Adults with metabolically healthy overweight or obesity present more brown adipose tissue and higher thermogenesis than their metabolically unhealthy counterparts

Lucas Jurado-Fasoli,[a,b,o,*] Guillermo Sanchez-Delgado,[a,c,d,e,o] Juan M. A. Alcantara,[a,d,f,g] Francisco M. Acosta,[a,h,i,j,k] Rocio Sanchez-Sanchez,[e,l] Idoia Labayen,[d,f,g] Francisco B. Ortega,[a,d,m] Borja Martinez-Tellez,[a,d,n,p] and Jonatan R. Ruiz[a,d,e,p,**]

[a]Department of Physical Education and Sports, Faculty of Sports Science, Sport and Health University Research Institute (iMUDS), University of Granada, Carretera de Alfacar s/n, 18071, Granada, Spain
[b]Department of Physiology, Faculty of Medicine, University of Granada, Granada, Andalucía, Spain
[c]Department of Medicine, Division of Endocrinology, Centre de Recherche du Centre Hospitalier Universitaire de Sherbrooke, Université de Sherbrooke, Sherbrooke, QC, Canada
[d]CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Granada, Spain
[e]Instituto de Investigación Biosanitaria, Ibs.Granada, Granada, Spain
[f]Department of Health Sciences, "Institute for Sustainability & Food Chain Innovation", Public University of Navarre, Pamplona, Spain
[g]Navarra Institute for Health Research, Pamplona, Spain
[h]Turku PET Centre, University of Turku, Turku, Finland
[i]Turku PET Centre, Turku University Hospital, Turku, Finland
[j]InFLAMES Research Flagship, University of Turku, 20014, Turku, Finland
[k]MediCity/PET Preclinical Laboratory, University of Turku, Turku PET Centre, Turku, Finland
[l]Servicio de Medicina Nuclear, Hospital Universitario Virgen de las Nieves, Granada, Spain
[m]Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland
[n]Department of Education, Faculty of Education Sciences and SPORT Research Group (CTS-1024), CERNEP Research Center, University of Almería, Almería, Spain

eBioMedicine
2024;100: 104948
Published Online xxx
https://doi.org/10.1016/j.ebiom.2023.104948

Summary

Background There is a subset of individuals with overweight/obesity characterized by a lower risk of cardiometabolic complications, the so-called metabolically healthy overweight/obesity (MHOO) phenotype. Despite the relatively higher levels of subcutaneous adipose tissue and lower visceral adipose tissue observed in individuals with MHOO than individuals with metabolically unhealthy overweight/obesity (MUOO), little is known about the differences in brown adipose tissue (BAT).

Methods This study included 53 young adults (28 women) with a body mass index (BMI) ≥25 kg/m^2 which were classified as MHOO (n = 34) or MUOO (n = 19). BAT was assessed through a static ^{18}F-FDG positron emission tomography/computed tomography scan after a 2-h personalized cooling protocol. Energy expenditure, skin temperature, and thermal perception were assessed during a standardized mixed meal test (3.5 h) and a 1-h personalized cold exposure. Body composition was assessed by dual-energy x-ray absorptiometry, energy intake was determined during an *ad libitum* meal test and dietary recalls, and physical activity levels were determined by a wrist-worn accelerometer.

Findings Participants with MHOO presented higher BAT volume (+124%, P = 0.008), SUVmean (+63%, P = 0.001), and SUVpeak (+133%, P = 0.003) than MUOO, despite having similar BAT mean radiodensity (P = 0.354). In addition, individuals with MHOO exhibited marginally higher meal-induced thermogenesis (P = 0.096) and cold-induced thermogenesis (+158%, P = 0.050). Moreover, MHOO participants showed higher supraclavicular skin temperature than MUOO during the first hour of the postprandial period and during the cold exposure, while no statistically significant differences were observed in other skin temperature parameters. We observed no statistically significant differences between MHOO and MUOO in thermal perception, body composition, outdoor ambient temperature exposure, resting metabolic rate, energy intake, or physical activity levels.

*Corresponding author. Department of Physical Education and Sports, Faculty of Sports Science, Sport and Health University Research Institute (iMUDS), University of Granada, Carretera de Alfacar s/n, 18071, Granada, Spain.
**Corresponding author. Department of Physical Education and Sports, Faculty of Sports Science, Sport and Health University Research Institute (iMUDS), University of Granada, Carretera de Alfacar s/n, 18071, Granada, Spain.
E-mail addresses: juradofasoli@ugr.es (L. Jurado-Fasoli), ruizj@ugr.es (J.R. Ruiz).
[o]These authors share the first authorship.
[p]These authors share the last authorship.

Interpretation Adults with MHOO present higher BAT volume and activity than MUOO. The higher meal- and cold-induced thermogenesis and cold-induced supraclavicular skin temperature are compatible with a higher BAT activity. Overall, these results suggest that BAT presence and activity might be linked to a healthier phenotype in young adults with overweight or obesity.

Funding See acknowledgments section.

Keywords: Brown fat; Adaptive thermogenesis; Thermoregulation; Cardiometabolic health; Metabolism

Research in context

Evidence before this study

There is a subset of individuals with overweight/obesity characterized by a lower risk of cardiometabolic complications, the so-called metabolically healthy overweight/obesity (MHOO) phenotype. Although individuals with MHOO have similar whole-body adiposity than those with metabolically unhealthy overweight/obesity (MUOO), fat partitioning is quite different. For instance, individuals with MHOO display higher levels of subcutaneous adipose tissue and lower visceral adipose tissue (VAT) compared to individuals with MUOO. However, little is known about the differences in brown adipose tissue (BAT) between MHOO and MUOO phenotypes.

Added value of this study

Here, we show that adults with MHOO presented higher BAT volume and activity than MUOO. Moreover, individuals with MHOO showed a higher meal-induced thermogenesis in the first and second hours of the meal test and a higher cold-induced thermogenesis than MUOO. Similarly, MHOO displayed an increased meal- and maintained cold-induced supraclavicular skin temperature (which has been used as a method for detecting BAT activity in humans) than their counterparts.

Implications of all the available evidence

Taken together, our findings suggest a role of BAT in conferring a healthy metabolic phenotype in young adults with overweight or obesity. Therefore, our study provides insights into the potential relevance of BAT in the MHOO phenotype and posits further evidence of a potential role of BAT in thermogenesis and cardiometabolic health in individuals with overweight and obesity.

Introduction

Overweight and obesity are associated with a higher risk of suffering chronic diseases such as cardiometabolic diseases, elevating the risk of mortality.[1] However, there is a subset of individuals with overweight/obesity characterized by a lower risk of cardiometabolic complications,[2–5] the so-called metabolically healthy overweight/obesity (MHOO) phenotype.[2–5] Individuals with MHOO display a relatively favourable phenotype without presenting dyslipidemia, hyperglycemia, or hypertension,[2,3] and with a lower risk of developing type 2 diabetes or cardiovascular diseases than their unhealthy counterparts.[6–11] Nevertheless, patients with MHOO have the potential risk of progressing into the metabolically unhealthy overweight/obesity (MUOO) phenotype.[12] Indeed, despite individuals with MHOO have a lower risk of all-cause mortality than MUOO, they still show a higher risk of all-cause mortality and cardiometabolic diseases than their metabolically healthy normal-weight counterparts.[13] Unravelling the physiological mechanisms behind the intriguing MHOO phenotype could be a promising approach to preventing the development of cardiometabolic diseases.

Although individuals with MHOO have similar whole-body adiposity than those with MUOO, fat partitioning is quite different. For instance, individuals with MHOO display higher levels of subcutaneous adipose tissue and lower visceral adipose tissue (VAT) compared to individuals with MUOO.[14–16] In mammals, adipose tissue can be mainly divided into white adipose tissue (WAT) which can store and release energy, and brown adipose tissue (BAT) which can dissipate energy as heat.[17] However, little is known about the differences in BAT between MHOO and MUOO phenotypes. A study involving over 134,000 individuals revealed that those patients with obesity and detectable BAT measured at room temperature [determined through ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography scans (^{18}F-FDG-PET/CT)], had a lower prevalence of dyslipidemia (7.9%), hypertension (7.9%), coronary artery disease (3%), and congestive heart failure (1.6%),[18] than individuals with obesity and without detectable BAT. Similarly, individuals with obesity and detectable BAT (measured through ^{18}F-FDG-PET/CT after a personalized cooling protocol) showed lower VAT mass, insulin resistance, and liver

steatosis markers than individuals with obesity and no detectable BAT.[19] Hence, from a biological perspective, it is plausible that individuals with MHOO exhibit elevated BAT volume and activity.

Human BAT seems to play a role in cold- and meal-induced thermogenesis and thermoregulation.[15,17] During cold exposure or after meal ingestion, there is a rise in energy expenditure above basal levels, in part to maintain core body temperature.[15,20,21] This increase in energy expenditure may be partially mediated by BAT thermogenesis and could be protective against weight gain and cardiometabolic diseases.[22,23] Consequently, if individuals with MHOO had higher levels of BAT volume and activity than their MUOO counterparts, their cold- and meal-induced thermogenesis (CIT and MIT, respectively) and thermoregulatory function might be greater than those individuals with MUOO.

In this study, our primary objective was to examine the role of BAT in the MHOO phenotype. To achieve this, we compared BAT volume, ^{18}F-FDG uptake, and radiodensity (an inverse indicator of BAT fat content) in adults with MHOO and MUOO. Additionally, we explored differences between MHOO and MUOO in thermogenesis, skin temperature, and thermal perception triggered by meal ingestion and cold exposure.

Methods

Participants and study design

A total of 53 young adults (18–25 years old, 52.8% females) participated in this cross-sectional study (Table 1). Participants were retrospectively selected among the participants of the ACTIBATE study[24,25] (clinicaltrials.gov: NCT02365129) for having a body mass index (BMI) ≥25 kg/m^2. Participants reported not smoking or taking any medication, having a stable body weight for the previous 3 months (<3 kg change), not presenting any cardiometabolic disease (e.g., hypertension, diabetes), and not being regularly exposed to cold. The data were collected in Granada (Spain) between September and November of 2015 and 2016.

