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Brown Adipose Tissue Volume and Fat Content Are Positively Associated With Whole-Body Adiposity in Young Men—Not in Women

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Human brown adipose tissue (BAT) volume has consistently been claimed to be inversely associated with whole-body adiposity. However, recent advances in the assessment of human BAT suggest that previously reported associations may have been biased. The present cross-sectional study investigates the association of BAT volume, mean radiodensity, and ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake (assessed via a static positron emission tomography [PET]–computed tomography [CT] scan after a 2-h personalized cold exposure) with whole-body adiposity (measured by DXA) in 126 young adults (42 men and 84 women; mean ± SD BMI 24.9 ± 4.7 kg/m^2). BAT volume, but not ^{18}F-FDG uptake, was positively associated with BMI, fat mass, and visceral adipose tissue (VAT) mass in men but not in women. These associations were independent of the date when the PET-CT was performed, insulin sensitivity, and body surface area. BAT mean radiodensity, an inverse proxy of BAT fat content, was negatively associated with BMI, fat mass, and VAT mass in men and in women. These results refute the widely held belief that human BAT volume is reduced in obese persons, at least in young adults, and suggest that it might even be the opposite in young men.

Brown adipose tissue (BAT) is the main tissue responsible for adaptive thermogenesis in rodents (1), in which it can be responsible for up to 60% of total energy expenditure (2,3). In humans, it has long been believed that BAT is only metabolically active in neonates and is either absent or irrelevant in adults (1). However, in the late 2000s, several independent research groups showed BAT to be present and metabolically active in human adults (4–9). Indeed, studies assessing BAT after cold stimulation have suggested that most, if not all, human adults possess some activable BAT (10). Despite the fact that the amount of BAT in humans is proportionally much smaller than in mice, it has been regarded as a promising target for therapies designed to tackle obesity and related comorbidities (10).

Early studies assessing human BAT via ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET)–computed tomography (CT) revealed an inverse association between BAT volume and whole-body adiposity (commonly defined as BMI, waist circumference, fat mass, fat mass percentage, or visceral adipose tissue [VAT] mass) (5,6,8). However, back in the 1970s it was shown that providing a hypercaloric cafeteria diet increased whole-body adiposity as well as BAT volume in rats (11). This classic study, and others

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(12–14), provided evidence of the obesity-induced hypertrophy/hyperplasia of BAT, at least in wild-type mice and rats. The inverse association between BAT volume and whole-body adiposity shown by the first human PET-CT studies thus conflicts with classic rodent studies. However, it should be considered that there is an ongoing debate on whether BAT studies conducted in murines at room temperature (i.e., below the animals' thermoneutrality) are translatable to humans (15,16). Thus, even if the previously reported increase in BAT volume in response to overfeeding in murine models kept at room temperature is a conserved mechanism in humans, it might still not translate to most modern humans, as we are rarely exposed to temperatures below thermoneutrality (17).

^{18}F-FDG–PET-CT scanning is currently considered the best available technique for human BAT volume quantification in vivo (18). However, despite the efforts for improving its validity for BAT assessment (19), this technique still presents considerable limitations and requires careful interpretation (10,18). Importantly, recent advances in ^{18}F-FDG–PET-CT scanning for human BAT assessment suggest that the inverse association between human BAT volume and whole-body adiposity could, at least in part, have been biased by methodological issues. Firstly, the initial (and indeed many of the later) studies on human BAT involved ^{18}F-FDG–PET-CT scanning under thermoneutral conditions or after a nonindividualized cold exposure. Noteworthily, current recommendations state that ^{18}F-FDG–PET-CT scanning should be performed after an individualized cold exposure (19,20). Conducting the ^{18}F-FDG–PET-CT scan under thermoneutral conditions likely makes functional BAT undetectable (19). On the other hand, using a fixed, nonindividualized cold exposure before a ^{18}F-FDG–PET-CT scan might lead to the over- or underestimation of BAT volume depending on a subject's insulation characteristics and cold tolerance, since participants might not be submitted to the same thermic stress (air at 17°C may maximize nonshivering thermogenesis in one person, yet only be a slight stimulus for the same in another) (20). Since obesity is thought to modify body insulation and/or the rate of heat production (21,22), not individualizing cold-exposure protocols could systematically bias the relationship recorded between BAT and whole-body adiposity (20). Secondly, the standardized uptake value (SUV), the unit used to express ^{18}F-FDG uptake, has traditionally been expressed relative to body mass (SUV_{BM}). However, it has now been established that the SUV should be expressed relative to lean body mass to help avoid body composition bias in glucose uptake assessments (23). Moreover, the SUV threshold, when defined as the minimal radiotracer uptake needed to quantify a voxel as BAT, should also be expressed relative to lean body mass (19)—something not done in most humans studies that have reported an inverse association between BAT and whole-body adiposity. Finally, the use of the glucose analog ^{18}F-FDG, the most frequently used radiotracer for BAT assessment, might also bias the relationship between BAT volume and whole-body adiposity. Insulin-resistant states, such as type 2 diabetes, impair BAT ^{18}F-FDG uptake without affecting its oxidative metabolism or fatty acid uptake (24). Therefore, the reported inverse association between BAT volume (assessed by ^{18}F-FDG uptake) and whole-body adiposity might also be biased by individuals who have age- or obesity-induced insulin resistance.

The present work investigated the association between BAT and whole-body adiposity in young, healthy adults by assessment of BAT volume, ^{18}F-FDG uptake, and mean radiodensity (an inverse proxy of BAT fat content [25]) after individualized cold exposure, with all other current methodological recommendations for human BAT analysis strictly followed (19).

RESEARCH DESIGN AND METHODS

Study Subjects

The study subjects were 126 young adults (Table 1). For this study, some of their baseline assessment data are used as recorded in the activating brown adipose tissue through exercise (ACTIBATE) study (26), an exercise-based randomized, controlled trial (NCT02365129, clinicaltrials.gov). All reported themselves sedentary (<20 min of moderate-to-vigorous physical activity on <3 days/week), not to smoke, to take no medication, to have had a stable body weight in the last 3 months (<3 kg change), and not to be exposed to cold regularly. All were classified as healthy in a comprehensive medical examination. All subjects provided their signed, informed consent to be included. The study was approved by the Human Research Ethics Committees of the University of Granada (no. 924) and Servicio Andaluz de Salud (Centro de Granada, CEI-Granada) and was performed with adherence to the latest version of the Declaration of Helsinki.

