Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults.

Francesco S. Celi¹, Robert J. Brychta¹, Joyce D. Linderman¹, Peter W. Butler¹,², Anna Teresa Alberobello¹, Sheila Smith¹, Amber B. Courville³, Edwin W. Lai², Rene Costello³, Monica C. Skarulis¹, Gyorgy Csako³, Alan Remaley³, Karel Pacak², and Kong Y. Chen¹

¹Clinical Endocrinology Branch, National Institute of Diabetes & Digestive & Kidney Diseases, ²Reproductive and Adult Endocrinology Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, ³Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda Maryland.

Running Head: Cold exposure and energy homeostasis

Correspondence and request for reprints:

Francesco Saverio Celi, M.D., MHSc.
Clinical Endocrinology Branch,
National Institute of Diabetes & Digestive & Kidney Diseases,
National Institutes of Health,
Building 10, CRC, RM 6-3940
10 Center Drive, MSC 1613
Bethesda, Maryland, 20892-1613
Tel: +301-435-9267
Fax: +301-480-4517
e-mail: fc93a@nih.gov
Abstract

**Objective.** Resting energy expenditure is a major contributor to the total energy expenditure and thus plays an important role in body weight regulation. Adaptive thermogenesis is a major component of energy expenditure in rodents, but little is known on the effects of exposure of humans to mild and sustainable reduction of environmental temperature.

**Design.** To characterize the dynamic changes in continuously measured resting energy expenditure, substrate utilization, and hormonal axes simultaneously in response to mild reduction of environmental temperature we performed a cross-over intervention.

**Methods.** Twenty-five volunteers underwent two 12-hour recordings of energy expenditure in whole-room indirect calorimeters at 24°C and 19°C with simultaneous measurement of spontaneous movements and hormonal axes.

**Results.** Exposure to 19°C resulted in an increase in plasma and urine norepinephrine levels (p<0.0001), and a 5.96 % (p<0.001) increase in energy expenditure without significant changes in spontaneous physical activity. Exposure to the lower temperature resulted in a significant increase in free fatty acid levels (p<0.01), fasting insulin levels (p<0.05), and a marginal decrease in postprandial glucose levels. A small but significant (p<0.002) increase in serum free T4 and urinary free cortisol (p<0.05) were observed at 19°C.

**Conclusions.** Our observations indicate that exposure to 19°C, a mild and tolerable cold temperature, results in a predictable increase in energy expenditure driven by a sustained rise in catecholamine, and the activation of counter-regulatory mechanisms.
**Key words:** Energy expenditure, substrate utilization, catecholamines, sympathetic nervous system, adaptive thermogenesis
Introduction

Obesity, which is the result of a sustained imbalance between energy intake and energy expenditure, has reached epidemic proportions. As a result, obesity-related complications contribute substantially to mortality and healthcare costs. Interestingly, even small but prolonged changes in the energy balance equation (energy gap), as low as 100 Kcal/day, may play a significant role in the gradual weight gain observed in the adult population. Most obesity-directed interventions, pharmacological and behavioral, are aimed at reducing energy intake. No interventions, except for increasing physical activity, are successful at increasing energy expenditure (EE) safely and effectively. Besides physical activity (voluntary, spontaneous, and shivering), the other components of total EE are the thermic effect of food, and resting EE. In humans, resting EE accounts for 65-85% of total EE and is mainly determined by body size and composition, but it also can be influenced by pathological states, such as hypo- or hyperthyroidism, fever and drugs.

Exposure to cold (16 °C), results in a predictable increase in resting EE (non-shivering thermogenesis) which may be partly explained by an activation of brown adipose tissue. However, such a level of cold exposure is not sustainable over a long period of time. Furthermore, to our knowledge, no study aimed at characterizing the changes in EE upon temperature manipulation within the range observed in climate-controlled buildings has been performed. Interestingly, the recent trend in regulating indoor climates has been proposed as a significant contributor to the increase in obesity prevalence. In this study we have accurately characterized the changes in EE, substrate utilization, heart rate variability, temperature, and hormonal homeostasis in healthy volunteers of both sexes exposed to 19 °C, well within the
range of tolerability.
Material and Methods

Participants and study design: The study was approved by the NIDDK-NIAMS IRB and conducted at the NIH Clinical Center in Bethesda, Maryland. The protocol was designed as a randomized, single blind, cross-over intervention study (ClinicalTrials.gov identifier number NCT00521729). Inclusion criteria for the enrollment in the study were an age range of 18-60 years and body mass index (BMI) 20-27 kg/m². Exclusion criteria were thyroid disease (by history or TSH <0.4 or >5.0 mIU/L at screening); hypertension (by history or blood pressure values >140/90 mmHg) or cardiovascular disease; pregnancy or use of hormonal contraception; diabetes mellitus; serum cholesterol ≥ 240 mg/dl; and/or serum triglycerides ≥ 220 mg/dl.