Participants were retrospectively classified as MHOO or MUOO following previously proposed criteria.[26] MUOO was defined as having a BMI ≥25 kg/m^2 and presenting at least one of the following comorbidities: a) Fasting triglyceride levels ≥150 mg/dL; b) Fasting high-density lipoprotein cholesterol (HDL-C) levels <40 mg/dL for men and <50 mg/dL for women; c) Resting systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg; d) Fasting glucose levels >100 mg/dL. MHOO was defined as having a BMI ≥25 kg/m^2 and not presenting any of the above-mentioned risk factors.

Procedures

Data presented in this study were collected in a total of 5 visits to the laboratory, as follows: 1) Resting and post-prandial energy expenditure, energy intake, and body composition; 2) Overnight fasting blood sample collection; 3) Shivering threshold assessment; 4) BAT assessment after a personalized cold exposure; 5) CIT measurement. The participants were instructed to commute to the research centre by bus or by car (i.e., with the minimum possible physical activity), after having slept as usual, and having refrained from stimulant beverages and any moderate (previous 24 h) or vigorous (previous 48 h) physical activity. Blood pressure was measured on three different days with an automatic sphygmomanometer (Omron M2, Omron Healthcare, Kyoto, Japan) and the average was calculated.

Resting and post-prandial energy expenditure (meal-induced thermogenesis)

Participants arrived at the lab early in the morning (8.15 am) after overnight fasting (12 h) and having consumed a standardized *ad libitum* dinner (i.e., boiled rice, tomato sauce, and egg omelette) the day before. Participants wore standardized clothes (sandals, shorts, and a T-shirt, clo value = 0.20). Participants were required to rest quietly in a slightly reclined bed for at least 30 min in a quiet and warm room (22-23 °C). Afterwards, the participant's resting metabolic rate (RMR) was measured through indirect calorimetry for 30 min, using either a CCM Express or a CPX UltimaCardiO2 metabolic cart (Medgraphics Corp, Minnesota, USA) equipped with either a ventilated Face-Tent or a neoprene face mask. Participants used the same metabolic cart and gas collection tool in each assessment (i.e., RMR, MIT, and CIT) following the manufacturer's recommendations. Immediately after the RMR measurement, participants ingested a standardized liquid breakfast (1.6 kcal/ml; 47% carbohydrates, 30% fat, 15% protein, 3% fibre; T-Diet Energy neutral flavour, Vegenat S. A., Badajoz, Spain) at 4 °C, with an energy intake equivalent to 50% of their RMR. Participants were instructed to consume the liquid breakfast within 10 min if possible. After consuming the breakfast, participants rested quietly, and indirect calorimetry was used again to assess the participant's respiratory gas exchange during three periods of 60 min, with 10 min of no measurement in between, for a total of 3 h and 20 min of MIT record. Whenever possible, the post-prandial indirect calorimetry started 15 min after starting the breakfast consumption. However, because some participants required longer to consume the breakfast provided, the start of the first indirect calorimetry measurement was delayed up to 15 min. Therefore, only the last 50 min of the first period were considered for further analyses.

Breath-by-breath indirect calorimetry data were averaged every minute and downloaded from the MGCDiagnostic®, Breeze Suite (8.1.0.54 SP7) software. For RMR, we selected the most stable 5-min period (i.e., the one with the lowest average of oxygen consumption, carbon dioxide production, minute ventilation, and

	ALL (n = 53)	MHOO (n = 34)	MUOO (n = 19)	P
Age (years)	22.2 (2.4)	21.8 (2.4)	22.9 (2.3)	0.461
Male (n, %)	25 (47%)	11 (32%)	14 (74%)	0.004
Female (n,%)	28 (53%)	23 (68%)	5 (26%)	
Weight (kg)	86.8 (15.0)	83.1 (12.3)	93.5 (17.4)	0.388
Height (m)	1.70 (0.10)	1.69 (0.08)	1.73 (0.10)	0.501
BMI (kg/m^2)	29.8 (3.6)	29.0 (3.4)	31.1 (3.6)	0.189
Lean mass (kg)[a]	47.5 (10.0)	45.2 (8.7)	52.3 (11.2)	0.810
Fat mass (kg)[a]	33.5 (7.1)	33.2 (7.2)	34.2 (7.2)	0.572
Fat mass (%)[a]	40.2 (6.4)	41.1 (6.9)	38.5 (5.0)	0.266
VAT mass (g)[a]	499.1 (147.2)	462.4 (145.9)	574.9 (121.8)	0.085
Glucose (mg/dl)	90.3 (7.9)	87.6 (6.5)	95.2 (8.0)	0.001
Insulin (µUl/ml) [Median (IQR)]	9.2 (8.5)	7.7 (7.2)	14.7 (13.2)	0.004
HOMA-IR [Median (IQR)]	1.9 (2.1)	1.6 (1.5)	3.2 (3.5)	0.004
Total cholesterol (mg/dl)	164.8 (34.2)	155.4 (21.6)	181.6 (45.4)	0.017
HDL-C (mg/dl)	48.3 (9.9)	51.2 (9.1)	43.0 (9.2)	0.042
LDL-C (mg/dl)	98.6 (28.7)	90.7 (17.5)	112.5 (38.7)	0.030
Triglycerides (mg/dl) [Median (IQR)]	74.5 (60.0)	65.0 (34.0)	114.0 (124.0)	<0.0001
Systolic blood pressure (mmHg)	122.4 (12.2)	116.8 (8.4)	132.1 (11.9)	0.0002
Diastolic blood pressure (mmHg)	73.9 (7.3)	71.3 (6.0)	78.5 (7.2)	0.002
Calendar day of the ^{18}F-FDG-PET/CT scan	308 (19)	311 (17)	306 (21)	0.280
Outdoor ambient temperature (°C)	22.5 (3.2)	22.1 (2.9)	23.3 (3.8)	0.368
Resting metabolic rate (kcal/day)[b]	1597.9 (317.9)	1559.9 (297.6)	1673.8 (357.6)	0.331
Ad libitum energy intake (kcal)[c]	942.2 (376.7)	853.3 (259.7)	1100.1 (494.8)	0.247
Energy intake from 24-h recalls (kcal/d)	1818.3 (413.7)	1798.8 (440.0)	1852.1 (372.8)	0.991
Sedentary time (min/day)	799.1 (72.4)	783.9 (77.0)	825.4 (56.0)	0.357
Light physical activity (min/day)	119.1 (30.3)	126.6 (31.1)	106.2 (24.6)	0.230
Moderate physical activity (min/day)	87.7 (34.3)	93.0 (37.4)	78.4 (26.5)	0.674
Vigorous physical activity (min/day) [Median (IQR)]	1.5 (2.5)	1.6 (2.9)	1.4 (2.0)	0.792

Data are presented as mean and standard deviation (SD) unless otherwise stated. The natural calendar day when the ^{18}F-FDG-PET/CT scan was performed is the number of days from January 1st of the same year (e.g., February 15th would be recorded as day 46). P values from analysis of covariance comparing MHOO vs. MUOO adjusting for sex or from chi-square test (sex variable). P value in resting metabolic rate and energy intake obtained from analysis of covariance adjusting for sex and lean mass.
Abbreviations: BMI: Body mass index; VAT: Visceral adipose tissue; HOMA-IR: Homeostasis model assessment of insulin resistance; HDL-C: High-density lipoprotein cholesterol; IQR: interquartile range; LDL-C: Low-density lipoprotein cholesterol. [a]Indicates variables with n = 49 (55% women), of which 33 (70% women) are MHOO and 16 (25% women) are MUOO. [b]Indicates outcome with n = 33, of which 22 (70% women) are MHOO, and 11 (25% women) are MUOO. [c]Indicates outcome with n = 52, of which 33 (67% women) are MHOO, and 19 (26% women) are MUOO.

Table 1: **Descriptive characteristics of participants with metabolically healthy overweight/obesity (MHOO) and metabolically unhealthy overweight/obesity (MUOO).**

respiratory exchange ratio, coefficients of variance), after excluding the first 5 min.[27] For MIT, we excluded the first 20 min of the first measurement period (10 min with incomplete data and the first 10 min of measurement), and the first 10 min of the second and third measurement periods. Subsequently, the data was averaged for each 5-min period.

Total urine production from the previous dinner to the breakfast, and from the standardized breakfast to the end of the MIT assessment were collected to measure total urine volume and urea concentration (Spinreact, Catalogue No. 283–17). Urinary nitrogen concentration was estimated from urea concentration following the equation "Nitrogen (g/l) = 0.0065* Urea (mg/dl) + 1.2598" obtained from a linear regression including urea and nitrogen measured concentrations from 20 participants.[28] Energy expenditure was estimated from oxygen consumption, carbon dioxide production, and estimated urinary nitrogen excretion rate, using Weir's abbreviated equation.[29] Unfortunately, due to problems with data collection (e.g., unavailability of the metabolic cart, error in calibration, etc.) only 26 participants (19 MHOO and 7 MUOO) had valid data and were therefore included in the analyses. We calculated the incremental area under the curve (trapezoidal rule) for the whole measurement, and for each measurement period separately, and expressed it as a percentage of the breakfast energy intake to estimate the total MIT.

Anthropometrics and body composition

We measured the participant's weight and height with a scale and stadiometer (Seca, model 799, Electronic

Column Scale, Hamburg, Germany) after an overnight fasting and BMI was calculated. Moreover, after assessing MIT, participants were moved into another room where a whole-body dual-energy x-ray absorptiometry scan (Discovery Wi, Hologic, Inc., Bedford, MA, USA) was performed to determine body composition.

