Temperature-acclimated brown adipose tissue modulates insulin sensitivity in humans

Paul Lee[1], Sheila Smith[1], Joyce Linderman[1], Amber B Courville[2], Robert J Brychta[1], William Dieckmann[3], Charlotte D Werner[1], Kong Y Chen[1], Francesco S Celi[1,4]

Diabetes, Endocrinology, Obesity Branch, National Institute of Diabetes and Digestive and Kidney Diseases[1], Department of Nutrition, Clinical Center[2], PET Department, Clinical Center[3], National Institutes of Health, Bethesda, USA and Division of Endocrinology and Metabolism[4], Virginia Commonwealth University, Richmond, USA

Correspondence author: Francesco S Celi, Division of Endocrinology and Metabolism[4], Virginia Commonwealth University, 1101 East Marshall Street, Sanger Hall, Room 7-007, Richmond, USA

Tel: 1 804 828 Email: fsceli@vcu.edu

None of the authors have any conflicts of interest to disclose

Abbreviated title: Temperature-acclimated human brown adipose tissue

Key words: white adipose tissue, beige adipose tissue, thermogenesis, adiponectin, leptin, CIDEA, GLUT4

Word count: 177 (Abstract); 3999 (Main Text)

Number of tables: 4 Number of figures: 4 Supplemental Data: 1

Diabetes Publish Ahead of Print, published online June 22, 2014

Abstract

In rodents, brown adipose tissue (BAT) regulates cold- (CIT) and diet-induced thermogenesis (DIT). Whether BAT recruitment is reversible and how it impacts on energy metabolism has not been investigated in humans. We examined the effects of temperature acclimation on BAT, energy balance and substrate metabolism in a prospective crossover study of 4-month duration, consisting of 4 consecutive blocks of 1-month overnight temperature acclimation [24°C (*month 1*) → 19°C (*month 2*) → 24°C (*month 3*) → 27°C (*month 4*)] of five healthy men in a temperature-controlled research facility. Sequential monthly acclimation modulated BAT reversibly, boosting and suppressing its abundance and activity in mild cold and warm conditions ($p<0.05$), respectively, independent of seasonal fluctuations ($p<0.01$). BAT-acclimation did not alter CIT but was accompanied by DIT ($p<0.05$) and post-prandial insulin sensitivity enhancement ($p<0.05$), evident only after cold-acclimation. Circulating and adipose tissue, but not skeletal muscle, expression levels of leptin and adiponectin displayed reciprocal changes concordant with cold-acclimated insulin sensitization. These results suggest regulatory links between BAT thermal plasticity and glucose metabolism in humans, opening avenues to harnessing BAT for metabolic benefits.

Introduction

Unhealthy diet and physical inactivity are the major culprits to the obesity crisis, although other environmental factors may also contribute (1). An overlooked component in energy balance is adaptive thermogenesis, which comprises diet-induced thermogenesis (DIT) and cold-induced thermogenesis (CIT). DIT is the portion of energy expended following food ingestion, beyond the energy cost of digestion/absorption (2). The CIT response defends core temperature during cold exposure (3). In rodents, both processes are chiefly regulated by brown adipose tissue (BAT). Through the action of uncoupling protein 1 (UCP1), energy is converted into heat, and represents a form of energy expenditure (EE) as energy is dissipated to the environment. BAT stimulation protects animals against diet-induced obesity and glucose intolerance (4).

In addition to "classic BAT" in the interscapuar region, cold exposure also induces the emergence of brown adipocyte-like cells (beige/brite adipocytes) within white adipose tissue (WAT) in animals (5; 6). Brown/beige fat generates heat from glucose/lipids and their high substrate utilization underlies protection against diet-induced insulin resistance in genetic, pharmacological and/or transplantation models of invigorated brown/beige fat status (7-9). In humans, histological examination had demonstrated the presence of BAT in adult in the 1970-80's (10-12), although BAT whole body abundance was not fully appreciated until its visualization was made possible by Positron Emission Tomography (PET)/CT (13-17). Not only is BAT inducible in humans (18; 19), it also exhibits oxidative capacity (20) and classic BAT/beige fat features (21; 22), thus forming the basis for the quest of BAT/beige fat-enhancing

strategies as anti-obesity treatments (23).

Acute cold exposure (hours) increases BAT activity (13; 15-17; 24), while longer-term exposure (days/weeks) expands BAT volume (25; 26). Because BAT recruitment could reduce adiposity (26), it suggests BAT may impact whole body energy homeostasis. The corollary is that reduced cold exposure could suppress BAT/beige fat function in humans, with potential obesogenic consequences (27). To date, cold exposure is the best-known activator (15-17) and recruiter (25; 26) of BAT, and associative data have linked higher BAT abundance with leanness and lower glycemia in humans (13; 15; 28; 29). Whether BAT withers under warm exposure and if BAT recruitment triggers compensatory metabolic and/or behavioral adaptations have not been investigated, but are integral to BAT physiology. Rodent studies have revealed a complex interplay between housing temperature, BAT recruitment and energy balance, which ultimately determines metabolic phenotype (30). To better appreciate the metabolic significance of human BAT, and the implications of BAT status on health, BAT recruitment interventions should be examined in the context of whole body energy metabolism.

In this study, we investigated the effects of long-term mild cold and warm exposure by minimal overnight manipulation of ambient temperature on individual BAT status and the corresponding energy/substrate homeostatic responses. We hypothesize human BAT exhibits plasticity and its activity modulates systemic energy metabolism.

METHODS

Subjects

Five healthy men were recruited through local advertisement and provided written informed consent. NIDDK-NIAMS IRB approved the study (ClinicalTrials.gov: NCT01730105). Supplemental Figure S1 summarizes recruitment, allocation and intervention.

Overall design

This is a prospective crossover study consisting of 4 consecutive blocks of 1-month duration [Supplemental Figure S2]: it incorporates i) sequential monthly thermal acclimation over 4 months, and ii) acute thermo-metabolic evaluations at the end of each study temperature regime. Volunteers were admitted to the Clinical Research Unit (NIDDK) in Bethesda, Maryland (April-November 2013) for the entire 4 months.

Monthly thermal acclimation

Volunteers resided in a temperature-adjusted private room, engaged in usual daily activities and returned to their room each evening. Room temperature was adjusted in the following sequence: 24°C(*month 1*)→19°C(*month 2*)→24°C(*month 3*)→27°C(*month 4*). Volunteers were exposed to the study temperature for at least 10 hours each night, wearing standardized hospital clothing with a combined thermal insulation value of 0.4 (clo). Only bed sheets were provided. Volunteers were asked to not deviate daily activity level over the study period. Each subject therefore acted as

his own control. At 08:00 at the end of each month, volunteers were admitted to a whole-room indirect calorimeter for thermo-metabolic evaluation.

Temperature monitoring

Volunteers wore two temperature data loggers (Extech RHT20, Nashua, NH), one "external to clothing" to track environmental temperature; the other "within clothing" to track immediate temperature changes in the "microenvironment" within clothing. We averaged individual exposed temperature every 30 minutes for the entire 4-month period, allowing us to record environmental temperature variations and "true temperatures" the individual was being exposed to.

Diet

All meals, including pre-packed lunches/snacks, were provided with the following composition: 50% carbohydrate, 20% protein, 30% fat. The first month was an equilibration period, during which volunteers followed a weight maintenance diet. After month 1, subjects ate according to hunger. Caloric/macronutrient content was calculated based on weight maintenance requirements, determined during equilibration month. Any unconsumed foods were returned/weighed for energy/macronutrient intake calculation. Subjects met study dieticians twice weekly to verify food diaries/compliance. Total intake/macronutrient was computed/analyzed using three-dimensional food models (ProNutra, version 3.4.0.0., Viocare Technologies, Princeton, NJ).

Appetite/hunger assessment

Subjects completed questionnaires assessing appetite twice a week before/after breakfast, by marking on a visual analogue scale (VAS) (10cm long) responses to the questions: 1) How hungry are you? 2) How full are you? 3) How much food can you consume? These questions gauged hunger, satiety and desire to eat before/after meals.

Before each monthly thermal-metabolic evaluation, volunteers underwent an *ad libitum* meal test, consisting of a selection of food items displayed in a vending machine. Subjects ate until they felt 'comfortably full'. Total energy/macronutrient intake were recorded, together with ratings of appetite (hunger, satiety, desire to eat) using the same weekly questionnaire at T=−10, 0, 60, 120, 180, 240 and 300 min where initiation of the meal was defined as T=0 min.

Acute thermo-metabolic evaluations

Thermo-metabolic evaluation was scheduled at the end of each month [Supplemental Figure S2], modeled on our previous published methods (24; 31), with total energy expenditure (EE) calculated as previously described (24; 31). Volunteers underwent two 24-hour sessions in a whole room calorimeter, exposed to first 24°C (day 1) then 19°C (day 3), with a resting 24-hour period in between. The temperature order was not randomized because our previous study did not reveal a sequence effect (31). Testing at the two temperatures allowed us to evaluate how monthly acclimation modulated EE/metabolism at both thermoneutral and mild cold conditions. Lunch (Boost Plus, Nestle Healthcare Nutrition, Inc., Vevey, Switzerland) and dinner (selected from Metabolic Menu) were provided at 13:00 and 19:00, consisting of 1/3 and 2/3 of daily caloric intake, respectively, based on calculation from equilibration month. CIT was calculated as difference in total EE between 24°C and 19°C, and DIT

as difference in pre- (08:00-13:00) and post-lunch (13:00-19:00) EE. As the test meal carried identical caloric and macronutrient content, we attributed any changes observed to arise from adaptive thermogenesis, because the facultative component (*i.e.* digestion/absorption) should be relatively unaltered. Shivering response was quantified by surface electromyography (EMG), as previously described (32), and volunteers reported perception to cold each month during EE testing using VAS. Hormonal/metabolic parameters were measured in venous samples. Post-prandial insulin sensitivity was calculated after a mixed meal (33), and adipose resistance index by product of free fatty acid × insulin. At the conclusion of thermo-metabolic study, body composition was measured, as previously published (24; 31).

PET/CT scanning

Positron-Emission Tomography (PET)-Computed Tomography (CT) was performed using Siemens Biograph mCT (Siemens Healthcare, Ill., USA) (32). PET/CT was undertaken at 08:00 the morning after the 19°C testing day, at the end of each acclimation month. Attenuation corrected PET-CT images were analyzed using custom software built with IDL (Excelis Visual Information Solutions, Inc., Boulder, CO). A 3-dimentional region of interest (ROI) was defined cranially by a horizontal line parallel to the base of the C4 vertebra, and caudally by an oblique line traversing the manubriosternal joint and T8 transverse process [Supplemental Figure S3]. BAT was defined as tissue with Hounsfield units -300 to -10 on CT (i.e. fat density) with a lean body mass standardized uptake value (SUV) of ≥2 (*i.e.* high glucose uptake). The chosen ROI captures major BAT depots in the cervical, supraclavicular, axillary, superior mediastinal and paravertebral areas. This ROI was chosen because spurious myocardial/renal excretory FDG uptake could not be reliably excluded from BAT.

198 This approach allowed examination of BAT evolution within a well-defined region of
199 adipose tissue across 4 months.
200
201 *PET-CT parameters*
202 The following parameters were analyzed: BAT volume, mean SUV and activity. BAT
203 volume, defined as the sum of the volume of all voxels that met HU-SUV criteria,
204 represents activated BAT. Mean SUV (normalized by FDG dose and lean body mass)
205 of ROI represents mean metabolic activity within BAT-harboring region. BAT
206 activity represents the total radioactivity (in MBq) within ROI and captures both
207 changes in volume and mean FDG uptake. Furthermore, because fat exhibits
208 metabolic activity as a continuum and the chosen SUV threshold of ≥2 is arbitrary,
209 and may potentially exclude more diffuse enhancement of adipose metabolic activity,
210 we also quantified mean SUV in the entire ROI within tissue of fat density (HU: -300
211 to -10). Mean SUV of whole fat depot estimates overall metabolic activity, and may
212 capture both BAT and diffuse beige fat activity. This is particular relevant in subjects
213 with lower BAT abundance [Supplemental Figure S5 and S7]. While BAT, defined
214 with a SUV threshold of ≥2, was not visually apparent in these two subjects, whole fat
215 activity followed same pattern of acclimated BAT changes. When a lower SUV was
216 used (≥1) [Supplemental Figure S8], adipose activity changes were visually
217 concordant with overall fat activity. Mean SUV uptake in liver and skeletal muscle
218 (rectus femoris) were quantified to compare temperature-acclimation impact on BAT
219 and other metabolic organs. All images were analyzed twice by an investigator (PL)
220 blinded to subject identity and acclimation temperature. Intra-scan coefficient of
221 variation of BAT volume, mean SUV, BAT activity and mean whole fat activity were
222 0.7%, 3.0%, 1.3% and 2.4%, respectively.

223

224 *Tissue biopsies*

225 Paired subcutaneous adipose/muscle biopsies were obtained at the end of each month,
226 from abdomen and rectus femoris, respectively (31). RNA extraction and cDNA
227 synthesis were performed using standard methods and genes governing thermogenesis
228 and glucose metabolism were examined using Taqman Gene Expression assays
229 (Applied Biosystems) [Supplementary Table S2].

230

231 *Laboratory measurements*

232 Plasma adiponectin, leptin and fibroblast growth factor 21 (FGF21) were measured by
233 ELISA (R&D Systems, Minneapolis, MN and BioVendor, Oxford, U.K.), according
234 to manufacturer's protocol, with intra-assay/inter-assay coefficients of variation
235 between 2.5 to 4.8%. Remaining tests were measured by Department of Laboratory
236 Medicine, NIH.

