Mild cold effects on hunger, food intake, satiety and skin temperature in humans

Langeveld M[1]*, Tan CY[1]*, Soeters MR[1]*, Virtue S[1], Watson LPE[1,2], Murgatroyd PR[1,2], Chatterjee VK[1], Vidal-Puig A[1] * shared first author

[1]University of Cambridge Metabolic Research Laboratories, Wellcome Trust-MRC, Institute of Metabolic Science, Addenbrookes Hospital, Cambridge, UK

[2]NIHR/Wellcome Trust Clinical Research Facility, Addenbrookes Hospital, Cambridge, UK

Key words: cold, thermogenesis, hunger

Word count abstract: 244, **word count text**: 3316

Corresponding author:

M.R. Soeters MD PhD

University of Cambridge, Metabolic ResearchLaboratories Level 4

Institute of Metabolic Science, Box 289, Addenbrooke's Hospital

Cambridge, CB2 0QQ, United Kingdom

Tel + 44-1223-769090, Email: mrsoeters@gmail.com

Abstract

Mild cold exposure increases energy expenditure and can influence energy balance, when appetite and energy intake do not increase at the same time. We exposed healthy volunteers to either a single episode of environmental mild cold and thermoneutrality. We measured hunger sensation and actual free food intake. To quantify dermal insulative cold response, we assessed thermal comfort and skin temperatures changes by infrared thermography.

After a thermoneutral overnight stay, five males and five females were exposed to either 18 degrees centigrade (mild cold) or 24 degrees centigrade (thermoneutrality) for 2.5 hours. Metabolic rate, vital signs, skin temperature, blood biochemistry, cold and hunger scores were measured at baseline and every 30 minutes during the temperature intervention. This was followed by an ad libitum meal to obtain actual *desired* energy intake after cold exposure.

We could replicate the cold induced increase in REE. But no differences in hunger, food intake or satiety after mild cold exposure compared to thermoneutrality were detected. After longer cold exposure, high cold sensation scores were reported, which were negatively correlated to thermogenesis. Skin temperature in the sternal area was tightly correlated to the increase in energy expenditure.

In conclusion, short-term mild cold exposure increases energy expenditure without changes in food intake. Mild cold exposure resulted in significant thermal discomfort, which was negatively correlated to the increase in energy expenditure. Moreover there is great between subject variability in cold response. These data provide further insight on cold exposure as an anti-obesity measure.

Abbreviations list

BAT, brown adipose tissue; NEFA, non-esterified fatty acids, NST, non-shivering thermogenesis; REE, resting energy expenditure; TSH: thyroid stimulating hormone; UEM, universal eating monitor

Introduction

At first sight obesity may appear as a condition that is easy to treat by either decreasing energy intake and/or increasing energy expenditure. In practice, long-term weight loss is very difficult to achieve. Since strategies that reduce energy intake fail in the majority of patients, increasing energy expenditure seems an attractive alternative. Energy dissipating drugs (e.g. thyroid hormone, ephedrine, dinitrophenol) have been used successfully to decrease body weight, but their use was discontinued because of unacceptable cardiovascular side effects (1-3). Exercise may be a healthier approach to increase energy expenditure, but the amount of exercise needed to significantly influence energy balance, as well as the accompanying increase in appetite, makes it an ineffective strategy for long-term body weight reduction (4).

Exposure to cold increases energy expenditure and is partly mediated by the activation of brown adipose tissue (BAT). Non-shivering thermogenesis (NST) is the increase in energy expenditure resulting from exposure to temperatures below the thermoneutral zone, but above the temperature threshold for shivering. By definition no physiological mechanisms for temperature regulation are active at thermoneutrality and thus no energy is spent on maintenance of temperature. For naked humans the thermoneutral zone is 27±2 ºC (5) and for lightly clothed humans it lies around 22 to 24°C, depending on the insulative properties of the clothing (6).

As described above, cold exposure induces physiological changes. More important, mild cold exposure may have a better adherence compared to profound cold exposure when used as an anti-obesity strategy to increase metabolic rate. The key question is whether increasing energy expenditure through mild cold exposure is accompanied by an increase in appetite and food intake. Cold exposure is known to increase food and energy intake in a wide range of animal species e.g. piglets, rats and birds (7,8,9), but not yet in humans.

Besides the increase in energy expenditure, cold exposure may also trigger an insulative response. Vasoconstriction, mediated via activation of alpha-adrenergic receptors, limits heat loss via the skin. Interestingly the vasoconstrictive response is highly variable between individuals and was shown to correlate negatively with the magnitude of NST in one study (10). Skin temperature changes during cold exposure may thus be a predictor of the metabolic response to cold exposure. This may also be the case for changes in temperature of the skin overlying the supraclavicular BAT depot, as suggested by two reports using infrared thermography to measure skin temperature in the supraclavicular region during a cold challenge (11,12). Moreover, it is the reduction in skin temperature during cold that is mediated by vasoconstriction, which is perceived as uncomfortable.

In this paper, we investigated the response to mild cold in healthy humans for this may be an attractive weight management strategy. More importantly, we focussed on changes in energy expenditure, food intake including appetite and satiety, and dermal temperature.

Methods

Subjects

Healthy volunteers were recruited through local advertisements in the East Anglian region of the United Kingdom. We recruited five lean males and five lean females, non-smokers, age between 22 and 60 years, who had no known medical conditions and were not taking any medications or supplements. To minimize seasonal variation of NST, which is known to exist, subjects were studied between April 2012 and September 2012 (13). All subjects provided written informed consent and the study conformed to the standards set by the latest revision of the Declaration of Helsinki. The study received approval from the Cambridge Central East of England Research Ethics Committee.