Ad libitum and self-reported energy intake

The participants were provided with an *ad libitum* meal 255 min after consuming the standardized breakfast. We offered them a plate of spaghetti (i.e., spaghetti, tomato sauce, pork tenderloin, and virgin olive oil), prepared immediately before. The lunch composition was 45.5% carbohydrates, 38.5% fat, and 16% proteins of the total energy, with an energy density of 1.54 kcal/g. The total amount offered was 1500 g for men and 1000 g for women. They were allowed to drink a total of 450 ml of water. The participants were instructed to eat until comfortably satiated and ate alone in a quiet, dimly lit room. The weight of food consumed was calculated from the initial and final plate weight, and energy intake was subsequently calculated. In addition, energy intake was estimated using three non-consecutive 24-h dietary recalls (one of them for a non-working day). Thereafter, the nutritional composition of the diet was obtained by the EvalFINUT software (http://www.finut.org/evalfinut/).

Sedentary time and physical activity levels

Sedentary time and physical activity levels were objectively measured with a wrist-worn accelerometer (ActiGraph GT3X+, Pensacola, FL) for 7 consecutive days. The accelerometers were initialized to store raw accelerations at a sampling frequency of 100 Hz.[30] The raw accelerations were exported and converted to the ".csv" format using ActiLife, version 6.13.3. software (ActiGraph). The raw ".csv" files were then processed using the GGIR package (version 1.6–0; https://cran.r-project.org/web/packages/GGIR/) in R (version 3.1.2, https://www.cran.r-project.org/). Sedentary time and physical activity (light, moderate, and vigorous) levels were estimated as reported elsewhere.[31]

Blood sample collection and cardiometabolic risk parameters

A blood sample was taken early in the morning after overnight fasting (12 h) from an antecubital vein and was immediately centrifugated, aliquoted, and stored at –80 °C until analyses. Serum glucose determination was performed in a Beckman Coulter AU5832 analyser using the reactive OSR6521 (Beckman Coulter Inc., CA, USA). Insulin levels were determined by chemiluminescent immunoassay in a DXI analyser (Beckman Coulter Inc., CA, USA). Serum triglycerides, total cholesterol, and HDL-C levels were determined by colorimetric methods in the Beckman Coulter AU5832 analyser using the reactive OSR61118, OSR6516, and OSR6587 (Beckman Coulter Inc., CA, USA), respectively. Low-density lipoprotein cholesterol (LDL-C) levels were estimated using the Friedewald equation.[32] Insulin resistance was estimated by the Homeostatic Model Assessment (HOMA-IR).[33]

Shivering threshold test

Participants attended to the research centre after at least 6 h of fasting and were equipped with the same standardized clothes used for RMR and MIT assessment. They rested in a warm room (22–23 °C) for 30 min. Later, they entered a mild cold room (19.5–20 °C) and dressed in a water-perfused vest (Polar Products Inc., Ohio, USA) set at ≈17 °C. Thereafter, the water temperature was slightly decreased (1.4 °C every 10 min) until shivering occurred (both self-reported and externally observed). The temperature of the shivering onset was considered the individual's shivering threshold (i.e., the lowest tolerable water temperature without externally observed or auto-reported shivering).[34]

Brown adipose tissue assessment after a personalized cold exposure

Forty-eight or 72 h after determining the individual's shivering threshold, participants came to the research centre after following the same instructions as for the shivering threshold test. Participants were equipped with the above-mentioned standardized clothes and rested for 30 min in a warm room. Later, they were moved to the mild cold room (19.5–20 °C), where they remained seated, and equipped with the water-perfused cooling vest set at 4 °C above their individual shivering threshold. After the first hour of cold exposure, the water temperature was increased by 1 °C to avoid shivering (5 °C above the individual shivering threshold). The water temperature was also increased by 1 °C if the participants started to shiver at any time. Participants remained cold-exposed for 2 h.

After the first hour of cold exposure, an intravenous bolus (≈185 MBq) of ^{18}F-FDG was injected through an antecubital catheter. At the end of the 2 h of cold exposure, a static PET-CT scan (Siemens Biograph 16 PET/CT, Siemens, Germany) from the *atlas vertebrae* to the mid-chest was performed.[25,35–37] The PET-CT images were analysed using the Beth Israel plugin for FIJI software.[38] An individualized, standardized uptake value (SUV) threshold (i.e., 1.2/[lean body mass/body mass]) and a fixed radiodensity range (–10 to –190 Hounsfield units) were used for BAT quantification.[35,36,39] The regions of interest (ROI) were semi-automatically outlined from the atlas vertebra to the fourth thoracic vertebra. BAT volume, SUVmean, SUVpeak, and radiodensity were quantified according to the BARCIST 1.0 recommendations.[39] All scans were visually examined to detect ^{18}F-FDG uptake in BAT-specific depots. BAT volume was determined as the number of pixels with an SUV value above the SUV threshold within –10 to –190 HU.

BAT SUV mean was determined as the mean SUV value in the aforementioned voxels and SUVpeak as the highest average SUV in a 1-ml spherical ROIs with the maximum SUV among BAT. BAT mean radiodensity was determined as the average radiodensity of those voxels meeting the aforementioned criteria in a single ROI covering the body (except the mouth) from the atlas to the thoracic vertebra 4. We also computed the SUVpeak of the descending aorta, the tricipital subcutaneous white adipose tissue (scWAT), and dorsocervical scWAT. We chose the dorsocervical scWAT because we previously observed that this area has higher glucose uptake than other subcutaneous areas.[37] SUV values were adjusted for lean mass for the statistical analyses.

Outdoor ambient temperature

The outdoor ambient temperature in the Granada metropolitan area was obtained from the Spanish National Meteorological Agency daily during the period of the study. Then, the average temperature of the 7 days before the PET-CT was taken as the individual's outdoor ambient temperature. Additionally, we registered the natural calendar day (1 = January first; 365 = December 31st) when the PET-CT was performed to account for seasonal differences on BAT detectability.

Cold-induced thermogenesis

Finally, 48–72 h after the PET-CT, CIT was assessed with participants receiving the same instructions mentioned above. After resting in the reclined bed for 20 min, the participant's resting energy expenditure was measured for 30 min (CCM Express/CPX Ultima-CardiO2, Medgraphics Cardiorespiratory Diagnostic, Saint-Paul, MN, USA) in the warm room (22–23 °C). Later, participants were moved into the mild cold room (19.5–20 °C) and equipped with the water-perfused cooling vest set at a temperature 4 °C above their shivering threshold. While quietly lying on the reclined bed, participants remained cold exposed for 65 min, during which indirect calorimetry was recorded in two 30-min periods, with 5-min breaks.

The indirect calorimetry data was averaged every minute and downloaded from the Breeze Suite (8.1.0.54 SP7) software. The resting energy expenditure in warm conditions was calculated as described earlier for RMR. For CIT, the first 5 min of each 30-min recording period were excluded. Subsequently, the remaining data was averaged every 5 min. Energy expenditure was estimated from oxygen consumption and carbon dioxide production using Weir's abbreviated equation,[29] assuming no nitrogen excretion. We calculated the incremental area under the curve (trapezoidal rule) to estimate total CIT.

Thermal perception and skin temperature

Participants' thermal perception during the month preceding the BAT assessment was reported by a visual analogue scale (0–100). Additionally, the thermal perception was assessed during the warm and cold periods of the personalized cooling protocol before BAT assessment with a numeric rate scale from 0 to 10. The thermal perception in different parts of the body was also measured before and during the assessment of MIT and CIT with a continuous 7-point thermal sensation interval scale (American Society of Heating, Refrigerating, and Air Conditioning Engineers, ASHRAE)[40,41] where −3 was cold, 0 was neutral and 3 was hot.

Skin temperature was measured before and during the assessment of MIT and CIT with 17 wireless thermometers (iButtons, DS-1922 L, Thermochron; resolution 0.0625 °C; Maxim, Dallas, USA), attached to the subjects' skin.[42] Skin temperature was recorded at 1 min intervals and a mean value for every 5 min was calculated for each thermometer. We calculated the mean,[43] proximal,[44] distal,[43] peripheral gradient (forearm minus fingertip),[43] and supraclavicular temperature.[45] All analysis and calculations were performed using the Temperatus® software (http://profith.ugr.es/temperatus)[46] as previously reported.[43,46,47] We also calculated a delta between the last 10 min of the first hour and 30 min of the postprandial period and cold exposure period respectively and the mean of the baseline period for each test.

Ethics

All participants provided written informed consent. The study was approved by the Ethical Committees for Human Research of the University of Granada (no. 924) and the *Servicio Andaluz de Salud* (*Centro de Granada, CEI-Granada, Spain*). The study protocol and experimental design were applied following the last revised ethical guidelines of the Declaration of Helsinki.

Sample size

This study includes cross-sectional analyses using baseline data from a randomized controlled trial aimed at determining the effects of a 24-week supervised exercise intervention on BAT volume and activity, which was originally powered to detect changes in BAT volume and activity.[25] Statistical power analyses served to set an enrollment target of 150 participants in the randomized controlled trial.[25] However, since the current study is based on secondary cross-sectional analyses using only baseline data, no a priori power calculation was performed. Participants with overweight or obesity with available BAT data were included in this cross-sectional analysis.

Statistical analyses

Descriptive data are expressed as mean (standard deviation) or median and interquartile range in skewed variables (mean/standard deviation >2). We compared sex frequency between participants with MHOO and MUOO using the Chi-square test. Differences between individuals with MHOO and MUOO in age,

anthropometric, body composition, and cardiometabolic risk variables were analysed using analysis of covariance (ANCOVA) adjusting for sex, whereas differences in RMR and energy intake were analysed using ANCOVA adjusting for sex and lean mass. All analyses were adjusted (chosen a priori) for sex due to the different distribution of males and females in the MHOO and MUOO groups. Differences in RMR and energy intake were further adjusted for lean mass (chosen a priori) since it is the strongest predictor of these outcomes.[48,49]

We also used ANCOVA models adjusted for sex to compare BAT volume, ^{18}F-FDG uptake, and mean radiodensity.[15,34,50,51] We additionally performed ANCOVA analysis adjusting for BMI, HOMA-IR, and age. We built a theoretical causal diagram based on the scientific literature to confirm the minimum sufficient adjustments for the total effect of MHOO or MUOO phenotype on BAT. Then, we used the online tool DAGitty[52] to build a directed acyclic graph (DAG),[53] and the covariates were consequently selected (Figure S1).

