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Contrasting short-term temperature effects on the profiling of metabolic and stress hormones in non-obese healthy adults: A randomized cross-over trial

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ABSTRACT

The manifestation of elevated and sustained air temperature gradient profiles in urban dwellings represents an emerging planetary health phenomenon. There is currently limited evidence about the effect of elevated air temperatures on metabolic health. The aim of this work was to assess changes in metabolic and stress hormonal profiles during a short-term stay in a mountainous, climate-cooler setting against those observed in the urban setting. A prospective, randomized, 2 x 2 cross-over trial of non-obese healthy adults in urban and mountainous areas of a Mediterranean country (Cyprus) was set up during summer, under real-life conditions. The intervention was a short-term stay (mean ± SD: 7 ± 3 days) in a mountainous, climate-cooler setting (altitude range: 650–1200 m), being ~1-h drive away from the main urban centres of Cyprus. The primary endpoint was the change in metabolic hormones levels (leptin and adiponectin) and stress hormone levels (cortisol) between the two settings. Personal air and skin temperature sensors were deployed while biospecimen were collected in each setting. A total of 41 participants between 20 and 60 years old were enrolled and randomized during July 2018, of whom 39 received the allocated intervention, 8 were lost to follow up or excluded from analysis and a total of 31 participants were analysed. A significant leptin reduction ($\beta = -0.255$; 95% CI: −0.472, −0.038; $p = 0.024$) was observed for non-obese healthy adults during their short-term stay in the mountainous environment. The intervention effect on adiponectin or cortisol levels was not statistically significant ($\beta = 0.058$; 95% CI: −0.237, 0.353; $p = 0.702$), and ($\beta = -0.026$; 95% CI: −0.530, 0.478; $p = 0.920$), respectively. In additional analyses, daily max skin temperature surrogate measures were significantly associated with leptin levels ($\beta = 0.34$; 95% CI: 0.051, 0.633; $p = 0.024$). During summer season, a short-term stay in climatologically cooler areas improved the leptin levels of non-obese healthy adults who permanently reside in urban areas of a Mediterranean country. A larger sample is needed to confirm the trial findings that could provide the rationale for such public health interventions in climate-impacted urban areas of our planet.

1. Introduction

The World Health Organisation recognises the overall health impacts of a changing climate as overwhelmingly negative (World Health Organization, 2017). Seasonal, long-sustained high temperature weather conditions appear to have a direct impact on human health by affecting the body's ability to regulate its internal temperature (Hajat et al., 2017; Hanna and McIver, 2018; Sarofim et al., 2016; Vardoulakis and Heaviside, 2012; Yang et al., 2014). The direct health impacts of exposure to heat are often assessed in terms of mortality risk (Gasparrini et al., 2015), including cardiovascular, respiratory, renal and infectious diseases, and neurological/psychiatric disorders (Basagaña et al., 2011; Xu et al., 2012; Åström et al., 2011; Checkley et al., 2009).

Evidence of community or regional measures to assess heat effects consist primarily of observational studies conducted in the US and Europe (White-Newsome et al., 2014; Lim and Spanger-Seigfried, 2004). Population vulnerability is defined on the basis of three components, namely, the exposure level, the extent of susceptibility and the adaptive capacity (Michelozzi et al., 2014). Groups of elderly people and individuals with impaired health status may be particularly vulnerable to climate change manifestations of higher temperatures, as they have diminished ability to thermo-regulate body core temperature, thus increasing medical co-morbidities or use of medications, while the

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social position of an individual could modify the extent of behavioural adjustments to sustained high temperatures (Bennett et al., 2014; Ishigami et al., 2008).

Earlier observational studies demonstrated the effect of heat stress on the cognitive function of elderly (mean age of 73 yrs old) using repeated measures of residential air temperature records (Gasparrini et al., 2015; Dai et al., 2016). Apart from clinical effects, excessive heat could adversely influence human performance and work capacity (Kjellstrom et al., 2009, 2016), or performance in exercise/sports (Brotherhood, 2008). Recently, the relationship between indoor environmental conditions, heat exposures, sleep and cognitive function was examined among young adults living in centrally-controlled air conditioned (AC) and non-AC residence halls on a university campus (Cedeño Laurent et al., 2018); authors found that students living in non-AC spaces during a heat wave in Boston experienced significant decrements on cognitive performance tests (reaction time, throughput, 2-digit visual addition/subtraction test) relative to AC residents at baseline (Cedeño Laurent et al., 2018).

There is currently limited evidence about the effect of elevated ambient air temperatures on metabolic outcomes. An experimental study showed that the adapted thermogenesis process was reduced in temperatures within the thermal comfort zone of the human being, while colder air exposures increased thermogenesis from the brown adipose tissue (BAT) and energy expenditure processes, resulting in lower body weight (Turner et al., 2016). Some evidence also exists about the increasing ambient temperature effects on age adjusted type II diabetes incidence rates in the US, however, these were premature results (ecological study) (Lee et al., 2014). Another experimental study conducted under well controlled conditions (n = 5) showed that a small elevation in ambient temperature (reaching 27 °C, for a month duration) increased leptin and decreased adiponectin levels (Blauw et al., 2017).

Therefore, the design and testing of cost-effective and sustainable health interventions for the public against the detrimental manifestations of climate change and hot weather are of paramount importance. We designed a pilot experimental trial (TEMP trial) in real-life conditions that examined changes in metabolic and stress hormonal profiles of healthy, non-obese adults in two study settings with distinctly different climatological characteristics: urban vs. higher in altitude (range: 650–1200 m, mean ± SD: 881 ± 200 m) mountainous setting (being 1-h away driving from main urban centres of Cyprus). In addition, the trial examined the relationship between personal air and skin temperatures and adipokines (leptin and adiponectin) between the urban and rural settings.