Procedures

Experimental work was performed in Granada (southern Spain) in eight waves between September and November of 2015 and of 2016. Subject data were collected over three visits to the laboratory (26). In the first, body composition (lean mass, fat mass, percentage fat mass, and VAT mass) was assessed by whole-body dual-energy X-ray absorptiometry (DXA) with a Discovery Wi device (Hologic, Inc., Bedford, MA). Height and weight were measured with subjects barefoot and wearing light clothing with a Model 799 electronic column scale (SECA, Hamburg, Germany). BMI was calculated as weight in kilograms divided by the square of height in meters. We calculated body surface area following the DuBois and DuBois formula: body surface area = (weight [kg]$^{0.425}$) × (Height [cm]$^{0.725}$) × 0.007184 (27). Waist circumference was measured twice in the minimum perimeter with an inextensible metallic

Table 1—Characteristics of the study subjects

	All (n = 126)		Men (n = 42)		Women (n = 84)		
	Mean	SD	Mean	SD	Mean	SD	P
Age (years)	21.9	2.2	22.3	2.2	21.8	2.1	0.156
BMI (kg/m^2)	24.9	4.7	27.3	5.5	23.7	3.7	**<0.001**
Waist circumference (cm)	80.8	14.2	90.6	15.4	75.8	10.5	**<0.001**
Lean mass (kg)	41.6	9.8	52.9	6.9	36.0	5.0	**<0.001**
Lean mass index (kg/m^2)	14.6	2.5	17.2	2.0	13.3	1.4	**<0.001**
Fat mass (kg)	25.1	8.9	26.1	11.2	24.6	7.6	0.420
Fat mass index (kg/m^2)	8.9	3.01	8.5	3.6	9.1	2.6	0.355
Fat mass (%)	35.1	7.5	30.7	7.7	38.5	5.9	**<0.001**
VAT mass (g)	335	179	433	179	285	158	**<0.001**
Body surface area (m^2)	1.79	0.22	1.99	0.19	1.70	0.16	**<0.001**
BAT volume (mL)	68.3	57.7	75.6	66.4	64.6	52.9	0.354
BAT SUVmean	2.18	1.11	2.02	0.82	2.26	1.22	0.181
BAT SUVpeak	6.49	4.74	6.02	4.50	6.72	4.86	0.436
BAT mean radiodensity (HU)*	−60.2	9.8	−59.7	9.9	−60.4	9.9	0.767
scWAT dorsocervical SUVpeak	0.29	0.15	0.32	0.14	0.28	0.15	0.111
scWAT tricipital SUVpeak	0.10	0.05	0.13	0.06	0.08	0.03	**<0.001**
Descending aorta SUVpeak	0.91	0.19	1.03	0.19	0.86	0.16	**<0.001**
HOMA	1.86	1.26	2.06	1.66	1.76	0.99	0.280

P value from Student t test. scWAT, subcutaneous white adipose tissue. *95 participants had valid data for this variable (30 men and 65 women). Bold numbers indicate significant differences.

tape, in the standing position, and the average value were considered for further analyses.

The shivering threshold was assessed, as previously described (28), on each subject's second visit. Briefly, after a fasting period (>6 h), the subjects were dressed in standardized clothes (clothing insulation value = 0.20) and entered a warm room for 30 min. They were then moved into a mild cold room (19.5–20°C) and fitted with a water-perfused cooling vest (Polar Products, Stow, OH) set at 16.6°C. The water temperature was progressively decreased until shivering started. We detected shivering visually and by asking the participants if they had begun to shiver.

On the third visit, 48–72 h after the shivering threshold test, BAT volume, mean radiodensity, and ^{18}F-FDG uptake were assessed as previously described (28). On arrival, still fasting (>6 h), the subjects put on the same standardized clothes and entered a warm room. After 30 min, they were moved to a mild cold room (19.5–20°C) and equipped with the same water-perfused vest set 4°C above their shivering threshold. Participants remained cold exposed for 2 h, during which time the water temperature was slightly increased if participants began to shiver. Of note, the water temperature target (4°C above the shivering threshold) was selected after we observed, in a previous unpublished pilot study, that most participants started shivering during 2 h of cold exposure at lower temperatures. One hour after the beginning of cold exposure, the subjects were injected with an intravenous ^{18}F-FDG bolus (∼185 MBq) and the water temperature increased by 1°C. After the 2 h of cold exposure, an ^{18}F-FDG–PET-CT scan was performed with a Siemens Biograph 16 PET-CT machine (Siemens, Berlin, Germany). We registered the date when the PET-CT was performed as the number of days from 1 January of the same year to the date when the PET-CT was actually performed (e.g., 15 February would be recorded as day 46).

^{18}F-FDG–PET-CT Analyses

PET-CT images were taken after cold stimulation from the atlas vertebrae (cervical 1) to the thoracic vertebra 6 (2 bed positions), approximately. The images were analyzed with use of the Beth Israel plugin for FIJI software (29). Six regions of interest (right and left laterocervical and supraclavicular areas, paraspinal region, and mediastinum) were semiautomatically outlined from the atlas vertebrae (cervical 1) to thoracic vertebra 4. Following current recommendations (19), we defined BAT as all voxels presenting a radiodensity between −190 and −10 Hounsfield units (HU) and an SUV higher than an individualized SUV threshold [1.2 / (lean body mass / body mass)]. Consequently, BAT volume was determined as the sum of all voxels meeting the aforementioned criteria, SUVmean was computed as the average SUV of all voxels classified as BAT, and SUVpeak was determined as the average SUV of all voxels contained in a 1cc sphere centered in the voxel presenting the highest SUV among those classified as BAT. Moreover, the radiodensity of all voxels

classified as BAT in a single region of interest covering the whole body, except the mouth, from the atlas to the thoracic vertebra 4 was averaged for obtaining BAT mean radiodensity (an indicator of the tissue fat content [25]). Thirty-one individuals presented some voxels classified as BAT out of the anatomical areas where BAT is located and were therefore excluded from BAT mean radiodensity analyses. SUVpeak values for the descending aorta, tricipital subcutaneous white adipose tissue, and dorsocervical subcutaneous white adipose tissue (behind thoracic vertebra 2) were also determined. All SUV values are expressed as a function of lean body mass.

