Brown adipose tissue volume and ^{18}F-fluorodeoxyglucose uptake are not associated with energy intake in young human adults

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ABSTRACT

Background: Several studies have explored the role of human brown adipose tissue (BAT) in energy expenditure. However, the link between BAT and appetite regulation needs to be more rigorously examined.

Objective: We aimed to investigate the associations of BAT volume and ^{18}F-fluordeoxyglucose (^{18}F-FDG) uptake after a personalized cold exposure with energy intake and appetite-related sensations in young healthy humans.

Methods: A total of 102 young adults (65 women; age: 22.08 ± 2.17 y; BMI: 25.05 ± 4.93 kg/m ^{2}) took part in this cross-sectional study. BAT volume, BAT ^{18}F-FDG uptake, and skeletal muscle ^{18}F-FDG uptake were assessed by means of static ^{18}F-FDG positron-emission tomography and computed tomography scans after a 2-h personalized exposure to cold. Energy intake was estimated via an objectively measured ad libitum meal and three nonconsecutive 24-h dietary recalls. Appetite-related sensations (i.e., hunger and fullness) were recorded by visual analog scales before and after a standardized breakfast (energy content = 50% of basal metabolic rate) and the ad libitum meal. Body composition was assessed by a whole-body DXA scan.

Results: BAT volume and ^{18}F-FDG uptake were not associated with quantified ad libitum energy intake (all $P > 0.088$), nor with habitual energy intake estimated from the 24-h dietary recalls (all $P > 0.683$). Lean mass was positively associated with both the energy intake from the ad libitum meal (β: 17.612, $R^2 = 0.213$; $P < 0.001$) and the habitual energy intake (β: 16.052, $R^2 = 0.123$; $P = 0.001$). Neither the interaction BAT volume × time elapsed after meal consumption nor that of BAT ^{18}F-FDG uptake × time elapsed after meal consumption had any significant influence on appetite-related sensations after breakfast or after meal consumption (all $P > 0.3$).

Conclusions: Neither BAT volume, nor BAT ^{18}F-FDG uptake after cold stimulation, are related to appetite regulation in young adults. These results suggest BAT plays no important role in the regulation of energy intake in humans. This trial was registered at clinicaltrials.gov as NCT02365129. *Am J Clin Nutr* 2020;111:329–339.

Keywords: brown fat, thermogenesis, appetite, energy balance, obesity

Introduction

Brown adipose tissue (BAT) is a highly thermogenic tissue whose main function is to produce heat to maintain mammals' body temperature (1). BAT plays a central role in the physiological regulation of body temperature in small mammals such as mice (2) and in newborn humans (1), but this tissue was long believed to be absent or metabolically irrelevant in adult humans. However, a decade ago, a number of independent research groups showed that BAT is in fact present and metabolically active in the latter (3–7). Initial observations suggested BAT volume and activity to be negatively associated with age, adiposity, and glycemia (4, 6), and positively associated with whole-body energy expenditure (3). Consequently, BAT has been proposed as a promising therapeutic target for the treatment of obesity and related comorbidities (8–11).

It has been hypothesized that BAT significantly contributes toward human energy expenditure (11–13). Indeed, initial studies showed positive associations between BAT and nonshivering thermogenesis in humans (3, 14–16). However, more recent studies examining human BAT function with more advanced

TABLE 1 Characteristics of the study participants[1]