237

238 *Statistical analysis*

239 Statistical analysis was performed using SPSS 20.0 (SPSS, Inc., Chicago, IL, USA).
240 Data are expressed as mean±standard deviations. Trend changes of physiologic and
241 hormonal parameters during temperature acclimation across 4 months, expressed as
242 fold change over baseline, were analyzed by one-way ANOVA with Bonferroni's
243 correction. Areas under the curve (AUC) were calculated using the trapezoidal rules
244 incorporating sampling-points across 24 hour-period from 0800 to 0700 the next
245 morning [Supplemental Figure S2]. Post-prandial glucose and insulin AUCs were
246 calculated in the period after lunch starting 1pm (T=0, 60, 120, 240, 360 minutes).

Pearson correlation coefficients were used to examine associations between variables. An α error of 0.05 was considered statistically significant.

RESULTS

Baseline acute thermo-metabolic evaluation

Five men (21±2 years old, BMI: 22±1 kg/m^2, body fat: 21±2%) participated in the study. Volunteers were first evaluated at baseline for BAT status and thermo-metabolic responses to temperature changes. Compared to 24°C, mild cold exposure at 19°C increased total EE by 6±4% ($p<0.05$), representing CIT response. Baseline cold-activated BAT volume was 55±61 ml with mean SUV of 3.2±0.8. EE at 19°C correlated positively with BAT volume ($R^2=0.82$, $p=0.03$). These results replicated findings in our previous overnight cold exposure studies (24; 31), and validated the methodology in the investigation of temperature acclimation-associated metabolic and physiologic consequences. Hereafter, we describe changes in physiologic and metabolic parameters at each monthly thermo-metabolic evaluation, with results stratified to either 19°C or 24°C testing condition to decipher impact of acclimation on metabolism under thermoneutrality and mild cold exposure.

Metabolic consequences of monthly acclimation

Tables 1-4 summarize changes in BAT, physiologic, dietary, body compositional and hormonal parameters across 4-month acclimation. Hormone/metabolite AUC results are shown in Table 4 and their fasting levels in Supplemental Table S3. Results from each domain are described in the following sub-sections.

BAT changes

Figure 1A-D demonstrates BAT evolution in one representative subject throughout 4-month sequential acclimation. Supplemental Figures S4-S7 show individual results. Figure 1E-H display mean changes in BAT volume and overall fat metabolic activity, which increased upon cold acclimation (19°C) by 42±18% ($p<0.05$) and 10±11% ($p<0.05$), respectively; decreased after the thermoneutral month (24°C) to nearly baseline level, and completely muted at the end of one-month warm exposure (27°C). BAT radio-density, measured in HU, responded to acclimation with the same pattern ($p<0.01$) [Table 1]. BAT HUs increased by 25±8% following cold acclimation, reversed after the thermoneutral month, and by the end of warm acclimation in month 4, HU was 18±11% lower than baseline values in month 1 [Table 1]. In contrast, mean SUV of skeletal muscle and liver remained unchanged during acclimation [Table 1]. Room ($p<0.05$) and individually exposed temperatures ($p<0.01$), but not outdoor temperatures, correlated with BAT changes during study period [Figure 1I, 1J and Supplemental Table S1].

Cold- and diet-induced thermogenesis

We next explored metabolic consequences of BAT acclimation. CIT response did not change significantly during temperature acclimation [Table 2]. In contrast, DIT measured at 19°C rose by 32±35% ($p=0.03$) following cold acclimation. Progressive re-warming suppressed 19°C DIT response at months 3 and 4 to nearly baseline level. DIT measured at 24°C was unaltered [Table 2].

Shivering response and cold sensitivity

296 Surface EMG recordings of muscle fasciculation/shivering measured at 19°C and
297 24°C were not different [Table 2], indicating absence of significant shivering and
298 validating our model in capturing non-shivering thermogenesis. Monthly acclimation
299 did not alter EMG recordings and subjects did not report changes in cold perception at
300 19°C during monthly calorimeter testing [Supplemental Figure S9].
301
302 *Diet and body composition*
303 Neither total caloric nor macronutrient content of intake changed during acclimation
304 [Table 3]. Biweekly hunger and satiety scores did not change significantly [Table 3];
305 however, volunteers reported an increase in desire to eat and reduction in satiety
306 during *ad libitum* meal test after cold acclimation, which reversed during the warm
307 months [Supplemental Figure S10]. Body composition was unaltered across study
308 period [Table 3].
309
310 *Pituitary-thyroid-adrenal axis*
311 To elucidate potential endocrine mediators of BAT acclimation, we profiled pituitary-
312 thyroid-adrenal axes [Table 4]. Cold acclimation increased free triiodothyronine (T3)
313 AUC measured at 24°C, but not at 19°C. Free T3 to free thyroxine (T4) ratio (an
314 indicator of peripheral T4 to T3 conversion (34)) was greater by 11±5% (p=0.01)
315 measured at 24°C. No significant changes were observed in TSH or the pituitary-
316 adrenal axis.
317
318 *Insulin sensitivity*
319 Total glucose and insulin AUCs did not change during acclimation [Table 4]. In
320 contrast, post-prandial insulin excursion measured at 19°C reached a nadir after cold

acclimation, without significant changes to glucose excursion [Figure 2A-B]. Indices of insulin sensitivity and resistance showed significant reciprocal changes during cold- and warm-acclimation, consistent with an improvement of post-prandial whole body insulin sensitivity following cold acclimation [Figure 2C-D]. These changes were absent during measurements at 24°C [Figure 3A-D].

Adipokine changes

Given our recent demonstration of BAT as an endocrine organ in humans (19; 32; 35), we probed adipokine changes during acclimation. Adiponectin AUC was augmented by 22±9% ($p<0.001$) after cold acclimation [Figure 2E]. Enhancement of adiponectin levels was observed not only at 19°C during acute thermo-metabolic evaluation, but similar increase occurred also at 24°C ($p<0.001$) [Figure 3E]. In contrast, cold acclimation reduced leptin AUC by 14±28% ($p<0.001$), evident at both 19°C [Figure 2F] and 24°C [Figure 3F]. These dichotomized changes returned almost to baseline during the thermoneutral third month, trending to the opposite directions at the end of the fourth month at 27°C ($p<0.05$). Changes in circulating adiponectin and leptin correlated negatively with changes in BAT activity after cold acclimation [Figure 2G-H and Figure 3G-H]. FGF21 AUC rose after cold acclimation, although overall trend did not reach significance [Table 4].

Fat and muscle gene expression

To explore sources of adipokine and origins of metabolic changes, fat and muscle biopsies were obtained from 4 volunteers at the end of each month. *Adiponectin* and *GLUT4* expression in adipose tissue [Figure 2I], but not muscle [Figure 3I], rose after cold acclimation while expression of *leptin* fell, and their respective trends reversed

after thermoneutral and warm acclimation months ($p<0.05$). Expression of *CIDEA*, a BAT gene governing lipid mobilization (36), increased following cold acclimation but decreased during re-warming [Figure 4]. No other BAT/beige fat gene changes were observed.

Discussion

The major finding of our study is the demonstration of BAT acclimation and its metabolic consequences by minimal manipulation of overnight temperature exposure, while allowing usual daily activities. Human BAT is inducible and suppressible by controlled mild cold and warm exposure, respectively, independent of seasonal fluctuations. BAT acclimation is accompanied by boosting of diet-induced thermogenesis and post-prandial insulin sensitivity. Mechanistically, this is associated with reciprocal changes of circulating adiponectin and leptin, mirrored by corresponding transcriptosomal changes in adipose tissue *ex vivo*. These results provide first evidence linking ambient temperature, BAT acclimation and whole body energy/substrate metabolism in humans.

Consistent with previous reports (25; 26), we confirmed BAT recruitability by cold exposure, but did not observe significant CIT response augmentation; the latter could be a type 2 error. Despite tentalizing associative data linking BAT abundance with favorable energy metabolism in humans, it remains unclear, to date, whether BAT recruitment is accompanied by metabolic benefits. We specifically sought to determine the significance of BAT recruitment, and revealed an association of BAT

371 acclimation with enhancement of post-prandial energy metabolism and insulin
372 sensitization. Within the allowance and feasibility of human research, we explored
373 underlying mechanisms through blood and tissue analyses.
374
375 First, within the pituitary-thyroid-adrenal axis, we observed an increase in T3/T4
376 ratio, which indicates enhanced T3 synthesis. Given the enrichment of BAT with type
377 2 deiodinase (37), and our previous report showing severe insulin resistance
378 amelioration by thyroid hormone-mediated BAT activation (38), we hypothesize
379 heightened T3 synthesis within BAT to be one plausible mechanism underlying
380 acclimated-BAT associated metabolic changes. Such pattern of increased thyroid
381 hormone turnover in the absence of TSH changes is reminiscent of cold adaptation
382 observed among Arctic residents (39).
383
384 Second, our adipokine profiling uncovered an intriguing relation between BAT,
385 adiponectin and leptin. Cold acclimation augmented circulating adiponectin but
386 decreased leptin. It is tempting to speculate cold-induced adiponectin, a potent
387 insulin-sensitizer, contributes to glucose metabolism improvement and leptin
388 reduction, the latter as a result of improved tissue sensitivity. Concordant gene
389 changes in adipose *adiponectin* and *leptin*, absent in muscle, argue adipose to be the
390 primary effector. Surprisingly, circulating adiponectin related negatively with BAT
391 activity, suggesting PET-detectable BAT was not the source of cold-induced
392 adiponectin. As BAT exhibits insulin-independent glucose uptake capacity (40),
393 lesser BAT expansion could have triggered alternative glucose utilizing pathways in
394 WAT during cold acclimation, evident by observed WAT *GLUT4* up-regulation.
395 Interestingly, such changes in circulating adiponectin and leptin were not limited to

cold-exposed condition [Figure 2], but persisted at thermoneutrality [Figure 3], indicating the temperature-acclimated hormonal milieu was not totally dependent on BAT activation. The corollary is that acclimated BAT could be serving beneficial metabolic functions not related to temperature regulation *per se*.

Third, newly identified cytokines, such as FGF21, may mediate temperature-acclimated tissue crosstalk. Recent identification of a FGF21-adiponectin feed-forward axis (41) led us to wonder if FGF21 augmentation following cold-acclimation could have brought forth the adiponectin rise. When BAT was muted at the end of warm acclimation, and adiponectin dwindled, FGF21 did not fall however, suggesting non-BAT FGF-secreting tissues might have compensated in states of relative BAT deficiency.

Fourth, although we did not observe an increase in beige fat gene expression, possibly due to the small sample size, we speculate fat browning to be a possibility. This is corroborated by finding an increased expression of the BAT gene *CIDEA* in adipose tissue following cold acclimation. Although ethical considerations prohibited serial neck fat biopsies in our volunteers, changes in radio-density within BAT by PET/CT have offered insight on tissue changes. Adipose tissue is typically characterized by HU between -10 to -300, in contrast to muscle tissue, whose HU is within the positive range. Compared to WAT, BAT has relatively less lipid, as it is filled with abundant mitochondria and blood vessels. This is exemplified by water-fat separated magnetic resonance imaging revealing lower fat fraction in activated BAT both in humans (42) and rodents (43). We speculate the rise and fall in BAT radio-density with cold and warm acclimation, respectively, could be reflections of WAT→BAT transformation

(or fat brown-ing). This is also supported by previous studies demonstrating cell-autonomous (44) and endocrine-mediated (19) cold-induced WAT browning in humans. Further studies are required to ascertain if WAT browning contributes to cold-acclimated BAT induced metabolic changes.

Collectively, our results infer a complex concerted BAT-WAT response to cold acclimation, which could involve interplay between CIDEA-mediated lipid mobilization (45; 46), GLUT4-enhanced glucose utilization and FGF21/adiponectin-induced insulin sensitization. Most importantly, all these changes occurred in the absence of measureable EE, caloric intake or body compositional alterations, suggesting such responses to be primary cold-induced metabolic sequelae, rather than compensatory physiologic adaptations. Nonetheless, because the desire to eat heightened after cold acclimation, we cannot exclude the possibility that appetite stimulation could diminish metabolic benefits of BAT recruitment if it increases caloric intake in longer-term studies.

The inducibility, suppressibility and plasticity of human BAT entail implications beyond thermoregulatory physiology. The translation of recently discovered BAT-activators in the laboratory to pharmacologic BAT stimulants available for clinical use is not a trivial process (23). Our study substantiates, in contrast, a simple BAT-modulating strategy: a mild reduction in environmental temperature is capable in recruiting BAT and yielding associated metabolic benefits; conversely, even a small elevation in ambient temperature could impair BAT, and dampen previously attained metabolic benefits. Such reversible metabolic switching, occurring within a temperature range achievable in climate-controlled buildings, therefore carries

446 therapeutic implications of BAT-acclimation, both on an individual and a public
447 health level. Bedroom temperature has gradually increased from 19°C to 21.5°C over
448 the last 3 decades in the US (47). The blunting of BAT function due to widespread
449 use of indoor climate control could be a neglected contribution to the obesity
450 epidemic. Moderate downward adjustment of indoor temperature could represent a
451 simple and plausible strategy in dampening the escalation of obesity on a population
452 level. Our volunteers reported satisfactory sleep during acclimation, although more
453 formal assessment of sleep quality is required in future studies.
454
455 Our findings should be viewed as a proof of concept illustrating human BAT
456 plasticity. We acknowledge the small sample size to be a limitation of our study.
457 Unfortunately, the conduct of long-term acclimation study necessitated substantial
458 resources and regrettably prohibited a large sample size. Despite a small study
459 population, the investigations were undertaken in a tightly monitored and controlled,
460 yet real life-simulating and applicable setting, encompassing the most comprehensive
461 spectrum of energy balance/metabolism to date to tackle a question fundamental to
462 human BAT research: *what is the significance of BAT recruitment?* The unveiled
463 positive relation between acclimated-BAT and glucose homeostasis is clinically
464 relevant. Glucose intolerance is an independent risk factor of cardiovascular mortality
465 and post-prandial hyperglycemia is its earliest manifestation (48). We emphasize a
466 causal linkage could not be definitely ascertained between BAT recruitment and post-
467 prandial insulin sensitivity improvement; however our study provides compelling
468 circumstantial evidence supporting a potential therapeutic role of BAT in impaired
469 glucose metabolism, and calls for the investigation of similar temperature acclimation
470 in individuals with impaired glycemia. Our observation of BAT recruitment

accompanied by insulin sensitization in the absence of significant weight loss echoes animal findings showing glucose homeostasis improvement following fat browning to be greater than expected from adiposity reduction alone (49). Whether it was indeed a result of fat phenotypic and/or adipokine changes merits further studies.