Insulin Resistance

Insulin resistance was estimated using HOMA: fasting insulin (mU/L) × fasting glucose (mmol/L) / 22.5 (30). Serum glucose and insulin concentrations were determined in a peripheral blood sample, taken after an overnight fast plus avoiding physical activity for the previous 48 h. Glucose determinations were performed with a Beckman Coulter AU5832 analyzer with reagent OSR6521 (Beckman Coulter, Inc., Brea, CA). The insulin concentration was determined by chemiluminescent immunoassay in a DxI analyzer (Beckman Coulter, Inc.).

Statistics

Results are presented as mean ± SD unless otherwise stated. Simple linear regression models were used to test the association between BAT-related and whole-body adiposity variables. Multiple linear regression models were used to test these associations, with adjustment for the date when the PET-CT was performed (model 1); the date when the PET-CT was performed and HOMA (model 2), since insulin resistance might mediate the associations between BAT and whole-body adiposity (10,31,32); the date when the PET-CT was performed and body surface area (model 3); and the date when the PET-CT was performed and the temperature of the water prefusing the cooling vest during the individualized cooling protocol (model 4). All calculations were made with SPSS, version 21.0 (SPSS Statistics; IBM Corporation, Armonk, NY). Significance was set at $P < 0.05$.

Data and Resource Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request and will be shared in accordance with appropriate data use agreements. No applicable resources were generated or analyzed during the current study.

RESULTS

Table 1 shows participants' characteristics. Significant sex interaction effects were detected in many of the association analyses, and, thus, results are presented in men (n = 42) and women (n = 84) separately. Sex differences in BMI are shown Supplementary Fig. 1.

BAT Volume and Whole-Body Adiposity

BAT volume was found to be weakly but positively associated with BMI, fat mass, and percentage fat mass in men but not in women (Fig. 1). We further adjusted the analyses for the date when the PET-CT scan was performed (a surrogate marker of outdoor temperature variation), since it has been shown to highly influence BAT-related parameters quantification (33,34). After this adjustment, all the associations in men remained significant (Table 2). Moreover, these associations were also independent of insulin resistance as assessed by HOMA and the body surface area (an important determinant of heat loss) (Table 2). With exclusion of the individuals without detectable BAT (PET negative) (8 men and 10 women), all the associations remained similar (data not shown).

BAT ^{18}F-FDG Uptake and Whole-Body Adiposity

Figure 2 shows the association between BAT SUVmean and whole-body adiposity. In women, an inverse association was detected between BAT SUVmean and BMI, whereas a trend toward an inverse association was seen for BAT SUVmean and fat and VAT mass. In contrast, no associations were detected in men (Fig. 2). This pattern remained after adjustment for the date when the PET-CT was performed, HOMA, and the cooling vest water temperature but disappeared after adjustment for body surface area (Table 3). All the associations remained when PET negative participants were excluded from the analyses (data not shown). No association was observed between BAT SUVpeak and whole-body adiposity variables in men or in women (Fig. 3). Similar results were obtained with exclusion of the PET negative participants (data not shown).

BAT Mean Radiodensity and Whole-Body Adiposity

Figure 4 shows the association between BAT mean radiodensity and whole-body adiposity. BAT mean radiodensity was inversely associated with BMI, fat mass, fat mass percentage, and VAT mass in men and women, and with waist circumference in men. Tables 2 and 3 show these associations with adjustment for the date when the PET-CT scan was performed, HOMA, body surface area, and the water temperature of the cooling vest used during the individualized cooling protocol.

Additionally, checks were made to determine whether the observed significant associations between BAT volume or SUVmean and whole-body adiposity were dependent on BAT mean radiodensity. In men, BAT volume and mean radiodensity were both independently associated with BMI (both $P < 0.032$) and fat mass (both $P < 0.049$), and trends toward significance were observed for the independent association of BAT volume and mean radiodensity with fat mass percentage and VAT in men. In

