SUPPLEMENTARY DATA

Supplementary Table 1. 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^^	

SUPPLEMENTARY DATA

Supplementary Table 2. 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

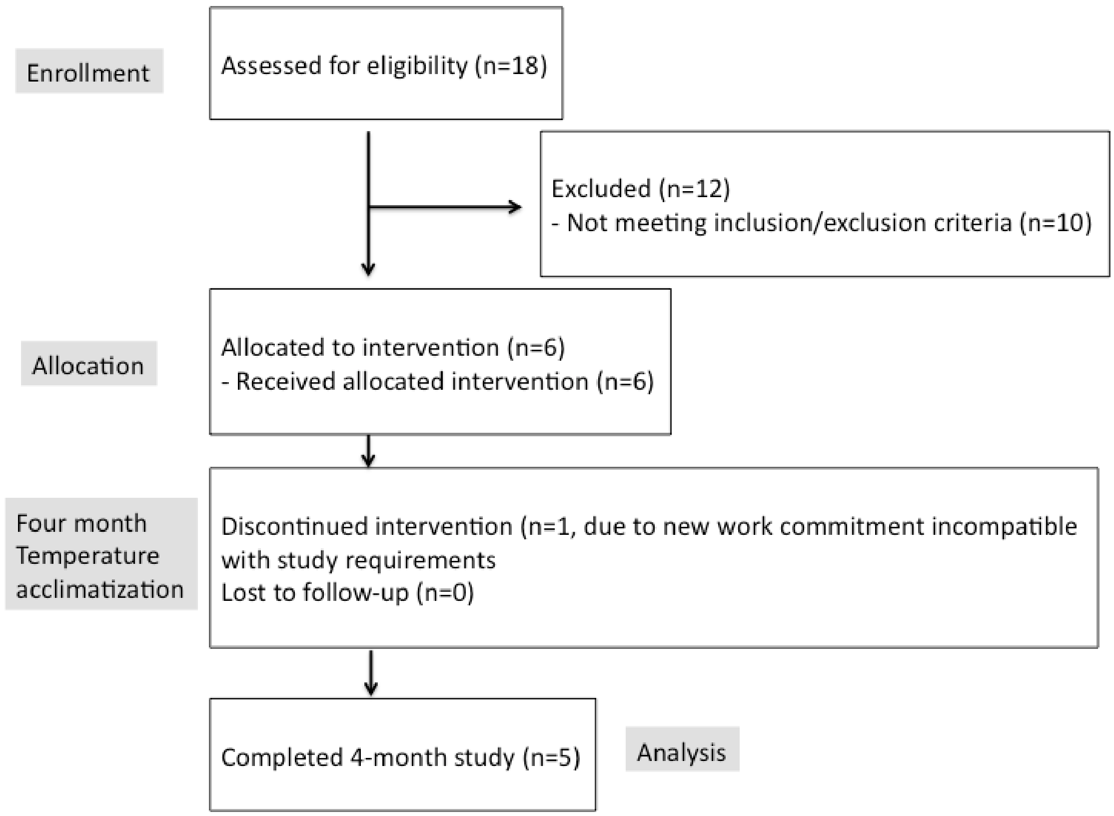
SUPPLEMENTARY DATA

Supplementary Table 3. 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

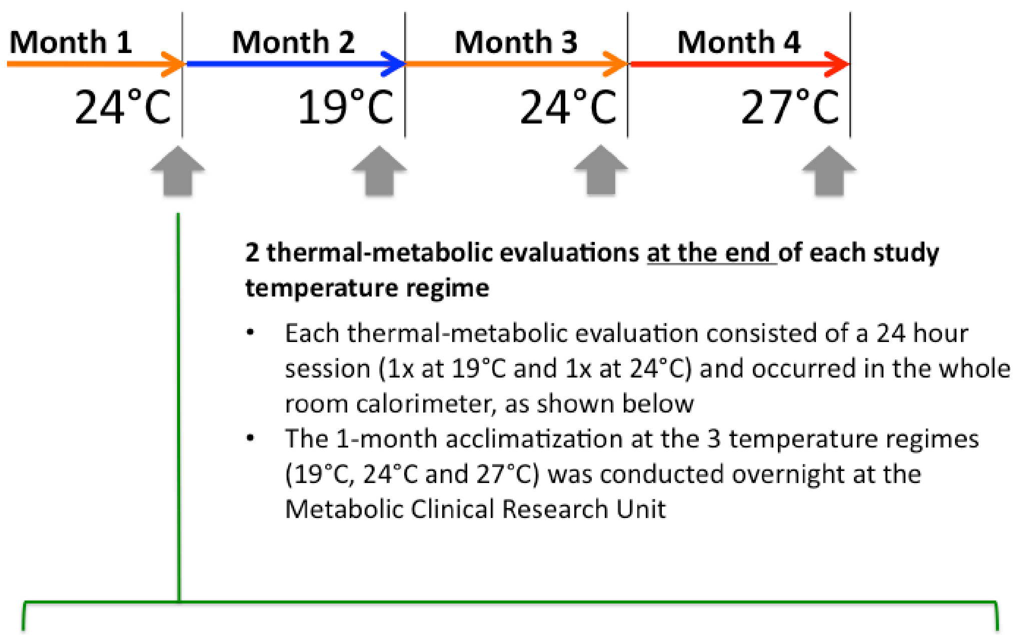
SUPPLEMENTARY DATA

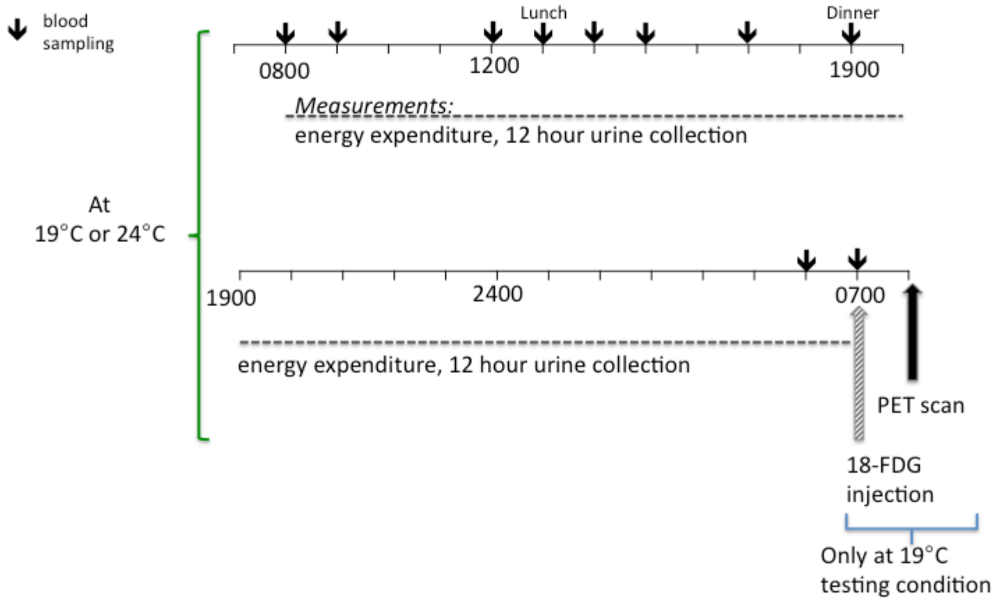
Supplementary Figure 1. Flow chart of volunteer recruitment, allocation and intervention.