We conducted ANCOVA adjusting for sex to compare descending aorta and scWAT ^{18}F-FDG uptake, the water temperature of the cooling vest during the personalized cooling protocol, thermal perceptions, MIT (%EI), and CIT (%RMR) between MHOO and MUOO. Two-way repeated-measures ANCOVA adjusting for sex (Huynh-Feldt correction) were used to investigate differences between MHOO and MUOO on the meal- and cold-induced kinetics of energy expenditure, skin temperature, and thermal perception (Factor 1 "time" [time after breakfast or time of cold exposure) and Factor 2 "Group" [MHOO or MUOO]). The assumptions of ANCOVAs and two-way repeated-measures ANCOVAs were checked by visual inspection of residuals, Levene's test for heteroscedasticity, and Shapiro–Wilk`s test, and visual inspection of Q–Q plots for normality. In addition, the lack of sex*group interaction was assessed with the ANCOVA analyses. Based on our relatively low sample size we used the Huynh-Feldt correction when the sphericity was not assumed.[54]

The analyses were conducted using the Statistical Package for Social Sciences (SPSS, v.27.0, IBM SPSS Statistics, IBM Corporation), and the level of significance was set at ≤0.05. Figures were built with GraphPad Prism software v.9 (GraphPad Software, San Diego, CA, USA).

Role of funding source

Funders did not participate in the study design, data collection, data analyses, interpretation, or writing of the manuscript.

Results

The descriptive characteristics of the participants are presented in Table 1. Participants with MHOO showed healthier levels of cardiometabolic risk factors than MUOO (i.e., lower glucose, insulin, HOMA-IR, total cholesterol, LDL-C, triglycerides, systolic and diastolic blood pressure, and higher HDL-C; all $P \leq 0.042$; Table 1). However, no statistically significant differences were observed in body composition (i.e., lean mass and adiposity), outdoor ambient temperature exposure, resting metabolic rate, energy intake, sedentary time, or physical activity levels between MHOO and MUOO (all $P \geq 0.085$; Table 1).

MHOO presented higher BAT volume (Mean difference = 60.5 ml, 95% CI, 16.672–104.329), SUVmean (Mean difference = 1.030, 95% CI, 0.465–1.595), and SUVpeak (Mean difference = 4.712, 95% CI, 1.654–7.770) than MUOO (all $P \leq 0.008$; Figs. 1 and 2), whereas BAT mean radiodensity was not different ($P = 0.354$; Fig. 2). The observed differences between MHOO and MUOO in BAT volume, SUVmean, and SUVpeak remained significant after including BMI ($P = 0.005$, $P = 0.002$, and $P = 0.005$, respectively) or HOMA-IR ($P = 0.001$, $P < 0.001$, and $P = 0.002$ respectively) as covariates together with sex. Similarly, the observed differences between MHOO and MUOO in BAT volume, SUVmean, and SUVpeak remained after adjusting for the minimum sufficient covariates obtained from the DAGs (i.e., sex, age, BMI and HOMA-IR; $P < 0.001$, $P < 0.001$, and $P = 0.003$ respectively), and after including both energy intake and physical activity levels as covariates (data not shown). The descending aorta SUVpeak, as well as the tricipital scWAT SUVpeak, were similar between MHOO and MUOO (both $P \geq 0.124$; Figure S2). In contrast, MHOO showed higher dorsocervical scWAT SUVpeak (a region that might present a brown-like signature[37]) than MUOO (Mean difference = 0.068, 95% CI, 0.019–0.116; $P = 0.007$; Figure S2).

We next investigated whether participants with MHOO were exposed to lower temperatures than MUOO. We observed that the water temperature of the cooling vests, just before BAT assessment, was slightly higher in individuals with MHOO than MUOO (Mean difference = 1.873 °C, 95% CI, –0.161 to 3.907; $P = 0.07$; Fig. 3a). However, there were no significant differences between MHOO and MUOO in the thermal perception feeling during the previous month to the BAT assessment, during the warm and cold periods of the personalized cold exposure test before the BAT assessment (all $P \geq 0.773$; Fig. 3b–d).

We observed no statistically significant differences between MHOO and MUOO in energy expenditure after meal intake (both $P \geq 0.096$; Fig. 4a and b), yet a trend toward higher MIT was observed in MHOO in the first (Mean difference = 2.848%, 95% CI, –0.051 to 5.746; $P = 0.054$) and second (Mean difference = 3.082%, 95% CI, –0.017 to 6.182; $P = 0.051$) hour of the meal test. We observed no statistically significant differences in the third hour of the MIT between MHOO and MUOO ($P = 0.487$). Energy expenditure

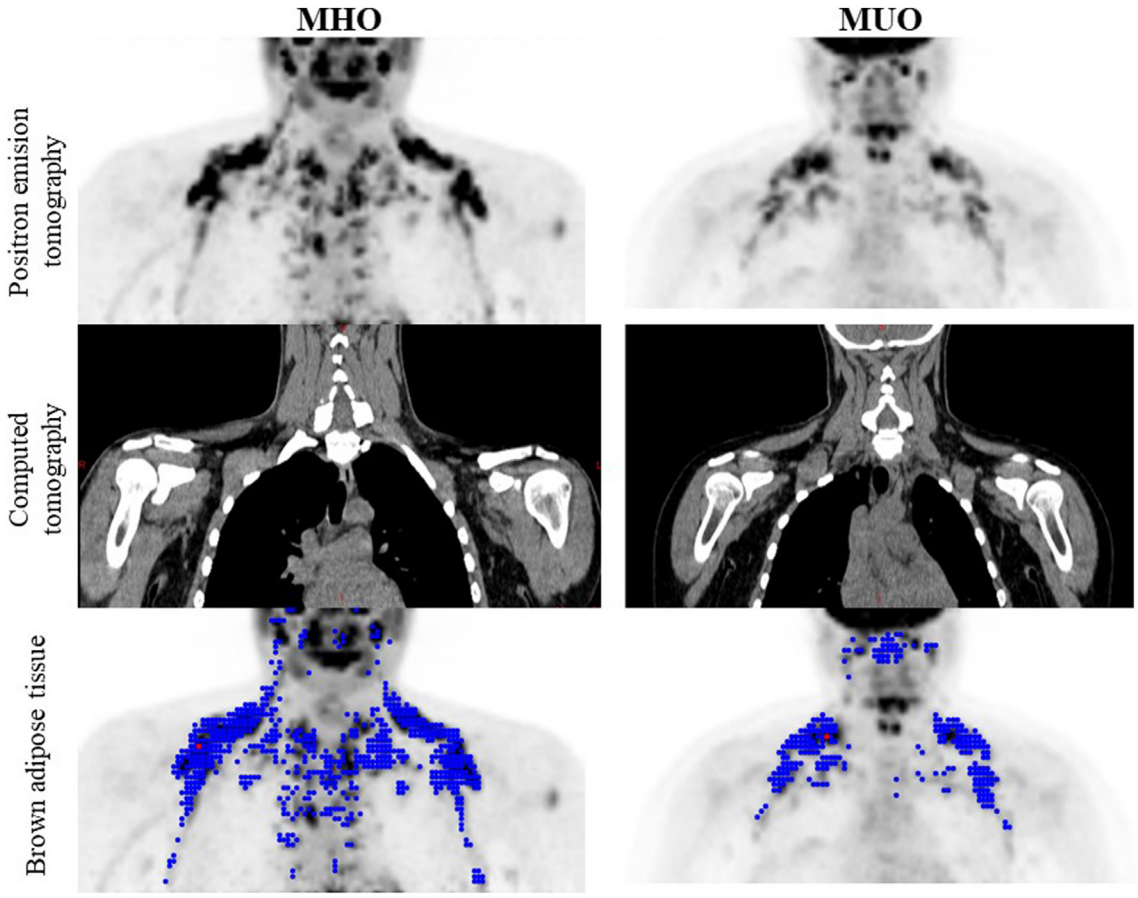

Fig. 1: **Images of two representative individuals, one with metabolically healthy and obesity (MHO) and another one with metabolically unhealthy and obesity (MUO).** Blue dots indicate brown adipose tissue volume and red dots indicate maximal brown adipose tissue activity (maximal standardized uptake value).

increased in both groups during cold exposure (η^2 = 0.163, P = 0.034, Fig. 4c), but MHOO exhibited higher CIT than MUOO (Mean difference = 10.920%, 95% CI, 0.012–21.828 P = 0.05; Fig. 4d).

In addition, we found no statistically significant differences between MHOO and MUOO in meal- and cold-induced changes in mean, proximal, distal, and peripheral gradient skin temperatures (all P interaction ≥0.089; Fig. 5a–h). In contrast, meal-induced changes in supraclavicular skin temperature were significantly different between groups (η^2 = 0.078, P interaction = 0.043; Fig. 5i). During the first 75 min of the post-prandial period, MHOO exhibited an increase in supraclavicular skin temperature (+0.4 °C), while MUOO did not change (+0.07 °C) (Mean difference = 0.349 °C, 95% CI, –0.058 to 0.755; P = 0.090). We also observed a near-significant trend in cold-induced changes in supraclavicular skin temperature (η^2 = 0.113, P interaction = 0.086; Fig. 5j). During the first 30 min of the cold exposure period, MHOO exhibited maintenance in supraclavicular skin temperature (–0.1 °C) whereas MUOO showed a decrease (–0.5 °C) (Mean difference = 0.329 °C, 95% CI, –0.123 to 0.781; P = 0.146). However, meal- and cold-induced changes in skin thermal perception in all regions (i.e., clavicular, abdomen, arm, hands, leg, feet, and body) were similar in MHOO and MUOO (all P interaction ≥0.176; Figure S3).