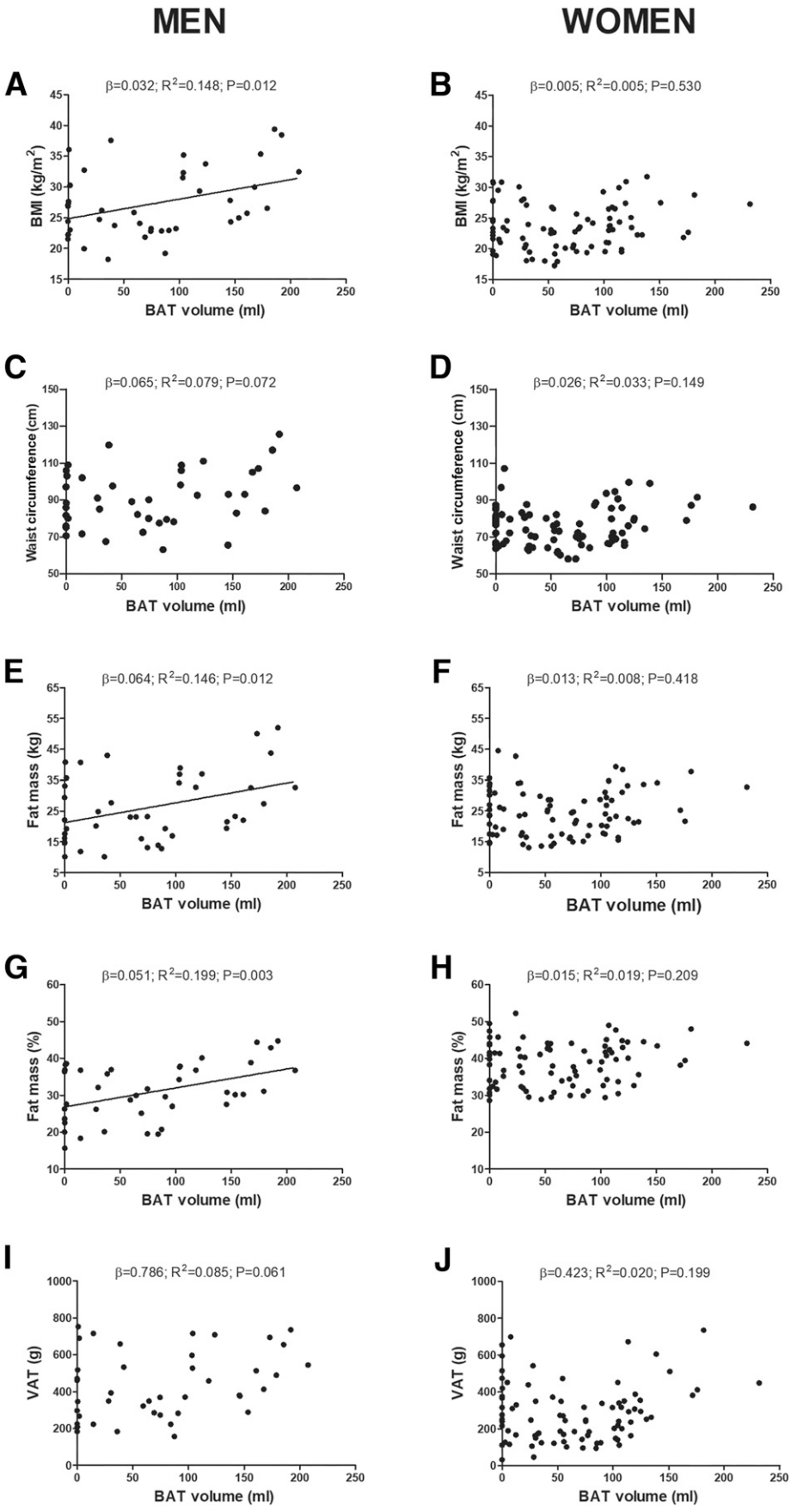

Figure 1—Associations between BAT volume and whole-body adiposity. β = unstandardized simple regression coefficient; R^2 = standardized coefficient of determination.

Table 2—Associations between BAT and whole-body adiposity in men (*n* = 42)

	BMI (kg/m^2)			Waist circumference (cm)			Fat mass (kg)			Fat mass (%)			VAT (g)		
	β	R^2	*P*	β	R^2	*P*	β	R^2	*P*	β	R^2	*P*	β	R^2	*P*
Model 1 (PET-CT date)															
BAT volume (mL)	0.040	0.170	**0.009**	0.079	0.087	0.069	0.077	0.160	**0.013**	0.061	0.215	**0.003**	1.122	0.122	**0.025**
BAT SUVmean	−0.417	0.010	0.747	−4.289	0.041	0.236	−0.744	0.014	0.777	0.421	0.020	0.815	−21.955	0.007	0.604
BAT SUVpeak	0.080	0.011	0.729	−0.408	0.016	0.529	0.057	0.013	0.902	0.168	0.026	0.599	0.308	0.000	0.967
BAT radiodensity (HU)	−0.306	0.301	**0.006**	−0.852	0.275	**0.007**	−0.534	0.250	**0.015**	−0.282	0.180	0.057	−6.414	0.145	0.068
Model 2 (PET-CT date and HOMA-IR)															
BAT volume (mL)	0.036	0.610	**0.001**	0.071	0.434	**0.041**	0.071	0.574	**0.002**	0.057	0.507	**0.001**	1.022	0.542	**0.006**
BAT SUVmean	−0.139	0.473	0.885	−3.602	0.393	0.218	−0.193	0.450	0.923	0.744	0.338	0.620	−13.109	0.443	0.684
BAT SUVpeak	0.112	0.479	0.509	−0.327	0.374	0.532	0.122	0.452	0.731	0.205	0.344	0.441	1.338	0.442	0.815
BAT radiodensity (HU)	−0.196	0.525	**0.041**	−0.538	0.505	0.051	−0.314	0.487	0.102	−0.160	0.347	0.253	−3.032	0.384	0.338
Model 3 (PET-CT date and BSA)															
BAT volume (mL)	0.024	0.757	**0.005**	0.035	0.693	0.178	0.043	0.835	**0.003**	0.042	0.643	**0.003**	0.627	0.683	**0.043**
BAT SUVmean	0.857	0.710	0.239	−0.855	0.679	0.690	2.018	0.806	0.100	2.019	0.584	0.101	17.990	0.651	0.488
BAT SUVpeak	0.255	0.731	**0.043**	0.068	0.678	0.858	0.435	0.813	**0.042**	0.385	0.589	0.075	5.806	0.662	0.201
BAT radiodensity (HU)	−0.056	0.757	0.434	−0.145	0.733	0.495	0.016	0.831	0.887	0.028	0.606	0.811	1.290	0.630	0.626
Model 4 (PET-CT date and vest T[a])															
BAT volume (mL)	0.054	0.413	**0.045**	0.056	0.209	0.512	0.082	0.374	0.164	0.041	0.339	0.329	1.304	0.249	0.195
BAT SUVmean	−3.674	0.404	0.052	−14.405	0.463	**0.009**	−8.768	0.477	**0.027**	−5.673	0.452	**0.044**	−128.953	0.326	0.062
BAT SUVpeak	−0.315	0.293	0.327	−1.607	0.316	0.092	−0.987	0.382	0.143	−0.666	0.379	0.160	−12.074	0.220	0.302
BAT radiodensity (HU)	−0.258	0.377	0.121	−0.567	0.290	0.258	−0.503	0.404	0.142	−0.279	0.377	0.237	−5.124	0.176	0.398

Unstandardized β, R^2, and *P* from multiple linear regressions. Model 1: adjustment for date when BAT assessment was performed. Model 2: model 1 adjustment plus HOMA of insulin resistance (HOMA-IR). Model 3: model 2 adjustments plus body surface area (BSA). Model 4: model 2 adjustments plus water temperature at the cooling vest (vest T[a]) prior to the PET-CT. Bold numbers indicate significant differences.