2. Methods

2.1. Trial oversight

The TEMP trial was a 2 x 2 cross-over pilot trial of healthy, non-obese adults (n = 41). The trial was conducted under real-life conditions (instead of controlled environment) to examine the effect of setting and personal ambient/skin temperature gradient on metabolic hormones levels (Fig. 1). Eligible participants were those who met the following criteria: healthy, non-obese adults between 20 and 60 years old having their permanent primary residence in one of the two largest cities of Cyprus (Nicosia and Limassol) (urban setting), who intended staying in the Troodos area (mountainous setting in Cyprus) for a short period of at least 5 consecutive days between July–September 2018 (majority of them had also a family house in the Troodos mountainous area). There was an approximate difference of 8–10 °C in ambient air temperature values (daily max) between the urban and the mountainous settings using the Cyprus Meteorology Service governmental data for all the months that the trial took place (Fig. S1). The research team was not responsible for any of participant expenses during their mountainous setting stay.

Data and sample collection took place in Cyprus from July until end of September 2018. The full trial protocol can be found in the Appendix (see Study Protocol). Recruitment was made possible via the dissemination of flyers, radio ads and telephone communication with governmental and other non-profitable organisations (Troodos Development Company, Troodos Tourist Board, etc.) and community leaders of the mountainous area. Moreover, personal visits to various camp sites in Troodos area were conducted, following approval from the camp site responsible person. Potential participants were informed about the study, screened for eligibility criteria and agreed to participate via verbal/telephone communication. Exclusion criteria were applied to those being pregnant or obese or suffering from a chronic condition (hypertension, diabetes, metabolic syndrome, cancer), or receiving pharmaceutical treatment for impaired glucose levels or hypertension or antidepressants or thyroxin medication for thyroid disorders, or those who traveled to another country in the week prior to the study initiation. The non-pharmacological health intervention was a short-term (mean ± SD: 7 ± 3 days) stay in the climate-cooler rural areas of the Troodos mountain for those Cypriot urban dwellers during the typically hot summer of the Mediterranean region.

2.2. Ethical statement

This trial was approved by the Cyprus National Bioethics Committee (ΕΕΒΚ/ΕΠ/2018/33) and a written informed consent was signed by the participants prior to the study initiation. The trial was registered in the US (ClinicalTrials.gov Identifier: NCT03625817). The complete trial protocol was submitted on July 13, 2018, four days before actual study start date, but it was first posted on August 10, because of an electronic delay in updating study outcome terminology based on ClinicalTrials.gov standardized language. The trial was performed in accordance with the principles of the Declaration of Helsinki (World Medical Association, 2013).

The authors assume responsibility for the accuracy and completeness of the data and analyses, as well as for the fidelity of the trial. Details of the analysis (i.e. datasets, scripts, workflows) can be found in the Appendix.

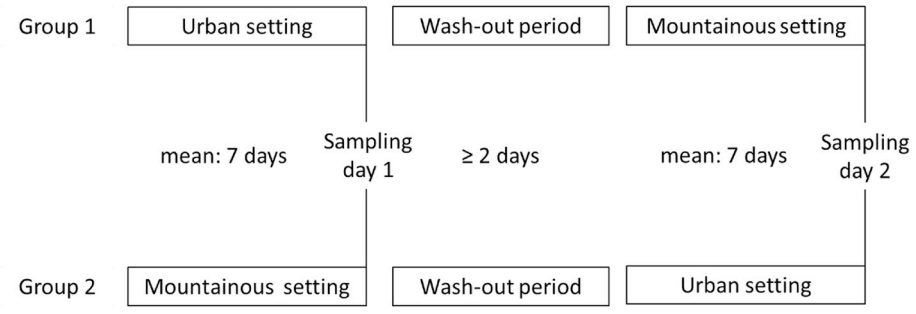

Fig. 1. Trial design and timeline.

2.3. Patient and public involvement

No participants were involved in setting the research question or the outcome measures. No participant was invited to contribute to the review process of the study. There are plans to directly disseminate the results of the research to study participants via a general announcement delivered to each participant's mailbox. The dissemination to the general population will be achieved through media outreach (e.g., press release) upon publication of the study results.

2.4. Randomization and masking

For the estimation of the sample size, the sample size program by the MGH Biostatistics Center was used (Schoenfeld, 2010). The input parameters were: significance level, within patient standard deviation, power and minimal detectable difference in means of leptin levels. Based on the literature, the within subject standard deviation of plasma leptin was assumed to be 3.53 μg/L for females and 0.01 μg/L for males (Saad et al., 1998). Thus, we hypothesized a minimal detectable difference in leptin levels between the urban and mountainous settings to be at least 1.9 μg/L, based on relevant data generated by other intervention studies (Hibi et al., 2017; Fontes-Villalba et al., 2016). Assuming a power of 80% and a two-sided 5% significance level, the sample size for this two-treatment crossover study was estimated to be 57 subjects. A few unexpected challenges during recruitment were presented that made it difficult to obtain the pre-calculated sample size, because most interested individuals expressed the interest to spend only a weekend in the mountainous setting, and the age group that typically expressed interest in staying longer in the mountain was ineligible (mostly $>$ 60 years old). Thus, the estimated size (n $=$ 57) was not feasible to reach during a summer's time, reaching a final size of 41 participants.