		All		Men		Women
Age, y	102	22.08 ± 2.17	37	22.20 ± 2.19	65	22.01 ± 2.17
BMI, kg/m^2	102	25.05 ± 4.98	37	27.40 ± 5.77	65	23.72 ± 3.93
Lean mass, kg	96	41.74 ± 9.82	35	52.17 ± 7.19	61	35.75 ± 4.82
Fat mass, kg	96	25.28 ± 9.45	35	26.17 ± 11.68	61	24.77 ± 7.96
Fat mass, %	96	35.90 ± 7.76	35	30.95 ± 7.85	61	38.74 ± 6.15
LMI, kg/m^2	96	14.66 ± 2.49	35	17.12 ± 2.12	61	13.25 ± 1.33
FMI, kg/m^2	96	8.95 ± 3.16	35	8.58 ± 3.80	61	9.16 ± 2.74
VAT, g	96	343.69 ± 183.44	35	434.39 ± 187.07	61	291.65 ± 160.92
BAT volume, mL	102	72.44 ± 60.91	37	89.26 ± 71.15	65	62.87 ± 52.44
BAT metabolic activity	102	356.28 ± 350.04	37	389.75 ± 371.96	65	337.23 ± 338.42
BAT SUVmean	102	3.85 ± 1.91	37	3.50 ± 1.41	65	4.05 ± 2.13
BAT SUVpeak	102	11.62 ± 8.43	37	11.02 ± 7.77	65	11.96 ± 8.83
All muscles SUVpeak	102	0.80 ± 0.20	37	0.80 ± 0.17	65	0.80 ± 0.21
Deep muscles SUVpeak	102	1.05 ± 0.29	37	1.05 ± 0.27	65	1.05 ± 0.31
Cervical muscles SUVpeak	102	1.07 ± 0.31	37	1.05 ± 0.27	65	1.07 ± 0.33
Cold-sensitive muscles SUVpeak	102	0.90 ± 0.29	37	0.87 ± 0.25	65	0.92 ± 0.31
Descending aorta SUVpeak	102	1.54 ± 0.34	37	1.65 ± 0.37	65	1.48 ± 0.31
Ad libitum energy intake, kcal	102	880.71 ± 380.14	37	1136.10 ± 423.08	65	735.34 ± 261.13
Ad libitum meal:rate of eating, kcal/min	100	76.91 ± 39.72	37	105.12 ± 47.12	63	60.34 ± 21.83
Habitual energy intake, kcal/d	102	1856.41 ± 453.73	37	2025.99 ± 4732.29	65	1759.87 ± 415.68

[1] Values are *n* or mean ± SD. All muscles: paracervical, sternocleidomastoid, scalene, longus colli, trapezius, parathoracic, supraspinatus, subscapular, deltoid, pectoralis major, and triceps brachii. Deep muscles: paracervical, scalene, longus colli, parathoracic, and subscapular. Cervical muscles: paracervical, sternocleidomastoid, scalene, and longus colli. Cold-sensitive muscles: sternocleidomastoid, scalene, longus colli, and pectoralis major. BAT, brown adipose tissue; FMI, fat mass index; LMI, lean mass index; SUV, standardized uptake value; VAT, visceral adipose tissue.

methodologies suggest that the direct contribution of BAT to whole-body energy expenditure in response to both cold (17–20) and overfeeding (17, 21, 22) is negligible. Nonetheless, the technological limitations associated with defining the total amount of BAT in humans (13), and the possible indirect role of BAT in thermogenesis [i.e., via activating muscle thermogenesis through endocrine mechanisms (18, 22)], still preclude any definitive conclusions being drawn with regard to BAT's role in human energy expenditure (13). Nevertheless, energy expenditure is considered to be a major determinant of energy intake (23–27) and, therefore, the link between BAT and appetite regulation needs to be rigorously examined to fully understand the contribution of BAT toward human energy balance (28).

The endocrine mechanisms connecting BAT activity to energy intake regulation have been partially elucidated in murine models (29–31), and some of these mechanisms are conserved in humans (31). In mice, BAT recruitment after cold acclimation is coupled with an increase in energy intake, resulting in no change in these animals' body composition (27). Importantly, human BAT volume is positively associated with fasting- and cold-induced concentrations of peptides involved in appetite regulation (32). Taken together, these findings suggest a possible role for BAT in human appetite regulation (32). However, energy intake regulation is the result of complex interactions between biological and psychological processes (25), and there is a need to directly study the relation of BAT with energy intake and appetite-related sensations, rather than its physiological predictors.

This study examines the associations of BAT volume and ^{18}F-fluordeoxyglucose (^{18}F-FDG) uptake after a personalized cold exposure, with energy intake and appetite-related sensations in young healthy adults. The associations of skeletal muscle ^{18}F-FDG uptake after this cold exposure with energy intake and appetite-related sensations are also investigated.