In summary, temperature acclimation modulates BAT abundance and activity, subsequently impacting energy and substrate metabolism in humans. BAT exhibits thermal plasticity intimately related to glucose homeostasis. Harnessing BAT by simple adjustment of ambient temperature could be a new strategy in the combat against obesity, diabetes and related disorders.

Acknowledgements

Paul Lee was supported by an Australian National Health Medical Research Council (NHMRC) Early Career Fellowship, the Diabetes Australia Fellowship and Bushell Travelling Fellowship, and the School of Medicine, University of Queensland. This study was supported by the Intramural Research Program Z01-DK047057-07 of NIDDK and the NIH Clinical Center. We thank Dr Peter Herscovitch and Dr Corina Millo, both from PET Department, Clinical Center, NIH, for advice on PET-CT scanning; Rachel Perron, Christopher Idelson, Sarah Smyth, Jacob Hattenbach and Juan Wang, all from Diabetes Endocrinology Obesity Branch, NIDDK, NIH, for technical assistance; Dilalat Bello and Oretha Potts, from Clinical Center, NIH, for dietary counseling/monitoring, and all nurses in the Clinical Metabolic Unit, NIH, for their nursing care.

P.L., S.S., J.L., A.B.C., R.J.B., K.Y.C., and F.S.C. participated in study concept, design, research, acquisition of data, analysis and discussion of results. W.D. and C.D.W. researched and anslyzed data, and contributed to discussion of results. P.L. wrote the article, and all authors participated in critical revision and approved the final version of the manuscript.

P.L. and F.S.C. are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

The funders have no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

No potential conflicts of interest relevant to this article were reported.

521 **Figure legends**

522

523 **Figure 1 Temperature-dependent BAT acclimation** Panels A-D display
524 representative PET-CT fused images of the cervical-supraclavicular region (left panel:
525 coronal view; right panel: transverse view) of one subject during monthly temperature
526 acclimation. BAT (Hounsfield units: -300 to -10 and SUV≥2) was shown in red.
527 Baseline BAT volume, mean SUV and activity were 26 ml, 2.65 and 0.238 MBq,
528 respectively [Panel A]. All parameters increased following one month of mild cold
529 acclimation (19°C) [Panel B], decreased to nearly baseline level after thermoneutral
530 month (24°C) [Panel C], and was nearly completely abolished at the end of 1-month
531 mild warm exposure in the final month (27°C) [Panel D]. Mean fold changes (N=5) of
532 BAT volume [Panel E], mean SUV [Panel F] and BAT activity [Panel G], relative to
533 month 1 (24°C), were significant across 4-month acclimation. Whole fat activity, as
534 defined by ^{18}F-fluodeoxyglucose uptake within tissue of fat density (Hounsfield units:
535 -300 to -10), followed the same pattern [Panel H], and interacted significantly with
536 temperature acclimation. Room [Panel I] and individual exposed temperatures [Panel
537 J], but not environmental seasonal fluctuations [Panel I], tracked BAT and whole fat
538 metabolic changes in the predicted temperature-dependent manner. Correlative
539 analysis between BAT parameters and temperature exposure is shown in
540 Supplemental Table S1. Individual PET-CT images and temperature profiles are
541 shown in Supplemental Figures S4-S7. *$p<0.05$ compared to month 1 (24°C);
542 #$p<0.05$ compared to month 2 (19°C).

543

544

Figure 2 Metabolic consequences of BAT-acclimation at 19°C Panels A and B compare post-prandial glucose and insulin excursions after mixed meal at 13:00 before and after cold acclimation, respectively, measured at 19°C. Glucose excursions were unchanged but insulin levels decreased, with a significant reduction in AUC, following mild cold acclimation (month 2). Accordingly, adipocyte insulin resistance (IR) was the lowest [Panel C], and Matsuda index (an indicator of insulin sensitivity) was the highest [Panel D] after cold acclimation (month 2). These changes in glucose metabolism were accompanied by an increase in circulating adiponectin [Panel E] and a decrease in circulating leptin [Panel F]. Cold acclimation-induced changes (month 1 to 2) in circulating adiponectin [Panel G] and leptin levels [Panel H] correlated negatively with changes in BAT activity. *Adiponectin* and *leptin* mRNA displayed concordant changes in subcutaneous adipose tissue biopsies with circulating levels and changes in *GLUT4* tracked those of *adiponectin* [Panel I]. $^{a}p<0.05$ compared to month 1 (24°C), $^{b}p<0.05$ compared to month 2 (19°C), $^{c}p<0.05$ compared to month 3 (24°C) and $^{d}p<0.05$ compared to month 4 (27°C).

Figure 3 Metabolic consequences of BAT-acclimatization at 24°C. Panels A and B compare post-prandial glucose and insulin excursions after mixed meal at 13:00 before and after cold acclimatization, respectively, measured at 24°C. Unlike measurements at 19°C [Figure 2A and B], no significant changes were observed in glucose or insulin excursions. Accordingly, adipocyte insulin resistance (IR) [Panel C] and Matsuda index (an indicator of insulin sensitivity) [Panel D] were unchanged. ~~However,~~ Circulating adiponectin increased [Panel E], while leptin decreased [Panel F], identical to measurements observed at 19°C [Figure 2E and F]. Cold acclimatization-induced changes (month 1 to 2) in circulating adiponectin [Panel G]

and leptin levels [Panel H] correlated negatively with changes in BAT activity. In contrast to those observed in adipose tissue [Figure 2I], *Adiponectin* and *GLUT4* mRNA did not change significantly in muscle [Panel I]. $^{c}p<0.05$ compared to month 3 (24°C) and $^{d}p<0.05$ compared to month 4 (27°C).

Figure 4 BAT and beige fat gene changes in adipose tissue biopsies across 4-month acclimatization. Panel A shows changes in general BAT gene expression (general BAT genes are defined as genes ascribed to general BAT function, and do not indicate their developmental origin). Expression of *CIDEA*, but not others, changed significantly (p=0.04) during acclimatization across 4-month period. Panel B shows changes in classic BAT gene expression. Classic BAT genes are defined as those expressed in interscapular BAT in animals or human infants (50). Panel C showed changes in beige fat gene expression. Beige fat genes are defined as those expressed in inducible brown adipocytes, also known as brite or beige adipocytes, found within WAT depots. No significant changes were observed in classic BAT and beige fat genes across temperature acclimation.

589 **Table 1 PET-CT parameters across 4 months of acclimation** At the end of each
590 testing month, subjects underwent acute thermo-metabolic evaluation at either 24°C
591 or 19°C. Results are reported as mean±standard deviation. [a]p<0.05 (month 1 *vs.* 2).
592

	Month 1 24°C	Month 2 19°C	Month 3 24°C	Month 4 27°C	Trend P value
PET-CT parameters					
BAT volume (ml)	55±61	78±84[a]	63±81	58±81	0.036
BAT mean SUV	3.2±0.8	3.8±1.3	3.4±1.0	3.4±0.8	0.35
BAT activity (MBq)	0.65±0.76	1.0±1.3[a]	0.8±1.1	0.7±1.0	0.038
BAT radiodensity (Houndsfield units)	-58.8±7.2	-44.2±6.8	-55.4±6.5	-69.2±6.8	<0.01
Whole fat mean SUV	0.61±0.13	0.68±0.18[a]	0.63±0.17	0.59±0.16	0.035
Muscle mean SUV	0.46±0.08	0.41±0.04	0.43±0.05	0.48±0.08	0.52
Liver mean SUV	1.68±0.08	1.50±0.14	1.61±0.15	1.67±0.16	0.15

593 **Table 2 Physiologic parameters across 4 months of acclimation** At the end of each testing month, subjects underwent acute thermo-metabolic
594 evaluation at either 24°C or 19°C. Results are reported as mean±standard deviation. [a]p<0.05 compared to 24°C during acute thermo-metabolic
595 evaluation each month; [b]p<0.05 (month 1 *vs.* 2), compared to matching measurement at same temperature performed at respective months as
596 indicated.
597

	Month 1 24°C		**Month 2 19°C**		**Month 3 24°C**		**Month 4 27°C**		**Trend P value**	
Physiologic parameters										
Calorimeter °C	24°C	19°C	24°C	19°C	24°C	19°C	24°C	19°C	24°C	19°C
Total EE (kcal)	2472±180	2624±198[a]	2366±358	2543±410[a]	2400±252	2555±346[a]	2341±255	2505±322[a]	0.45	0.46
Respiratory quotient	0.84±0.03	0.84±0.01	0.84±0.02	0.83±0.02	0.85±0.02	0.84±0.02	0.84v0.03	0.85±0.02	0.72	0.47
Total activity (units)	8.4±1.6	8.4±2.8	7.6±2.9	7.1±2.4	6.8±3.7	7.1±4.1	7.9±5.0	7.2±4.7	0.54	0.38
Surface electromyography ($x10^{-6}$ RMS)	2.8±0.4	2.5±1.3	2.7±0.3	2.6±0.2	2.8±0.5	2.7±0.2	2.8±0.3	2.6±0.4	0.98	0.83
CIT (%)	6.2±4.1		7.4±3.1		6.2±3.9		6.8±3.2		0.16	
DIT (%)	10.3±13.1	33.4±18.2	19.0±15.4	42.2±17.4[a,b]	19.1±16.0	37.1±19.6[a]	22.5±11.0	34.4±19.2[a]	0.30	0.36

598
599

600 **Table 3 Nutritional and body compositional parameters across 4 months of acclimation** At the end of each testing month, subjects
601 underwent acute thermo-metabolic evaluation at either 24°C or 19°C. Results are reported as mean±standard deviation. [a]p<0.05 (month 1 *vs.* 2),
602 [b]p<0.05 (months 2 *vs*. 4), compared to matching measurement at same temperature performed at respective months as indicated.
603

	Month 1 24°C	**Month 2 19°C**	**Month 3 24°C**	**Month 4 27°C**	**Trend P value**
Dietary intake					
Caloric (kcal/d)	2530±321	2620±412	2623±342	2514±359	0.32
Protein (g/d)	126±16	131±19	131±15	127±18	0.35
Fat (g/d)	88±9	91±13	93±11	86±10	0.16
Carbohydrate (g/d)	319±42	331±51	329±44	320±48	0.44
Appetite/hunger visual analogue scale					
Hunger AUC	13.1±6.3	20.7±7.0	17.5±6.1	15.5±3.5	0.13
Satiety AUC	35.8±4.6	25.7±8.5	25.5±5.4	27.9±7.9	0.09
Desire to eat AUC	14.6±4.3	20.1±3.4[a,b]	17.9±4.2	16.5±5.3	0.003
Body composition					
Body weight (kg)	74.4±7.3	74.8±7.5	74.9±7.4	74.7±7.7	0.72
Lean mass (kg)	55.8±6.0	56.3±6.1	56.6±6.3	56.1±6.4	0.56
Fat mass (kg)	14.6±0.5	14.5±0.8	14.6±1.4	14.7±1.7	0.95
% body fat	20.92±2.00	20.62±1.64	20.64±2.22	20.88±2.51	0.99

604
605

606 **Table 4 Hormonal and metabolic parameters across 4 months of acclimation** At the end of each testing month, subjects underwent acute
607 thermo-metabolic evaluation at either 24°C or 19°C. Results are reported as mean±standard deviation. [a]p<0.05 compared to 24°C during acute
608 thermo-metabolic evaluation each month; [b]p<0.05 (month 1 *vs.* 2), [c]p<0.05 (months 2 *vs*. 4) and [d]p<0.05 (months 1 *vs*. 4), compared to matching
609 measurement at same temperature performed at respective months as indicated.
610

	Month 1 24°C		**Month 2 19°C**		**Month 3 24°C**		**Month 4 27°C**		**Trend P value**	
Hormonal parameters										
Calorimeter °C	24°C	19°C	24°C	19°C	24°C	19°C	24°C	19°C	24°C	19°C
Sympathoadrenal										
Urinary epinephrine (ug/d)	7.5±5.0	8.0±4.8	7.5±3.9	8.3±5.6	7.5±4.4	7.7±4.6	8.3±6.1	7.7±5.1	0.97	0.94
Urinary norepinephrine (ug/d)	46±29	53±14	56±30	64±20	35±9	61±18[a]	37±5	58±24	0.42	0.56
Glucocorticoid axis										
ACTH AUC (pg.min/ml)	207±61	199±73	199±51	197±65	203±74	176±61	209±71	199±83	0.20	0.40
Cortisol AUC (µg.min/ml)	0.96±0.14	0.91±0.13	0.87±0.10	0.95±0.12	0.81±0.13	0.84±0.17	0.90±0.15	0.88±0.22	0.14	0.39
Urinary cortisol (µg/d)	49±9	36±13	49±27	39±9	39±11	38±11	39±22	49±21	0.42	0.25
Thyroid axis										
TSH AUC (µIU.min/ml)	7.8±3.5	8.0±2.8	7.5±3.6	7.3±2.1	8.6±4.3	7.3±2.2	8.8±3.5[c]	7.6±1.9	0.38	0.39