Study outline

Outline of the study design is depicted in Figure 1. Subjects were studied twice during two days, about two weeks apart, one of the days the subjects were tested under thermoneutrality and the other day under mild cold exposure, they were blinded to the setting and tests were performed in random order. Subjects were asked to refrain from strenuous physical activity, alcohol and caffeine for 24h before their visit. Each participant arrived at the Clinical Research Facility around 16.00 hours (hrs) on day 0 and remained until 14.00 hrs on day 1. Height, weight and body composition (DXA (GE Lunar Prodigy GE Healthcare, Madison, WI, USA; software version 12.2)) were measured. At 18.00 hrs, a standardised dinner was served. The energy content of the meal was 1/3 of a participant's daily requirements estimated from predicted resting metabolic rate, using the Schofield equation, multiplied by an activity factor of 1.35. Meal composition was 30–35% fat, 12–15% protein and 50–55% carbohydrate by energy. The participants retired to bed in the temperature controlled room (24°C) at 23.00

hrs and were provided with standardized light clothing and bedding. The temperature-controlled room is a habitual room including a desk, television, computer, sink and toilet. The participant was woken the next morning at 07.00 hrs and stayed in bed in semi-supine position (upper part of the bed at 45°) without bedding. All participants were asked to remain awake and inactive. To enable thermal imaging, male subjects had a bare torso and women wore a boob tube for the remainder of the experiment. Baseline indirect calorimetry, vital signs, cold and hunger scores and blood tests were taken at 7.30 hrs. Next, the subjects either stayed in this room at 24°C or were moved to the mild cold room (18°C). Subsequently, thermal imaging, vital signs, indirect calorimetry, cold and hunger scores and blood tests were repeated every 30 minutes (figure 1) during 2,5 hours. In between the measurements subjects were allowed to read or watch TV but did not leave the bed except for toileting. Blood samples were taken via a large indwelling venous catheter without the use of a heated hand box or blanket to prevent local warming. Afterwards the universal eating monitor was used to assess speed of eating the ad libitum meal and other parameters related to appetite and food intake.

Indirect calorimetry

REE was measured by ventilated canopy respiratory gas exchange (GEM; GEMNutrition, Daresbury, UK) in supine position. Measurements were recorded during 12-minute intervals every 30 minutes. Energy expenditure was calculated from the macronutrient respiratory quotients and energy equivalents of oxygen published by Elia and Livesey (14).

Cold and hunger scores

Participants were asked to rate the sensation of cold of the whole body and hands separately on a 1 to 10 scale, with ratings as following; 1 was rated as *not at all cold* and 10 was the *coldest one had ever felt*. Similarly for the degree of hunger, with ratings as: 1 for *not hungry at all*, and 10 was rated as *the most hungry one had ever felt*.

Blood biochemistry

Glucose was measured using the Hexokinase method on a Siemens Dimension RXL AutoAnalyser. Reagents and Calibrators were purchased from Siemens. Free Fatty Acids were measured using the Roche Free Fatty Acid kit. This assay was modified to run in microtitre plate format. Thyroid stimulating hormone (TSH), free thyroxin (fT4) and iodothyronin (T3) were measured by time-resolved fluorescence immunoassay on an AutoDELFIA analyser (Perkin Elmer) using kits from Perkin Elmer. Cortisol was measured by fluorescence immunoassay on the Siemens Centaur Autoanalyser. A minimum of two quality control samples were run in each assay.

Universal Eating Monitors (UEM)

The UEM (The Sussex Meal Patterning System) was used. Subjects ate an homogenous test meal (e.g. pasta) containing normal energy percent ratios (~30% carbohydrates, ~30% protein and ~40% fat). Test meal intake was continuously monitored using the UEM equipment (19). Here, food is served and eaten from a plate placed on weighing scales connected to a computer. Generated intake data contained the amount eaten and the seconds spend eating. The monitors allow automated combinations of appetite ratings by visual analogue scales and intake data. The VAS scales rate feelings of hunger, sickness, fullness and desire to eat on a 0 to 100 scale (15).

157

158

159

160 *Thermography*

161 Skin temperature images were obtained with a ThermaCam 3000 , and images were analysed
162 by ThermaCAM Researcher Pro 2.9 software (both FLIR systems, Boston, United States).
163 Camera settings were temperature dependent: at 24ºC; emissivity: 0.98, humidity: 45%,
164 distance: 1.2 m, external and reflected temperature: 24ºC, at 18ºC, emissivity: 0.98, humidity:
165 40%, distance: 1.2 m, external and reflected temperature: 18ºC. Two skin regions were
166 defined; first the supraclavicular area (bordered by the acromioclavicular joint,
167 sternoclavicular joint and the sternocleidomastoid trapezoid angle) and second, the sternal
168 area (the top 10 centimetres above of the sternum). For recognition of these anatomical
169 landmarks on the thermal images, we placed metal markers on the skin. At each time point,
170 per area the average of three images was taken for analysis.

171

172 *Statistic analysis*

173 All analysis was performed on SPSS 21. Time series data were analysed using repeated
174 measures ANOVA. Each ANOVA model was built using 'time' as within subject effect,
175 'temperature' as independent factor and 'time*temperature' as the interacting term. A
176 significant 'time*temperature' effect is interpreted as a significant effect of mild cold
177 exposure on the rate of change over time. Each term in the ANOVA model was analysed for
178 sphericity (Muachly's Test) and if found to be violated, within subject effects was determined
179 by the Greenhouse-Geisser test. For all statistical test a P values <0.05 was considered

significant. All paired data were analysed by Student's T-test. Correlations were assessed using a Pearson's test. Data are presented as mean±SD.

Results

Metabolic response to mild cold exposure

We studied 10 Caucasian healthy subjects, 5 males and 5 females, age ranging from 22 to 60 years old, BMI ranging from 20.8 to 24.9 kg/m^2. Subject characteristics are included in table 1. Mild cold exposure significantly increased energy expenditure without visible shivering compared to thermoneutrality (repeated measurement ANOVA for cold effect p=0.01). Over 150 minutes of exposure to 18°C a total of 48±14 kJ (range 13 to 127 kJ) was expended above baseline energy expenditure at 24°C (fig. 2A). Respiratory exchange ratio (RER) dropped during the experiment under both conditions (repeated measurement ANOVA for the effect of time p<0.01). There was no significant effect of temperature on RER (fig. 2B; repeated measurement ANOVA for the effect of temperature p=0.195).

