human BAT, the results have been contradictory. Thyroidectomized patients subjected to ^{18}F -FDG PET/CT imaging after cold exposure, both in a hypothyroid state (high TSH, low FT4) and during a thyrotoxic state (suppressed TSH, high FT4), showed significantly higher BAT activation during thyrotoxicosis (29). Nine patients with Graves' hyperthyroidism underwent ^{18}F -FDG PET/CT imaging at room temperature both during hyperthyroidism (suppressed TSH, high FT4) and euthyroidism (normal TSH, normal FT4). The only active BAT was seen in a patient in a euthyroid state (30). Finally, an 11.5-year-old girl with severe primary hypothyroidism (high TSH, very low FT4) was reported to display active BAT with infrared thermal imaging and magnetic resonance imaging in a hypothyroid state and diminished BAT activity in a euthyroid state (normal TSH, normal FT4). Interestingly, the

authors raised the possibility of either TSH or TRH having a stimulatory effect on BAT (31). Based on these observations, the effect of circulating thyroid hormones on BAT activity has not been established yet.

Animal studies indicate that TRH plays a major role in both the sympathetic and the neuroendocrine stimulation of BAT. During cold exposure, cold-sensitive TRH neurons in the hypothalamic PVN are activated. These neurons directly activate spinal preganglionic neurons which in turn project to BAT via the superior cervical ganglion (10). In the hypothalamus–pituitary–thyroid axis, the cold-induced increase in TRH stimulates TSH secretion by the pituitary, which in turn stimulates thyroid hormone release from the thyroid (11). Research in various species showed that both core body temperature (32, 33) and BAT-specific temperature (13) are increased by central or peripheral administration of TRH. Intracerebroventricular infusion of TRH in rodents was found to activate BAT via sympathetic neurons, an effect that could be blocked by pre-treatment with TRH-receptor type 1 antibodies and attenuated by sympathetic denervation of BAT depots (13, 14).

While these studies support the concept that the increase in BAT activity in the present study was caused by central effects of TRH, we cannot exclude a role for peripheral TRH or TSH at present. Indeed, TSH receptors are expressed in rat BAT (34), and bovine TSH was shown to increase thermogenesis in mouse brown adipocytes *in vitro* (35). Whether TRH receptors are expressed in BAT is unknown. Future studies should address the mechanism of TRH-induced BAT activation in humans.

A limitation of our study is that we obtained approval from the medical ethics committee to study only 15 healthy men. When none of the first six subjects showed any BAT activity after TRH or placebo administration, we obtained permission to expose the subsequent nine subjects to mild cold during 1 h prior to scanning, but not to include additional subjects. Due to the small number of subjects and safety considerations limiting the number of PET/CT scans to two per subject, our study has limited statistical power.

In summary, we show that intravenous administration of TRH can increase the activity of cold-stimulated BAT in healthy adult men. The activity is mediated either by TRH or increased plasma TSH following TRH administration. The results exclude a primary role for plasma T4 or T3. Our findings add to our understanding of the regulation of BAT in humans and may assist in the development of future pharmacological strategies aimed at modulating BAT activity in the management of obesity.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-17-0966>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

C A H coordinated the study. C A H, Z Z, L P K, A B, A K, P H B, A S P v T, H J V, J B and E F designed the study. C A H screened and enrolled participants and arranged informed consent. C A H, Z Z, T C d W, E P, M Y, A K, P H B, H J V, J B and E F performed or supported data analysis, including the statistical analysis. C A H, A B, A S P v T, H J V, J B and E F interpreted the results. C A H and E F wrote the manuscript. All authors critically reviewed the report. No writing assistance was provided. C A H, A S P v T and E F had access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses.

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