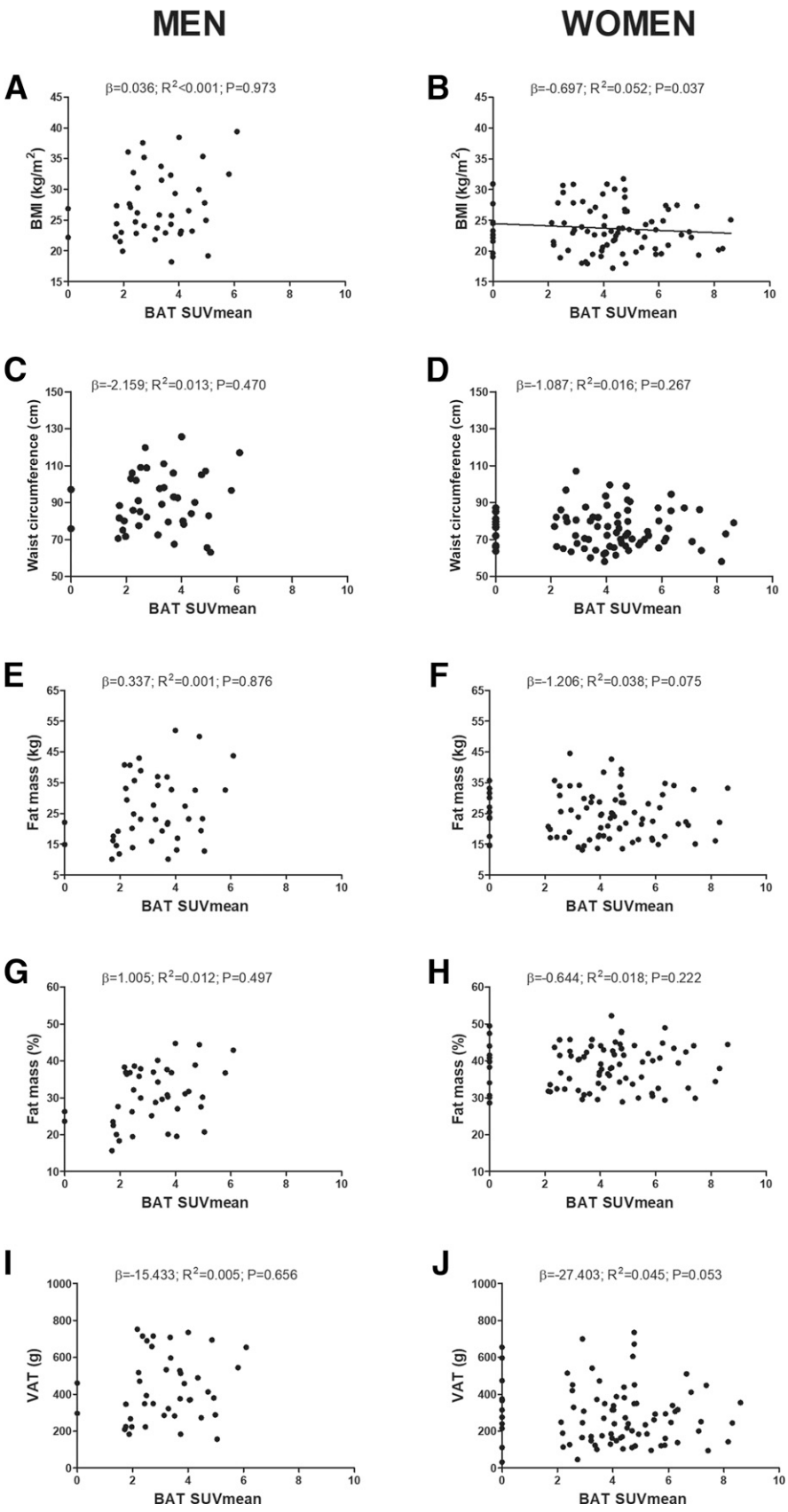

Figure 2—Associations between BAT mean ^{18}F-FDG uptake and whole-body adiposity. β = unstandardized simple regression coefficient; R^2 = standardized coefficient of determination.

Table 3—Associations between BAT and whole-body adiposity in women (*n* = 84)