Per the trial protocol, random allocation of the intervention was made *a priori* to two groups which differed in the sequence of the treatments; this was done in blocks in order to keep the sizes of treatment groups similar. Group 1 included volunteers initially staying in their permanent house in the urban setting and then moving to the rural/mountainous setting for at least 5 days; Group 2 included individuals who initially stayed in the mountainous setting for at least 5 days and then returned to their permanent residence in the urban setting. Block randomization was performed using the function RAND () in Excel with a randomly generated list by a research investigator with no trial involvement. Details on the block randomization are available in the Supplementary Appendix (see Study Protocol). The initial allocation to the two groups was equal; however, during recruitment, a few participants (n $=$ 8) reported that due to major personal/family reasons they opted to continue the study in the other group than that initially allocated to, resulting to unequal group sizes. We clarify here that these 8 participants completed both treatment periods, but in the reverse order; a sensitivity analysis done without these 8 participants, showed the same trends of metabolic hormones as those presented in the main analysis (Table S15). Due to the inherent non-pharmacological nature of the trial, blinding of the participants to group assignment was not possible. The blinding of the researchers to the subjects' identity was achieved by the coding of all study materials (urine containers, questionnaires, and diaries). The personnel who delivered the intervention did not take outcome measures. All outcome assessors/analysts were kept masked to the allocation.

2.5. Trial procedures

Participants spent their short-term stay in the following rural communities of the mountainous Troodos area in Cyprus: Agros, Alona, Platania, Kakopetria, Kalopanayiotis, Louvaras, Moniatis, Oikos, Platanistasa (altitude range: 650–1200 m, mean $\pm$ SD: 881 $\pm$ 200 m). The researcher visited the participant one day before the first sampling day ($\geq$4 days after being in the allocated setting). Upon signing the informed consent, the participant completed the baseline questionnaires (questionnaire A on demographics, lifestyle/behavior, etc., and the Munich ChronoType Questionnaire). Study materials (coded vials for urine sampling in plastic bag, 24-h recall food diary for all time-stamped daily activities, personal air and skin temperature sensors, instructions for urine collection and handling of temperature sensors) were provided. Participants were asked to keep (to the extent possible) their sleeping and eating habits, in both the urban and mountainous settings, constant. The researcher explained to participants that they had to collect separately all urine voids, noting down their day's activities in the activity diary and to wear the personal temperature sensors continuously until mid-day (noon) of the following to the sampling day. Participants were also asked to temporarily store the urine vials in their home freezer until collection on the following day by the researchers. During the second visit, researchers arranged with the participant the next visit that would take place $\geq$4 days after being at the other setting (urban or mountainous/rural, depending on the group allocation scheme). Based on the study protocol, an at least 2 days washout period between settings was deemed necessary (mean $\pm$ SD: 16 $\pm$ 9 days, range: 2–36 days) (Fig. 1). Participants, during the washout period, stayed in their regular urban environment.

2.6. Sensor data

Per the trial protocol, sensors of personal air and skin temperature, including activity tracking (as a surrogate marker of physical activity) (e-TACT, BodyCAP Medical, France) were worn by each participant. The personal air temperature sensor was worn as a tag on the participant's chest area, while the skin sensor was attached to the armpit. For the skin temperature sensor, only data collected with temperatures $\geq$34 °C were included in the final data analysis, because we assumed that the participant wasn't wearing the sensor, if skin temperature data were $<$ 34 °C. Skin/personal air temperature peaks characteristic of unique temperature microenvironments were identified across the 24-h period using the PeakFinder function, a noise tolerant fast peak detection algorithm available through MATLAB (PeakFinder.m, Mathworks file ID: 25500, Supplementary Appendix). For each sensor, we specified the temperature measurement period (1 min), sampling frequency (50 Hz), the actimetry period (1 min), the accelerometer sensitivity threshold to consider an activity as important or not (0.1 g) and the measurement range (2G). The activity sensor tracking mode used the classic TAT method (time above threshold) as typically used for physical activity measurements (Jean-Louis et al., 2001).

2.7. Outcomes

Per the trial protocol, the primary outcomes were the metabolic (leptin and adiponectin) and stress (cortisol) hormone profiling in the two different climatological settings. Urinary leptin and adiponectin protocol measurements have been used in previous health studies as a non-invasive protocol to assess the levels of these adipokines (Zaman et al., 2003; Jeon et al., 2013). Metabolic hormones (leptin and adiponectin) were quantified using immunoassay kits that had been validated for human urine (immunoassays, Elabscience, US). The human adiponectin ELISA kit had a sensitivity of 0.47 ng/mL, and detection range of 0.78–50 ng/mL with a coefficient of variation being $<$ 10%. Similarly for the human leptin ELISA kit, its sensitivity was 46.9 pg/mL with detection range of 78–5000 pg/mL, and a coefficient of variation of $<$ 10% (immunoassays, Elabscience, US). Urinary cortisol levels were determined using an adaption of a GC-MS/MS method, with a limit of detection equal to 0.17 μg/L (Moon et al., 2009). Creatinine was quantified using the Jaffe method with a limit of detection equal to 0.25 g/L (Angerer and Hartwig, 2010).