Female participants were studied in the early follicular phase of the menstrual cycle (days 2-10). After enrollment, study volunteers were admitted to the Metabolic Clinical Research Unit and after a two-day equilibration diet (see below), they were randomly assigned to a 12-hour stay in a whole-room indirect calorimeter (respiration chamber) at either 24°C (75°F) or 19°C (68°F). After a 36-hour recovery period volunteers repeated the 12-hour stay at the second study temperature (Fig. 1a). Two days prior to testing and throughout the duration of the study, volunteers received a caffeine-free weight maintenance diet providing 50% carbohydrate, 20% protein, and 30% fat. Resting energy requirements for the equilibration diet were calculated from the subject’s height, weight, and age using the Mifflin – St. Jeor equation 11. An activity factor associated with the subjects’ self-reported physical activity was used to calculate their total daily energy needs12. The day prior the respiration chamber recording study volunteers finished dinner by 19:00, i.e. twelve hours before the study. Six hours into the respiration chamber stays volunteers received one standard liquid meal (Boost Plus, Nestle Healthcare Nutrition, Inc.) representing 30% of their calculated energy needs as part of the equilibration diet. After the
completion of the 12-hour chamber recording, volunteers were offered a meal containing the remaining 70% calorie content of the estimated energy needs. Throughout the recordings the volunteers wore standard clothing (hospital scrubs) and no blanket was available. Volunteers were asked to limit their physical activity to a minimum and were encouraged to watch TV or use a computer while sitting on a recliner or staying in bed. Blood samples were collected at -15, 0, 30, 60, 90, 120, 180, 240, 300, 360, 390, 420, 450, 480, 540, 600, 660, and 720 min. via one of the air tight sampling ports while the volunteer was sitting in the recliner. Microdialysis samples (15 µl) were collected at baseline and every 30 minutes throughout the recordings (Fig. 1b); microdialysis vials were passed via one of the air tight sampling ports and stored at minus 20 until processed.

**Respiration chamber:** Each study volunteer underwent continuous recordings of EE and respiratory quotient in a whole-room indirect calorimeter. A similar calorimeter was described previously, and can be operated at any stable interior (room) temperature ranging 16.5 - 25.2±0.5°C, and at a relative humidity of 35-55%. All temperature, humidity, and barometric pressure are continuously measured (Optica, GE Sensing, Billerica, MA). Air tight sampling ports for blood draws, and a four-way air-locking food and specimen passage, were designed to allow blood draws and specimens retrievals with minimal disturbance to the chamber environment. The accuracy and precision of the respiration chambers were evaluated initially using a series of propane calibration tests at 19°C and 24°C. The results of the tests demonstrated no significant difference in the accuracy of the VO₂ and VCO₂ measurements between 19°C and 24°C when the temperature was maintained within 0.2°C of the set-point
temperature. Each recording started at 08:00 and ended at 20:00. Although the chambers have a small window exposed to south, most of the lighting is provided by fluorescent bulbs.

**Weight, Height, and Body Composition:** Each volunteer was weighed daily using a digital balance (Scale-Tronix 5702, Carol Stream IL) under fasting conditions. Height was measured using a stadiometer (Seca 242, Hanover MD). Body composition was recorded by a dual energy x-ray absorptiometry scanner (Lunar iDXA, GE Healthcare, Madison WI) and analyzed with proprietary software.

**Physiological Monitoring:**

*Vital signs:* Blood pressure and heart rate values were recorded in the morning of the study upon awakening, and immediately after the completion of the respiration chamber stay.

*Heart Rate Variability:* A Holter monitor (Del Mar-Reynolds, Irvine, CA) was used to record ECG waveform data throughout each study. The inter-beat-interval data was analyzed in a custom program created in Matlab® (The Mathworks, Natick, MA). Heart rate variability determination was performed using established methods. Thirty-minute segments overlapping by 50% were used to compute the power in the very low frequency, low frequency and high frequency bands, representing autonomic influence on the heart via hormonal regulation, the sympathetic system, and the parasympathetic system, respectively.

*Core and Skin Temperature:* Core and skin temperatures were measured continuously using ingestible capsules and dermal patches (Philips Respironics, Bend, OR). The dermal patches
were placed on the posterior aspect of the neck, abdomen (1 inch above the navel), and dominant thigh.

**Activity Monitors:** Actical™ accelerometers (Philips-Respironics, Bend, OR) worn on the wrist, ankle, and hip were used to record the subjects’ physical activity every 15 seconds.