Discussion

In this study, we show that adults with MHOO present higher BAT volume (+124%), SUVmean (+63%), and SUVpeak (+133%), measured after a personalized cold exposure, than individuals with MUOO. Additionally, MHOO shows higher MIT immediately after consuming the meal, higher CIT, and higher cold-induced supraclavicular skin temperature during the post-prandial period and cold exposure than their counterparts. Taken together, our findings provide insights into the possible relevance of BAT in the MHOO phenotype and posit further evidence of a potential role

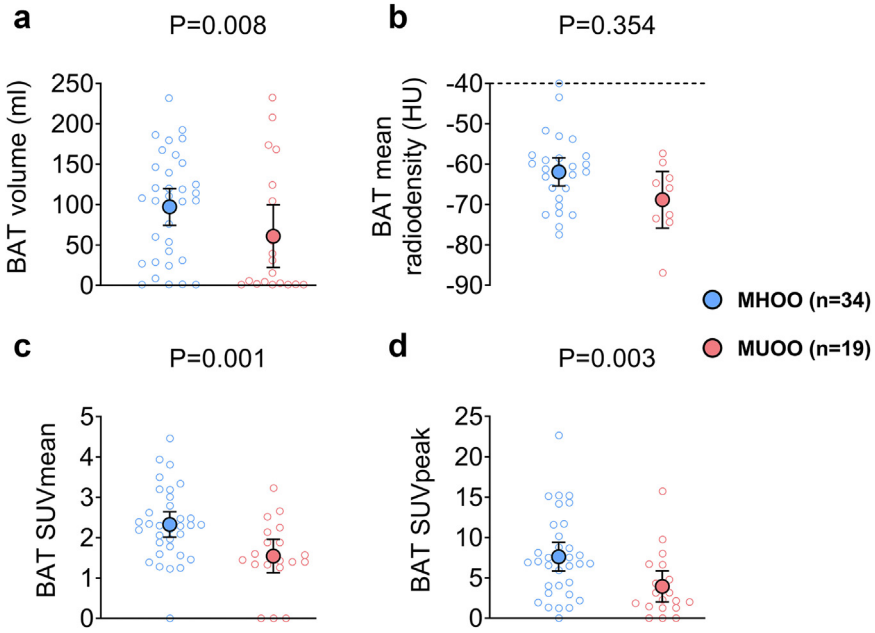

Fig. 2: **Differences between adults with metabolically healthy overweight/obesity (MHOO) and metabolically unhealthy overweight/obesity (MUOO) in brown adipose tissue volume, ^{18}F-FDG uptake and mean radiodensity after a personalized cold exposure**. a) BAT volume; b) BAT mean radiodensity (MHOO, n = 27; MUOO, n = 9); c) BAT SUVmean; and d) BAT SUVpeak. SUV values are shown relative to lean mass. Data represent mean and 95% confidence interval. P values obtained from analysis of covariance adjusting for sex. *Abbreviations*: BAT: brown adipose tissue; HU: Hounsfield Unit; SUV: Standardized uptake value.

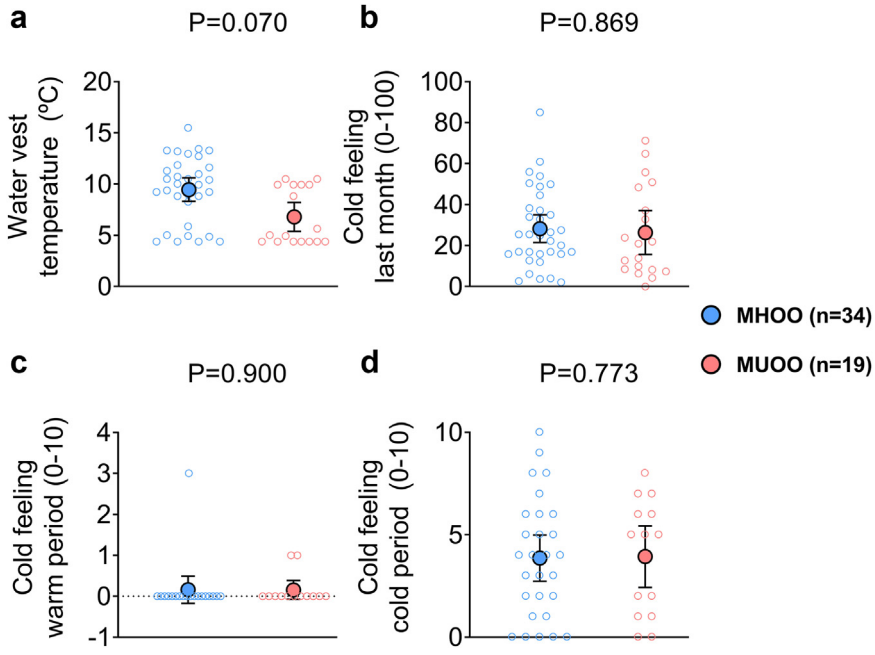

Fig. 3: Differences between adults with metabolically healthy overweight/obesity (MHOO) and metabolically unhealthy overweight/obesity (MUOO) in the temperature of the water irrigating the cooling vest before the brown adipose tissue assessment (a), thermal perception during the previous month (b), thermal perception during the warm period before the personalized cold exposure before the PET-CT scan (c; MHOO, n = 19 and MUOO, n = 13), and thermal perception during the cold period of the personalized cold exposure before the PET-CT scan (d; MHOO, n = 28 and MUOO, n = 15). Panels b, c, and d, the greater the value the greater the cold perception. Data represent mean and 95% confidence interval. P values obtained from analysis of covariance adjusting for sex.

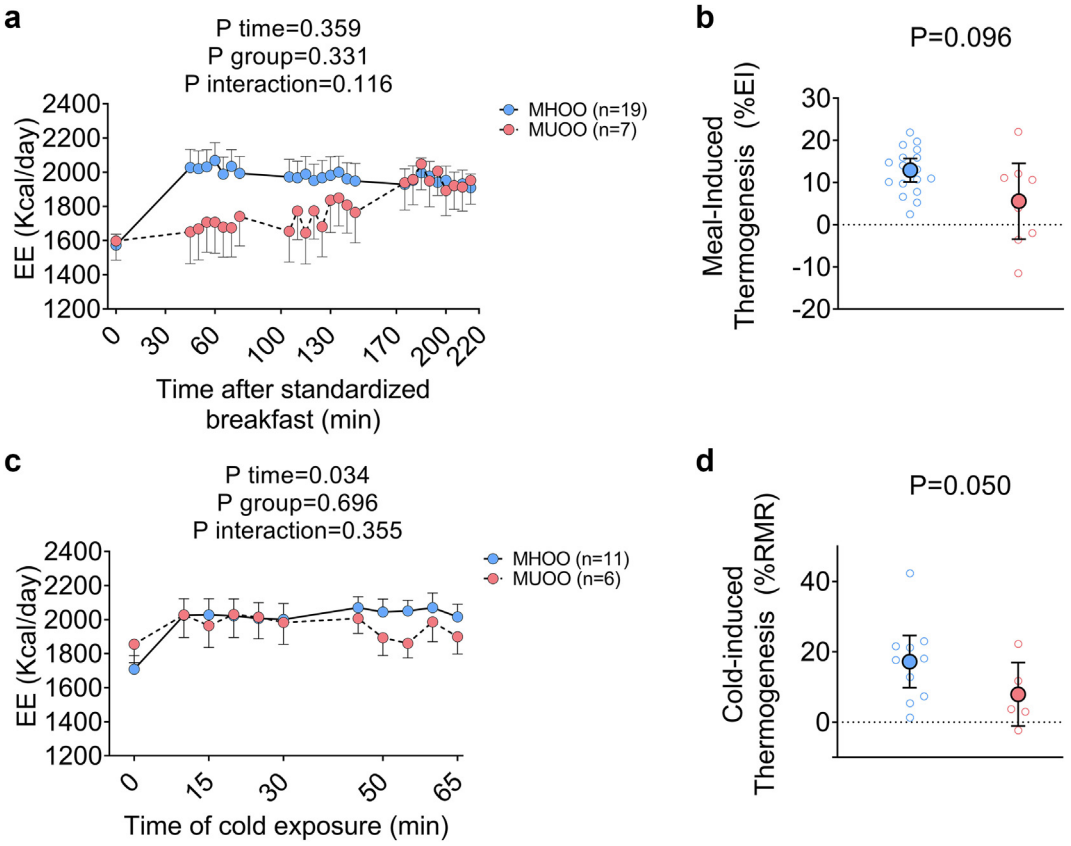

Fig. 4: Differences between adults with metabolically healthy overweight/obesity (MHOO) and metabolically unhealthy overweight/obesity (MUOO) in meal- (a, b) and cold-induced thermogenesis (c, d). Data represent mean and 95% confidence interval. Panels a and c: P values for time, group, and *Time* × *Group* interaction effects obtained from two-way repeated-measures analysis of covariance (ANCOVA) adjusting for sex (Huynh-Feldt correction). Timepoint 0 represents the resting metabolic rate value. Panels b and d: P values obtained from analysis of covariance adjusting for sex. *Abbreviations*: EE: Energy expenditure; EI: Energy intake; min: minutes; RMR: Resting metabolic rate.

of BAT in thermogenesis and cardiometabolic health in individuals with overweight or obesity.