Vital signs

Heart rate remained stable at thermoneutrality at 56.7±4.2 (T=0) to 58.1±4.2 (T=150 min) beats per minute (bpm). Heart rate decreased in response to mild cold exposure from an average of 59.5±4.4 (t=0) to 56.7±4.5 bpm (t=150 min)(supplemental fig. 1A; repeated measurement ANOVA for the effect of time*temperature p=0.025). Systolic blood pressure remained stable between 109±2 (T=0) and 113±2 mmHg (T=150 min) at thermoneutrality and

increased from 107±2 (T=0) to 120±4 mmHg (T=150 min) during mild cold exposure (figure 3; repeated measures ANOVA for the effect of time*temperature p=0.007). Diastolic blood pressure remained stable during both the stay at thermoneutrality and during mild cold exposure (figure 3).

Biochemistry

Plasma glucose and fT4 increased similarly, and plasma cortisol, TSH and T3 decreased similarly under both thermal conditions (figure 4). The only biochemical parameter that responded to a difference in ambient temperature was the plasma non-esterified fatty acid (NEFA) level (figure 4B). NEFA levels increase under both conditions, but this increase was more at mild cold exposure (211±62 umol/L) compared to thermoneutrality (132±59 umol/L) (repeated measures ANOVA for the effect of temperature p<0.001).

Cold sensation

At thermoneutrality the score for whole body cold sensation remained stable between 2.1±0.5 and 2.3±0.5 whereas during mild cold exposure the score significantly increased from 3.0±0.5 to 7.2±0.3, (repeated measures ANOVA for the effect of temperature p<0.001)(figure 5A). For hands the same pattern was observed, at thermoneutrality no significant change, but during mild cold exposure the cold score increased significantly from 3.0±0.6 to 7.5±0.5 (figure 5B, repeated measures ANOVA for the effect of temperature p<0.001). There was a significant negative correlation between the cold score for hands after 150 minutes of mild cold exposure and the cumulative increase in energy expenditure above baseline during this time (figure 5C, r^2=0.481, Pearson p<0.001). There was no correlation between the cold score

for whole body at 150 minutes and the cumulative increase in energy expenditure in response to mild cold (r^2=0.000).

Hunger, food intake and satiety

Feelings of hunger increased during both situations over 150 minutes. A trend towards a higher hunger score during cold was observed (figure 6A, repeated measurement ANOVA for the effect of time p=0.021, for the effect of temperature p=0.064). Prior to the meal, feelings of fullness, hunger, sickness and desire to eat were similar after exposure to both thermal conditions (figure 6B). During the meal, the same amount of food was consumed after thermoneutrality and mild cold exposure (2740±567 vs 2878±492 kJ paired t-test p=0.69)(figure 6C). There were no differences in the time spent eating during both situations; thermoneutrality (714±124 seconds) versus cold (778±115 seconds)(paired t-test p=0.14)(figure 6D). There was no correlation between the amount of food consumed and basal metabolic rate and between the amount of food consumed and the increase in energy spent during 150 minutes of mild cold exposure (r^2=0.076, p=0.271 and r^2=0.00, p=0.962 Pearson test). Feelings of fullness, hunger and desire to eat after the test meal were not different after either situation; cold versus thermoneutrality (figure 6B). Feeling of sickness was significantly greater after mild cold exposure (figure 6B).

Skin temperature changes assessed by thermography

During both situations (thermoneutrality and cold exposure), the supraclavicular skin temperature was higher compared to the sternal skin area (repeated measurement for the effect

of location ANOVA $p<0.01$). At 24°C skin temperature in both areas remained stable (figure 7A). In response to mild cold exposure, skin temperature dropped in both areas during the first 30 minutes and stayed stable thereafter. This temperature drop was greater in the sternal compared to the supraclavicular area (figure 7A). The temperature difference between both areas, increased in response to mild cold exposure and remained stable at thermoneutrality (figure 7B; repeated measurement ANOVA for the effect of temperature*time interaction $p<0.01$). There was a tight positive correlation between skin temperature of the sternal area and the increase in energy expenditure (figure 7D; $r^2=0.787$, $p=0.001$, Pearson test). Supraclavicular temperature was not correlated to the increase in energy expenditure (figure 7E; $r^2=0.286$, $p=0.103$, Pearson test).

Discussion

In this paper, we investigated the response to mild cold in healthy humans with a focus on changes in energy expenditure, food intake and dermal temperature. As described in previous studies, exposure to mild cold induced a small but significant increase in energy expenditure. We showed that, at least in short-term, energy intake does not increase since two and a half hours of mild cold exposure had no influence on the amount of food eaten and time spent eating. During cold exposure, feelings of hunger showed a trend to increase to a level above what was observed during thermoneutrality. Once out of the cold, the difference disappeared and no differences could be found in pre-meal feelings of hunger, desire to eat, fullness or sickness after exposure to the two different thermal conditions. We cannot exclude the possibility that excess energy expenditure in cold is compensated by increased food-intake later. Whether prolonged mild cold exposure, with meals consumed in the cold, would increase energy intake remains to be determined. Historical data obtained under harsher

thermal conditions show a negative correlation between outdoor temperature (ranging from -30 to +35°C) and food intake (9). To our knowledge, there are no data on the effect of prolonged mild cold exposure on hunger and food intake.

Skin temperature falls in response to a drop in ambient temperature. In studies published so far, the supraclavicular temperature either increased (12,16) or decreased to a lesser extend compared to other body areas (11,17). A recently published study shows a positive correlation between the skin temperature in the supraclavicular region and clavicular BAT volume and activity during cold exposure (16). Based on these data we hypothesised that the change in the temperature difference between the supraclavicular region and the sternal area would be positively correlated to the increase in energy expenditure in response to mild cold exposure. This was not the case, nor was the supraclavicular temperature in itself related to the metabolic response to mild cold. We conclude that skin temperature measurements of area's overlying superficial BAT depots are not helpful in predicting metabolic responses to cold.