Methods

Participants

A total of 102 subjects (65 women) took part in the study (**Table 1**). All were enrolled in the ACTIBATE study (33), a

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Supplemental Table 1 and Supplemental Figures 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Data described in the article, code book, and analytic code will be made available upon request pending application and approval.

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Abbreviations used: BAT, brown adipose tissue; PAL, physical activity level; PET-CT, positron-emission tomography and computed tomography; RMR, resting metabolic rate; ROI, region of interest; SUV, standardized uptake value; VAS, visual analog scale; 18F-FDG, 18F-fluorodeoxyglucose.

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randomized controlled trial (NCT02365129) aiming to study the effect of a 24-wk exercise training program on BAT volume and ^{18}F-FDG uptake after cold-exposure. The present study includes the baseline measurements of the ACTIBATE study, which were performed in September, October, and November of 2015 and 2016. All participants having valid data for both BAT and ad libitum energy intake (primary variables) were included in the analyses (see the flowchart in **Supplemental Figure 1**). Table 1 shows the descriptive characteristics of the participants. All participants were young (18–25 y old) and self-reported to be healthy, sedentary (<20 min moderate-to-vigorous-intensity physical activity on <3 d/wk), nonsmokers, not taking any medication, had a stable body weight over the preceding 3 months (<3 kg change), and not be regularly exposed to cold.

All participants gave their written informed consent to be included. The study protocol and design, which were approved by the Human Research Ethics Committee of the University of Granada (n°924) and the Servicio Andaluz de Salud, adhered to the Declaration of Helsinki (last revision 2013).

Procedures

The data reported in this study were collected over 3 subject-visits to our center, all within a period of 3 wk (33). Subjects were required to arrive by bus or by car (i.e., undertaking the minimum physical activity possible), after having slept as usual, and having refrained from stimulant beverages and any moderate physical activity in the previous 24 h, or any vigorous physical activity in the previous 48 h. Moreover, no physical activity was allowed on the testing days, and participants remained still (either lying or sitting) during the assessment.

Figure 1 shows the timeline of the procedures on experimental day 1. Subjects arrived at 08:15 am after an overnight fast (12 h), having consumed a standardized dinner (i.e., boiled rice, tomato sauce, and egg omelet) the evening before. After voiding, they were dressed in standardized clothes (clothing insulation value: 0.20) and entered a warm room (22.78 ± 0.94°C; 43.84 ± 6.74% humidity). Their postabsorptive resting metabolic rate (RMR) was then measured using a CCM Express or Ultima CardiO2 metabolic cart (Medical Graphics Cardiorespiratory Diagnostics) (34, 35) while reclining for 30 min on a bed, as per current methodological recommendations (36). The average of the most stable 5-min period was selected to be representative of the individuals' RMR (34). Immediately after the 30-min RMR measurement, all subjects were provided with a standardized liquid breakfast which was consumed within a maximum period of 10 min. After finishing, they rested quietly on the bed for another 3 h 30 min (they were allowed to use the toilet, and, in this case, asked to limit their physical activity to light walking). They were then moved into another room in which a whole-body DXA scan was performed. Finally, 4 h 15 min after consuming the breakfast, the subjects were provided with an ad libitum lunch to objectively quantify their energy intake.

On experimental day 2, the subjects' shivering threshold was assessed following a previously described method (37). Briefly, in fasted conditions for ≥6 h, the subjects entered a cool room (19.5–20°C) wearing the same standardized clothes as previously and a water-perfused cooling vest (Polar Products Inc.). The water temperature was then progressively reduced until shivering began (self-reported and externally observable). The water temperature at the onset of shivering was recorded as the shivering threshold (5.4 ± 2.2°C and 6.3 ± 2.2°C for men and women, respectively; range: 3.9–12.2°C for both men and women).