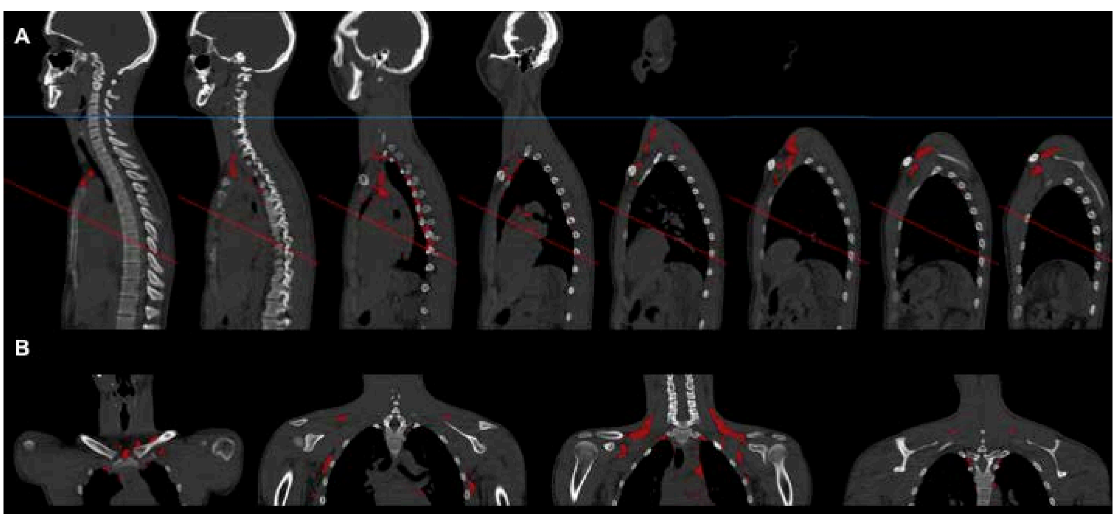
SUPPLEMENTARY DATA

Supplementary Figure 2. 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.