Relative mild cold exposure (18° C) resulted in significant thermal discomfort but no visible shivering in our study (figure 4A and B). That this thermal discomfort is also perceived in daily life is likely, since the average living room temperature in the UK has increased from approximately 18 to 21°C over the last three decades (17). Mild cold exposure is uncomfortable due to the perception of the reduction in skin temperature, which is mediated by vasoconstriction. We not only show that a negative correlation between the metabolic and the vasoconstrictive response to mild cold exists, but also that the metabolic response to mild cold is negatively correlated to the cold score for hands. Therefore, the lower the metabolic response to mild cold exposure, the colder one feels. At extreme ends, individuals could be

296 classified as ‘vasoconstrictors’; those with a strong insulative response and a low metabolic
297 response to mild cold exposure and ‘metabolisers’; those with a high metabolic response that
298 allow a relatively high rates of peripheral heat loss by maintaining a higher skin temperature.
299 Hypothetically, vasoconstrictors would be at greater risk to develop overweight or obesity,
300 since they will take behavioural measures to avoid the negative sensation elicited by mild
301 exposure and spent less energy on thermoregulation compared to metabolisers.

302

303 As described earlier, mild cold exposure resulted in an increase in plasma free fatty acid
304 (FFA) concentrations (e.g. 19,20,21). Studies on more extreme cold exposure have shown an
305 increased rate of lipolysis in humans (22), making this the most likely source of increased
306 FFAs during mild cold exposure. Mild cold exposure in humans increases systolic blood
307 pressure (e.g. 19,23, current study) and may increase LDL cholesterol concentrations (24, 25).
308 Taken together, short-term mild cold exposure results in unfavourable changes in several
309 cardiovascular risk factors. Lower temperatures are known to increase the incidence of
310 cardiovascular events such as myocardial infarction and stroke, which have an increased
311 incidence rate in winter, even in countries with milder climates (26). Whether the changes in
312 cardiovascular risk factors that occur when lowering temperature in the range from around
313 22°C to around 16-18°C also results in a higher cardiovascular disease incidence remains to
314 be established.

315 We aimed to standardize the experimental circumstances as much as possible. Therefore,
316 subjects were admitted the afternoon before the study and exposed to exact identical meals,
317 temperatures and sleep time. However, some limitations remain. Due to the intensive study
318 protocol we only included 10 subjects, which may have prevented us from finding smaller
319 effects. This may also explain why we did not find differences between men and women (data

not shown) although the latter both may have more BAT and insulative capacity (27,28,29). Also the time spent in the cold, and the temperature in which the meal was consumed may have led to results that are not generalizable. Additionally, we did not measure BAT FDG uptake but our primary aim was to investigate skin temperature in relation to energy expenditure. Finally, we included healthy non-obese subjects and our results may not apply to obese subjects who may have larger insulative capacity due to more subcutaneous adipose tissue.

In conclusion, short-term mild cold exposure results in an increase in energy expenditure that is not directly compensated by an increase in energy intake and may thus be used to alter energy balance. The increase in energy expenditure is small, but if maintained throughout longer periods could be used to prevent weight gain or even promote modest weight loss. However, lower temperatures lead to thermal discomfort, especially in those with a low metabolic response to cold and may induce unfavourable cardiovascular and metabolic changes. Moreover, the response to cold is variable and not all subjects may show an increase in energy expenditure during cold exposure. Long-term studies measuring the effect of lowering ambient temperature on thermal comfort, body weight, adiposity and cardiovascular risk factors will have to establish whether mild cold exposure is an effective anti-obesity measure.

Competing interests

None of the authors have competing interests

Funding

The study was funded by NIHR, BRC Seed Fund, individual grants: ML and MS: Marie Curie Fellowship, CYT: Welcome Trust Fellowship, SV: MRC, BHF and BBSRC, AVP: BBSRC.

Acknowledgements

We thank Katie Bird, Liz Blower, Cathy Baker and many others of the Addenbrookes Clinical Research Facility Staff for excellent assistance during the studies.

References

1. Haller CA, Benowitz NL. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. New Eng J Med 2000;343:25:1833-1838.
2. Yen M, Burns Ewald M. Toxicity of weight loss agents. J Med Toxicol 2012;8:145–152.
3. Comerma-Steffensen S, Grann M, Andersen CU, Rungby J, Simonsen U. Cardiovascular effects of current and future anti-obesity drugs. Curr Vasc Pharmacol. 2014;12(3):493-504.
4. Thomas DM, Bouchard C, Church, Slentz TC, Kraus WE, Redman LM, Martin CK, Silva AM, Vossen M, Westerterp K, Heymsfield SB. Why do individuals not lose more weight from an exercise intervention at a defined dose? An energy balance analysis. Obes Rev 2012:13; 835–847.
5. Erikson H, Andersen KL, Scholander PF. The critical temperature in naked man. Acta Physiol Scand 1956;37(1):35-39.
6. Kingma B, Frijns A, van Marken Lichtenbelt W. The thermoneutral zone: implications for metabolic studies. Front Biosci;2012;4:1975-1985.
7. Macari M, Dauncey MJ, Ingram DL. Changes in food intake in response to alterations in the ambient temperature: modifications by previous thermal and nutritional experience. Pflugers Arch 1983;396(3):231-237.
8. Brobeck JR. Food intake as a mechanism of temperature regulation. Yale J Biol Med 1948; 20(6): 545–552.
9. Blaxter K. Energy metabolism in animals and man. Cambridge: Cambridge University Press; 1989; 204-206.