**Data Processing:** Data recorded by the physiological monitors (heart rate, core and skin temperature, physical activity monitors) and respiration chamber were processed in identical fashion for the 24°C and 19°C studies. The first hour of recording was excluded from the analysis to allow for equilibration of the respiration chamber equipment and for the volunteer to be acclimated to the chamber environment. The last hour of recording was also excluded from the analysis to prevent contamination of the data from anticipatory activity. The intervening ten-hour recording from minute 61 to 660 was analyzed. The hour preceding and following the meal were excluded from the pre- and post-prandial analyses only, to prevent feeding-related anticipation or activity from confounding the data.

**Microdialysis:** Subcutaneous adipose tissue microdialysis was performed using a CMA 60 (CMA Microdialysis, Stockholm, Sweden) catheter with a flow rate of 0.5 µl min⁻¹ using Ringer’s solution. Study volunteers were instructed in changing the vials every 30 min. Glucose and glycerol were measured by microanalyzer (CMA Microdialysis, Stockholm, Sweden).
Subcutaneous adipose tissue biopsy: Seven volunteers consented to subcutaneous adipose tissue biopsy from the periumbilical area within 30 minutes of the completion of each respiration chamber recording.

Laboratory assessment: Plasma specimens collected in chilled tubes were separated within 30 min, whereas serum specimens were separated within one hour of collection. For the measurement of urine analytes (urea, myoglobin, cortisol, and catecholamines) 12-hour specimens were collected by splitting them in non-preservative and preservative (acetic acid) containers as appropriate for the test. Urinary myoglobin and urinary free cortisol concentrations were measured at the Mayo Medical Laboratories (Rochester, MN). Plasma catecholamine concentrations were determined in the laboratories of the Reproductive Biology & Medicine Branch of NICHD \(^\text{18}\), whereas all other tests were performed in the NIH Clinical Center Department of Laboratory Medicine.

Real-time PCR: Total RNA was extracted from subcutaneous adipose tissue samples using the RNAzol\(^\text{®}\) method and converted to cDNA using a reverse transcriptase kit from Marligen (Ijamsville, MD). The cDNA was then amplified in a TaqMan\(^\text{®}\) 7000 real-time PCR apparatus using Cyclophilline, UCP-1, PRDM-16, PGC-1 and D2 TaqMan\(^\text{®}\) gene expression assays (Applied Biosystems, Foster City, CA).

Statistical analysis: Statistical analysis was performed using Prism 5\(^\text{®}\) (GraphPad, La Jolla, CA) using two-tailed paired \(t\)-test. Non-parametric data were analyzed using Wilcoxon signed rank sum test. Where indicated, data were log transformed. The area under the curve was calculated
using the trapezoidal rule. Results are expressed as mean ± 1 SD; an \( \alpha \) error of 0.05 was considered the threshold for statistical significance. A power calculation was performed \textit{a priori} based on the primary outcome of EE. Assuming a delta of 7\% in EE for a 6 °C difference in environmental temperature \(^{19}\), a sample size of 10 subjects from each gender would provide an 80\% statistical power at a significance of p<0.05.
Results

Patient recruiting and characteristics: Twenty-five eligible individuals (Table I) completed the study (Fig. 2).

Physiology parameters: A summary of the physiology recordings is reported in Table II.

Respiration Chamber Results: Exposure to 19°C resulted in an increase in energy expenditure in 18 of 21 subjects (Fig. 3), with a group mean of 5.96% (p<0.001) and a trend toward a decrease in pre-prandial respiratory quotient (p=0.09). At 19°C, the increase in EE was observed both pre- and post-prandially (Fig. 4, Table II). No differences were observed between sexes.

Physical Activity: No differences in physical activity were observed in subjects at the two temperature exposures (Table II).

Core body and skin temperature: There was no significant change in core body temperature, but skin temperature at the neck, abdomen, and thigh were reduced at 19°C (all p<0.001). The core and thigh temperatures demonstrated a significant increase during the post-prandial period at 24°C and 19°C (both p<0.01).

Heart rate and heart rate variability: Heart rate was significantly reduced at 19°C (p=0.004). The magnitude of all three heart rate variability components significantly increased at 19°C (p<0.001).
Blood pressure: No significant difference was observed at baseline in either systolic (24° C 116.1±11.8 vs. 19° C 118.3±10.0 mmHg, p=n.s.) or diastolic (24° C 66.8±7.3 vs. 19° C 68.8±7.9 mmHg, p=n.s.) blood pressure. Conversely at 19° C upon completion of the study both systolic (24° C 125.8±12.7 vs. 19° C 130.6±13.5 mmHg, p<0.05) and diastolic (24° C 70.4±6.7 vs. 19° C 76.4±11.9 mmHg, p<0.02) blood pressure values were significantly increased.