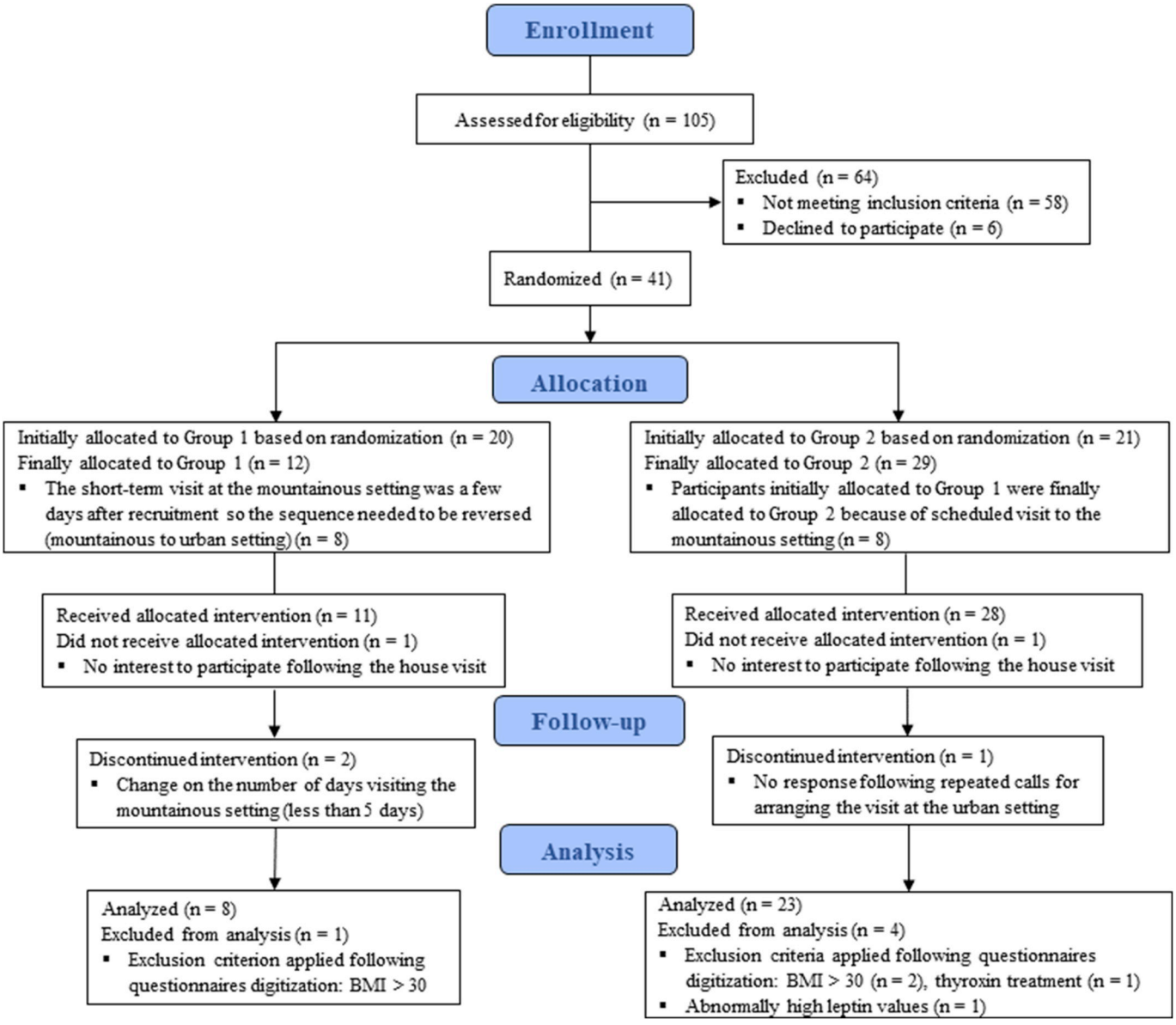

Fig. 2. Flow chart of participants included in the analysis.

2.8. Urine sample collection

Two days of urine sampling per participant were obtained, one day in the end of the ≥ 5-day period in the urban setting and one day in the end of the ≥ 5-day period in the mountainous/rural setting. On the sampling day, the first morning void urine sample was discarded and the first morning void of the following day was obtained. All bladder content was emptied into separate vials, whenever participants urinated. The time stamp of each urination and its volume were recorded in a diary. All samples were stored in the freezer until handed to the laboratory, where aliquoting was performed and samples were stored at −80 °C until analysis. Due to budgetary constraints, only two samples per setting were included in this analysis: the first morning void and the last before sleep samples. In cases where the first morning void wasn't available, then the first sample of the sampling day was used (n = 6, collection time mean (range): 11:00 am, (05:30–15:30)).

2.9. Statistical analysis

The baseline characteristics were presented overall and by study group. Categorical variables were described with sample size and percentages and compared by the chi-square test. Approximately normally-distributed continuous variables were described with means and standard deviations (SD), and compared by the *t*-test. Non-normal continuous variables were described with medians and interquartile ranges (IQR) and compared by the Wilcoxon test. The caloric intake in each sampling day was calculated by estimating portion sizes as recorded in the 24-h self-reported food recall diary (Supplementary Appendix). Variables with skewed distributions were log transformed to approximate normality. All outcome variables were adjusted for creatinine to account for urine dilution. Hormone and creatinine values below the LOD were imputed to LOD/2.

For the analysis of the primary outcomes, linear mixed effect models were fitted for the adipokines and cortisol levels (log-transformed) as a function of the treatment/setting (mountainous vs urban) and sampling time (first morning vs. last before night sleep sample) of the diurnal hormonal measurements, accounting for both between and within subject variability. All models included participant-level (repeated measures within person) random intercepts with an unstructured covariance matrix. The treatment group effect was addressed via the

inclusion of random terms effect for participants, since participants were allocated to the treatment order; urban - mountainous and mountainous – urban groups.