BAT has been postulated as a potential target to improve cardiometabolic health.[55] Since cold exposure is the main activator of BAT in humans,[15,17] a different cold stimulus could bias the interpretation of our results. However, the differences in BAT volume and BAT ^{18}F-FDG uptake observed in our study after a personalized cold exposure cannot be attributed to a stronger cold stimulation in the MHOO group. Indeed, the water temperature during the cooling protocol tended to be warmer in MHOO than in MUOO. In the same vein, the outdoor ambient temperature the week before the enrolment in the study was similar between groups. In addition, MHOO showed higher scWAT dorsocervical ^{18}F-FDG uptake after a personalized cold exposure than MUOO. We previously showed that ^{18}F-FDG uptake in the dorsocervical area was higher than in other scWAT areas, and positively correlated with supraclavicular BAT volume, suggesting a potential brown-like signature in this area.[37] Taken together, our results suggest that adults with MHOO have higher BAT volume and BAT ^{18}F-FDG uptake, which may confirm the potential of BAT recruitment and activation in preventing metabolic abnormalities development in people with overweight or obesity. Our results partially support previous studies reporting that humans with detectable BAT, even with overweight or obesity, have healthier cardiovascular profiles[18,19] and, therefore, might be at a lower risk of cardiometabolic disease.[18]

These findings should, however, be interpreted with caution. ^{18}F-FDG PET-CT scan after a personalized cold exposure is the most used method for human BAT *in vivo* quantification,[15,56] but it suffers some important limitations. This method for BAT quantification relies on glucose uptake, and therefore, it does not measure tissue thermogenesis or quantify lipid metabolism within the tissue.[15] Importantly, impaired insulin sensitivity may decrease ^{18}F-FDG uptake in BAT and underestimate BAT prevalence and BAT thermogenic capacity in individuals with insulin resistance.[15,57] For instance, individuals with type 2 diabetes mellitus

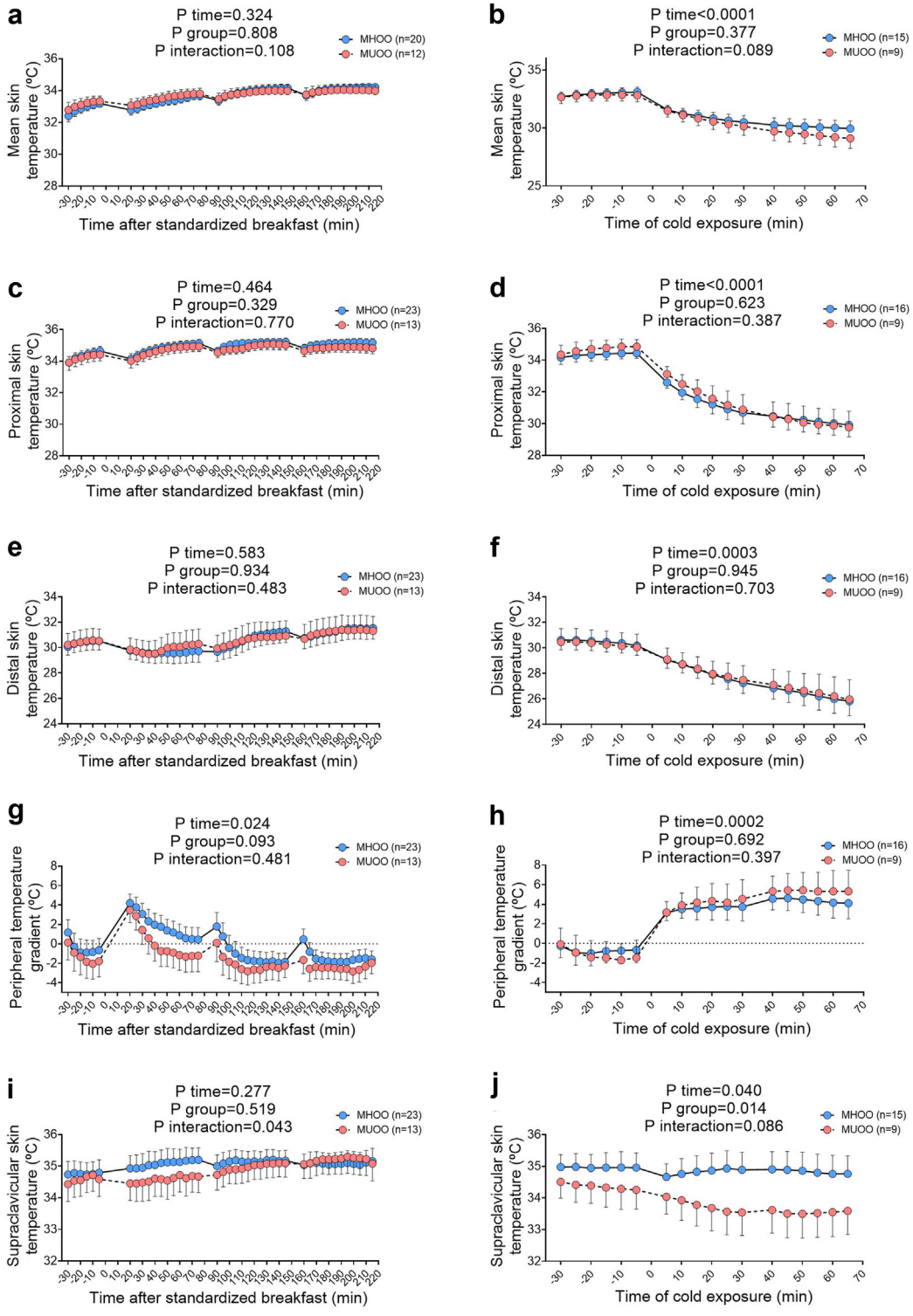

Fig. 5: Differences between adults with metabolically healthy overweight/obesity (MHOO) and metabolically unhealthy overweight/obesity (MUOO) in meal- (a, c, e, g, i) and cold-induced (b, d, f, h, j) changes in mean, proximal, distal, peripheral gradient, and supraclavicular skin temperatures. In meal-induced changes in temperature (a, c, e, g, i), negative values in the X axes represent the resting metabolic rate period, whereas in cold changes in temperature (b, d, f, h, j) represent the warm room period. Data represent mean and 95% confidence interval. In meal-induced changes (a, c, e, g, i), time point 0 represents the start of the meal intake, whereas in cold-induced changes (b, d, f, h, j) represents the start of the cold exposure. P values for time, group, and *Time* × *Group* interaction effects obtained from a two-way repeated-measures analysis of covariance (ANCOVA) adjusting for sex (Huynh-Feldt correction).

present an impairment of BAT glucose metabolism despite showing similar BAT levels than healthy counterparts when using a derived fatty acid tracer or similar BAT oxidative capacity.[57] Since MHOO are more insulin sensitive than MUOO, as reflected in our study in lower levels of glucose and insulin, we cannot discard that differences in insulin sensitivity might have biased the results on BAT volume and ^{18}F-FDG uptake. Nonetheless, we repeated the MHOO vs. MUOO comparisons of BAT volume and ^{18}F-FDG uptake adjusting the analyses for HOMA-IR and observed similar results. This suggests that our findings may be independent of differences in insulin sensitivity. Nevertheless, future studies should use PET radiotracers other than glucose analogues (e.g., ^{11}C-acetate, ^{15}O-oxygen, or ^{18}F-fluoro-thia-heptadecanoic acid) to replicate these findings.

The higher BAT volume and glucose uptake in individuals with MHOO concurs with a marginally higher MIT, a higher CIT, and higher supraclavicular skin temperature after meal ingestion and during cold exposure. In humans, a large part of BAT is located in the supraclavicular region,[58,59] which has a higher thermogenic capacity than other regions.[60] The supraclavicular skin temperature has been used as a method for detecting BAT activity in adult humans[44,61] and is significantly increased upon cold exposure,[44] in contrast to proximal and distal temperature, which decreases in response to cold exposure.[45] Altogether, these results reinforce the interpretation that BAT is more active in MHOO participants.

There are several potential mechanistic explanations for the link between BAT and a healthier cardiometabolic profile. BAT activation could increase the uptake of circulating glucose and fatty acids, as well as of fatty acids from triglyceride-rich lipoproteins as fuel, alleviating hyperglycemia,[55] and hyperlipidemia,[62–65] and increasing HDL-C levels.[64,66] Additionally, activated BAT releases endocrine factors (e.g., fibroblast growth factor 21), the so-called "batokines".[67] These batokines can regulate whole-body metabolism through autocrine, paracrine, and endocrine functions and act in different organs and tissues (e.g., WAT, heart, skeletal muscle).[68] Indeed, the batokines' endocrine function might underlie the association between BAT volume and activity and a healthy cardiometabolic profile.[68] Batokines enhance peripheral insulin sensitivity and lower blood lipids improving systemic glucose and lipid homeostasis and protecting against cardiometabolic diseases in mice.[67] Indeed, humans with detectable BAT showed lower glucose and triglyceride levels and higher HDL-C levels than those without detectable BAT.[18] It is thus conceivable that the higher BAT volume and ^{18}F-FDG uptake observed in MHOO display an insulin-sensitizing and lipid-lowering stimulus which might confer a protective metabolic phenotype against abnormalities. Further studies are warranted to confirm the direction of this hypothesis, since this may point to BAT transplantation and/or pharmaceutical enhancement as a possible therapy in humans, as it has been shown in animal models.[69–71] In addition, the difference between MHOO and MUOO groups in glucose and lipid homeostasis or batokines in response to meal ingestion and cold exposure could not be investigated. Thus, future studies should investigate whether individuals with MHOO have higher BAT, and whether this higher BAT concurs with a healthier glycaemic and lipid response and a different "batokines" response upon meal ingestion and cold exposure. Lastly, evidence linking BAT with metabolic health is derived from observational studies, and no causality can be established. Thus, it could be hypothesized that there could be other variables explaining this association (i.e., insulin sensitivity, white adipose tissue functionality) that might influence our observed results.

Limitations

Several limitations need to be acknowledged. The observational design of the study does not allow to infer any causal relationship. Since the present study included young adults, the findings cannot be extrapolated to older adults or unhealthy populations. The relatively low sample size does not allow us to analyse the data in women and men separately. Moreover, the MHOO concept is inherently relative, and its formulation may directly influence the prevalence of MHOO and MUOO.[13,72] Lastly, our results should be interpreted with caution, considering the study design and the potential influence of unmeasured confounding variables such as specific BAT fat content, BAT activity assessed by metabolic flux, and BAT insulin resistance. Prospective studies are required to better understand the role of BAT in the MHOO phenotype.