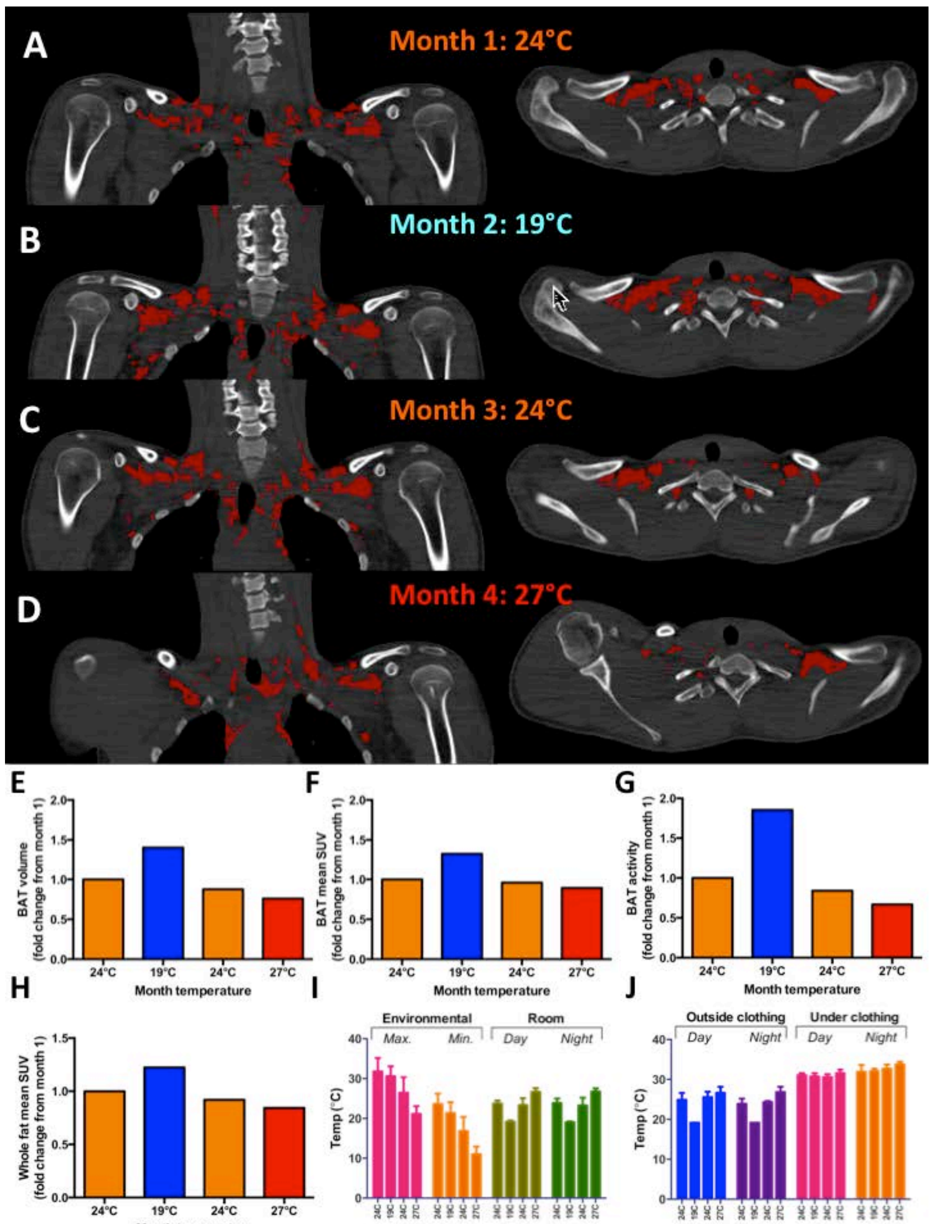


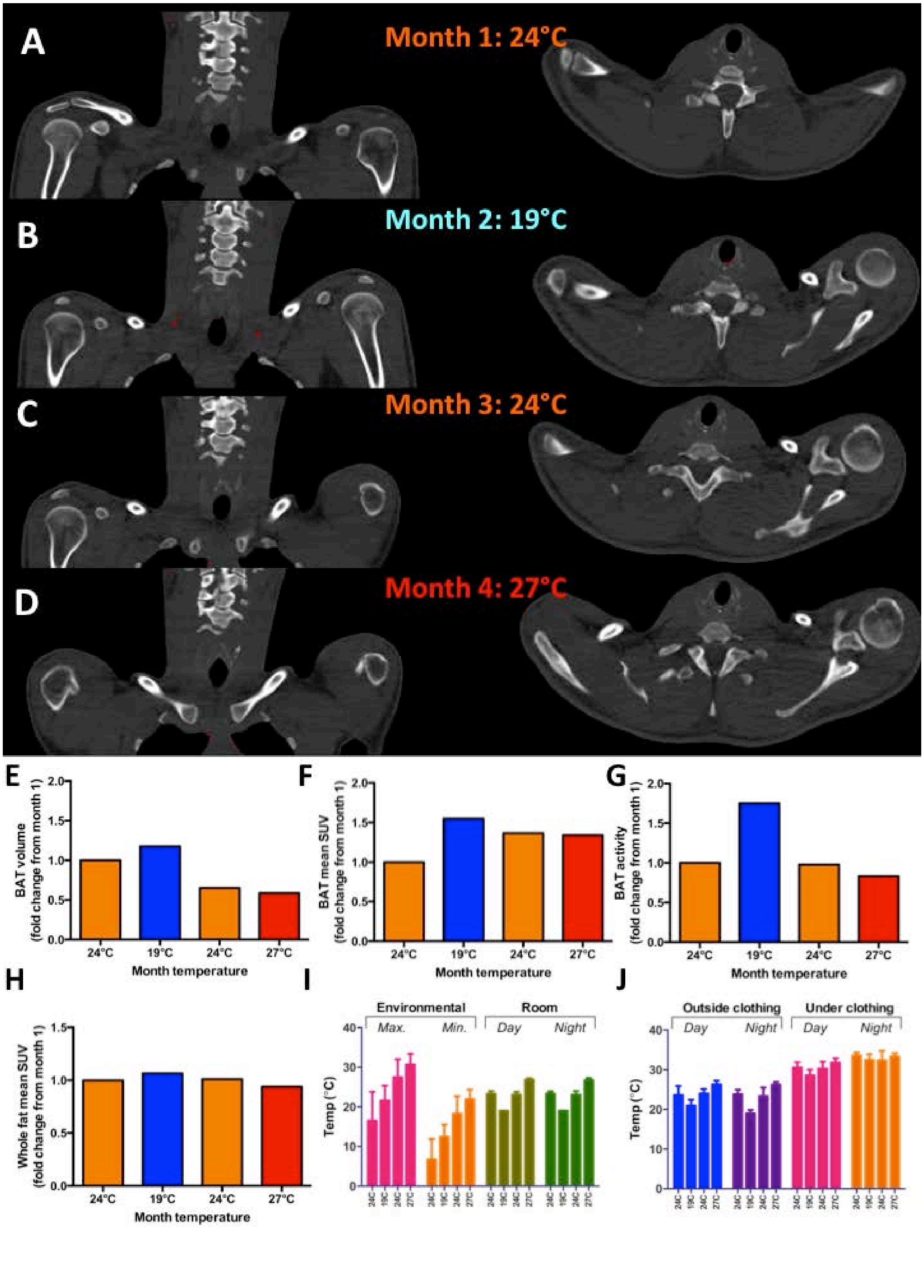
Supplementary Figure 3. Three-dimensional region of interest (ROI) constructed for comparison of BAT volume and activity across 4-month period. The region was defined cranially by a horizontal line (blue) parallel to the base of C4 vertebra, and caudally by an oblique line (maroon) traversing the manubriosternal 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.