In additional analyses, we used mixed-effect linear regression models to evaluate the effect of possible confounders, such as sex, BMI, age, physical activity (using the surrogate of daily cumulative activity from skin sensors tracking), dietary habits (daily caloric intake) and psychosocial stress (urinary cortisol measurements, in models where the outcome was leptin or adiponectin). In these additional models, fixed effect terms were the treatment and one of the aforementioned possible confounder variables. In another set of additional models, we evaluated the effect of the temperature sensor data on the adipokines and cortisol levels; the treatment (setting) variable wasn't included as a fixed effect term as it was inherently correlated with air temperature. Another set of sensitivity analysis was undertaken based on the additional analysis of the outcomes for the hormones of leptin and adiponectin with linear mixed-effect models adjusted for sex, since sex is modifying the magnitude of metabolic hormone levels. The number of fixed effect terms in the models was intentionally limited, due to the small sample size of the trial and due to the strict adherence to the study protocol and its statistical analysis plan.

Statistical tests and confidence intervals were two-sided with the statistical significance level set at 5%. Wherever possible, 95% confidence intervals were presented. All analyses were performed in R (v.3.6.1) with RStudio (v.1.1.463). The full list of packages used in the analysis and the script can be found in the Supplementary Appendix.

3. Results

3.1. Participant characteristics

A total of 41 subjects were recruited and agreed to participate; and upon house visit, 2 of them declined participation (Fig. 2). During follow-up, 3 participants were excluded, since they only provided urinary samples in one of the two settings. During analysis, 5 additional participants were excluded, because exclusion criteria applied following questionnaires digitization [obese status (n = 3) and ongoing thyroxin treatment (n = 1)] and because of abnormally high leptin levels in all four samples of a participant (mean ± SD: 3926 ± 209 ng/L). Eventually, thirty one non-obese adult volunteers (36% males, 41.5 ± 11 years old, BMI: 24.5 ± 3 kg/m^2) were included in the main analysis; 23 of them were allocated in Group 2 (first mountainous and then urban setting) and the rest in Group 1 (first urban setting and then crossing over to the mountainous setting) (Table 1). High educational attainment was noted, with the majority holding at least a university/college degree (71%). At baseline, most participants were non-smokers (71%) with intermediate chronotype (62%) and spending about 5.5 h per day on electronic screens. About half of them reported that they never/rarely consume alcohol (42%) and not regularly exercising (45%). Baseline characteristics of the 8 participants who dropped out are presented in the Supplementary Appendix (Table S1).

3.2. Follow-up and outcomes

Data from the wearable air and skin temperature sensors was collected for a number of participants (n = 29 for air temperature sensors with n = 14 in both settings, and n = 27 for skin sensors with n = 14 in both settings) due to the following constraints: i) sensor availability: most participants visited the mountainous setting at around the same period and the available number of sensors was inadequate, ii) technical issues: few sensors stopped recording before the end of the sampling day (n = 2), and iii) personal choices of a few participants who didn't want to use the sensor at the urban setting (n = 2). Personal air temperature sensors produced diurnal data points in the range of 21,620–41,260 data counts; median [IQR] temperature was about 2 °C lower (p < 0.001) in the mountainous vs. the urban setting (26.7 °C [25.4, 28.7] vs. 28.6 °C [27.1, 30.5]) (Table 2). Skin temperature sensors generated data that ranged between 12,240 and 27,080 data counts; median skin temperature was similar between the two settings (35.1 °C [34.6, 35.6] vs. 35.1 °C [34.6, 35.7]). Median [IQR] maximum personal air temperatures were lower, though not statistically different (p = 0.07), in the mountainous setting (31.9 °C [30.6, 33.5] vs. 34.2 °C [31.8, 35.9] in the urban setting), and slightly higher in the urban setting for the skin temperature (36.4 °C [36.3, 37.0] vs. 36.6 °C [36.3, 36.8]). The median [IQR] number of diurnal skin temperature peaks (denoting a drastic change in temperature slope within a short time interval) was lower (p = 0.026) in the mountainous setting compared to that for the urban areas (6.0 [3.3, 7.0] vs. 7.0 [6.3, 9.0]) (Table S2), presumably because of larger reported percentage of indoor environments with AC use in the urban setting (kitchen: 32% vs 3% in mountain, and living room: 77% vs. 10% in mountain) (Table S7). All samples had leptin and adiponectin values above LOD, while only 9% and 12% of the samples had values below LOD for creatinine and cortisol, respectively.

In mixed-effect regression models, during the short-term stay in the mountainous setting, participants in both groups had on average significantly lower levels of leptin (β = −0.26; 95% CI: −0.472, −0.038; p = 0.024) (Table 3). No significant effect of the treatment (setting) was observed for adiponectin despite a positive trend (β = 0.058; 95% CI: −0.237, 0.353; p = 0.702). No significant difference in cortisol levels was observed between the mountainous and urban settings

Table 1
Demographics and baseline characteristics of the study population (overall and by group).