10. Van Marken Lichtenbelt WD, Schrauwen P,van de Kerckhove S, and Westerterp-Plantenga MS. Individual variation in body temperature and energy expenditure in response to mild cold. Am J Physiol Endocrinol Metab 2002;282: E1077–E1083.
11. Lee P, Ho KKY, Greenfield JR. Hot fat in a cool man: infrared thermography and brown adipose tissue. Diab Obes Metab 2010;13:92–93.
12. Symonds ME, Henderson K, Elvidge L, Bosman C, Sharkey D, Perkins AC, Budge H. Thermal imaging to assess age-related changes of skin temperature within the supraclavicular region co-locating with brown adipose tissue in healthy children. J Pediatr 2012;161:892-898.
13. Nishimura T, Motoi M, Egashira Y, Choi D, Aoyagi K, Watanuki S. Seasonal variation of non-shivering thermogenesis (NST) during mild cold exposure. J Physiol Anthropol 2015;34:11.
14. Elia M, Livesey G. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. World Rev Nutr Diet. 1992;70:68-131.
15. Dovey TM, Clark-Carter D, Boyland EJ, Halford JCG. A guide to analysing Universal Eating Monitor data: Assessing the impact of different analysis techniques. Physiol Behav 2009:96;78–84.
16. Boon MR, Leontine Bakker LEH, van der Linden RAD, Arias-Bouda LP, Smit F, Verberne HJ, van Marken Lichtenbelt WD, Jazet IM, Rensen PCN. Supraclavicular skin temperature as a measure of 18F-FDG uptake by BAT in human subjects. PloSOne 2014;9:6:e98822.
17. Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y, Saito M. Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. Obesity 2011;19:13-16.

18. Johnson F, Mavroggiani A, Ucci M, Vidal-Puig A, Wardle J. Could increased time spent in a thermal comfort zone contribute to population increases in obesity? Obes Rev 2011;12:543-551.

19. 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, Che KY. 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;163:863–872.

20. Blondin DP, Labbé SM, Tingelstad HC, Noll C, Kunach M, Phoenix S, Guérin B, Turcotte EE, Carpentier AC, Richard D, Haman F. Increased brown adipose tissue oxidative capacity in cold-acclimated humans. J Clin Endocrinol Metab 2014;99(3):E438–E446

21. Ouellet V, Labbé SM, Blondin DP, Phoenix S, Guérin B, Haman F, Turcotte EE, Richard D, Carpentier AC. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J Clin Invest. 2012;122(2):545–552.

22. Koska J, Ksinantova L, Sebokova E, Kvetnansky R, Klimes I, Chrousos G, Pacak K. Endocrine regulation of subcutaneous fat metabolism during cold exposure in humans. Ann. N.Y. Acad. Sci. 2002;967:500–505.

23. Kingma BRM, Frijns AJH, Saris WHM, Steenhoven AA and van Marken Lichtenbelt WD. Increased systolic blood pressure after mild cold and rewarming: relation to cold-induced thermogenesis and age. Acta Physiol 2011;203:419–427.

24. Hong YC, Kim H, Oh SY, Lim YH, Kim SY,Yoon HY, Park M. Association of cold ambient temperature and cardiovascular markers. Sci Tot Environm 2012;435–436:74–79.

25. Dong M, Yang X, Lim S, Cao Z, Honek J, Lu H, Zhang C, Seki T, Hosaka K, Wahlberg E, Yang J, Zhang L, La T, Sun B, Li X, Liu Y, Zhang Y, Cao Y. Cold exposure promotes atherosclerotic plaque growth and instability via UCP1-dependent lipolysis. Cell Metab 2013;18:118–112.
26. The Eurowinter Group. Cold exposure and winter mortality from ischaemic heart disease, cerebrovascular disease, respiratory disease, and all causes in warm and cold regions of Europe. Lancet 1997;349:1341-1346.
27. 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(15):1509-17.
28. van den Beukel JC, Grefhorst A, Hoogduijn MJ, Steenbergen J, Mastroberardino PG, Dor FJ, Themmen AP. Women have more potential to induce browning of perirenal adipose tissue than men. Obesity 2015;23(8):1671-9.
29. Solianik R, Skurvydas A, Vitkauskienė A, Brazaitis M. Gender-specific cold responses induce a similar body-cooling rate but different neuroendocrine and immune responses. Cryobiology. 2014;69(1):26-33.

443 **Figure legends**

444 **Figure 1**. Study design. Black bars represent measurements including indirect calorimetry,
445 cold and hunger scores, vital signs and blood test. UEM denotes universal eating monitor.

446

447 **Figure 2**. (A) Cumulative energy expended above basal metabolic rate over 150 minutes of
448 thermal challenge. * $p < 0.05$ compared to 24°C. (B) Change in respiratory exchange ratio
449 (RER) compared to baseline.

450

451 **Figure 3**. Average heart rate (A), systolic blood pressure (B) and diastolic blood pressure (C)
452 over 150 minutes of thermal challenge. *$p<0.05$ for 24°C versus 18°C.

453

454 **Figure 4**. Plasma glucose (A), non-esterified free fatty acid (NEFA)(B), cortisol (C), thyroid
455 stimulating hormone (TSH)(D), free thyroxine (FT4)(E) and free tri-iodothyronine (FT3)(F)
456 over 150 minutes of exposure to 24°C and 18°C.

457

458 **Figure 5**. Score for perception of cold (cold score) for whole body (A) and hands (B). (C)
459 Correlation between cold score for hands and EE over baseline after 150 minutes of mild cold
460 exposure.

461

462 **Figure 6**. Score for perception of hunger over 150 minutes of thermal challenge (A). Visual
463 analogue scale for hunger and satiety before and after UEM test meal (B). Amount of food

464 consumed (KJ)(C) and time spent eating (seconds)(D) during UEM test meal. *p<0.05 for
465 24°C versus 18°C.

466 **Figure 7**. Surface temperature over the supraclavicular and sternal area as measured by
467 thermal imaging at 24°C and 18°C (A) over 150 minutes of thermal challenge. (B)
468 Temperature difference between supraclavicular area and sternal area. (C) Correlation
469 between cumulative energy expended (EE) over baseline after 150 minutes of mild cold
470 exposure and the change in temperature difference between supraclavicular and sternal areas
471 after 150 minutes. (D) Correlation between EE over baseline after 150 minutes of mild cold
472 exposure and sternal temperature after 150 minutes. (E) Correlation between EE over baseline
473 after 150 minutes of mild cold exposure and supraclavicular temperature after 150 minutes.

474

475

Table 1. Subject characteristics.