Free T4 AUC (pg.min/ml)	95±13	95±9	93±12	92±12	93±9	93±11	90±9	94±9	0.39	0.54
Free T3 AUC (pg.min/ml)	26±1	27±1	29±2[b]	28±1	28±3	27±2	26±2[d]	28±2	0.16	0.59
Free T3/free T4 AUC	2381±490	2410±345	2642±574[b]	2556±393	2513±449	2511±466	2515±481	2491±403	0.06	0.41
Glucose and lipid metabolism										
Total glucose AUC (mg.min/ml)	7.38±0.64	7.32±0.37	7.22±0.51	7.25±0.63	7.22±0.73	7.32±0.63	7.14±0.51	7.11±0.74	0.75	0.71
Post prandial glucose AUC (mg.min/ml)	2.73±0.27	2.68±0.34	2.59±0.13	2.68±0.29	2.64±0.40	2.57±0.29	2.64±0.15	2.53±0.27	0.79	0.63
Total insulin AUC (IU.min/L)	170±102	210±83	198±97	143±49	212±118	186±123	171±87	182±128	0.08	0.44
Post prandial insulin AUC (IU.min/L)	106±64	133±57	111±52	77±22	132±85	114±80	103±55	109±79	0.19	0.31
Total free fatty acid AUC (mEq.min/L)	3.53±0.70	3.76±1.05	3.36±0.31	3.37±1.16	2.73±0.86	3.20±0.40	3.65±1.09	3.76±0.41	0.36	0.68
Fasting total cholesterol (mg/dL)	120±24		132±24		117±16		136±11		0.13	
Fasting LDL (mg/dL)	71±21		75±21		62±13		76±10		0.20	
Fasting TG (mg/dL)	57±16		68±27		68±15		65±22		0.31	
Fasting HDL (mg/dL)	38±7		44±7		41±7		46±4[d]		0.03	
Adipokine										
Leptin AUC (ng.min/ml)	16±5	15±6	14±3[b]	12±2[a,b]	29±15	26±11	25±12	25±11[c]	0.01	0.002

Adiponectin AUC (pg.min/ml)	99±38	103±37	117±51[b]	127±49[a,b]	78±31	77±32	74±32[c]	82±38[c]	0.0007	0.0003
FGF21 AUC (pg.min/ml)	333±57	411±104	343±46	460±91[a,b]	350±25	400±80	370±37	435±75	0.28	0.10

611

612

613
614
615
616

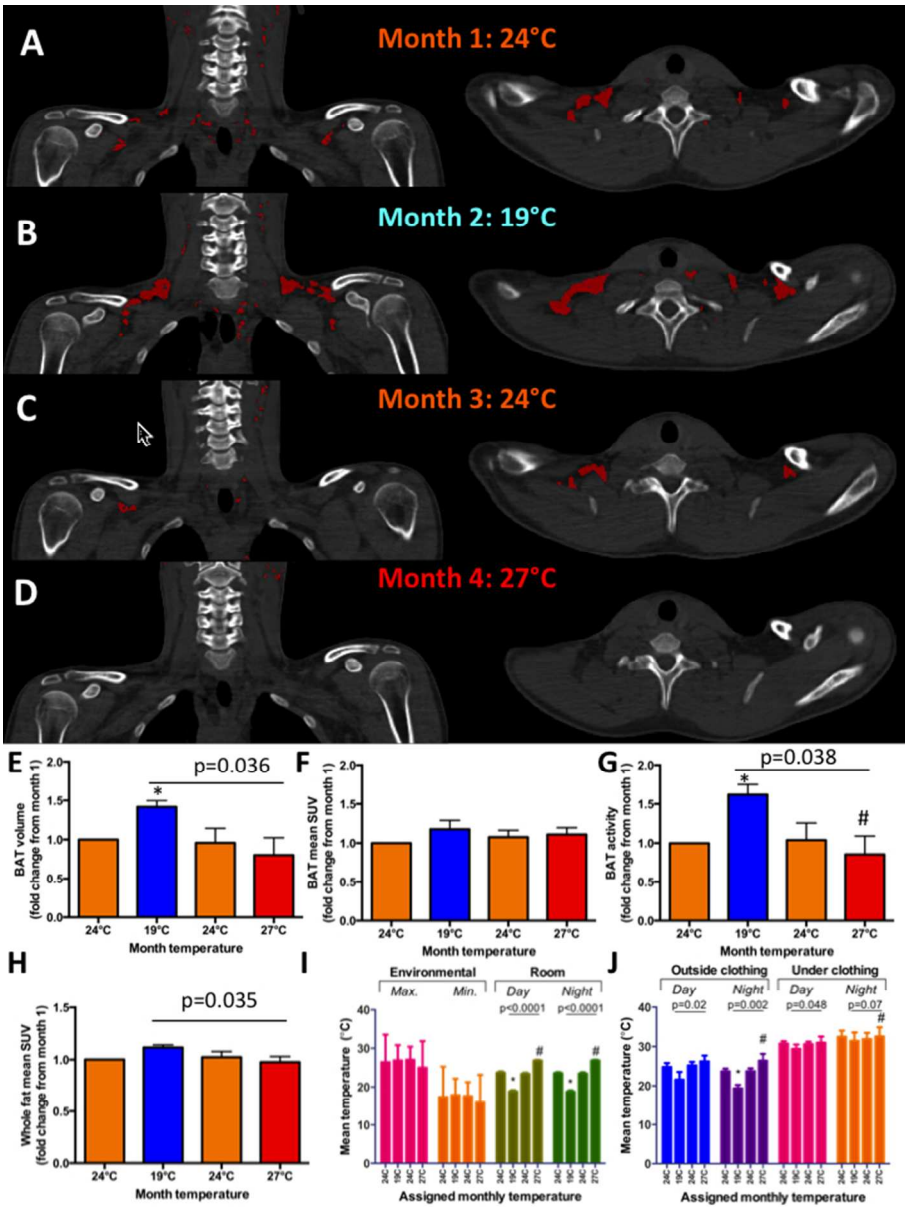
References

1. Keith SW, Redden DT, Katzmarzyk PT, Boggiano MM, Hanlon EC, Benca RM, Ruden D, Pietrobelli A, Barger JL, Fontaine KR, Wang C, Aronne LJ, Wright SM, Baskin M, Dhurandhar NV, Lijoi MC, Grilo CM, DeLuca M, Westfall AO, Allison DB: Putative contributors to the secular increase in obesity: exploring the roads less traveled. Int J Obes (Lond) 2006;30:1585-1594
2. Lowell BB, Bachman ES: Beta-Adrenergic receptors, diet-induced thermogenesis, and obesity. J Biol Chem 2003;278:29385-29388
3. Saito M: Brown adipose tissue as a regulator of energy expenditure and body fat in humans. Diabetes Metab J 2013;37:22-29
4. Cannon B, Nedergaard J: Brown adipose tissue: function and physiological significance. Physiol Rev 2004;84:277-359
5. Petrovic N, Shabalina IG, Timmons JA, Cannon B, Nedergaard J: Thermogenically competent nonadrenergic recruitment in brown preadipocytes by a PPARgamma agonist. Am J Physiol Endocrinol Metab 2008;295:E287-296
6. Wu J, Cohen P, Spiegelman BM: Adaptive thermogenesis in adipocytes: Is beige the new brown? Genes Dev 2013;27:234-250
7. Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng YH, Goodyear LJ: Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest 2013;123:215-223
8. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM: PRDM16 controls a brown fat/skeletal muscle switch. Nature 2008;454:961-967
9. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP, Spiegelman BM: A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481:463-468
10. Heaton JM: The distribution of brown adipose tissue in the human. J Anat 1972;112:35-39
11. Bouillaud F, Combes-George M, Ricquier D: Mitochondria of adult human brown adipose tissue contain a 32 000-Mr uncoupling protein. Biosci Rep 1983;3:775-780
12. Tanuma Y, Tamamoto M, Ito T, Yokochi C: The occurrence of brown adipose tissue in perirenal fat in Japanese. Arch Histol Jpn 1975;38:43-70
13. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR: Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009;360:1509-1517
14. Lee P, Zhao JT, Swarbrick MM, Gracie G, Bova R, Greenfield JR, Freund J, Ho KK: High prevalence of brown adipose tissue in adult humans. J Clin Endocrinol Metab 2011;96:2450-2455
15. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M: High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 2009;58:1526-1531

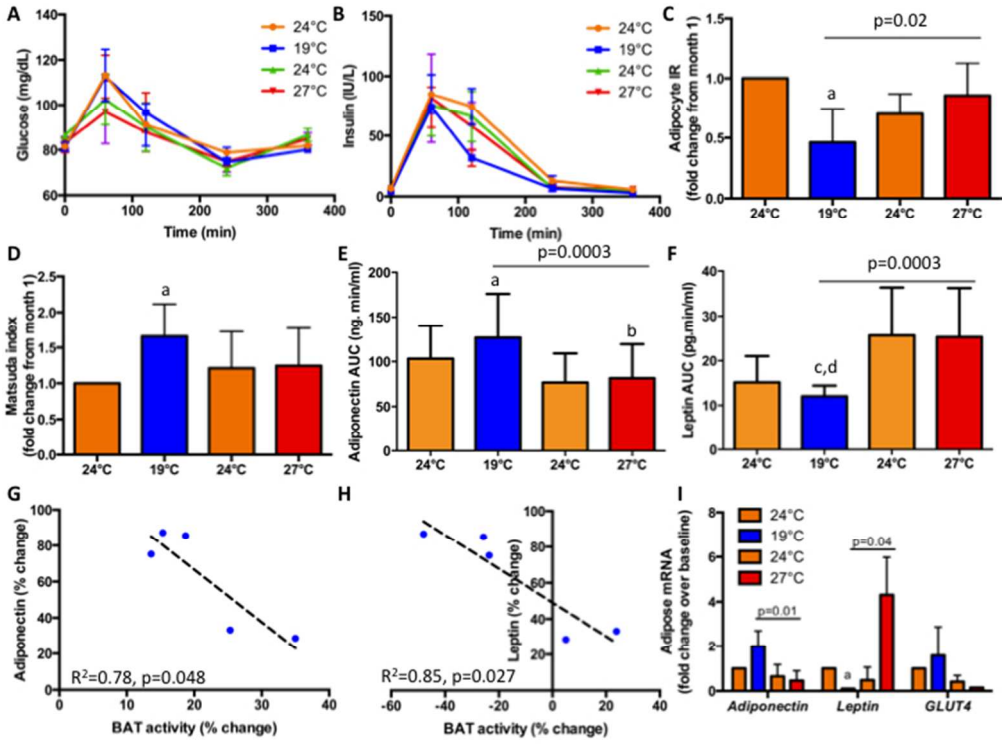
16. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ: Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009;360:1500-1508
17. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P: Functional brown adipose tissue in healthy adults. N Engl J Med 2009;360:1518-1525
18. Lee P, Swarbrick MM, Zhao JT, Ho KK: Inducible brown adipogenesis of supraclavicular fat in adult humans. Endocrinology 2011;152:3597-3602
19. Lee P, Werner CD, Kebebew E, Celi FS: Functional thermogenic beige adipogenesis is inducible in human neck fat. Int J Obes (Lond) 2014;38:170-176
20. Ouellet V, Labbe SM, Blondin DP, Phoenix S, Guerin 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;122:545-552
21. Sharp LZ, Shinoda K, Ohno H, Scheel DW, Tomoda E, Ruiz L, Hu H, Wang L, Pavlova Z, Gilsanz V, Kajimura S: Human BAT Possesses Molecular Signatures That Resemble Beige/Brite Cells. PLoS One 2012;7:e49452
22. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM: Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 2012;150:366-376
23. Lee P, Swarbrick MM, Ho KK: Brown adipose tissue in adult humans: a metabolic renaissance. Endocr Rev 2013;34:413-438
24. Chen KY, Brychta RJ, Linderman JD, Smith S, Courville A, Dieckmann W, Herscovitch P, Millo CM, Remaley A, Lee P, Celi FS: 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:E1218-1223
25. van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ, Hansen J, Jorgensen JA, Wu J, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD: Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J Clin Invest 2013;123:3395-3403
26. 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;123:3404-3408
27. Saito M: Human brown adipose tissue: regulation and anti-obesity potential [Review]. Endocrine journal 2014;
28. Lee P, Greenfield JR, Ho KK, Fulham MJ: A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 2010;299:E601-606
29. 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) 2013;
30. Feldmann HM, Golozoubova V, Cannon B, Nedergaard J: UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. Cell Metab 2009;9:203-209
31. Celi FS, Brychta RJ, Linderman JD, Butler PW, Alberobello AT, Smith S, Courville AB, Lai EW, Costello R, Skarulis MC, Csako G, Remaley A, Pacak K, Chen KY: Minimal changes in environmental temperature result in a significant