Laboratory data The laboratory data obtained are reported in Table III.

Catecholamines: While baseline plasma norepinephrine levels were similar between the two temperature exposures, a significant increase in the area under the curve (AUC) was observed at 19°C (p<0.0001) (Fig. 4). Concordantly, plasma dihydroxyphenylglycine (DHPG) and 3,4-Dihydroxyphenylacetic acid (DOPAC), and 12-hour urinary catecholamine showed significant increases (all p≤0.05). The observed changes were similar in men and women.

Glucocorticoid axis: While no significant changes were observed in both plasma ACTH and serum cortisol AUCs, a small but significant increase in urinary free cortisol was observed at 19°C (p<0.05). When the analysis was performed according to the gender a small but significant increase in serum cortisol AUC (p<0.04) was observed in males but not in females. Conversely, the increase in urinary free cortisol excretion at 19°C was significant in females (p<0.02), but not in males.
Thyroid hormones axis: As compared to 24°C, exposure to 19°C resulted in small, non-significant increases in total T3 and TSH AUCs and a significant increase in serum free T4 (p=0.03). When the analysis was performed according to the gender, a small but significant increase in serum T3 AUC was observed in males (p<0.05) but not in females. Similarly, while the change in free T4 was highly significant in males (p<0.002) no significant change was observed in females.

Glucose and insulin: While no statistical difference was observed in the 12-hour AUCs for plasma glucose, when the analysis was limited to the three hours following the meal (times 360-540 min.), a significant reduction in the AUC was observed at 19°C (p<0.05). Conversely, although no significant change was observed in postprandial insulin AUC, exposure to 19°C resulted in a small but significant increase in fasting (times -15 - 300 min.) insulin AUC (p=0.04) without differences between sexes.

Free fatty acids and β-hydroxybutyrate: 19°C exposure resulted in a significant increase in free fatty acids (p<0.01) and a non-significant increase in β-hydroxybutyrate AUCs without differences between sexes.

Subcutaneous adipose tissue real-time PCR and microdialysis: No transcript was observed for UCP-1, and D2, and a weak signal was observed for PRDM-16 and PGC-1. No significant change was observed in the expression levels of these two genes (data not shown). A trend toward an increase in the glycerol AUC was observed in the microdialysate (p=0.07) at 19°C.
Conversely, a non-significant reduction in the glucose AUC was observed in the same group (p=0.07), without differences between sexes.
Discussion

The primary aim of this study was to quantify the dynamic changes in EE, and investigate their associations with changes in hormonal axes, substrate utilization, and autonomic nervous system parameters in healthy subjects of both sexes exposed to a sustainable reduction in environmental temperature. We thus targeted whole-body adaptive thermogenesis, irrespective of the anatomical or tissue distribution. The two experimental conditions (19-24°C) were chosen because they are tolerable and closely represent the range of temperatures in climate-controlled buildings and homes. In order to accurately measure the changes in hormonal homeostasis and substrate utilization we performed multiple blood sampling concomitant to the EE measurement. We further explored the acute effects of a standard meal on these physiological parameters.

Our data demonstrate that a 5°C reduction in the environmental temperature resulted in a consistent increase of about 6% in EE without differences between sexes. The observed changes are due to an increase in non-shivering thermogenesis without any measurable increase in spontaneous physical activity. The relative magnitude of this increase in EE was generally similar to those measured by others upon exposure to colder temperatures for longer periods.

Not surprisingly the exposure to 19°C resulted in a decrease in skin temperature due to superficial vasoconstriction, which was consistent with previous findings. Contrary to earlier observations performed at a colder temperature (16°C), we did not observe changes in core temperature. A post-meal increase in temperature, indicating a measurable thermic effect of food, was present in both the abdomen and neck region at 24°C and 19°C. However, this
response was not observed at 19°C in either the thigh, or the core temperatures, suggesting that the thermic effect of food is somewhat blunted upon exposure to 19°C.

We postulate that the increase in sympathetic nervous system activity, as evidenced by the relative elevation of plasma and urinary catecholamine, and by the increase in the low frequency heart rate variability, is a major effector for the increase in EE in response to 19°C. The dynamic changes in plasma concentrations of DOPAC and DHPG mirrored that of norepinephrine, indicating that the overall catecholamine turnover was up-regulated. Consistent with previous findings, the observed reduction in heart rate and the increase in blood pressure are most likely secondary to peripheral vasoconstriction.

Contrary to previous observations, we did not observe any substantial difference in substrate utilization, although at 19°C we observed a trend toward a reduction in fasting RQ. This discrepancy could be explained by differences in experimental conditions, since our lower temperature was milder. The increase in plasma free fatty acids is consistent with catecholamine-stimulated lipolysis. The marginal increase in glycerol levels in the subcutaneous adipose tissue further supports this explanation.