	Males	**Female**
Age (years)	44.7±5.2	33.7±6.9
BMR (J/min)	4445.5±225.9	3808±196.6
Height (m)	1.76±0.03	1.65±0.04
Weight (kg)	69.5±3.3	62.6±4.1
BMI (kg/m^2)	22.4±0.8	22.9±0.9
Fat (kg)	12.8±2.7	20.2±2.4
Lean (kg)	53.1±1.8	39±2.2
FFM (kg)	55.8±1.9	41.5±2.4

BMR basal metabolic rate; BMI body mass index; FFM fat free mass.

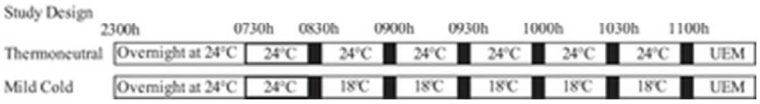

Figure 1
32x6mm (300 x 300 DPI)

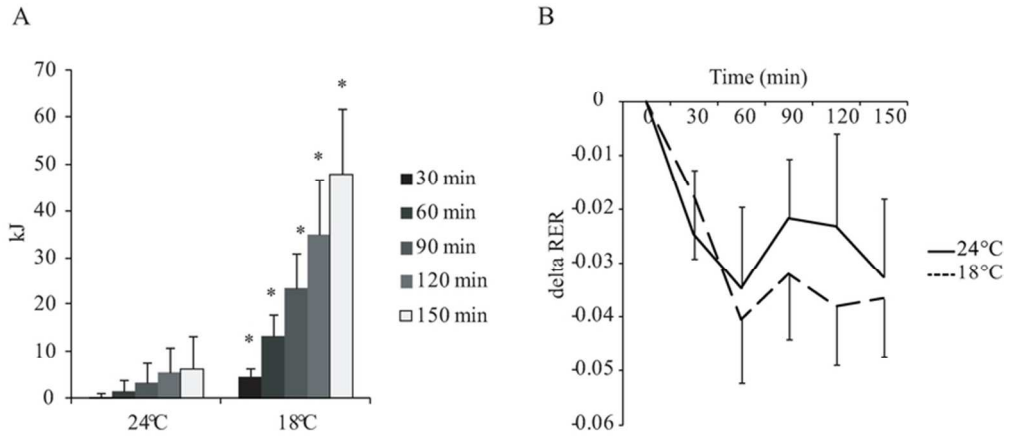

Figure 2
72x32mm (300 x 300 DPI)

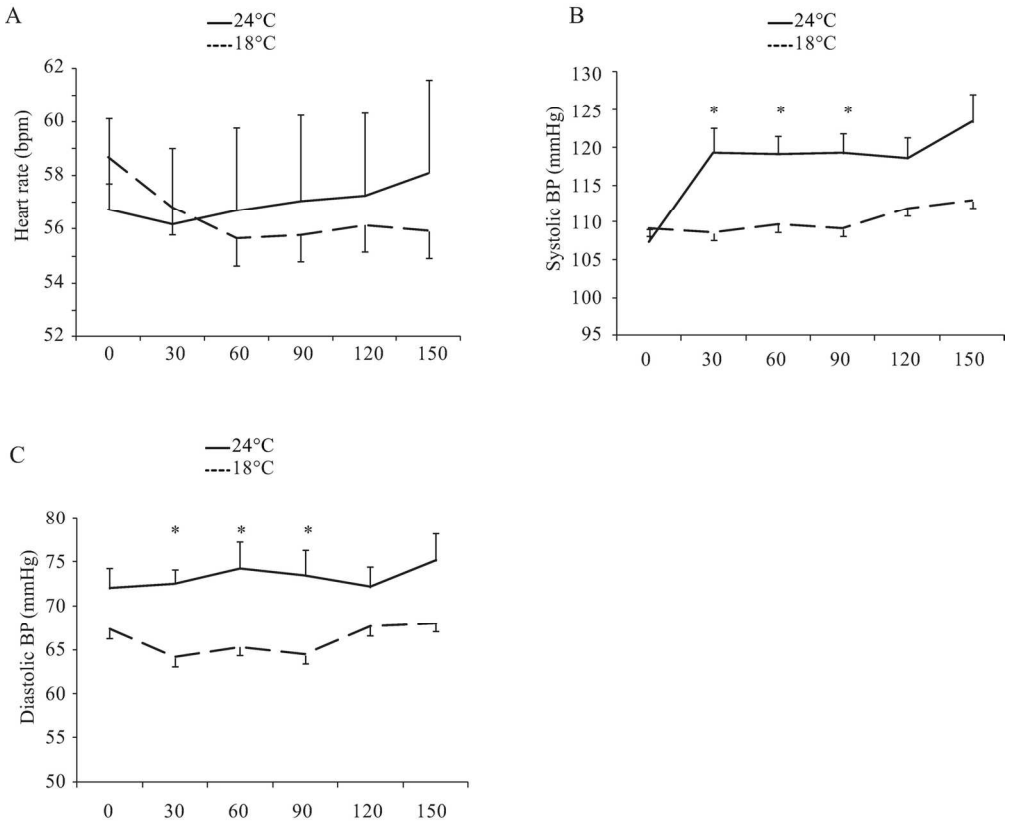

Figure 3
146x121mm (300 x 300 DPI)