713 increase in energy expenditure and changes in the hormonal homeostasis in
714 healthy adults. Eur J Endocrinol 2010;163:863-872
715 32. Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, Perron RM,
716 Werner CD, Phan GQ, Kammula US, Kebebew E, Pacak K, Chen KY, Celi FS: Irisin
717 and FGF21 Are Cold-Induced Endocrine Activators of Brown Fat Function in
718 Humans. Cell Metab 2014;19:302-309
719 33. Cobelli C, Dalla Man C, Toffolo G, Basu R, Vella A, Rizza R: The oral minimal
720 model method. Diabetes 2014;63:1203-1213
721 34. Maia AL, Kim BW, Huang SA, Harney JW, Larsen PR: Type 2 iodothyronine
722 deiodinase is the major source of plasma T3 in euthyroid humans. J Clin Invest
723 2005;115:2524-2533
724 35. Lee P, Brychta RJ, Linderman J, Smith S, Chen KY, Celi FS: Mild cold exposure
725 modulates fibroblast growth factor 21 (FGF21) diurnal rhythm in humans:
726 relationship between FGF21 levels, lipolysis, and cold-induced thermogenesis. J
727 Clin Endocrinol Metab 2013;98:E98-102
728 36. Barneda D, Frontini A, Cinti S, Christian M: Dynamic changes in lipid droplet-
729 associated proteins in the "browning" of white adipose tissues. Biochim Biophys
730 Acta 2013;1831:924-933
731 37. Celi FS: Brown adipose tissue--when it pays to be inefficient. N Engl J Med
732 2009;360:1553-1556
733 38. Skarulis MC, Celi FS, Mueller E, Zemskova M, Malek R, Hugendubler L,
734 Cochran C, Solomon J, Chen C, Gorden P: Thyroid hormone induced brown
735 adipose tissue and amelioration of diabetes in a patient with extreme insulin
736 resistance. J Clin Endocrinol Metab 2010;95:256-262
737 39. Andersen S, Kleinschmidt K, Hvingel B, Laurberg P: Thyroid hyperactivity
738 with high thyroglobulin in serum despite sufficient iodine intake in chronic cold
739 adaptation in an Arctic Inuit hunter population. Eur J Endocrinol 2012;166:433-
740 440
741 40. Orava J, Nuutila P, Lidell ME, Oikonen V, Noponen T, Viljanen T, Scheinin M,
742 Taittonen M, Niemi T, Enerback S, Virtanen KA: Different metabolic responses of
743 human brown adipose tissue to activation by cold and insulin. Cell Metab
744 2011;14:272-279
745 41. Holland WL, Adams AC, Brozinick JT, Bui HH, Miyauchi Y, Kusminski CM,
746 Bauer SM, Wade M, Singhal E, Cheng CC, Volk K, Kuo MS, Gordillo R,
747 Kharitonenkov A, Scherer PE: An FGF21-adiponectin-ceramide axis controls
748 energy expenditure and insulin action in mice. Cell Metab 2013;17:790-797
749 42. Hu HH, Wu TW, Yin L, Kim MS, Chia JM, Perkins TG, Gilsanz V: MRI detection
750 of brown adipose tissue with low fat content in newborns with hypothermia.
751 Magn Reson Imaging 2014;32:107-117
752 43. Hu HH, Smith DL, Jr., Nayak KS, Goran MI, Nagy TR: Identification of brown
753 adipose tissue in mice with fat-water IDEAL-MRI. J Magn Reson Imaging
754 2010;31:1195-1202
755 44. Ye L, Wu J, Cohen P, Kazak L, Khandekar MJ, Jedrychowski MP, Zeng X, Gygi
756 SP, Spiegelman BM: Fat cells directly sense temperature to activate
757 thermogenesis. Proc Natl Acad Sci U S A 2013;110:12480-12485
758 45. Nordstrom EA, Ryden M, Backlund EC, Dahlman I, Kaaman M, Blomqvist L,
759 Cannon B, Nedergaard J, Arner P: A human-specific role of cell death-inducing
760 DFFA (DNA fragmentation factor-alpha)-like effector A (CIDEA) in adipocyte
761 lipolysis and obesity. Diabetes 2005;54:1726-1734

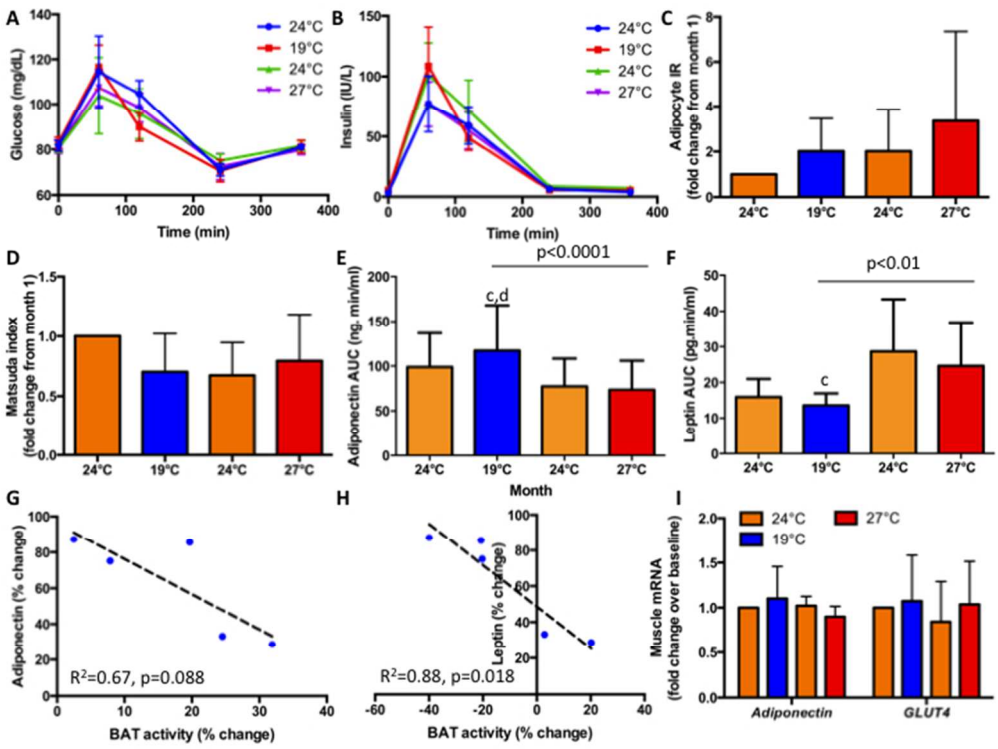
762 46. Wu L, Zhou L, Chen C, Gong J, Xu L, Ye J, Li D, Li P: Cidea controls lipid droplet
763 fusion and lipid storage in brown and white adipose tissue. Science China Life
764 sciences 2014;57:107-116
765 47. Johnson F, Mavrogianni A, Ucci M, Vidal-Puig A, Wardle J: Could increased
766 time spent in a thermal comfort zone contribute to population increases in
767 obesity? Obes Rev 2011;12:543-551
768 48. Glucose tolerance and mortality: comparison of WHO and American Diabetes
769 Association diagnostic criteria. The DECODE study group. European Diabetes
770 Epidemiology Group. Diabetes Epidemiology: Collaborative analysis Of
771 Diagnostic criteria in Europe. Lancet 1999;354:617-621
772 49. Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, Cohen P, Cinti
773 S, Spiegelman BM: Prdm16 determines the thermogenic program of
774 subcutaneous white adipose tissue in mice. J Clin Invest 2011;121:96-105
775 50. Rosen ED, Spiegelman BM: What we talk about when we talk about fat. Cell
776 2014;156:20-44
777



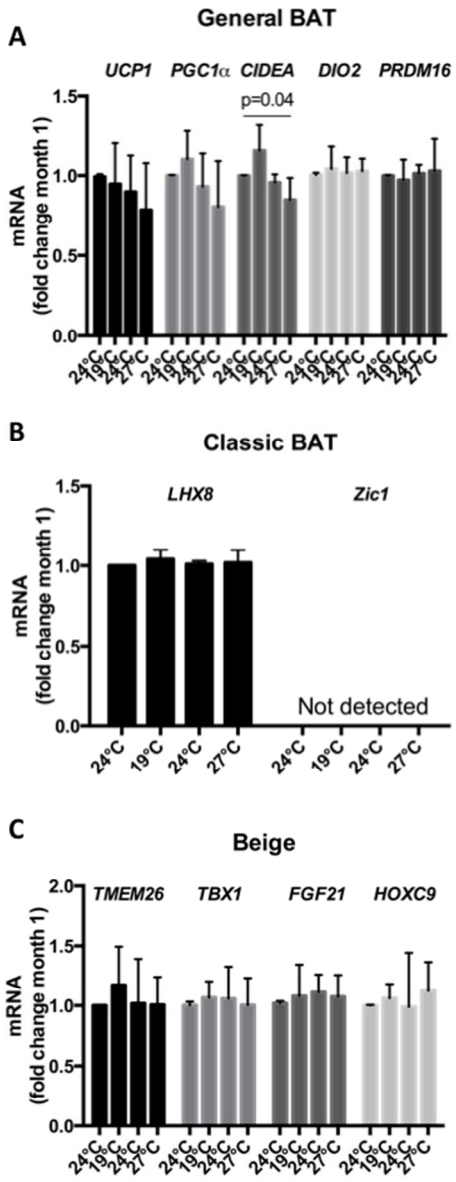
Temperature-dependent BAT acclimation
254x338mm (72 x 72 DPI)



Metabolic consequences of BAT-acclimation at 19°C
254x190mm (72 x 72 DPI)



Metabolic consequences of BAT-acclimatization at 24°C
254x190mm (72 x 72 DPI)



BAT and beige fat gene changes in adipose tissue biopsies across 4-month acclimatization
254x338mm (72 x 72 DPI)

SUPPLEMENTAL DATA

Supplement to: **Lee *et al.* Temperature-acclimated brown adipose tissue modulates insulin sensitivity in humans**

Contents

Table S1 Correlation coefficients between BAT parameter fold changes and temperature exposure over 4-month acclimatization. No relationships were seen between BAT parameters and environmental seasonal fluctuations. In contrast, strong correlations were observed between controlled room temperature and individual exposed temperatures with BAT and whole fat metabolic activity. *p<0.05, #p<0.01 and ^^p<0.10.

Pearson Correlation coefficients	BAT volume	Mean SUV	BAT activity	Whole fat activity	Environmental Temp		Room Temp		Outside clothing Temp		Under clothing Temp	
					Max	Min	Day	Night	Day	Night	Day	Night
BAT volume		0.56	0.99*	0.97*	0.63	0.76	-0.98*	-0.98*	-1.00#	-0.99*	-0.98*	-0.85
Mean SUV	0.56		0.67	0.68	0.02	0.06	-0.48	-0.47	-0.57	-0.50	-0.69	-0.66
BAT activity	0.99*	0.67		0.99#	0.60	0.71	-0.97*	-0.97*	-0.99*	-0.98*	-1.00#	-0.89
Whole fat activity	0.97*	0.68	0.99#		0.65	0.74	-0.97*	-0.97*	-0.97*	-0.97*	-0.99#	-0.94^^
Environmental maximum temp	0.63	0.02	0.60	0.65		0.97*	-0.77	-0.77	-0.60	-0.73	-0.58	-0.76
Environmental minimum temp	0.76	0.06	0.71	0.74	0.97*		-0.87	-0.87	-0.74	-0.84	-0.70	-0.78
Room day temp (day)	-0.98*	-0.48	-0.97*	-0.97*	-0.77	-0.87		1.00#	0.97*	1.00#	0.96*	0.90^^
Room temp (night)	-0.98*	-0.47	-0.97*	-0.97*	-0.77	-0.87	1.00#		0.97*	1.00#	0.96*	0.89
Outside clothing temp (day)	-1.00#	-0.57	-0.99*	-0.97*	-0.60	-0.74	0.97*	0.97*		0.98*	0.98*	0.84
Outside clothing temp (night)	-0.99*	-0.50	-0.98*	-0.97*	-0.73	-0.84	1.00#	1.00#	0.98*		0.97*	0.89
Under clothing temp (day)	-0.98*	-0.69	-1.00#	-0.99#	-0.58	-0.70	0.96*	0.96*	0.98*	0.97*		0.90^^
Under clothing temp (night)	-0.85	-0.66	-0.89	-0.94^^	-0.76	-0.78	0.90^^	0.89	0.84	0.89	0.90^^	

Table S2 Taqman gene expression assays

Gene name	Catalogue number
Adiponectin	Hs00605917_m1
Leptin	Hs00174497_m1
GLUT4	Hs00168966_m1
UCP1	Hs00222452_m1
PRDM16	Hs00922674_m1
PGC1α	Hs01016719_m1
CIDEA	Hs00154455_m1
DIO2	Hs00988260_m1
ZIC1	Hs00602749_m1
LHX8	Hs00418293_m1
TBX1	Hs00271949_m1
TMEM26	Hs00415619_m1
HOXC9	Hs00396786_m1
FGF21	Hs00173927_m1
TBP	Hs00427620_m1

Table S3 Fasting hormonal and metabolic parameters across 4 months of acclimation At the end of each testing month, subjects underwent acute thermo-metabolic evaluation at either 24°C or 19°C. Results of measurements obtained at 7am after 24 hour exposure to either 24°C or 19°C are reported as mean±standard deviation. [a]p<0.05 compared to 24°C during acute thermo-metabolic evaluation each month; [b]p<0.05 (month 1 *vs.* 2), [c]p<0.05 (months 2 *vs*. 4), [d]p<0.05 (months 1 *vs*. 4), and [e]p<0.05 (months 2 *vs*. 3), compared to matching measurement at same temperature performed at respective months as indicated.

	Month 1 24°C		**Month 2 19°C**		**Month 3 24°C**		**Month 4 27°C**		**Trend P value**	
Hormonal parameters										
Calorimeter °C	24°C	19°C	24°C	19°C	24°C	19°C	24°C	19°C	24°C	19°C
Glucocorticoid axis										
ACTH (pg/ml)	25.2±9.9	29.4±13.6	35.0±16.4	28.0±8.4	24.8±10.2	29.6±13.4	28.1±17.4	23.8±11.9	0.60	0.21
Cortisol (µg/dl)	15.5±2.7	15.0±4.1	16.7±4.6	15.9±5.0	14.0±1.9	14.9±5.6	12.7±1.6	12.2±3.9	0.26	0.53
Thyroid axis										
TSH (µIU/ml)	1.1±0.5	1.3±0.5	1.1±0.6	1.3±0.5	1.3±0.6	1.5±0.7	1.3±0.6	1.3±0.4	0.29	0.59
Free T4 (ng/dL)	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.2	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1	0.74	0.93
Free T3 (µg/dl)	312±29	334±12	340±11	337±14	313±6	346±49	341±48	328±24	0.14	0.66
Glucose and lipid metabolism										
Glucose (mg/ml)	85.8±4.2	85.2±3.7	82.6±6.4	85.0±5.7	90.0±6.0	84.4±16.3	84.6±6.1	86.8±3.3	0.19	0.98

Insulin (IU/L)	6.2±3.5	8.4±2.5	10.6±5.0	7.6±2.6	7.6±3.0	8.4±4.0	8.2±3.9	8.4±4.0	0.26	0.95
Free fatty acid (mEq/L)	0.3±0.2	0.3±0.1	0.4±0.1	0.3±0.1	0.3±0.1	0.2±0.1	0.4±0.2	0.3±0.1	0.35	0.22
Adipokine										
Leptin (ng/ml)	2.7±1.1[d]	2.6±1.1[d]	2.2±0.8 [c]	1.9±0.6[c,e]	4.6±2.1	4.1±1.8[a]	4.8±2.1	4.1±1.8[a]	<0.01	<0.01
Adiponectin (pg/ml)	10.6±4.1	10.6±4.2	12.7±5.4	13.7±5.8[a,b,c,e]	8.0±3.3	8.2±3.5	8.0±3.8	8.5±3.9	<0.01	<0.01

Figure legends

Figure S1 Flow chart. Flow chart of volunteer recruitment, allocation and intervention.