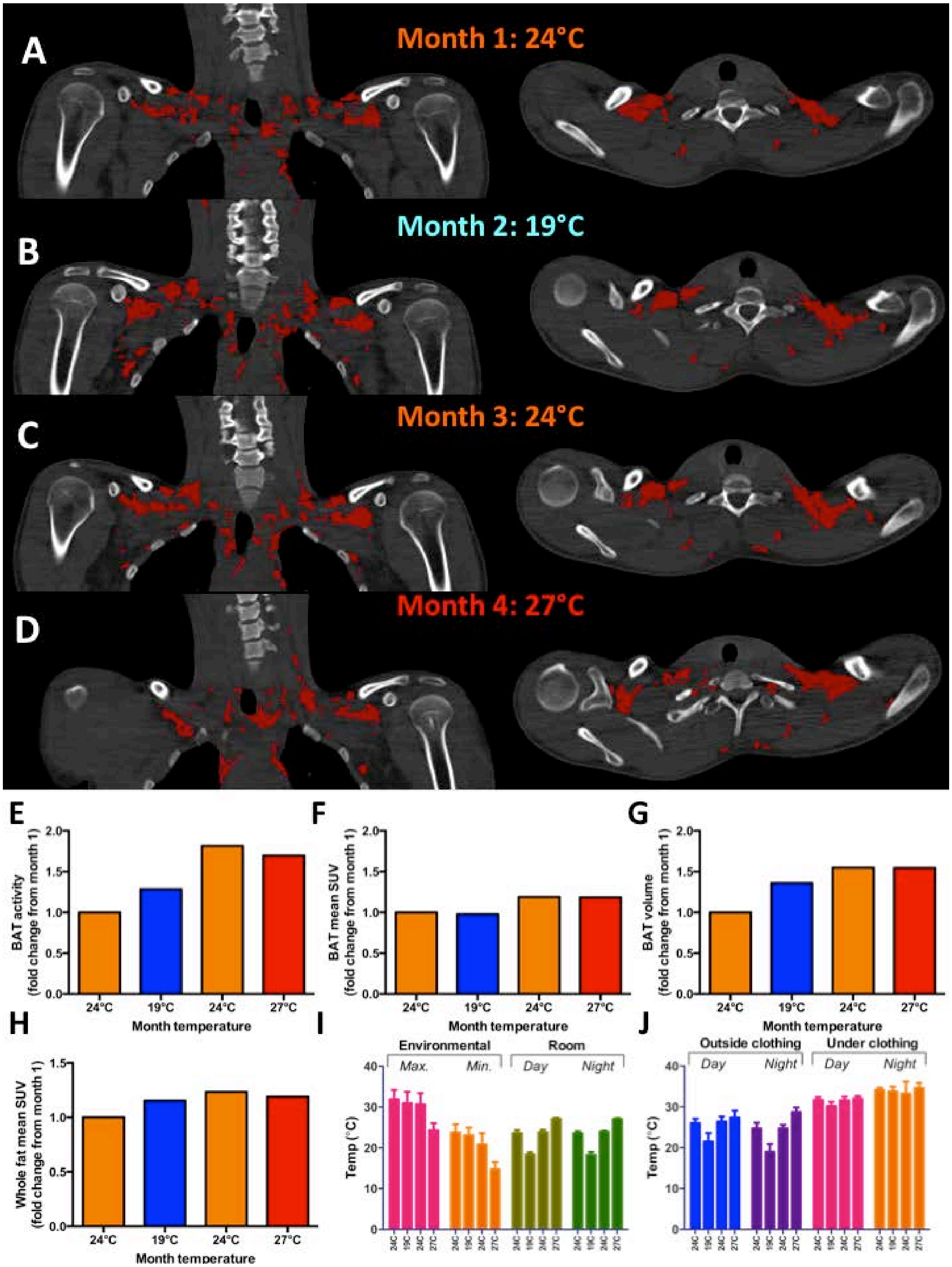
SUPPLEMENTARY DATA

Supplementary Figures 4-7. 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.