	BMI (kg/m^2)			Waist circumference (cm)			Fat mass (kg)			Fat mass (%)			VAT (g)		
	β	R^2	*P*	β	R^2	*P*	β	R^2	*P*	β	R^2	*P*	β	R^2	*P*
Model 1 (PET-CT date)															
BAT volume (mL)	0.006	0.007	0.469	0.039	0.034	0.107	0.015	0.010	0.367	0.016	0.019	0.227	0.511	0.026	0.148
BAT SUVmean	−0.769	0.056	**0.031**	−1.146	0.016	0.273	−1.336	0.042	0.065	−0.797	0.026	0.157	−29.867	0.048	**0.048**
BAT SUVpeak	−0.140	0.029	0.125	−0.200	0.008	0.455	−0.209	0.016	0.258	−0.115	0.009	0.423	−5.140	0.022	0.182
BAT radiodensity (HU)	−0.103	0.089	**0.021**	−0.246	0.066	0.062	−0.238	0.110	**0.010**	−0.229	0.168	**0.001**	−4.797	0.109	**0.009**
Model 2 (PET-CT date and HOMA-IR)															
BAT volume (mL)	0.003	0.183	0.712	0.035	0.212	0.111	0.010	0.134	0.555	0.012	0.103	0.349	0.384	0.211	0.229
BAT SUVmean	−0.772	0.239	**0.018**	−0.978	0.197	0.303	−1.358	0.174	**0.047**	−0.821	0.119	0.131	−28.781	0.242	**0.033**
BAT SUVpeak	−0.154	0.217	0.064	−0.171	0.191	0.483	−0.238	0.151	0.172	−0.137	0.104	0.323	−5.636	0.223	0.102
BAT radiodensity (HU)	−0.079	0.223	0.064	−0.178	0.154	0.164	−0.200	0.191	**0.027**	−0.209	0.206	**0.003**	−3.692	0.206	**0.034**
Model 3 (PET-CT date and BSA)															
BAT volume (mL)	0.004	0.537	0.505	0.038	0.529	**0.025**	0.010	0.733	0.259	0.013	0.341	0.226	0.425	0.468	0.107
BAT SUVmean	−0.311	0.543	0.217	0.448	0.500	0.561	−0.234	0.730	0.549	−0.230	0.330	0.630	−12.100	0.457	0.297
BAT SUVpeak	−0.069	0.541	0.277	0.090	0.499	0.643	−0.039	0.729	0.690	−0.027	0.329	0.822	−2.377	0.454	0.414
BAT radiodensity (HU)	−0.061	0.630	**0.035**	−0.133	0.481	0.181	−0.141	0.791	**0.002**	−0.180	0.459	**0.002**	−3.400	0.467	**0.018**
Model 4 (PET-CT date and vest T[a])															
BAT volume (mL)	−0.006	0.112	0.621	0.030	0.080	0.364	−0.013	0.161	0.568	−0.010	0.095	0.583	−0.279	0.172	0.562
BAT SUVmean	−1.561	0.277	**0.004**	−2.223	0.109	0.149	−2.586	0.280	**0.012**	−1.871	0.193	**0.028**	−72.890	0.383	**0.001**
BAT SUVpeak	−0.279	0.210	**0.028**	−0.333	0.081	0.353	−0.411	0.214	0.087	−0.297	0.138	0.136	−13.231	0.301	**0.008**
BAT radiodensity (HU)	−0.163	0.285	**0.005**	−0.290	0.136	0.104	−0.297	0.305	**0.010**	−0.250	0.257	**0.007**	−5.313	0.340	**0.016**

Unstandardized β, R^2, and *P* from multiple linear regressions. Model 1: adjustment for date when BAT assessment was performed. Model 2: model 1 adjustment plus HOMA of insulin resistance (HOMA-IR). Model 3: model 2 adjustments plus body surface area (BSA). Model 4: model 2 adjustments plus the water temperature at the cooling vest (vest T[a]) prior to the PET-CT. Bold numbers indicate significant differences.

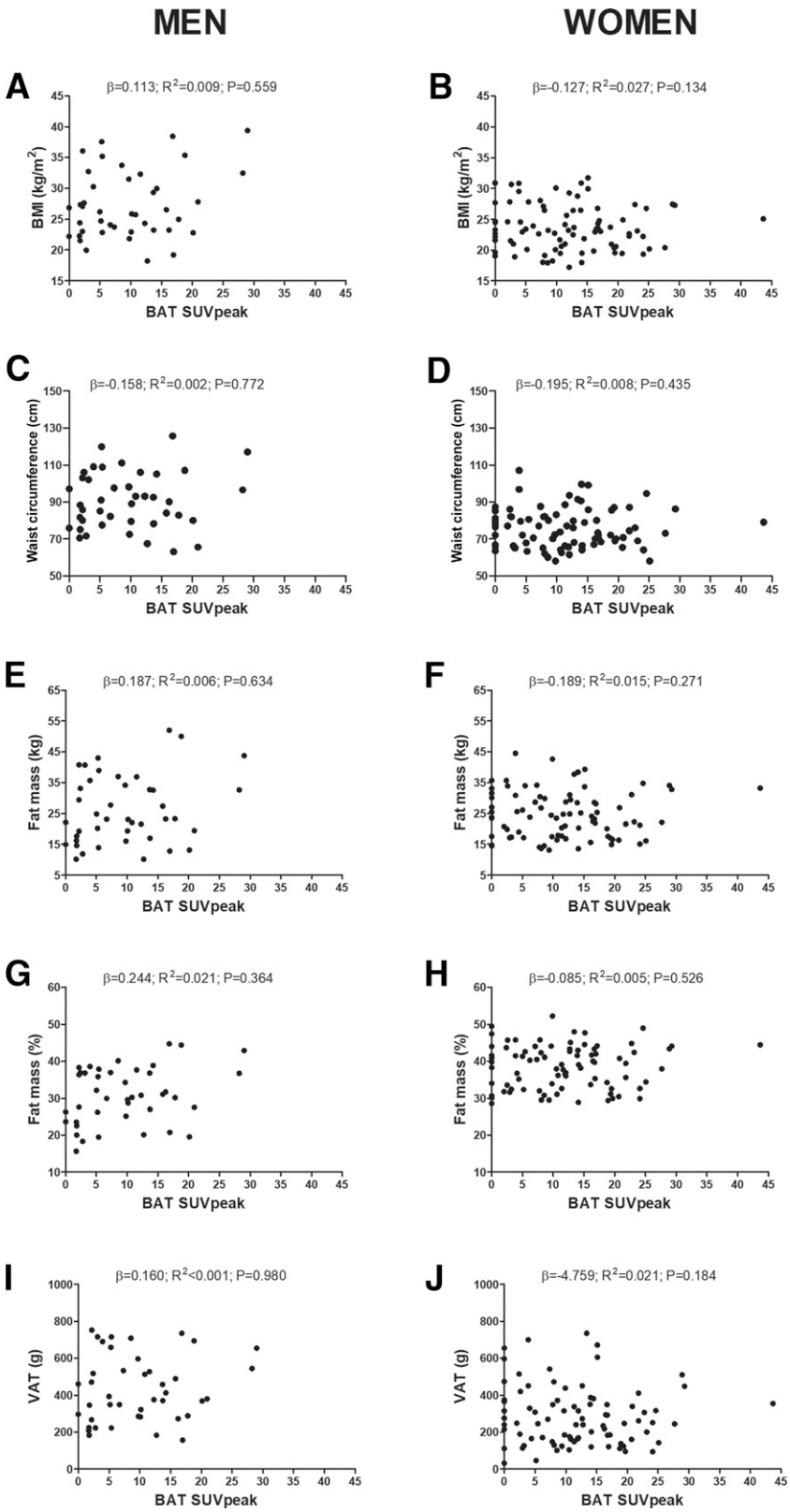

Figure 3—Associations between BAT peak ^{18}F-FDG uptake and whole-body adiposity. β = unstandardized simple regression coefficient; R^2 = standardized coefficient of determination.