Figure S2 Overall outline of acclimatization and thermo-metabolic evaluation protocol This is a prospective crossover study consisting of 4 consecutive blocks of studies of 1-month duration each. It incorporates i) sequential monthly thermal acclimatization over a 4-month period, and ii) acute thermo-metabolic evaluations at the end of each study temperature regime. Procedures undertaken during each acute thermo-metabolic evaluation were listed. Volunteers underwent two 24-hour sessions of observation, while exposed to first 24°C and then 19°C, with a resting non-testing period of 1 day in between. PET-CT scanning was performed after the 19°C testing day at the end of each acclimatization month. Each subject undertook a total of four PET-CT scans during the entire study. During the two 24-hour sessions, volunteers wore standardized hospital clothing with a combined thermal insulation value of 0.4 (clo). Subjects were fasted in the morning (8 hours from previous night) to allow fasting blood samples to be collected. The meals served during study were caffeine-free with fixed macronutrient contents (Lunch and dinner: one-third and two-thirds of daily caloric intake, respectively). Volunteers were informed to minimize physical activity during testing. Hormonal and metabolic parameters were measured in the calorimeter, at time points as indicated, to allow AUC computation (FGF21 AUC was calculated incorporating 5 time points (0, 1, 4, 5, 9 hours); AUC of other hormones/substrates incorporated all 10 time points.) The following procedures were undertaken during the two 24-hour thermo-

metabolic evaluations: real-time energy expenditure, RQ, spontaneous movements, blood sampling for hormonal/substrate measurements, 12-hour urine collection for catecholamine and cortisol, and optional subcutaneous adipose tissue and/or muscle biopsy.

Figure S3 Three-dimensional region of interest (ROI) constructed for comparison of BAT volume and activity across 4-month period. The region was defined cranially by a horizontal line (blue) parallel to the base of C4 vertebra, and caudally by an oblique line (maroon) traversing the manubriosternal joint and superior aspect of the T8 transverse process. Panel A displayed sagittal sections of PET-CT of one subject, showing the defined ROI extending from midline to the arm (left to right). Coronal sections are displayed, extending anteriorly from sternoclavicular joint to the vertebral column posteriorly in Panel B (left to right). The ROI captures all major BAT depots (in red) in humans: cervical, supraclavicular, superior mediastinal, axillary and paravertebral depots, as shown in the sagittal and coronal images.

Figure S4-S7 Temperature-dependent BAT acclimatization of individual subjects Panels A-D in each Figure show representative PET-CT fused images of the cervical-supraclavicular region (left panel: coronal view; right panel: transverse view) of one subject during monthly temperature acclimatization. BAT (Hounsfield units: -300 to -10 and SUV>2) was shown in red. Panels E-G show fold changes of BAT volume [Panel E], mean SUV [Panel F] and BAT activity [Panel G] relative to month 1 (24°C) across 4 months of acclimatization. Fold change in whole fat activity, as defined by ^{18}F-

fluodeoxyglucose uptake within tissue of fat density (Hounsfield units: -300 to -10), is displayed in [Panel H]. Two subjects [Figure S5 and S7] had little visible BAT using a SUV threshold of ≥2.0. However, both subjects manifested an increase in whole fat activity, indicating enhancement of fat metabolism [Panel H], but the overall level did not reach the SUV threshold. This is illustrated in Figure S8, showing PET-CT images with lower SUV threshold (≥1.0), and the temperature-dependent effects on fat activity.

Figure S8 Temperature-dependent fat activity of subjects with low BAT status using lower SUV threshold. Panel A and Panel B show re-analysis PET-CT images of two subjects with little visible BAT using a SUV threshold of ≥2 (see Figure S5 and S7) using a SUV threshold of ≥1. Adipose tissue (Hounsfield unit: -300 to -10) is shown in green in coronal CT sections on the left column, which display the major supraclavicular fat depot at the sterno-clavicular joint. Adipose tissue with SUV≥1 is shown in orange in the corresponding PET images in the right column. This "low-activity" BAT increased following one month of cold acclimatization (19°C), decreased to nearly baseline level after thermoneutral month (24°C), and was completely abolished at the end of 1 month warm exposure in the final month (27°C) in both subjects. These results are concordant with overall changes in fat metabolic activity during acclimatization, shown in Panel H of Figure S5 and S7.

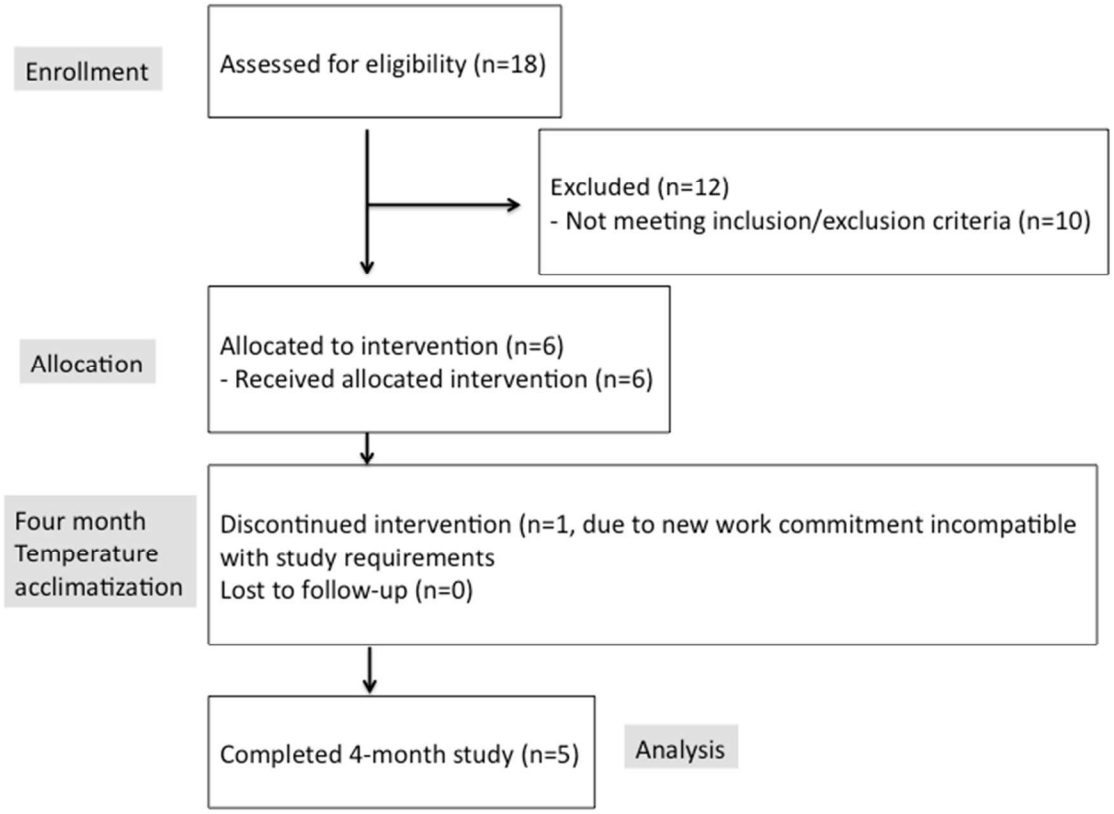
Figure S9 Summary of individual perception to cold sensation during study period

Participants reported subjectively their perception to cold sensation (how cold do you feel) at the end of each month after 24 hour exposure to 19°C on a visual analogue scale

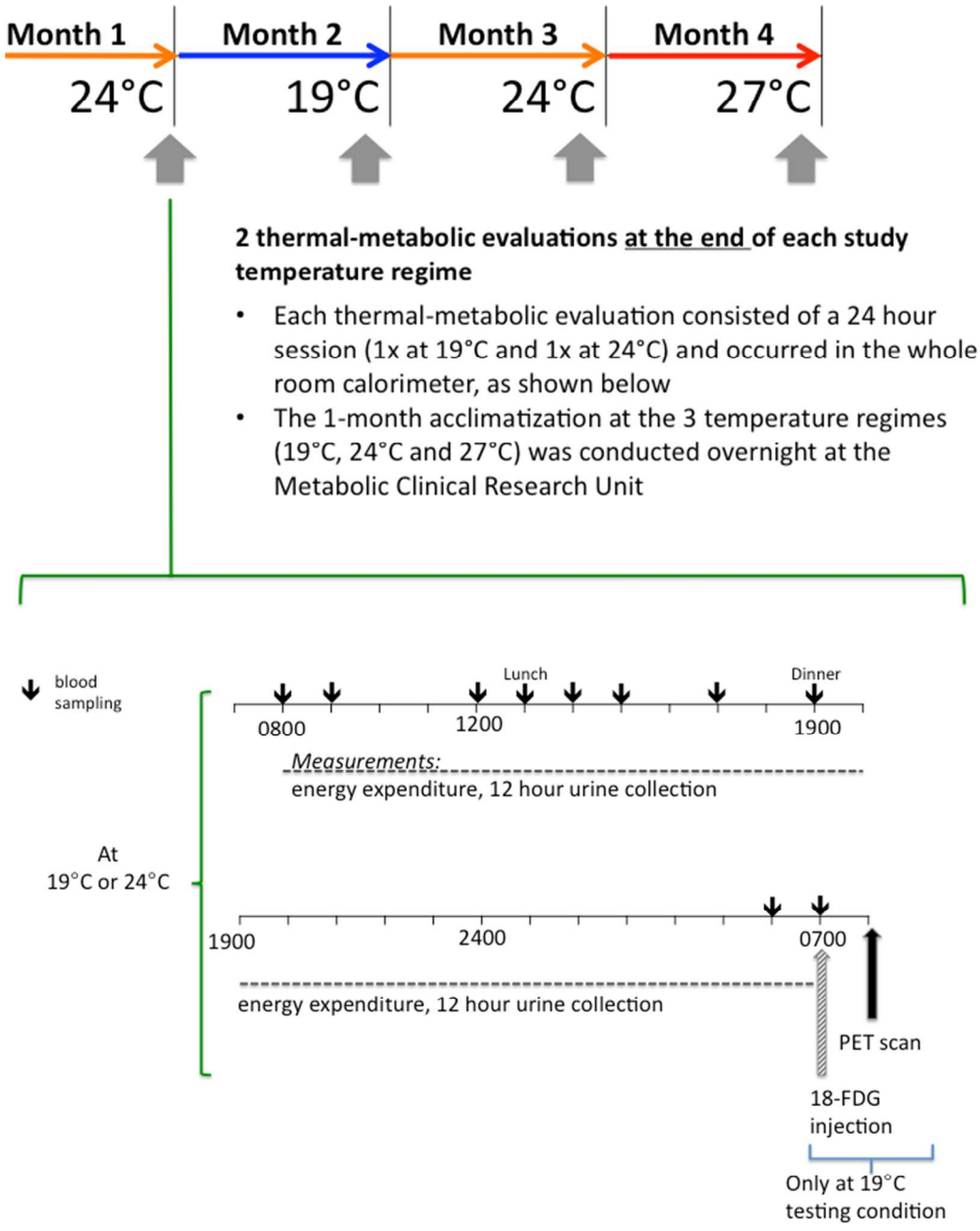
from 1 (not cold at all) to 10 (extremely cold). Perception to cold did not change during monthly acclimation.

Figure S10 Summary of appetite visual analogue scale questionnaires Panels A-C (pre-meal) and D-F (post-meal) show mean scores of the three displayed questions obtained from bi-weekly questionnaires. Panels G-I show AUC scores to the three questions obtained during monthly *ad libitum* meal test. No significant trends were observed in biweekly questionnaires. However, during *ad libitum* meal test, there was a strong trend for subjects to report increased hunger [Panel G] and decreased satiety [Panel H] following cold acclimatization (month 2), with scores returning to baseline following sequential re-warming during months 3 and 4. Scores reflecting changes in desire to eat were concordant to hunger and satiety scores, reached significance across 4 month acclimatization [Panel I]. *$p<0.05$, compared to month 1 and $^{\#}p<0.05$, compared to month 2.