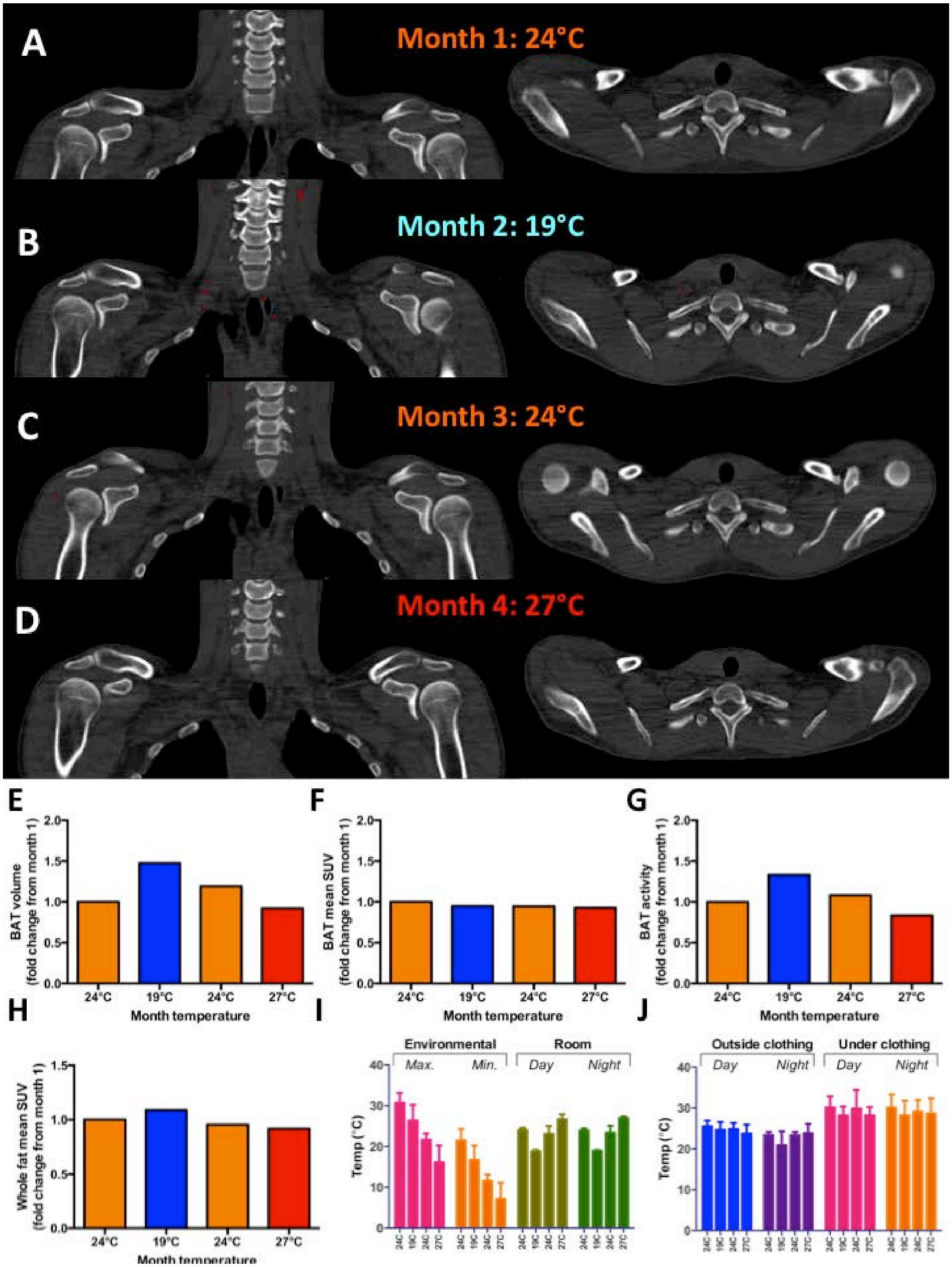




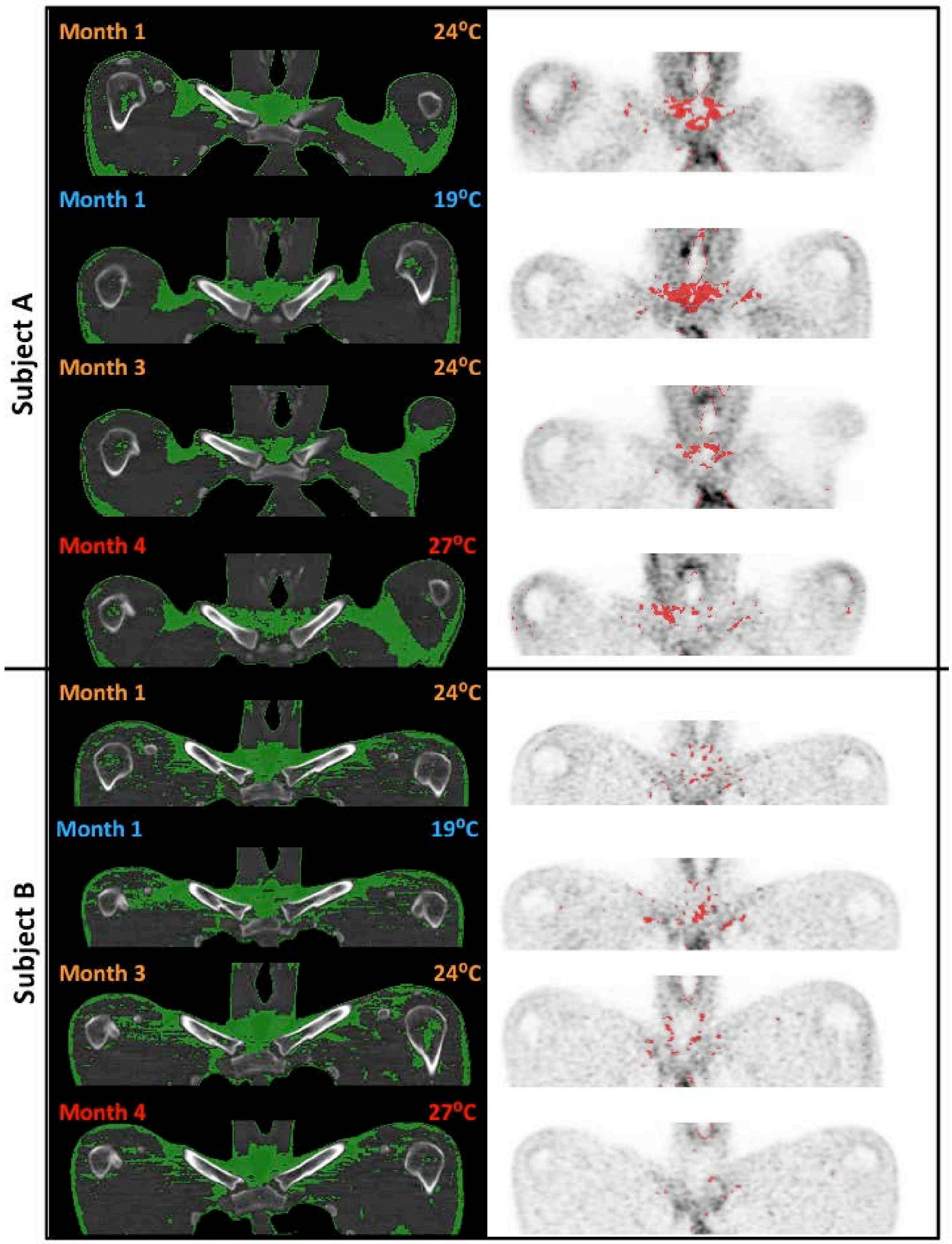
Month 1: 24°C
Month 2: 19°C
Month 3: 24°C
Month 4: 27°C
BAT volume (fold change from month 1)
BAT mean SUV (fold change from month 1)
BAT activity (fold change from month 1)
Whole fat mean SUV (fold change from month 1)
Month temperature
24°C
19°C
27°C
Environmental
Room
Max.
Min.
Day
Night
Outside clothing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