Experimental day 3 took place 48–72 h after the shivering threshold test (also after a fasting period of ≥6 h). Subjects underwent a static ^{18}F-FDG positron-emission tomography and computed tomography (PET-CT) scan using a Siemens Biograph 16 PET-CT machine to assess their BAT volume and BAT and skeletal muscle ^{18}F-FDG uptake after a personalized cold exposure based on their shivering threshold (37). Briefly, all subjects entered a cool room (19.5–20°C) with the water in the cooling vest set at 4°C above the individual shivering threshold. After the first hour of cold exposure, they received an injection of ∼185 MBq ^{18}F-FDG and the water temperature was increased by 1°C. After a further hour at this temperature, the PET-CT scan was performed. Self-reported menstrual cycle phase of the female participants was recorded at each visit.

PET-CT analysis

^{18}F-FDG-PET-CT scans were performed and analyzed in agreement with current methodological recommendations (38), following the protocol described elsewhere (37, 39). Images were analyzed using the Beth Israel plug-in for FIJI software (40). The PET-CT images were obtained from cervical vertebra 1 to thoracic vertebra 6 (approximately). For assessing BAT volume and ^{18}F-FDG uptake, voxels with a radiodensity between −190 and −10 Hounsfield units and an ^{18}F-FDG uptake greater than the individualized standardized uptake value (SUV) threshold of 1.2/(lean body mass/body mass) were taken into account (38). BAT volume, BAT metabolic activity (BAT volume × BAT SUVmean), BAT SUVmean, and BAT SUVpeak were all calculated (38). The SUVpeak values of the paracervical, sternocleidomastoid, scalene, longus colli, trapezius, parathoracic, supraspinatus, subscapular, deltoid, pectoralis major, and triceps brachii muscles were also determined from 1-slice regions of interest (ROIs), and the values obtained were averaged for all muscles on either side of the body. These muscles were also grouped as deep (paracervical, scalene, longus colli, parathoracic, subscapular), cervical (paracervical, sternocleidomastoid, scalene, longus colli), and cold-sensitive (sternocleidomastoid, scalene, longus colli, pectoralis major) muscles (20). A ROI (1 slice) was also defined in the descending aorta to be used as reference tissue.

Standardized breakfast and ad libitum meal

For the standardized liquid breakfast, subjects received a smoothie (Tdiet® energy, Vegenat S.A.) at 4°C. This product has an energy density of 1.6 kcal/mL; carbohydrates made up 47% of the meal's total energy content, fat 35%, proteins 15%, and fiber 3% (http://vegenatnutricion.es/index.php?r=nutricion/producto&id=10). This breakfast was eaten while seated on the same bed in which the RMR was measured. Subjects consumed a quantity equivalent to 50% of their RMR. They were free to drink water during their breakfast, and were allowed to do so over the next 2 h 30 min.

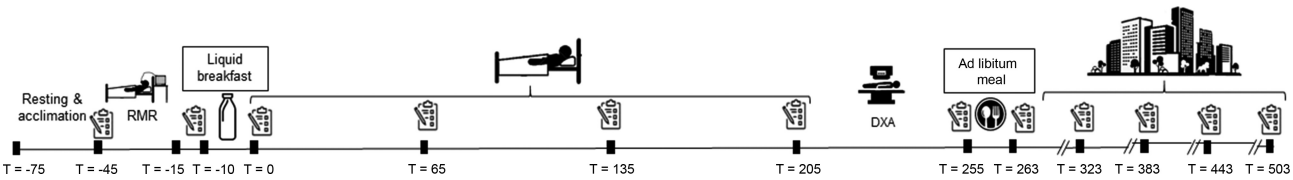

FIGURE 1 Day 1 experimental procedure. Numbers indicate time in minutes. Pen and paper symbols represent visual analogue scales. RMR, resting metabolic rate.

For the ad libitum meal, subjects received a plate of spaghetti with tomato sauce, pork tenderloin, and virgin olive oil, prepared in the research center immediately before its being served. Carbohydrates provided 45.5% of the meal's total energy content, fat 38.5%, and proteins 16%; the energy density was 1.54 kcal/g. The total amount offered was 1500 g for men and 1000 g for women. The subjects were ushered into a quiet, dimly lit room where they found their plate together with a glass of water (450 mL); they were then left alone without external distractions while eating. The participants were instructed to eat until comfortably satisfied (41). Food intake was measured as the difference in the weight of the plate plus food before and after the meal. The energy intake was subsequently calculated.