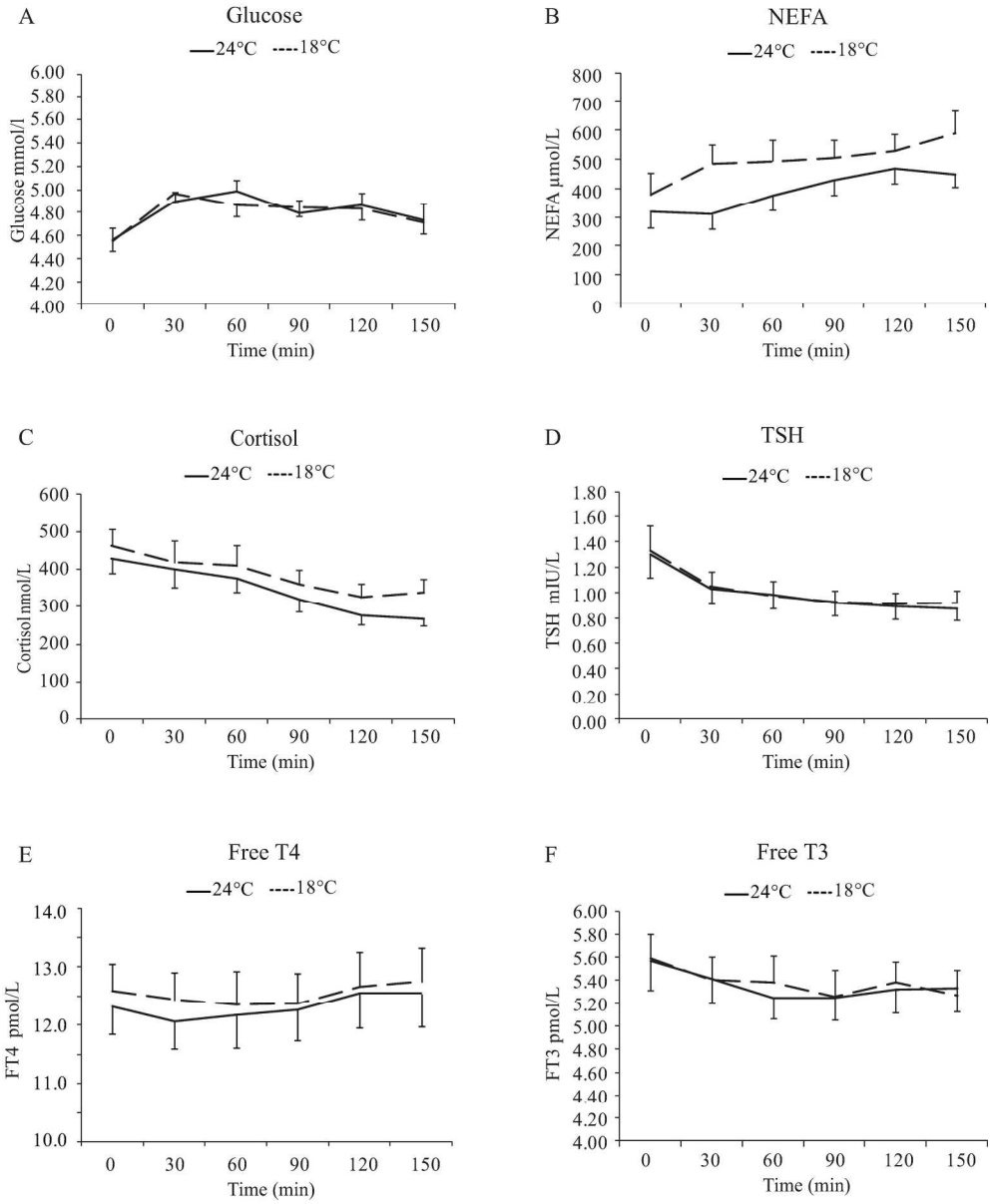

Figure 4
218x266mm (300 x 300 DPI)

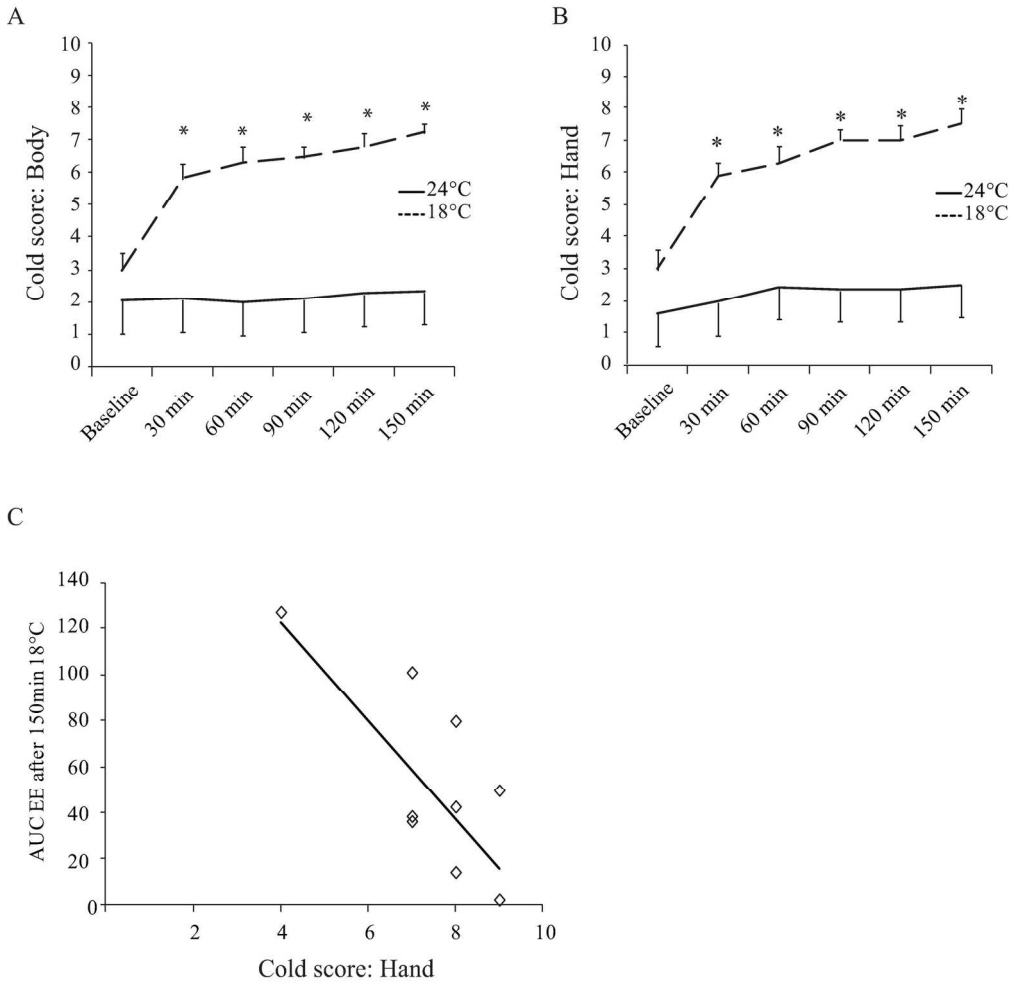

Figure 5
165x161mm (300 x 300 DPI)