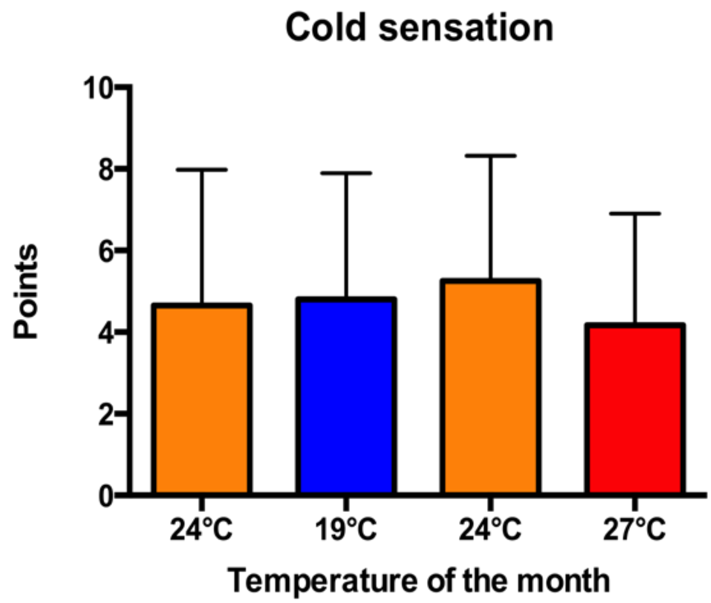
A
Month 1: 24°C
B
Month 2: 19°C
C
Month 3: 24°C
D
Month 4: 27°C
E
BAT activity (fold change from month 1)
F
BAT mean SUV (fold change from month 1)
G
BAT volume (fold change from month 1)
Month temperature
24°C
19°C
24°C
27°C
H
Whole fat mean SUV (fold change from month 1)
I
Environmental
Room
Max.
Min.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