Appetite sensations

On experimental day 1, subjects' appetite-related sensations (i.e., hunger and fullness) were recorded using visual analog scales (VASs) at several times. All were asked to provide this information before the RMR assessment, immediately before the standardized breakfast, immediately after it, 65, 135, and 205 min after it, immediately before the ad libitum meal, immediately after it, and 1, 2, 3, and 4 h after it (Figure 1). Every VAS assessment was completed in the laboratory, except for those after the ad libitum meal, which were completed in free-living conditions (subjects were instructed not to eat for the 4 h after consuming their ad libitum meal). The original version of the VAS has been validated as a means of analyzing appetite-related sensations after a meal (42), as has the Spanish version actually employed (43).

Body composition

Height and weight were measured before the RMR assessment, without shoes and with light clothing, with a model 799 Seca scale and stadiometer. Lean mass, fat mass, fat mass percentage, and visceral adipose tissue mass were assessed by a whole-body DXA scan (Discovery Wi, Hologic, Inc.). Body, lean, and fat mass indexes were calculated as kg/m^2.

Dietary recalls

Habitual energy intake was estimated using 3 nonconsecutive 24-h dietary recalls (1 of them for a nonworking day). For this, subjects were interviewed by dieticians, recording all the foods and drinks consumed on the previous day. A book with pictures of different food servings and sizes was used to help subjects estimate the amount of food consumed. The nutritional composition of the diet was obtained using EvalFINUT software (FINUT; http://www.finut.org/evalfinut/). The habitual consumption of water and salt was not recorded. To avoid bias, the subjects were not informed when their diets were going to be recorded. Further, they were classified as plausible or nonplausible reporters using the Goldberg cutoff method (44). For this, habitual physical activity was assessed using an ActiGraph GT3X+ wrist-worn accelerometer for 7 consecutive days (24 h/d) (45). Subjects were given detailed information on how to wear the accelerometer. The raw data were processed and analyzed as explained elsewhere (45), and the mean Euclidean Norm Minus One (mG) during the time spent awake was obtained as an overall indicator of physical activity. Only data for those subjects who wore the accelerometer for $\geq$10 h/d and $\geq$4 h/night for $\geq$4 d were included in analyses (46). The most active subject was arbitrarily assigned a physical activity level (PAL) of 1.8, whereas the least active was assigned a PAL of 1.4. For each of the remaining subjects a proportional value between 1.4 and 1.8 was calculated.

Statistical analyses

Descriptive statistics are presented as mean $\pm$ SD, unless otherwise stated. Simple linear regression was used to test the associations of BAT volume, BAT and skeletal muscle ^{18}F-FDG uptake, and body composition variables with energy intake. Sex interaction was tested by multiple linear regression models entering the variable of interest, the sex, and the product of variable of interest $\times$ sex.

BAT-related variables have been reported to vary across the year (47, 48), with sex (4, 37), and with body composition (4, 49). Therefore, multiple linear regression models were used to test the associations of BAT volume and BAT and skeletal muscle ^{18}F-FDG uptake with energy intake after adjusting for the date when the PET-CT scan was performed (Model 1), as per Model 1 plus sex (Model 2), and as per Model 2 plus fat mass and lean mass (Model 3). One-factor repeated-measures ANCOVA was used to study the relation of BAT volume and BAT and skeletal muscle ^{18}F-FDG uptake with appetite-related sensations over time elapsed since meal consumption (standardized breakfast and ad libitum meal). Subjects were divided into BAT volume and BAT and skeletal muscle ^{18}F-FDG uptake tertiles, and appetite-related sensations over time elapsed since meal consumption for the highest and the lowest tertiles were compared by 2-factor (tertile and time elapsed since meal consumption) ANOVA.

All analyses were conducted using the Statistical Package for the Social Sciences version 21.0 (IBM SPSS Statistics, IBM Corporation). Significance was set at $P < 0.05$.