	Overall	Group 1	Group 2	p-value[a]
N	31	8	23	
Age (mean (SD))	41.5 (10.8)	40.9 (8.0)	41.7 (11.6)	0.868
Sex (%)				0.771
Female	20 (64.5)	6 (75.0)	14 (60.9)	
Male	11 (35.5)	2 (25.0)	9 (39.1)	
BMI (mean (SD))	24.5 (3.0)	25.9 (3.7)	24.0 (2.6)	0.117
BMI categories (%)				0.571
Normal	20 (64.5)	4 (50.0)	16 (69.6)	
Overweight	11 (35.5)	4 (50.0)	7 (30.4)	
Education level (%)				0.736
Master/PhD	11 (35.5)	2 (25.0)	9 (39.1)	
University/college	11 (35.5)	3 (37.5)	8 (34.8)	
Secondary	9 (29.0)	3 (37.5)	6 (26.1)	
Chronotype categories^ (%)				0.034
Early	5 (23.8)	4 (57.1)	1 (7.1)	
Intermediate	13 (61.9)	2 (28.6)	11 (78.6)	
Late	3 (14.3)	1 (14.3)	2 (14.3)	
Smoking status (%)				0.829
Smoker	6 (19.4)	2 (25.0)	4 (17.4)	
Non-smoker	22 (71.0)	5 (62.5)	17 (73.9)	
Former smoker	3 (9.7)	1 (12.5)	2 (8.7)	
Alcohol consumption frequency (%)				0.648
Weekly	12 (38.7)	2 (25.0)	10 (43.5)	
Monthly	6 (19.4)	2 (25.0)	4 (17.4)	
Rarely/Never	13 (41.9)	4 (50.0)	9 (39.1)	
Physical exercise (%)				0.017
Yes	17 (54.8)	1 (12.5)	16 (69.6)	
No	14 (45.2)	7 (87.5)	7 (30.4)	
Screen time (hours/day) (mean (SD))	5.5 (3.6)	5.9 (3.5)	5.4 (3.7)	0.747
Days in mountainous setting (mean (SD))	7.3 (2.7)	7.0 (1.2)	7.4 (3.1)	0.485
Washout period days (mean (SD))	15.6 (8.9)	11.9 (7.3)	16.9 (9.2)	0.172

^the chronotype categories cut-offs are: early (< 3:00), intermediate (3:00–5:00) and late (> 5:00) (Roenneberg, 2012).

[a] The above variables were tested for differences between the two groups by chi-square tests for categorical variables and t-tests for normally distributed continuous variables.

Table 2
Summary table of temperature and activity variables based on the ambient air and skin sensor data.

	Mountainous		Urban		p-value[a]
	n	median [IQR]	n	median [IQR]	
Ambient air temperature (°C)	21,615	26.7 [25.4, 28.7]	41,259	28.6 [27.1, 30.5]	< **0.001**
Skin temperature (°C)	12,239	35.1 [34.6, 35.6]	27,078	35.1 [34.6, 35.7]	0.726
Activity^	12,239	0.0 [0.0, 55.0]	27,078	0.0 [0.0, 47.8]	0.639
Min ambient air temperature (°C)	14	23.4 [20.7, 24.6]	29	24.9 [23.9, 26.3]	**0.032**
Max ambient air temperature (°C)	14	31.9 [30.6, 33.5]	29	34.2 [31.8, 35.9]	0.074
Max skin temperature (°C)	14	36.4 [36.3, 37.0]	27	36.6 [36.3, 36.8]	0.858
Median skin temperature (°C)	14	35.0 [34.7, 35.3]	27	35.0 [34.8, 35.2]	0.967
Max activity[b]	14	902 [752, 1020]	27	898 [706, 1199]	0.923
Cumulative activity[b]	14	48,085 [25,489, 79,134]	27	54,216 [35,729, 78,291]	0.630

[a] Based on the Wilcoxon rank sum test.
[b] Based on the skin sensors data.

($\beta = -0.026$; 95% CI: −0.530, 0.478; $p = 0.920$). The intraclass correlation coefficient (ICC) was moderate in the leptin model (0.52) and a bit lower in the adiponectin and cortisol models (0.41 and 0.23, accordingly). In additional analyses, we evaluated the effect of air temperature sensor data with the adipokines and cortisol levels. We observed that all skin temperature surrogate measures (max, median, mean) were significantly associated with the leptin levels, as well as the max personal air sensor data (Table S8). The mean and median skin temperature levels were also significantly associated with the adiponectin levels (Table S9) whereas the mean and max personal air temperature data and the max skin temperature data were significantly negatively associated with the cortisol levels (Table S10).

The separate addition of possible confounders (sex, BMI, age, cortisol levels) in the metabolic hormone models did not alter the significance of the treatment effect on leptin (Table S11). However, when adding daily cumulative activity or daily caloric intake, the effect of setting on leptin levels was not significant at the $\alpha = 0.05$ level, i.e., $\beta = -0.29$ (95% CI: −0.63, 0.04; $p = 0.09$) and $\beta = -0.24$ (95% CI: −0.49, 0.001; $p = 0.06$), respectively (Table S11). Having urinary creatinine as a fixed effect term in the hormone models (the response variables were log-transformed but not creatinine-adjusted in this set of models) did not result in deviations from trends observed in the main analysis (Table S14).

4. Discussion

Extending the work published in other hyperthermia human studies, and to the best of our knowledge, this is the first, 2 x 2 prospective, randomized, cross-over, non-pharmacological trial that investigated the alterations of metabolic and stress hormones associated with a short-term stay (mean duration of 7 days) in climatologically cooler areas than those observed in geographically adjacent urban settings (~1-h driving distance). A robust significant leptin reduction ($p = 0.024$) during the short-term stay of non-obese healthy adults in the climate-cooler environment was observed.

These metabolic hormones, or adipokines (leptin, adiponectin) were selected as primary outcomes for this trial, because they are secreted by the adipose tissue and play a major physiological role in regulating appetite, food intake and metabolism. Hence, they are involved in the early pathogenesis of obesity and metabolic syndrome, both being high risk factors for type II diabetes. Leptin resistance is a possible player in the roadmap to the metabolic syndrome and type II diabetes (Unger and Scherer, 2010), while adiponectin appears to play an important role in the perturbation of metabolic pathways associated with diabetogenesis, due to its anti-inflammatory, anti-atherogenic, and insulin-sensitizing properties (Ghoshal and Bhattacharyya, 2015).