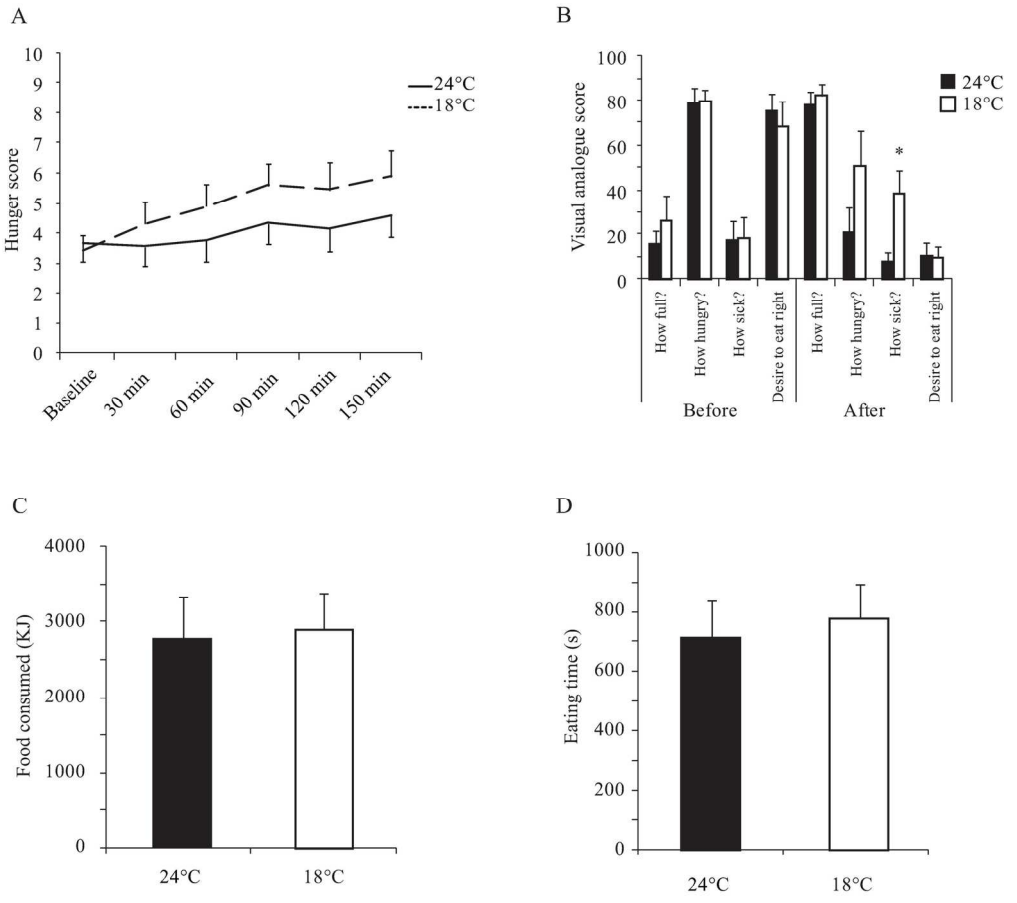

Figure 6
162x145mm (300 x 300 DPI)

Figure 3

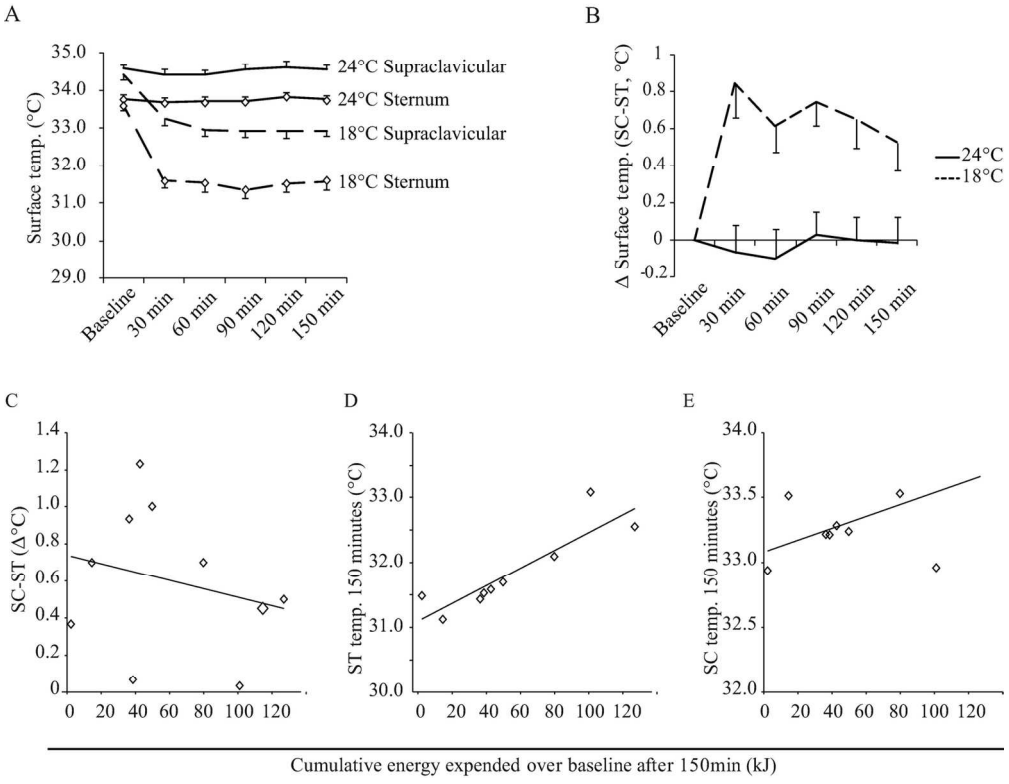

Figure 7
146x119mm (300 x 300 DPI)