Figure S1


Enrollment
Assessed for eligibility (n=18)
Excluded (n=12)
- Not meeting inclusion/exclusion criteria (n=10)
Allocation
Allocated to intervention (n=6)
- Received allocated intervention (n=6)
Four month Temperature acclimatization
Discontinued intervention (n=1, due to new work commitment incompatible with study requirements
Lost to follow-up (n=0)
Completed 4-month study (n=5)
Analysis

137 **Figure S2**

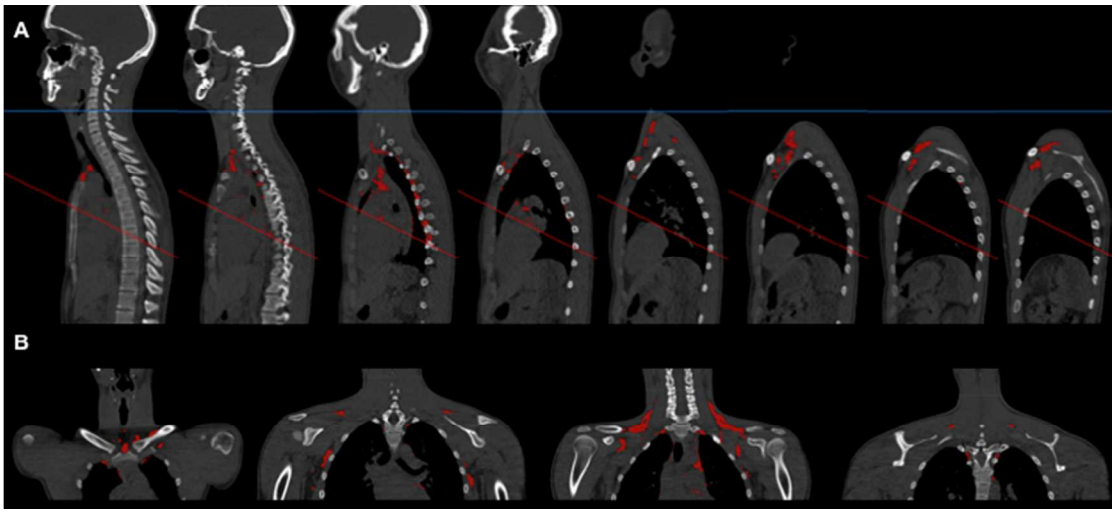


138

139

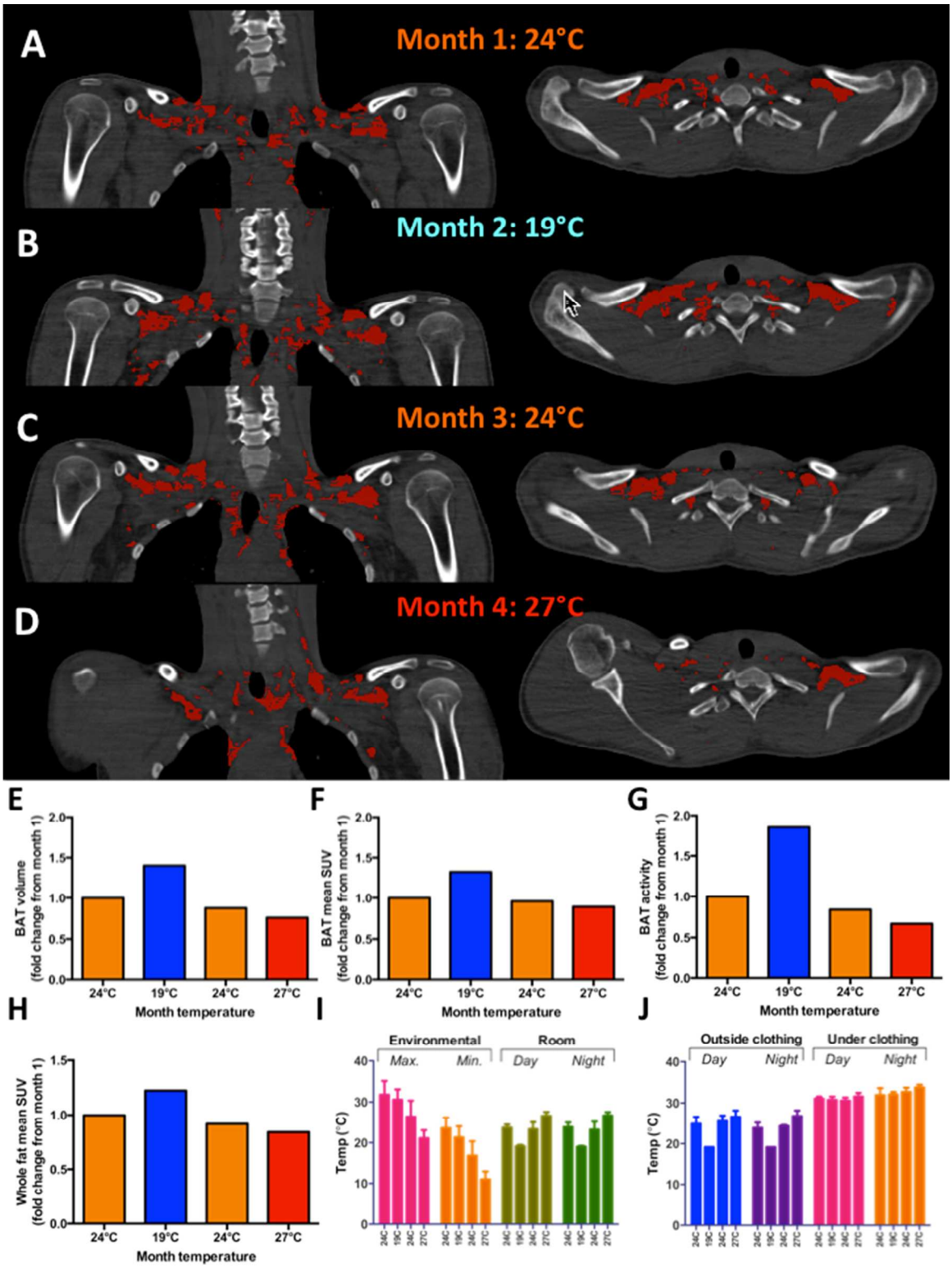
140

Figure S3



146 **Figure S4**

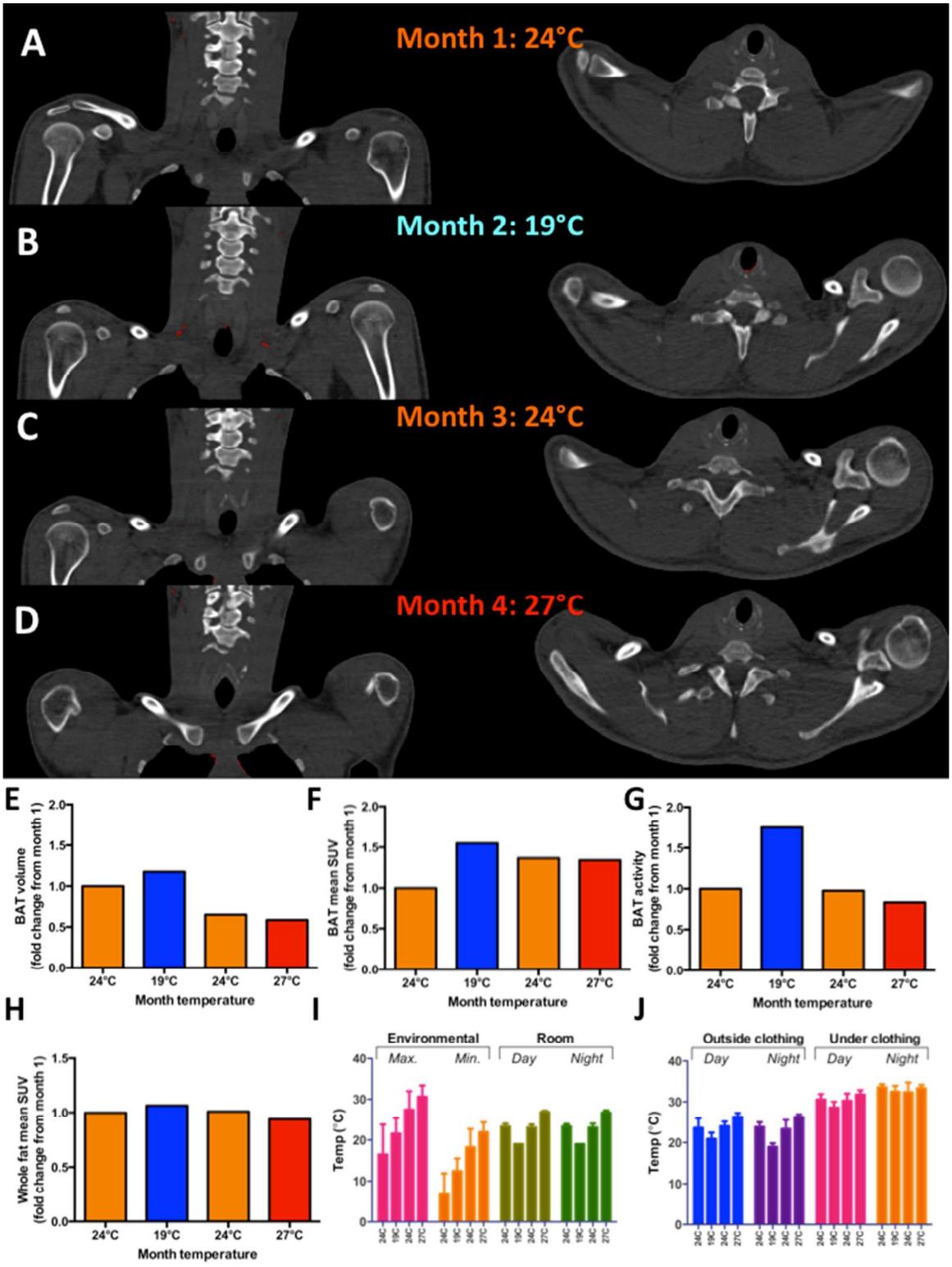
147



148

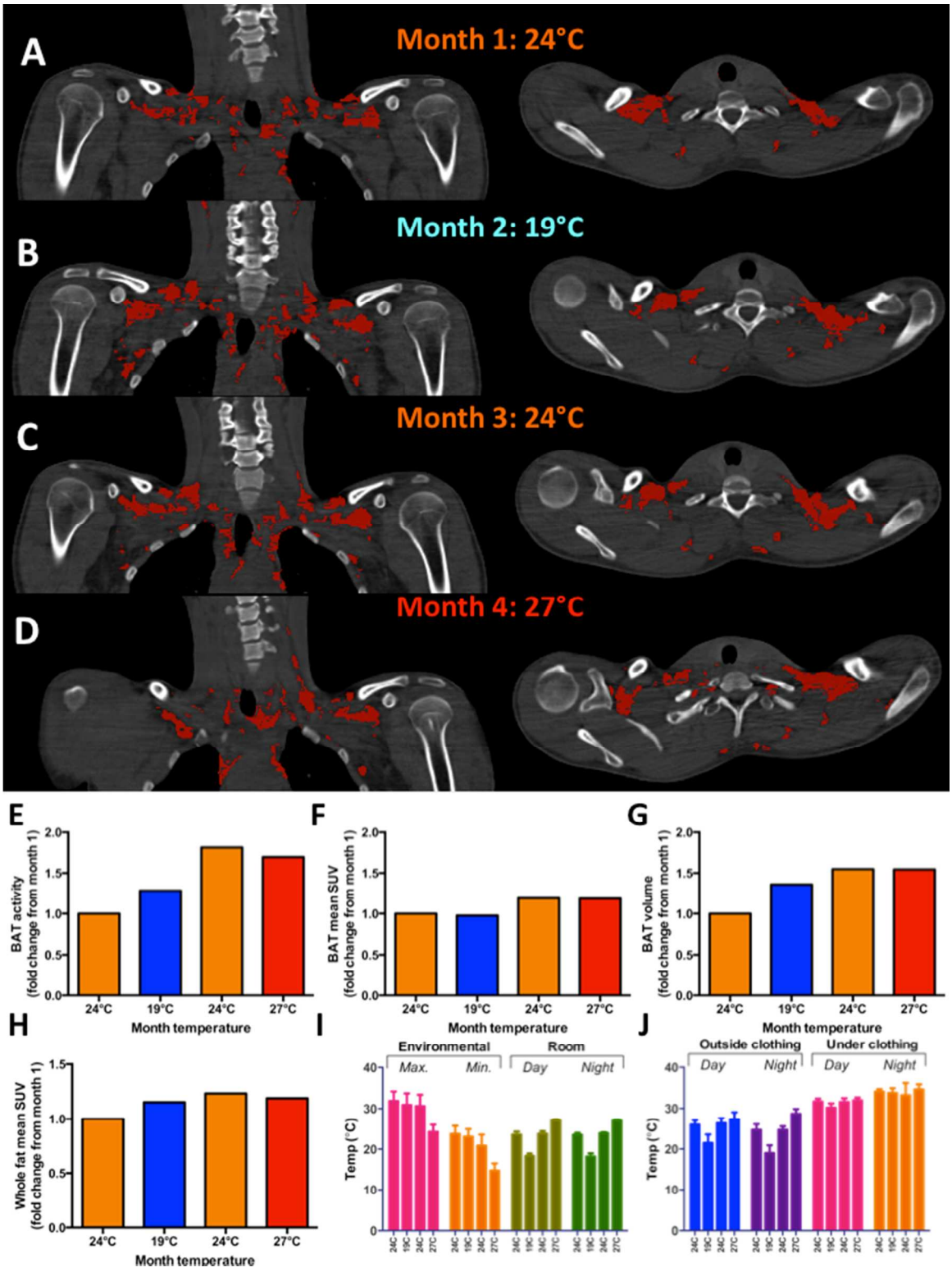
149

Figure S5


A
Month 1: 24°C
B
Month 2: 19°C
C
Month 3: 24°C
D
Month 4: 27°C
E
BAT volume (fold change from month 1)
F
BAT mean SUV (fold change from month 1)
G
BAT activity (fold change from month 1)
24°C
19°C
24°C
27°C
Month temperature
H
Whole fat mean SUV (fold change from month 1)
I
Environmental
Max.
Min.
Room
Day
Night
Temp (°C)
J
Outside clothing
Under clothing
Day
Night