Conclusions

Adults with MHOO show higher BAT volume and ^{18}F-FDG uptake than MUOO. This concurs with a higher meal- and cold-induced thermogenesis and with a higher supraclavicular skin temperature after a meal and during cold exposure. Taken together, our findings suggest a role of BAT in conferring a healthy metabolic phenotype in young adults with overweight or obesity. Future intervention studies should investigate the effects of BAT activators (e.g., cold exposure, pharmaceutical agents) in preventing metabolic abnormalities and reducing the risk of cardiometabolic disease in individuals with overweight or obesity.

Contributors

Conceptualization, LJF, GSD, RSS, IL, FBO, BMT, and JRR; Methodology, GSD, JMAA, FMA, IL, FBO, BMT, and JRR; Validation, GSD, JMAA, FMA, BMT, and JRR; Formal Analysis, LJF, GSD, and BMT; Data collection, GSD, JMAA, FMA, RSS, and BMT; Data Curation, LJF and GSD; Writing—Original Draft, LJF, GSD, JRR, and BMT; Writing—Review & Editing, all authors; Supervision, BMT and JRR. All authors commented on the manuscript and approved the final version of the

manuscript. JRR. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request, as the study consists of a high number of participants and outcomes and requires specific knowledge for data interpretation.

Database and data handling procedures

The study has a data management plan that strictly follows the regulations of the University of Granada, which ensures data protection for all the project. The data are treated according to EU regulation 2016/679 (e.g., pseudo anonymization, security measures to prevent unauthorized access) and national legislation (Seventeenth additional provision, Health data treatments) of Spanish Organic Law 3/2018, of December 5th, on the Protection of Personal Data and guarantee of digital rights. All the data and research material are stored following the guidelines of the University of Granada and archived in a Central biobank. Our data management plan also follows the H2020 FAIR principle, as we are making our research data findable, accessible, interoperable (i.e., allowing data exchange between researchers), and reusable (while keeping in mind the embargo policies).

Declaration of interests

None.

Acknowledgements

The authors would like to thank all the participants of this study for their time and effort. This study is part of a PhD thesis conducted in the Biomedicine Doctoral Studies of the University of Granada, Spain.

The study was supported by the Junta de Andalucía, Consejería de Transformación Económica, Industria, Conocimiento y Universidades Dirección General de Investigación y Transferencia del Conocimiento (ref. P18-RT-4455, ref. SOMM17/6107/UGR, and DOC 01151) and European Regional Development Funds (ERDF), the Spanish Ministry of Economy and Competitiveness via the Fondo de Investigación Sanitaria del Instituto de Salud Carlos III (PI13/01393), and PTA-12264, Retos de la Sociedad (DEP2016-79512-R), the Spanish Ministry of Education (FPU13/04365, FPU15/04059 and FPU19/01609), the Fundación Iberoamericana de Nutrición (FINUT), the Redes Temáticas de Investigación Cooperativa RETIC (Red SAMID RD16/0022), the AstraZeneca HealthCare Foundation, the University of Granada Plan Propio de Investigación 2016 -Excellence actions: Unit of Excellence on Exercise and Health (UCEES). G.SD is supported by a Marie Skłodowska-Curie Actions-Individual Fellowship (101028941; Horizon 2020, European Commission). B.MT is supported by a grant for the requalification of the Spanish university system from the Ministry of Universities of the Government of Spain, funded by the European Union, NextGeneration EU (María Zambrano program, reference RR _C_2021_04). J.M.A.A. is supported by the Juan de la Cierva-Formación Grant FJC2020-044453-I funded by MCIN/AEI/10.13039/501100011033 and "European Union NextGeneration EU/PRTR".

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104948.

References

1 Bentham J, Di Cesare M, Bilano V, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet*. 2017;390:2627–2642. https://doi.org/10.1016/S0140-6736(17)32129-3.
2 Karelis AD. Metabolically healthy but obese individuals. *Lancet*. 2008;372:1281–1283.
3 Primeau V, Coderre L, Karelis AD, et al. Characterizing the profile of obese patients who are metabolically healthy. *Int J Obes*. 2011;35:971–981. https://doi.org/10.1038/ijo.2010.216.
4 Bell JA, Hamer M, Batty GD, Singh-Manoux A, Sabia S, Kivimäki M. Incidence of metabolic risk factors among healthy obese adults: 20-year follow-up. *J Am Coll Cardiol*. 2015;66:871–873.
5 Gómez-Ambrosi J, Silva C, Galofré JC, et al. Body mass index classification misses subjects with increased cardiometabolic risk factors related to elevated adiposity. *Int J Obes*. 2012;36:286–294.
6 St-Pierre AC, Cantin B, Mauriège P, et al. Insulin resistance syndrome, body mass index and the risk of ischemic heart disease. *CMAJ*. 2005;172:1301–1305. https://doi.org/10.1503/cmaj.1040834.
7 Calori G, Lattuada G, Piemonti L, et al. Prevalence, metabolic features, and prognosis of metabolically healthy obese Italian individuals: the cremona study. *Diabetes Care*. 2011;34:210–215. https://doi.org/10.2337/dc10-0665.
8 Voulgari C, Tentolouris N, Dilaveris P, Tousoulis D, Katsilambros N, Stefanadis C. Increased heart failure risk in normal-weight people with metabolic syndrome compared with metabolically healthy obese individuals. *J Am Coll Cardiol*. 2011;58:1343–1350. https://doi.org/10.1016/j.jacc.2011.04.047.
9 Mclaughlin T, Abbasi F, Lamendola C, Reaven G. Heterogeneity in the prevalence of risk factors for cardiovascular disease and type 2 diabetes mellitus in obese individuals effect of differences in insulin sensitivity. *Arch Intern Med*. 2007;167:642–648.
10 Bonora E, Kiechl S, Willeit J, et al. Insulin resistance as estimated by homeostasis model assessment predicts incident symptomatic cardiovascular disease in caucasian subjects from the general population: the Bruneck study. *Diabetes Care*. 2007;30:318–324. https://doi.org/10.2337/dc06-0919.
11 Meigs JB, Wilson PWF, Fox CS, et al. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. *J Clin Endocrinol Metab*. 2006;91:2906–2912. https://doi.org/10.1210/jc.2006-0594.
12 Blüher M. Metabolically healthy obesity. *Endocr Rev*. 2020;41:405–420. https://doi.org/10.1210/endrev/bnaa004.
13 Zhou Z, Macpherson J, Gray SR, et al. Are people with metabolically healthy obesity really healthy? A prospective cohort study of 381,363 UK Biobank participants. *Diabetologia*. 2021;64:1963–1972. https://doi.org/10.1007/s00125-021-05484-6/Published.
14 Tchernof A, Després JP. Pathophysiology of human visceral obesity: an update. *Physiol Rev*. 2013;93:359–404. https://doi.org/10.1152/physrev.00033.2011.
15 Carpentier AC, Blondin DP, Haman F, Richard D. Brown adipose tissue—a translational perspective. *Endocr Rev*. 2022;44:143–192. https://doi.org/10.1210/endrev/bnac015.
16 Tanriover C, Copur S, Gaipov A, et al. Metabolically healthy obesity: misleading phrase or healthy phenotype? *Eur J Intern Med*. 2023;111:5–20. https://doi.org/10.1016/j.ejim.2023.02.025.
17 Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev*. 2004;84:277–359. https://doi.org/10.1152/physrev.00015.2003.
18 Becher T, Palanisamy S, Kramer DJ, et al. Brown adipose tissue is associated with cardiometabolic health. *Nat Med*. 2021;27:58–65. https://doi.org/10.1038/s41591-020-1126-7.
19 Herz CT, Kulterer OC, Prager M, et al. Active Brown adipose tissue is associated with a healthier metabolic phenotype in obesity. *Diabetes*. 2022;71:93–103. https://doi.org/10.2337/db21-0475.
20 Celi FS, Le TN, Ni B. Physiology and relevance of human adaptive thermogenesis response. *Trends Endocrinol Metabol*. 2015;26:238–247. https://doi.org/10.1016/j.tem.2015.03.003.
21 Brychta RJ, Chen KY. Cold-induced thermogenesis in humans. *Eur J Clin Nutr*. 2017;71:345–352. https://doi.org/10.1038/ejcn.2016.223.
22 Carneiro IP, Elliott SA, Siervo M, et al. Is obesity associated with altered energy expenditure? *Adv Nutr*. 2016;7:476–487. https://doi.org/10.3945/an.115.008755.
23 Smith RL, Soeters MR, Rob CIW. Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. *Endocr Rev*. 2018;39:489–517. https://doi.org/10.1210/er.2017-00211.
24 Sanchez-Delgado G, Martinez-Tellez B, Olza J, et al. Activating brown adipose tissue through exercise (ACTIBATE) in young adults: rationale, design and methodology. *Contemp Clin Trials*. 2015;45:416–425. https://doi.org/10.1016/j.cct.2015.11.004.
25 Martinez-Tellez B, Sanchez-Delgado G, Acosta FM, et al. No evidence of brown adipose tissue activation after 24 weeks of supervised exercise training in young sedentary adults in the ACTIBATE randomized controlled trial. *Nat Commun*. 2022;13:5259.

26 Ortega FB, Lavie CJ, Blair SN. Obesity and cardiovascular disease. *Circ Res*. 2016;118:1752–1770. https://doi.org/10.1161/CIRCRESAHA.115.306883.

27 Sanchez-Delgado G, Alcantara JMA, Ortiz-Alvarez L, et al. Reliability of resting metabolic rate measurements in young adults: impact of methods for data analysis. *Clin Nutr*. 2017;37(5):1618–1624. https://doi.org/10.1016/j.clnu.2017.07.026.