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
Day
Night
Under clothing
Day
Night
Temp (°C)

Supplementary Figure 8. 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.

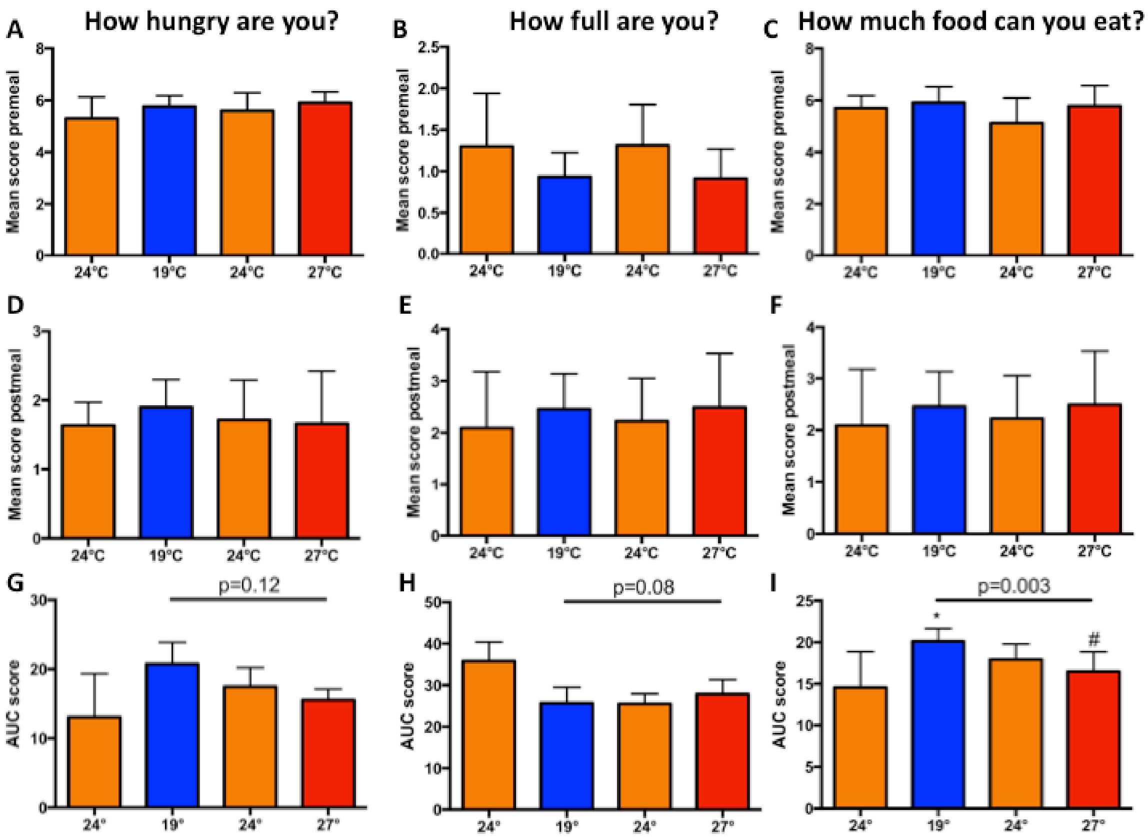


Supplementary Figure 9. Summary of individual perception to cold sensation during study period Participants reported subjectively their perception to cold sensation (how cold do you feel) at the end of each month after 24 hour exposure to 19°C on a visual analogue scale from 1 (not cold at all) to 10 (extremely cold). Perception to cold did not change during monthly acclimation.



SUPPLEMENTARY DATA

Supplementary Figure 10. 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.



SUPPLEMENTARY DATA

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