The effects of catecholamines on glucose metabolism are more complex. The increase in fasting insulin observed at 19°C is likely secondary to catecholamine-mediated hepatic gluconeogenesis; however, it did not extend into the post-prandial period and the glucose AUC was lower during this part of the study. Furthermore, the raise in FFA likely contributes to the state of relative insulin resistance observed during fasting. Taken together, these findings can be
interpreted as a state of relative insulin resistance and catecholamine-stimulated hepatic
gluconeogenesis, with an overall increase in total consumption of metabolic fuels. The marginal
decrease in subcutaneous adipose tissue glucose levels further strengthens this explanation.

At 19 °C we also observed an increase in free T4 (and serum T3 in men), consistent with a shift
toward activation of the pituitary-thyroid axis. Alternatively, one could speculate that the
increase in free T4 (as measured by analog assay) is at least in part secondary to an interference
due to the relative increase in FFA levels. Nonetheless it is worth noting that sustained
exposure to beta-adrenergic stimulation generates an increase in serum T3 levels, in keeping
with an activation of the type-2 deiodinase mediated T4 to T3 conversion. It is thus
conceivable that prolonged exposure to mild cold will result in a significant activation of the
thyroid axis (with a further increase in EE) as a long-term compensatory response to cold
exposure. Finally, although no changes were demonstrated in ACTH levels, the small, but
significant increase in urinary free cortisol (and serum cortisol in men), implies activation of the
hypothalamic-pituitary-adrenal axis consistent with a stress response. The changes in the
glucocorticoid and thyroid axes were particularly evident in males. Although we cannot rule out
gender as a primary cause of these differences, it is possible that the observed changes are at
least in part attributable to the higher proportion of fat-free mass in males. Conversely, the
greater increase in urinary cortisol observed in females is probably related to the lower fat free
mass (and hence relatively lower urinary creatinine), since the excretion was measured as
cortisol/creatinine ratio.
Our data indicate that subtle modulations in the environmental temperature, well within the limits of tolerability, result in significant changes in EE and in many hormonal axes. The magnitude of the observed increase in EE, is remarkable since it is similar or superior to pharmacological interventions aimed at increasing EE and ultimately to weight loss\textsuperscript{27-30}. Indeed, projecting the point estimate increase in EE from this study over the 24-hour period would represent 20\% of the negative energy balance commonly prescribed in weight loss interventions\textsuperscript{31, 32}. It is worth noting that the strict adherence to such regimens is uncommon, and that the self-assessment of the dietary intake is usually biased toward under-reporting\textsuperscript{33}; hence the actual deficit in energy balance required to achieve a sustained weight loss would probably be significantly less than what is commonly prescribed, remarkably similar to the one we observed\textsuperscript{2}.

It is possible that compensatory mechanisms, such as an increase in energy intake (appetite) would prevent weight loss\textsuperscript{34}. Furthermore, our data indicate that exposure to 19\,\textdegree C leads to a mild increase in blood pressure, a state of relative insulin resistance, and a marginal increase in cortisol whose long-term consequences could potentially trump the beneficial effects of increasing resting EE.

This study is a proof-of-concept that manipulations in the environmental temperature, well within the range of tolerability, result in a significant increase in EE and in measurable changes in hormonal homeostasis. One plausible mechanism is catecholamine induction of BAT-like activity. While no changes were observed in the BAT-specific transcripts in subcutaneous adipose tissue, it is possible that longer duration of exposure to 19\,\textdegree C could stimulate transcription of BAT-specific genes\textsuperscript{35, 36}. However, the actual contribution of BAT to the maintenance of energy balance in humans has not been empirically demonstrated, and it is
possible that other tissues such as skeletal muscle may play a major role in adaptive thermogenesis in adult humans\textsuperscript{37}.

The findings of this study are particularly robust since, to the best of our knowledge, this is the first simultaneous characterization of the changes in energy expenditure, sympathetic nervous system activity, and hormonal axes by frequent blood sampling in response to minimal perturbations of the environmental temperature in a relatively large number of volunteers of both sexes. The study design and the use of a dietary controlled run-in period virtually eliminate the possibility of bias due to carry-over effect, and any confounder due to anticipation. Furthermore, we carefully calibrated our whole-room indirect calorimeters at each temperature to ensure accurate measurements of physiological changes in energy expenditure and substrate oxidation. One obvious limitation of the study is represented by the brevity of the intervention; we were thus unable to demonstrate any difference in clinically significant endpoints, such as BMI, body composition and carbohydrate metabolism parameters. Further, we could not evaluate the effects of counter-regulatory mechanisms. Since our study population was limited to non-obese, relatively young individuals, it is also possible that subjects with a higher percentage of fat mass, such as overweight and elderly individuals may have a blunted response to this intervention\textsuperscript{38}.