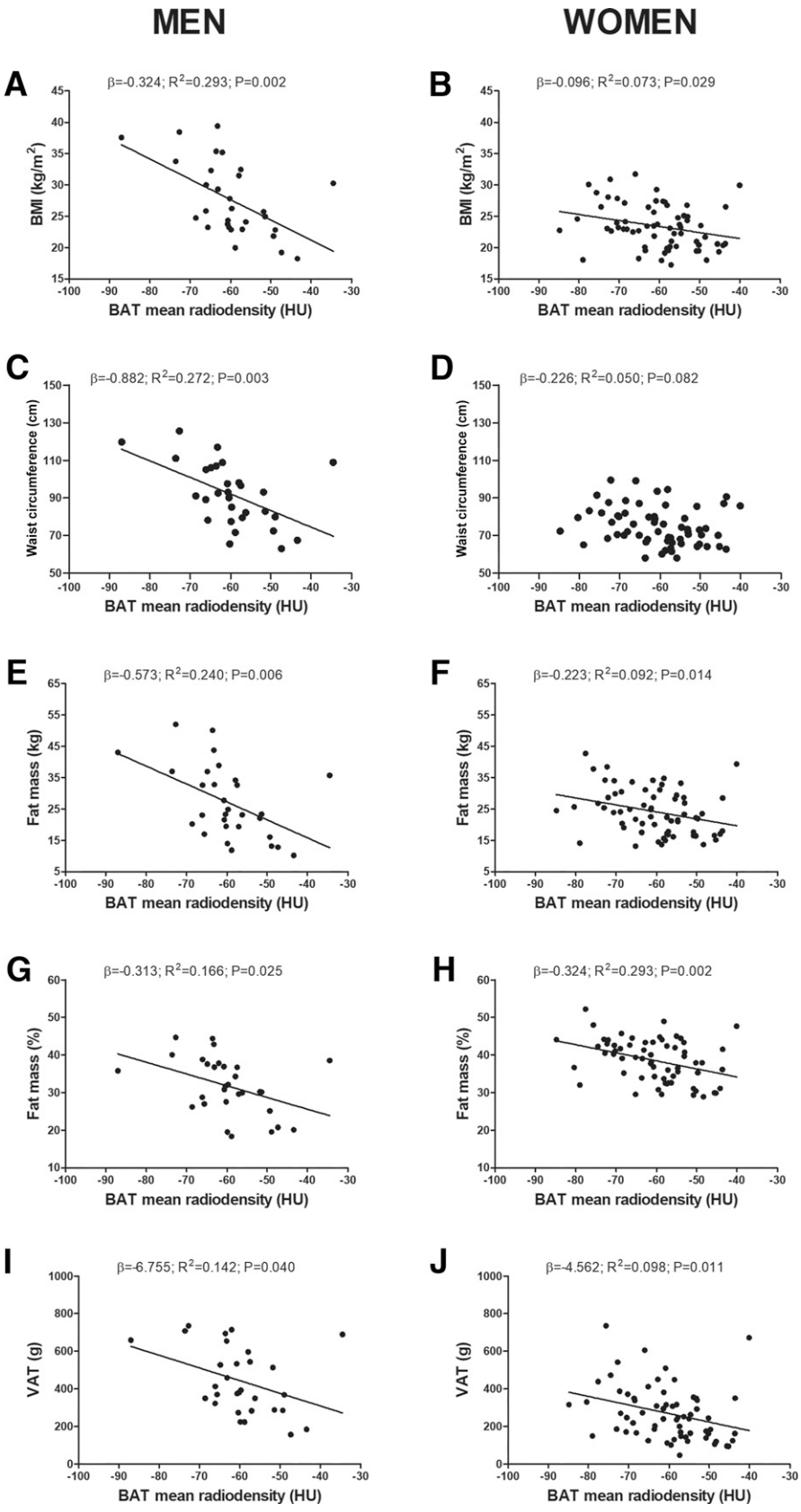

Figure 4—Associations between BAT mean radiodensity and whole-body adiposity. β = unstandardized simple regression coefficient; R^2 = standardized coefficient of determination.

women, adjustment for BAT mean radiodensity did not alter the lack of association between BAT volume and whole-body adiposity or the pattern of association between BAT SUVmean and whole-body adiposity.

BAT-related variables were not associated with body surface area (Supplementary Fig. 2) or with lean mass (Supplementary Fig. 3), except for BAT mean radiodensity in men. Supplementary Tables 1 and 2 show the associations between subcutaneous white adipose tissue/descending aorta ^{18}F-FDG uptake and whole-body adiposity.

DISCUSSION

This is the first study analyzing the association between BAT volume, assessed with an ^{18}F-FDG–PET-CT scan after individualized cold exposure and strict following of all current methodological recommendations (19), and whole-body adiposity in a large group (>100) of young individuals (likely preventing the ^{18}F-FDG from being biased by age-induced insulin resistance or BAT dysfunction). Our results show a weak positive relationship of BAT volume and fat content with whole-body adiposity in young men. In women, BAT volume is not associated with whole-body adiposity, despite our detection of positive associations between BAT fat content and whole-body adiposity. This refutes the widely held belief that BAT volume is inversely related to whole-body adiposity and suggests that it might even be the opposite, at least in young men.