Time of the day patterns have been observed in plasma leptin and adiponectin concentrations, with leptin rising and adiponectin decreasing during night, following the circadian rhythm of the adipose function (Shostak et al., 2013), hence, the inclusion of both morning and night samples in the mixed effect regression models (as fixed terms). The leptin reduction observed in the mountainous setting could be attributable to both a decrease in thermal load and the combined influence of other environmental (cleaner air) and/or behavioural factors (e.g. sleep, diet, activity) that could package the effect of the climatologically cooler intervention in these real-life settings; this beneficial effect was also observed for adiponectin and cortisol, albeit not significant in the mixed effect regression models ($p > 0.05$).

The cooler ambient air temperature profile of the mountainous setting vs that of the urban setting during the summer period was *a priori* observed (before study initiation) and post-confirmed with governmental data obtained from the Cyprus Meteorological Service; daily max air temperature data in each setting (one station in Troodos mountain, one in Nicosia and another in Limassol urban centres)

Table 3
Linear mixed-effect models of log-transformed biomarkers (leptin, adiponectin and cortisol) as a function of mountainous setting (in comparison to the urban setting) and first morning sample (in comparison to the last before sleep sample) accounting for the repeated measurements.

	Leptin (ng/g)			Adiponectin (ug/g)			Cortisol (ug/g)		
	Estimate	CI	p-value	Estimate	CI	p-value	Estimate	CI	p-value
Mountainous setting	−0.255	−0.472–−0.038	**0.024**	0.058	−0.237–0.353	0.702	−0.026	−0.530–0.478	0.920
First morning sample	−0.121	−0.338–0.096	0.276	−0.275	−0.570–0.020	0.071	1.383	0.879–1.886	< **0.001**
Within participant variance	0.38			0.70			2.05		
Between participant variance	0.41			0.48			0.62		
ICC	0.52			0.41			0.23		
Observations	124			124			124		

Models details.
(a) Leptin, adiponectin and cortisol are creatinine adjusted and log-transformed.
(b) Random intercepts for the repeated samples within participants with unstructured covariance matrix.
Abbreviations: CI: confidence interval; ICC: intraclass-correlation coefficient.

confirmed that the average max daily air temperatures followed the pattern of urban > mountainous settings, e.g., Nicosia (35 °C) > Limassol (33 °C) > Troodos (25 °C) (Fig. S1). The personal air temperature data obtained at the individual level with our sensors showed a smaller temperature difference of about 2 °C between urban and mountainous areas that participants spent time during the study period; a much smaller difference in temperature was observed with the skin temperature data. The smaller temperature gradient with the sensor data was indicative of the individual behaviors, such as longer indoors stays often accompanied with air conditioning (Table S7) that attenuated the larger ambient outdoors air temperature difference observed with stationary governmental data. Nevertheless, per our trial, we observed consistent positive associations between individual personal air and skin temperature sensor data and the two studied metabolic hormones. The biological plausibility of the observed cooler climatic effectiveness driving the beneficial effects on the metabolic hormones requires further mechanistic investigation.

To date, most studies on the metabolic effects of environmental temperature gradient exposures have been performed in non-human subjects. In humans, there are few studies that provided the first evidence linking ambient air temperature, brown adipose tissue (BAT) acclimation, and whole-body energy/substrate metabolism (Turner et al., 2016; Lee et al., 2014; Blauw et al., 2017). It is well known that BAT activation occurs at lower air temperatures, while the adapted thermogenesis phenomenon may be reduced in environments with temperatures within the thermal comfort zone (Turner et al., 2016). In colder air exposures, BAT-induced thermogenesis potential and energy expenditure may be both increased, suggesting regulatory links between BAT thermal recruitment and glucose metabolism (Turner et al., 2016). An experimental study conducted under well-controlled indoor settings (n = 5) showed that even a small elevation in ambient temperature (reaching 27 °C, for a month duration) impaired BAT, increased leptin and decreased adiponectin levels (Lee et al., 2014). A global analysis showed the positive association between mean annual temperature and country-wise age-adjusted, sex-adjusted and income-adjusted prevalence of raised fasting blood glucose, with Eastern Mediterranean and Western Pacific being the two WHO regions with the highest fasting blood glucose prevalence rates (Blauw et al., 2017). The focus of the above mentioned human studies was on early-stage pathophysiological and metabolic markers, being part of biological pathways that could, under certain circumstances, lead to diabetogenesis.

Several factors may be implicated with fluctuations in the adipokines' circulating patterns, such as sleep, diet, psychosocial stress or physical activity; these factors were *a priori* accounted for in this trial design and most of them were added as possible confounders in additional models. Diary-based 24-h recall meal information was analysed for all participants showing that caloric intake among the two settings did not significantly differ (p = 0.16, Table S11); in effect, median [IQR] caloric intake was 1358 kcal [919, 1692] vs. 1253 kcal [1015, 1638] in the mountainous vs. urban settings, respectively (Table S2). When daily caloric intake was added as a confounder, the effect of setting on the magnitude of leptin levels was not significant any more at the α = 0.05 level (p = 0.055) (Table S11). Cortisol is a well-established biomarker of psychosocial stress; lower cortisol levels were observed in the mountainous setting, although the effect was not significant (β = −0.026; 95% CI: −0.530, 0.478; p = 0.920) (Table 3); however, when cortisol was added as a covariate in the regression models, it was not associated with either leptin or adiponectin (S11, S12). Furthermore, the activity tracking feature of personal sensors was used as a surrogate of physical activity. Activity tracking analysis showed that the median [IQR] cumulative activity in the mountainous vs. urban settings did not significantly (p = 0.63) differ (48,090 activity units [25,490, 79,130] vs. 54,220 [35,730, 78,290]) (Table S2) and when daily cumulative activity was added as a confounder in the mixed effect models, there was no association with either hormone levels (S11–S13); however, the effect of setting on leptin levels was not significant at the α = 0.05 level (p = 0.09) (Table S11).