When we explored the individual changes in EE with respect to fat mass and changes in plasma norepinephrine, we observed a non-significant negative trend which supports this hypothesis (\textit{data not shown}). Although we performed multiple comparisons on our dataset, one should consider that the primary endpoint was defined \textit{a priori} in the setting of the statistical power analysis and that the findings in the various secondary endpoints are in keeping with the study hypothesis and primary endpoint findings. Thus the possibility of type-1 error appears extremely unlikely.
In conclusion our study demonstrates that minimal modulation of the environmental temperature results in a significant and potentially clinically relevant increase in energy expenditure. Further studies are needed to investigate the long term effects of mild cold exposure on clinically relevant end-points and its applicability as an intervention aimed to promote weight loss.
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Disclosures: The authors have nothing to disclose.
References:


30. Yoshioka M, Doucet E, Drapeau V, Dionne I & Tremblay A. Combined effects of red pepper and caffeine consumption on 24 h energy balance in subjects given free access to foods. *Br J Nutr* 2001 85 203-211.

Study protocol, overview

19°C

Enrollment → Metabolic Unit admission → Equilibration diet → 12-hour respiration chamber

24°C

Equilibration diet → 12-hour respiration chamber → Equilibration diet → 12-hour respiration chamber

Equilibration diet: 2 days

12-hour Respiration Chamber stay, procedures and specimens sampling protocol

Core temperature, Heart rate, Blood pressure recording

Microdialysis (samples q30’)

Meal

Blood Sampling

12-hour Urine collection

Microdialysis probe insertion

= 1 hour

Microdialysis probe removal
Adipose tissue biopsy (voluntary)

Figure 1
Assessed for Eligibility (n=60)

Excluded (n=31)
- Not meeting inclusion criteria (n=21)
- Refused to participate (n=10)

Randomized (n=29)

Allocated to 19°C/24°C intervention (n=14)
- Received allocated intervention (n=12)
- Did not receive allocated intervention (n=2)
  - unable to participate (n=1)
  - chamber failure (n=1)

Allocated to 24°C/19°C intervention (n=15)
- Received allocated intervention (n=13)
- Did not receive allocated intervention (n=2)
  - unable to participate (n=1)
  - vaso-vagal episode at start of study (n=1)

Respiratory chamber recordings analyzed for EE (n=21)

Excluded from respiratory chamber recordings for EE (n=1) due to non compliance to physical activity restriction

Excluded from respiratory chamber recordings for EE (n=3) due to recording equipment failure

Serial blood samples analyzed (n=22)

Excluded due to poor venous access (n=3)

Figure 2
Figure 3

- **Energy Expenditure (kcal/hr)**
  - 60
  - 70
  - 80
  - 90
  - 100
  - 110

- **24°C**
  - Males: Circles
  - Females: Open circles

- **19°C**
  - Males: Circles
  - Females: Open circles

- **Significance:**
  - **p < 0.001**

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Figure 4
### Table I: Subject characteristics (mean ± standard deviation)

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<th>Male (N=15)</th>
<th>Female (N=10)</th>
<th>Total (N=25)</th>
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<tr>
<td>Height (cm)</td>
<td>174.7 ± 7.6</td>
<td>165.8 ± 6.3</td>
<td>171.1 ± 8.3</td>
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<tr>
<td>Weight (kg)</td>
<td>73.1 ± 9.6</td>
<td>61.0 ± 5.3</td>
<td>68.2 ± 10.0</td>
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<td>BMI (kg/m²)</td>
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<td>22.2 ± 1.9</td>
<td>23.3 ± 2.2</td>
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<tr>
<td>Age (yrs)</td>
<td>26.1 ± 5.8</td>
<td>32.3 ± 10.6</td>
<td>28.6 ± 8.5</td>
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<tr>
<td>Body Fat (%)*</td>
<td>19.7 ± 6.2</td>
<td>26.4 ± 3.5</td>
<td>22.7 ± 6.1</td>
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<tr>
<td>Caloric Content of Meal in</td>
<td>837 ± 163</td>
<td>630 ± 78</td>
<td>754 ± 169</td>
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<tr>
<td>Respiration Chamber (kcal)**</td>
<td>8 Caucasian</td>
<td>6 Caucasian</td>
<td>14 Caucasian</td>
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<tr>
<td></td>
<td>2 Black</td>
<td>3 Black</td>
<td>5 Black</td>
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<tr>
<td></td>
<td>4 Asian</td>
<td>1 Asian</td>
<td>5 Asian</td>
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<tr>
<td></td>
<td>1 Hispanic</td>
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* N = 22 (10 Female)