The findings of this study contrast with the results of many previous human studies that reported negative associations between BAT volume and whole-body adiposity (5,6,8). However, it should be noted that the inverse relationship between BAT volume and whole-body adiposity has not been unanimously reported in previous literature and has only been found in approximately one-half of the studies published. Moreover, most of the studies reporting negative associations between BAT volume and whole-body adiposity were conducted with ^{18}F-FDG PET-CT methodologies that did not match the most recent recommendations for BAT assessment and, more importantly, did not involve individualized cold exposure before scanning (19). It is now accepted that performing ^{18}F-FDG PET-CT after individualized cold exposure is a reliable technique (35), considered the gold standard for in vivo human BAT volume quantification (18,19,36). Not individualizing cold exposure before the PET-CT scan likely results in the over- or underestimation of BAT volume, leading to different levels of bias with respect to participants of different body composition (20). This is indeed somehow corroborated by our own data, since the water temperature when shivering occurred in an incremental cooling protocol performed 48–72 h before the PET-CT was inversely associated with adiposity in women (Supplementary Fig. 4). It might indicate that, at least in women, the higher the adiposity, the coldest the water temperature needed for stimulating BAT (21).

Another relevant factor likely explaining the discrepancy between our data and data of many previous studies is the age of the participants. Indeed, it might be that the inverse association between BAT volume and adiposity shown by others is, at least in part, explained by the subjects' age. For example, it has repeatedly been reported that aging reduces the volume and activity of BAT (6,10,37–39), and aging is also commonly associated with a progressive increase in whole-body and central adiposity, as well as with the development of insulin resistance (40,41). Therefore, the smaller BAT volume observed by others in obese individuals may actually be real (i.e., not explained by biased measurements) in middle-aged adults but not in young adults. Age-related insulin resistance may also be accompanied by catecholamine resistance (42–45), and in fact, norepinephrine transporter availability in BAT was recently shown to be reduced in obesity (46).

Besides refuting the inverse association between BAT volume and whole-body adiposity, we observed an intriguing positive association between BAT volume and whole-body and central adiposity in men but not in women. This sexual dimorphism in the relation between BAT volume and whole-body adiposity has not been reported before. Unfortunately, we did not control the phase of the menstrual cycle in female participants, and therefore this might have biased the results and prevent us from observing a positive association in females. Future studies are needed to corroborate this sexual dimorphism in young individuals.

Other authors have previously reported positive associations between BAT volume and whole-body adiposity in small samples ($n < 25$) (47–49), although not using a prescan individualized cold exposure. Other studies have also documented positive associations between UCP1 expression levels and whole-body adiposity (50,51). In our study, the weak association between BAT volume and whole-body adiposity observed in men persisted after accounting for BAT mean radiodensity, suggesting that increases in BAT volume are not driven purely by lipid accumulation (i.e., brown adipocyte hypertrophy). Thus, it might be that, at least in young men, both BAT hypertrophy (i.e., increased brown adipocytes size driven by increased fat content) and hyperplasia (i.e., the recruitment of new brown adipocytes) occur in parallel with increases in body fat. This is very speculative at this point and needs further testing. However, it would be in agreement with murine studies conducted at room temperature (12–14). Nonetheless, even if it is assumed that positive energy balance induces BAT hypertrophy and hyperplasia in humans, its importance remains to be determined. A failure in white adipose tissue expansion seems to be the critical factor in determination of white adipocytes function and, consequently, when lipotoxicity occurs in other organs (52). Similarly, different grades of BAT hypertrophy and hyperplasia in response to positive energy balance might determine brown adipocytes' function, and it is tempting to speculate that those individuals presenting higher BAT adipogenesis capacity would better preserve BAT thermogenic function in response to overfeeding.

Nonetheless, it should be noted that having similar or even higher BAT volume does not necessarily imply that fatter individuals present similar or higher BAT thermogenic capacity. Indeed, the opposite is suggested by the observed inverse association between BAT mean radiodensity (a proxy of fat content [the lower the radiodensity, the higher the fat content], which has been shown to be related to impaired BAT thermogenesis [25]) and whole-body adiposity. Moreover, we observed a negative association between BAT SUVmean and whole-body adiposity in women. Despite ^{18}F-FDG presenting important limitations for assessing BAT thermogenic activity (10,31), this might indicate that fatter women indeed have lower BAT thermogenic capacity. In line with that, Jespersen et al. (53) recently showed that BAT from individuals with obesity has lower UCP1 expression and that many other genes involved in cellular respiratory pathways are downregulated. In summary, the lack of association, or even the positive relationship, between BAT volume and whole-body adiposity observed in our study is not necessarily in conflict with those studies reporting an impaired BAT thermogenic capacity in obese individuals.

The present results should be considered with some caution. First, the cross-sectional design of this study precludes the establishment of any causal relationship. Second, although significant, many of the detected associations had relatively low R^2 values, suggesting the need to confirm these results. Further, as mentioned before, we did not control the menstrual cycle of female participants, which might have biased their BAT assessment. Moreover, despite the need for individualizing the cold exposure before the PET-CT is widely recognized and shivering threshold is the most commonly used method, individualizing criteria other than shivering threshold might be more adequate. Finally, although the ^{18}F-FDG PET-CT is now considered the gold standard for assessing BAT volume in vivo, it has some limitations. For example, it does not allow the observation of small brown fat deposits within the white adipose tissue. Consequently, this study should be replicated if a new technology for BAT volume assessment in vivo becomes available.

In summary, the present work shows that BAT volume and fat content but not its glucose uptake capacity (i.e., SUVmean and SUVpeak)—as determined by ^{18}F-FDG PET-CT scanning following an individualized cold exposure—are positively associated with whole-body adiposity in young men, whereas BAT fat content but not volume is associated in young women. These results suggest that the widely held belief that BAT volume is reduced in obese individuals could be wrong—at least in young individuals. Moreover, these findings suggest that BAT expandability might take place in parallel to fat mass increase in young men.

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Author Contributions. G.S.-D., B.M.-T., A.G., and J.R.R. designed the study. G.S.-D., B.M.-T., F.M.A., J.M.L.-E., and J.R.R. conducted the experiments. G.S.-D., B.M.-T., F.M.A., and S.V. analyzed the data. G.S.-D. drafted the manuscript. G.S.-D. and J.R.R. are primarily responsible for the manuscript's final content. G.S.-D., B.M.-T., F.M.A., S.V., A.V.-P., A.G., J.M.L.-E., and J.R.R. discussed the results and approved the final version of the manuscript. G.S.-D. and J.R.R. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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