The risk of bias in intervention assignment was minimized using central randomization. Thus, the results, i.e. reduction of metabolic hormone levels upon short-term stay in the specific mountainous area could be considered generalizable for the specific population group (i.e. non-obese healthy adults residing in an urban area of a Mediterranean city with similar characteristics of the studied mountainous setting). Given that participating rural areas were randomly selected from all the rural communities of the broader Troodos mountainous area, i.e., the tested intervention setting, we do not expect that background population characteristics, such as the neighborhoods' socioeconomic backgrounds, have influenced the observed data.

Our trial has several strengths. Abiding by the principles of the human exposome framework as set by Dr. Wild in 2005, our trial combined a series of methods and tools (biomarker measurements, questionnaire and diary responses, temperature sensors, etc.) covering all three domains of the human exposome concept to better characterize the intervention treatment effects in a non-pharmacological trial. It is often the case that treatment effects in non-pharmacological trials may be attributed to a number of active components and not to a single active substance/component/drug, as typically is the case in pharmacological trials; as such, the collection of sensor-based temperature data for each participant was continuous and overcame the typical shortcoming of other experimental studies that set temperature measurements at only a few specific discrete time points or measuring stations. The fact that temperature measurements were taken with the sensors at the individual level was a novelty of this trial. To the best of our knowledge, no other non-pharmacological trial has used such sensors to track personal air temperature gradient fluctuations. Our sensors set-up could allow for the differentiation of metabolic hormonal profiling due to changes in a range of personal air/skin temperatures. Observational studies collect air temperature data from meteorological stations; hence, the spatiotemporal coverage and exposure misclassification bias is a concern. Furthermore, this trial was conducted in real-life conditions, moving away from well-controlled environments that typically expose participants to a discrete set of temperatures for a pre-specified period. These experimental studies have focused on cognitive performance, whereas our trial is the first one globally, focusing on metabolic hormonal alterations due to elevated air temperatures in urban settings. Other strengths include the repeated measures design that was accounted for in the statistical analysis plan.

This trial has few limitations. The trial size was smaller than it was originally calculated due to recruitment challenges and hence the post hoc power of the study was estimated to be 0.53; nevertheless, it was able to provide the first evidence on the association between air/skin temperature profiling and metabolic hormones. A wide time window of several hours during the collection of the first morning void sample, introduced additional variability in the magnitude of metabolic hormones diurnal patterns, that was not accounted for. Another limitation was that the purpose of the stay in the mountainous setting was mostly recreational, hence it was possible that a package of factors other than those ascribed to the setting (i.e. physical activity, diet, sleep, psychosocial stress) may have contributed to the treatment effect, even though participants were instructed to maintain their habits throughout the study period. Measurements for most of these confounders were taken; however, they couldn't all be simultaneously accounted for as fixed effect terms in the linear mixed effect models due to limited sample size. Also, observed trends in this trial dealt with short-term adipokines effects that may not be applicable to the disease process of chronic metabolic outcomes (e.g. type II diabetes), that require longer time windows to develop.

The present study provides rationale for the implementation of short-term mobility measures for sensitive subpopulation groups to nearby geographic locations with climate cooler characteristics during hot Mediterranean summers. This non-pharmacological trial provides the first evidence of the possible adverse effects of elevated summer

temperatures on metabolic hormone profiling in urban settings. A short-term mean stay of 7 days in climatologically cooler areas during the Mediterranean summer improved the profile of a metabolic hormone (leptin) for non-obese healthy adults who permanently reside in urban areas of a Mediterranean country. More data is needed to elucidate the biological pathways driving the metabolic adipokines regulation under the climatic cooler scenario versus the heat wave scenario in urban areas of Mediterranean cities. Such beneficial effects on metabolic hormones are warranted to be tested with a larger sample; the implications of such a non-pharmacological intervention trial could be of paramount public health importance for sensitive subpopulation groups, e.g. elderly, who during summertime in the city, could spend a few short-term periods in such nearby climatologically cooler peri-urban environments with the goal to relieve some of the adverse temperature effects experienced in the urban Mediterranean summer. Overall, the influence of elevated air temperatures and its urban temperature microenvironments on relevant end points of disease have not yet been extensively tackled. The manifestation of elevated and sustained air temperature gradient profiles (due to indoor/outdoor conditions and other microenvironments) in urban dwellings represents an emerging planetary health phenomenon worth of studied in detail.

Contributors

KCM conceived, designed and coordinated the trial. AP, CK and KCM conducted the field work and data collection. CK conducted the sample analyses. CK executed the primary data analysis and ABZ the secondary analysis, both with guidance from KCM and CAC. All authors interpreted the results and drafted the manuscript. KCM obtained internal funding. All authors revised the article for important intellectual content. KCM is the principal investigator and guarantor of the study.

Declaration of competing interest

No competing interests: All authors declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Transparency declaration

The manuscript's guarantor affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned (and, if relevant, registered) have been explained.

Financial disclosure - role of the funding source

Internal funds of the principal investigator (KC Makris) were used for this study. No external funding was obtained for this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing

The authors commit to making the relevant anonymized patient level data available via open access.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2019.109065.

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