**30% of calculated daily energy needs
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<th></th>
<th>N</th>
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<td><strong>Calorimetry</strong></td>
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<tr>
<td>Energy Expenditure (kcal/hr)</td>
<td>21</td>
<td>82.09</td>
<td>86.98</td>
<td>77.94</td>
<td>83.38</td>
<td>86.70</td>
<td>90.52</td>
<td>p&lt;0.001</td>
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<tr>
<td>Respiratory Quotient</td>
<td>21</td>
<td>0.82</td>
<td>0.82</td>
<td>0.80</td>
<td>0.79</td>
<td>0.85</td>
<td>0.84</td>
<td>p=0.09</td>
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<tr>
<td>Wrist Activity (activity counts)</td>
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<td>69,442</td>
<td>72,434</td>
<td>27,402</td>
<td>30,823</td>
<td>27,798</td>
<td>26,884</td>
<td>p=0.97</td>
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<td>Hip Activity (activity counts)</td>
<td>21</td>
<td>3,897</td>
<td>3,609</td>
<td>1,554</td>
<td>1,586</td>
<td>1,657</td>
<td>1,412</td>
<td>p=0.21</td>
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<tr>
<td>Ankle Activity (activity counts)</td>
<td>22</td>
<td>11,662</td>
<td>10,690</td>
<td>4,324</td>
<td>4,384</td>
<td>5,691</td>
<td>4,917</td>
<td>p=0.53</td>
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<tr>
<td><strong>Body Temperature</strong></td>
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<tr>
<td>Core Body Temperature (°C)</td>
<td>10</td>
<td>36.90</td>
<td>36.87</td>
<td>36.79</td>
<td>36.82</td>
<td>37.04</td>
<td>36.96</td>
<td>p=0.06</td>
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<td>Neck Temperature (°C)</td>
<td>17</td>
<td>35.12</td>
<td>31.05</td>
<td>34.96</td>
<td>33.82</td>
<td>35.28</td>
<td>34.32</td>
<td>p=0.001</td>
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<tr>
<td>Abdominal Temperature (°C)</td>
<td>22</td>
<td>34.32</td>
<td>32.99</td>
<td>34.12</td>
<td>32.63</td>
<td>34.47</td>
<td>33.40</td>
<td>p=0.0016</td>
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<tr>
<td>Thigh Temperature (°C)</td>
<td>21</td>
<td>32.62</td>
<td>30.63</td>
<td>32.39</td>
<td>30.39</td>
<td>32.93</td>
<td>30.98</td>
<td>p=0.001</td>
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<tr>
<td><strong>Heart Rate Variability</strong></td>
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<tr>
<td>Heart Rate (BPM)</td>
<td>20</td>
<td>65.26</td>
<td>62.33</td>
<td>61.89</td>
<td>59.52</td>
<td>68.78</td>
<td>65.43</td>
<td>p=0.005</td>
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<tr>
<td>Very Low Frequency HRV (msec^2)</td>
<td>20</td>
<td>4,146</td>
<td>5,432</td>
<td>4,803</td>
<td>6,311</td>
<td>3,487</td>
<td>4,413</td>
<td>p=0.14</td>
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<tr>
<td>Low Frequency HRV (msec^2)</td>
<td>20</td>
<td>1737</td>
<td>2233</td>
<td>1972</td>
<td>2491</td>
<td>1485</td>
<td>1938</td>
<td>p=0.021</td>
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<tr>
<td>High Frequency HRV (msec^2)</td>
<td>20</td>
<td>663</td>
<td>962</td>
<td>744</td>
<td>1056</td>
<td>575</td>
<td>837</td>
<td>p=0.0043</td>
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</tbody>
</table>

*p<0.05 compared to pre-prandial period of the same day
**p<0.01 compared to pre-prandial period of the same day
## Table III Laboratory data