28 Sanchez-Delgado G, Alcantara JMA, Acosta FM, et al. Energy expenditure and macronutrient oxidation in response to an individualized nonshivering cooling protocol. *Obesity*. 2020;28:2175–2183.

29 Weir JBDB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol*. 1949;109:1–9.

30 Migueles JH, Ulf CC, Nystro CD. Accelerometer data collection and processing criteria to assess physical activity and other outcomes: a systematic review and practical considerations. *Sports Med*. 2017;47:1821–1845. https://doi.org/10.1007/s40279-017-0716-0.

31 Acosta FM, Martinez-Tellez B, Sanchez-Delgado G, et al. Association of objectively measured physical activity with brown adipose tissue volume and activity in young adults. *J Clin Endocrinol Metab*. 2019;104:223–233.

32 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.

33 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.

34 Martinez-Tellez B, Sanchez-Delgado G, Garcia-Rivero Y, et al. A new personalized cooling protocol to activate brown adipose tissue in young adults. *Front Physiol*. 2017;8:863. https://doi.org/10.3389/fphys.2017.00863.

35 Martinez-Tellez B, Sanchez-Delgado G, Boon MR, Rensen PCN, Llamas-Elvira JM, Ruiz JR. Distribution of Brown adipose tissue radiodensity in young adults: implications for cold [18F]FDG-PET/CT analyses. *Mol Imaging Biol*. 2019;22:425–433. https://doi.org/10.1007/s11307-019-01381-y.

36 Martinez-Tellez B, Nahon KJ, Sanchez-Delgado G, et al. The impact of using BARCIST 1.0 criteria on quantification of BAT volume and activity in three independent cohorts of adults. *Sci Rep*. 2018;8:1–8. https://doi.org/10.1038/s41598-018-26878-4.

37 Martinez-Tellez B, Sanchez-Delgado G, Alcantara JMA, et al. Evidence of high 18 F-fluorodeoxyglucose uptake in the subcutaneous adipose tissue of the dorsocervical area in young adults. *Exp Physiol*. 2019;104:168–173. https://doi.org/10.1113/EP087428.

38 Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods*. 2012;9:676–682. https://doi.org/10.1038/nmeth.2019.

39 Chen KY, Cypess AM, Laughlin MR, et al. Brown adipose reporting criteria in imaging STudies (BARCIST 1.0): recommendations for standardized FDG-PET/CT experiments in humans. *Cell Metab*. 2016;24:210–222. https://doi.org/10.1016/j.cmet.2016.07.014.

40 Paliaga G, Schoen LJ, Alspach PF, et al. *Thermal environmental conditions for human occupancy*. ASHRAE Sta:58. Ashrae; 2013. ISSN 1041-2336.

41 *American society of heating refrigerating and air-conditioning Engineers*. ASHRAE HANDBOOK FUNDAMENTALS I-P edition supported by ASHRAE Research; 2005.

42 Ortiz-Alvarez L, Ruiz JR, Gil A, et al. Skin temperature response to a liquid meal intake is different in men than in women. *Clin Nutr*. 2018;38(3):1339–1347. https://doi.org/10.1016/j.clnu.2018.05.026.

43 Martinez-Tellez B, Sanchez-Delgado G, Acosta FM, et al. Differences between the most used equations in BAT-human studies to estimate parameters of skin temperature in young lean men. *Sci Rep*. 2017;7. https://doi.org/10.1038/s41598-017-10444-5.

44 Boon MR, Bakker LEH, Van Der Linden RAD, et al. Supraclavicular skin temperature as a measure of18F-FDG uptake by BAT in human subjects. *PLoS One*. 2014;9. https://doi.org/10.1371/journal.pone.0098822.

45 Martinez-Tellez B, Garcia-Rivero Y, Sanchez-Delgado G, et al. Supraclavicular skin temperature measured by iButtons and 18F-fluorodeoxyglucose uptake by brown adipose tissue in adults. *J Therm Biol*. 2019;82:178–185. https://doi.org/10.1016/j.jtherbio.2019.04.006.

46 Martinez-Tellez B, Quesada-Aranda A, Sanchez-Delgado G, Fernández-Luna JM, Ruiz JR. Temperatus ® software: a new tool to efficiently manage the massive information generated by iButtons. *Int J Med Inform*. 2019;126:9–18. https://doi.org/10.1016/j.ijmedinf.2019.03.007.

47 Acosta FM, Martinez-Tellez B, Sanchez-Delgado G, et al. Physiological responses to acute cold exposure in young lean men. *PLoS One*. 2018;13. https://doi.org/10.1371/journal.pone.0196543.

48 Blundell JE, Caudwell P, Gibbons C, et al. Body composition and appetite: fat-free mass (but not fat mass or BMI) is positively associated with self-determined meal size and daily energy intake in humans. *Br J Nutr*. 2012;107:445–449.

49 Lam YY, Redman LM, Smith SR, et al. Determinants of sedentary 24-h energy expenditure: equations for energy prescription and adjustment in a respiratory chamber. *Am J Clin Nutr*. 2014;99:834–842.

50 Cypess AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med*. 2009;360:1509–1517. https://doi.org/10.1056/NEJMoa0810780.

51 Wang Q, Zhang M, Xu M, et al. Brown adipose tissue activation is inversely related to central obesity and metabolic parameters in adult human. *PLoS One*. 2015;10:1–13. https://doi.org/10.1371/journal.pone.0123795.

52 Textor J, Van der Zander B, Gilthorpe MS, Liśkiewicz M, Ellison GTH. Robust causal inference using directed acyclic graphs: the R package 'dagitty. *Int J Epidemiol*. 2016;45:1887–1894.

53 Tennant PWG, Murray EJ, Arnold KF, et al. Use of directed acyclic graphs (DAGs) to identify confounders in applied health research: review and recommendations. *Int J Epidemiol*. 2021;50:620–632.

54 Field A. *Discovering statistics using IBM SPSS statistics*. SAGE Publications; 2013.

55 Ruiz JR, Martinez-Tellez B, Sanchez-Delgado G, Osuna-Prieto FJ, Rensen PCN, Boon MR. Role of human Brown fat in obesity, metabolism and cardiovascular disease: strategies to turn up the heat. *Prog Cardiovasc Dis*. 2018;61:232–245. https://doi.org/10.1016/j.pcad.2018.07.002.

56 Carpentier AC, Blondin DP, Virtanen KA, Richard D, Haman F, Turcotte ÉE. Brown adipose tissue energy metabolism in humans. *Front Endocrinol*. 2018;9. https://doi.org/10.3389/fendo.2018.00447.

57 Blondin DP, Labbé SM, Noll C, et al. Selective impairment of glucose but not fatty acid or oxidative metabolism in brown adipose tissue of subjects with type 2 diabetes. *Diabetes*. 2015;64:2388–2397.

58 Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab*. 2007;293:E444–E452. https://doi.org/10.1152/ajpendo.00691.2006.

59 van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, et al. Cold-activated Brown adipose tissue in healthy men. *N Engl J Med*. 2009;360:1500–1508. https://doi.org/10.1056/nejmoa0808718.

60 Ouellet V, Labbé SM, Blondin DP, et al. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J Clin Invest*. 2012;122:545–552.

61 van der Lans AAJJ, Vosselman MJ, Hanssen MJW, Brans B, van Marken Lichtenbelt WD. Supraclavicular skin temperature and BAT activity in lean healthy adults. *J Physiol Sci*. 2016;66:77–83.

62 Berbée JFP, Boon MR, Khedoe P, et al. Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. *Nat Commun*. 2015;6:1–11.

63 Bartelt A, John C, Schaltenberg N, et al. Thermogenic adipocytes promote HDL turnover and reverse cholesterol transport. *Nat Commun*. 2017;8:1–10.

64 Bartelt A, Bruns OT, Reimer R, et al. Brown adipose tissue activity controls triglyceride clearance. *Nat Med*. 2011;17:200–205.

65 Khedoe PPSJ, Hoeke G, Kooijman S, et al. Brown adipose tissue takes up plasma triglycerides mostly after lipolysis. *J Lipid Res*. 2015;56:51–59.

66 Acosta FM, Sanchez-Delgado G, Martinez-Tellez B, et al. A larger brown fat volume and lower radiodensity are related to a greater cardiometabolic risk, especially in young men. *Eur J Endocrinol*. 2022;187:171–183.

67 Villarroya F, Cereijo R, Villarroya J, Giralt M. Brown adipose tissue as a secretory organ. *Nat Rev Endocrinol*. 2017;13:26–35. https://doi.org/10.1038/nrendo.2016.136.

68 Ziqubu K, Dludla PV, Mabhida SE, et al. Brown adipose tissue-derived metabolites and their role in regulating metabolism. *Metabolism*. 2023;150:155709. https://doi.org/10.1016/j.metabol.2023.155709.

69 Kikai M, Yamada H, Wakana N, et al. Transplantation of brown adipose tissue inhibits atherosclerosis in apoE-/- mice: contribution of the activated FGF-21-adiponectin axis. *Cardiovasc Res*. 2018;114:i1–i13. https://doi.org/10.1093/cvr/cvx212.

70 Liu H, Liu X, Wang L, Sheng N. Brown adipose tissue transplantation ameliorates male fertility impairment caused by diet-induced obesity. *Obes Res Clin Pract*. 2017;11:198–205. https://doi.org/10.1016/j.orcp.2016.06.001.

71 Yuan X, Hu T, Zhao H, et al. Brown adipose tissue transplantation ameliorates polycystic ovary syndrome. *Proc Natl Acad Sci U S A*. 2016;113:2708–2713. https://doi.org/10.1073/pnas.1523236113.

72 Smith GI, Mittendorfer B, Klein S. Metabolically healthy obesity: facts and fantasies. *J Clin Invest*. 2019;129:3978–3989. https://doi.org/10.1172/JCI129186.