153 **Figure S6**

154

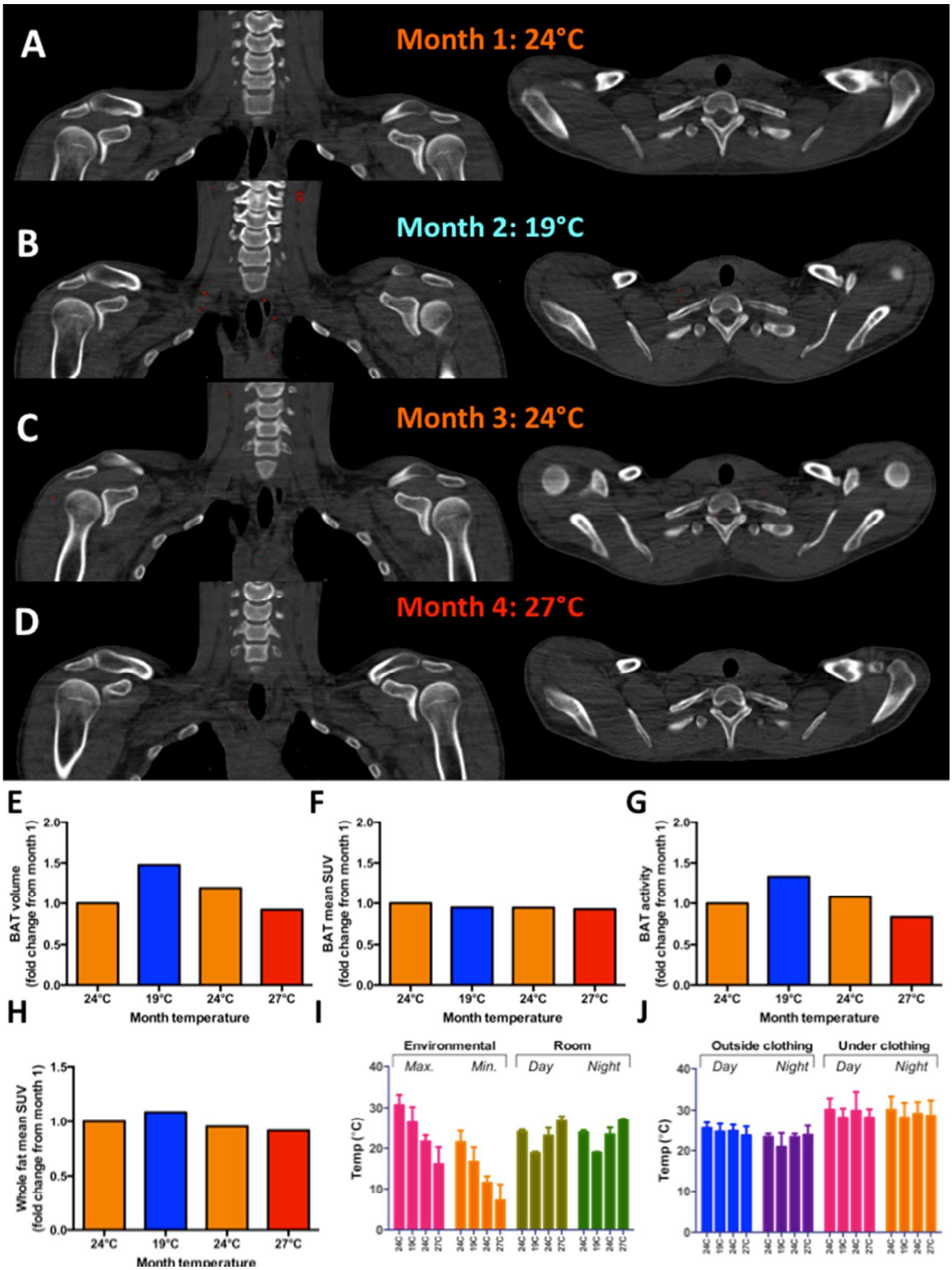


155

156

157 **Figure S7**

158



159

Figure S8

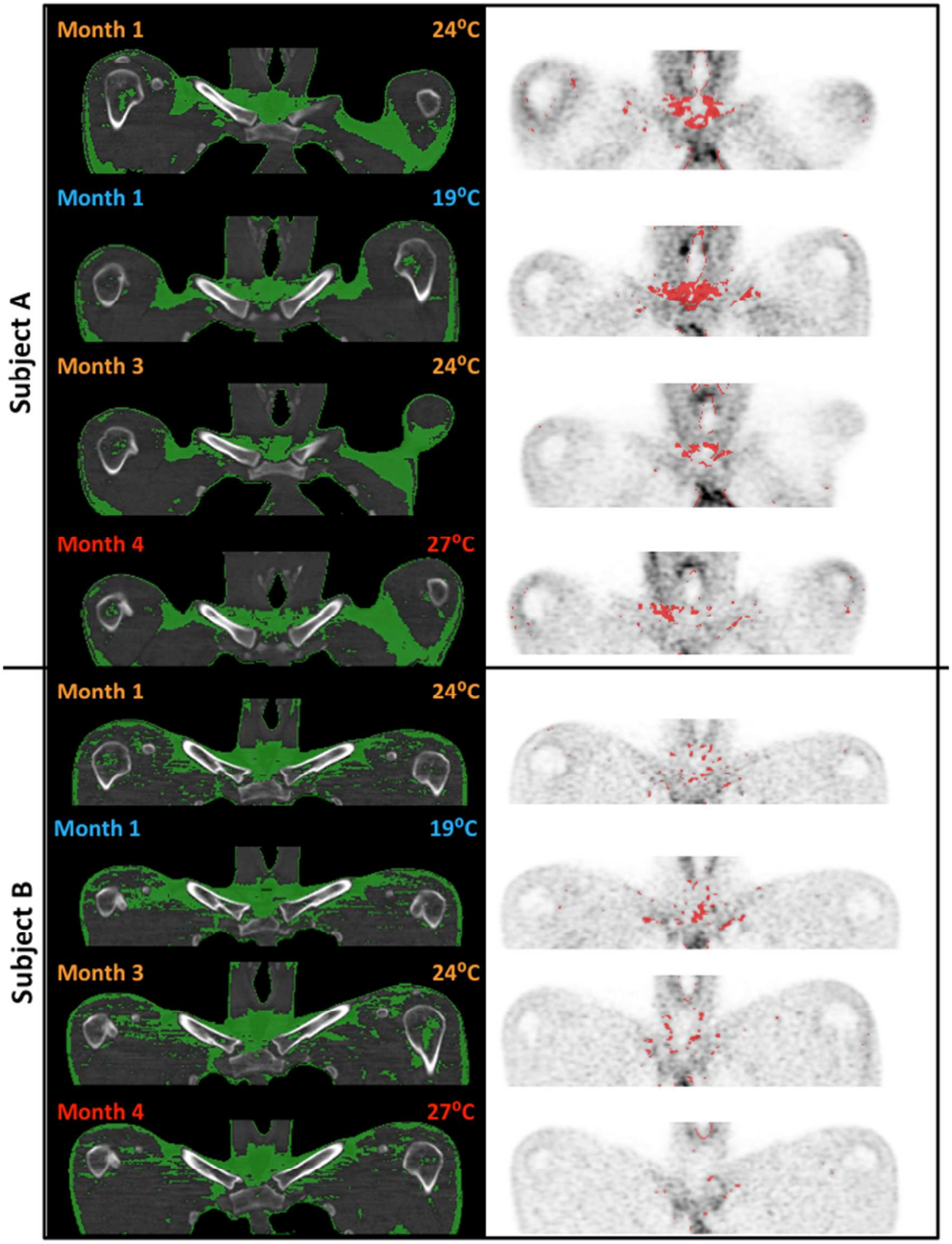

Subject A
Month 1
24°C
Month 1
19°C
Month 3
24°C
Month 4
27°C
Subject B
Month 1
24°C
Month 1
19°C
Month 3
24°C
Month 4
27°C

Figure S9

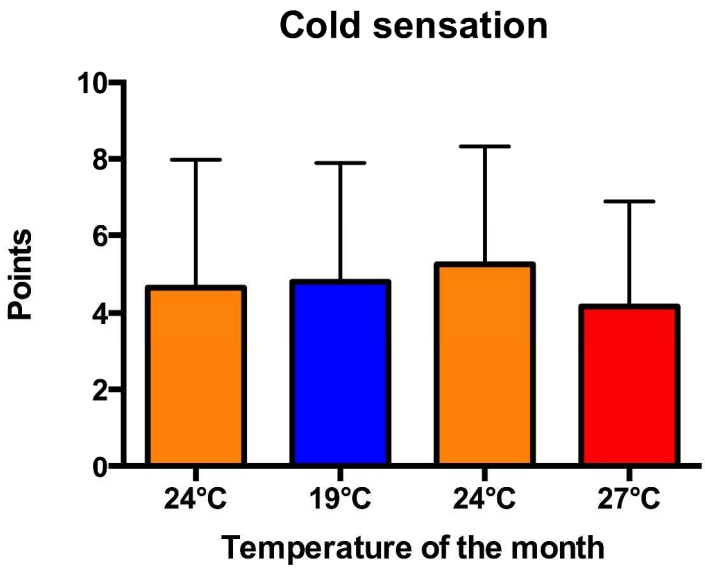
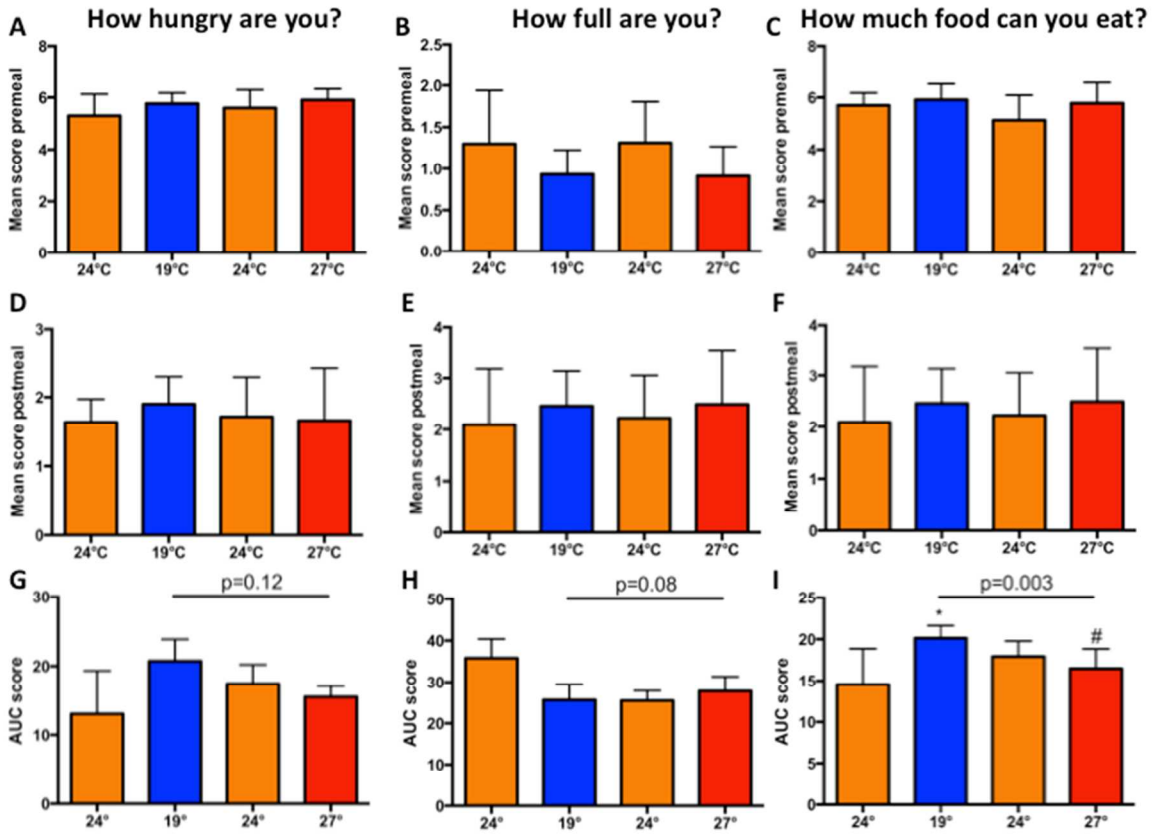

Cold sensation
Points
10
8
6
4
2
0
24°C
19°C
24°C
27°C
Temperature of the month

Figure S10


A
How hungry are you?
Mean score premeal
24°C
19°C
24°C
27°C
B
How full are you?
C
How much food can you eat?
D
Mean score postmeal
E
F
G
p=0.12
AUC score
H
p=0.08
I
p=0.003
*
#

References:

1. Celi FS, Brychta RJ, Linderman JD, et al. Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults. Eur J Endocrinol 2010;163:863-72.
2. Brychta RJ, Rothney MP, Skarulis MC, Chen KY. Optimizing energy expenditure detection in human metabolic chambers. Conf Proc IEEE Eng Med Biol Soc 2009;2009:6864-8.
3. de Jonge L, Nguyen T, Smith SR, Zachwieja JJ, Roy HJ, Bray GA. Prediction of energy expenditure in a whole body indirect calorimeter at both low and high levels of physical activity. Int J Obes Relat Metab Disord 2001;25:929-34.
4. Chen KY, Acra SA, Donahue CL, Sun M, Buchowski MS. Efficiency of walking and stepping: relationship to body fatness. Obes Res 2004;12:982-9.
5. Chen KY, Sun M. Improving energy expenditure estimation by using a triaxial accelerometer. J Appl Physiol 1997;83:2112-22.
6. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 2007;293:E444-52.
7. Lee P, Greenfield JR, Ho KK, Fulham MJ. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 2010;299:E601-6.
8. Tarnopolsky MA, Pearce E, Smith K, Lach B. Suction-modified Bergstrom muscle biopsy technique: experience with 13,500 procedures. Muscle Nerve 2011;43:717-25.