<table>
<thead>
<tr>
<th></th>
<th>24°C</th>
<th>19°C</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td><strong>Catecholamines</strong></td>
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</tr>
<tr>
<td>Plasma norepinephrine AUC (x10⁵ pg.min/mL)</td>
<td>2.74 ± 1.11</td>
<td>4.68 ± 2.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma norepinephrine 420' (pg/mL)</td>
<td>464.0 ± 264.7</td>
<td>895.7 ± 565.9</td>
<td>&lt;0.001</td>
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<tr>
<td>Urine epinephrine (µg/12-hour)</td>
<td>5.10 ± 2.415</td>
<td>6.41 ± 3.636</td>
<td>0.05</td>
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<tr>
<td>Urine norepinephrine (µg/12-hour)</td>
<td>20.70 ± 8.659</td>
<td>29.44 ± 11.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urine DOPA (µg/12-hour)</td>
<td>118.1 ± 47.80</td>
<td>136.3 ± 42.08</td>
<td>0.03</td>
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<tr>
<td>DOPAC AUC (x10⁵ pg.min/mL)</td>
<td>0.97 ± 0.45</td>
<td>1.14 ± 0.69</td>
<td>0.04</td>
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<tr>
<td>DHPG AUC (x10⁵ pg.min/mL)</td>
<td>8.12 ± 3.59</td>
<td>9.35 ± 4.25</td>
<td>&lt;0.01</td>
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<tr>
<td><strong>Glucocorticoid Axis</strong></td>
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<td></td>
</tr>
<tr>
<td>ACTH AUC (x10⁴ pg.min/mL)</td>
<td>1.25 ± 0.42</td>
<td>1.29 ± 0.54</td>
<td>0.49</td>
</tr>
<tr>
<td>Serum cortisol AUC (x10³ µg.min/dL)</td>
<td>6.69 ± 1.26</td>
<td>7.03 ± 1.26</td>
<td>0.21</td>
</tr>
<tr>
<td>Urine cortisol (µg/g Creatinine)</td>
<td>28.5 ± 17.4</td>
<td>38.5 ± 33.2</td>
<td>&lt;0.02</td>
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<tr>
<td><strong>Thyroid Hormone Axis</strong></td>
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<tr>
<td>TSH AUC (x10³ µUI.min/mL)</td>
<td>0.98 ± 0.57</td>
<td>1.04 ± 0.53</td>
<td>0.22</td>
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<tr>
<td>T3 AUC (x10⁵ ng.min/dL)</td>
<td>7.02 ± 1.14</td>
<td>7.22 ± 1.31</td>
<td>0.07</td>
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<tr>
<td>Free T4 AUC (x10³ ng.min/dL)</td>
<td>0.98 ± 0.13</td>
<td>1.02 ± 0.15</td>
<td>0.02</td>
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<tr>
<td><strong>Serum Glucose and Insulin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose AUC (x10⁴ mg.min/dL)</td>
<td>65.90 ± 4.32</td>
<td>65.03 ± 4.20</td>
<td>0.29</td>
</tr>
<tr>
<td>Fasting glucose AUC (-15'-300') (x10³ mg.min/dL)</td>
<td>26.63 ± 1.95</td>
<td>26.62 ± 2.04</td>
<td>0.99</td>
</tr>
<tr>
<td>Post-meal glucose AUC (360'-540') (x10³ mg.min/dL)</td>
<td>19.71 ± 2.55</td>
<td>18.69 ± 2.61</td>
<td>0.04</td>
</tr>
<tr>
<td>Insulin AUC (x10³ mIU.min/mL)</td>
<td>8.53 ± 3.44</td>
<td>10.77 ± 6.34</td>
<td>0.07</td>
</tr>
<tr>
<td>Fasting insulin AUC (-15'-300') (x10³ mIU.min/mL)</td>
<td>0.65 ± 0.86</td>
<td>0.83 ± 0.98</td>
<td>0.04</td>
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<tr>
<td>Post-meal insulin AUC (360'-540') (x10³ mIU.min/mL)</td>
<td>5.78 ± 2.19</td>
<td>7.39 ± 5.11</td>
<td>0.13</td>
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<tr>
<td><strong>Subcutaneous Adipose Tissue Microdialysis</strong></td>
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<tr>
<td>Glycerol AUC (x10⁵ µmol.min/mL)</td>
<td>1.12 ± 0.50</td>
<td>1.35 ± 0.62</td>
<td>0.07</td>
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<tr>
<td>Glucose AUC (x10³ mmol.min/mL)</td>
<td>2.84 ± 0.60</td>
<td>2.56 ± 0.60</td>
<td>0.07</td>
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<tr>
<td><strong>Other Measurements</strong></td>
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<tr>
<td>Free fatty acids AUC (x10³ µEq.min/L)</td>
<td>2.88 ± 0.93</td>
<td>3.24 ± 1.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>β-hydroxybutyrate AUC (x10⁴ µmol.min/L)</td>
<td>7.70 ± 4.27</td>
<td>9.58 ± 6.95</td>
<td>0.50</td>
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<tr>
<td>Creatine kinase AUC (x10⁵ U.min/L)</td>
<td>1.02 ± 0.77</td>
<td>1.08 ± 0.82</td>
<td>0.62</td>
</tr>
<tr>
<td>Urine myoglobin</td>
<td>Not detected</td>
<td>Not detected</td>
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</table>