Results

No significant association was seen linking BAT volume and BAT and skeletal muscle ^{18}F-FDG uptake with the energy intake from the objectively measured ad libitum meal (all $P > 0.088$; **Figure 2**); this result persisted after adjusting for the date when the PET-CT was performed, and for sex, lean mass, and fat mass (**Table 2**). Nor was any significant association found linking BAT volume and BAT and skeletal muscle ^{18}F-FDG uptake with habitual energy intake as estimated from the subjects' dietary recalls (all $P < 0.683$; **Supplemental Figure 2**). This finding persisted after adjustment for the date when the PET-CT was performed, for sex, lean mass, and fat mass, and when excluding nonplausible reporters (17 under-reporters, data not shown). The results also persisted when excluding participants with no PET-detectable BAT ($n = 20$, 19.6%). Moreover, we did not detect any significant sex interaction and the results persisted after adjusting for menstrual cycle phase (data not shown).

Figure 3 shows the associations of lean and fat mass with the energy intake from the ad libitum meal. Lean mass was positively associated with the energy intake from the ad libitum meal (β: 17.612, $R^2 = 0.213$; $P < 0.001$) and from the 24-h dietary recalls (β: 16.052, $R^2 = 0.123$; $P = 0.001$; **Supplemental Figure 3**). In contrast, fat mass was neither associated with the energy intake from the ad libitum meal (β: 2.710, $R^2 = 0.005$; $P = 0.508$), nor with the energy intake from the 24-h dietary recalls (β: −0.081, $R^2 < 0.001$; $P = 0.987$; Supplemental Figure 3).

The interactions BAT volume × time elapsed after meal consumption and BAT ^{18}F-FDG uptake × time elapsed after meal consumption had no significant influence on appetite-related sensations after breakfast or after the ad libitum meal ($P > 0.3$ in both cases; **Figure 4**, **Supplemental Table 1**). Neither did the interactions all skeletal muscle ^{18}F-FDG uptake × time elapsed after meal consumption, deep skeletal muscle ^{18}F-FDG uptake × time elapsed after meal consumption, and cervical skeletal muscle ^{18}F-FDG uptake × time elapsed after meal consumption have any significant influence on appetite-related sensations after breakfast or after the ad libitum meal (all $P > 0.28$; **Figure 5**, Supplemental Table 1). However, the interaction cold-sensitive skeletal muscle ^{18}F-FDG uptake × time elapsed after meal consumption did have a marginally significant influence on these appetite-related sensations ($P = 0.06$; Supplemental Table 1). The latter relation was corroborated when comparing, by 2-factor ANOVA, the highest and the lowest cold-sensitive skeletal muscle ^{18}F-FDG uptake tertiles over time after meal consumption ($P = 0.034$; Figure 5). Moreover, the AUC for fullness was positively correlated with all ($r = 0.281$, $P = 0.011$), deep ($r = 0.261$, $P = 0.018$), cervical ($r = 0.281$, $P = 0.011$), and cold-sensitive ($r = 0.257$, $P = 0.020$) muscle ^{18}F-FDG uptakes (data not shown).

Finally, a significant association was observed between the energy intake from the ad libitum meal and the habitual energy intake (β: 0.229, $R^2 = 0.083$; $P = 0.001$; **Supplemental Figure 4**), a result that persisted after excluding the nonplausible reporters (i.e., 17 participants). In addition, we tested whether fasted and pre–ad libitum meal sensations of hungry and fullness were associated with the ad libitum energy intake, finding no association (all $P > 0.06$).

Discussion

The present results show that BAT volume and ^{18}F-FDG uptake are not associated with energy intake assessed by an ad libitum meal and by 24-h dietary recalls. It is noteworthy that both methods for assessing energy intake were sensitive enough to detect an already well-established positive association between lean mass and energy intake (50). No role for BAT was detected with respect to the regulation of appetite-related sensations either before or after the 2 meals the subjects ate. This suggests that BAT has little to do with human appetite regulation. Similarly, skeletal muscle ^{18}F-FDG uptake was not associated with energy intake, yet the results suggest that some skeletal muscle groups are associated with the regulation of appetite-related sensations after a meal. More work is needed to confirm this.

The present results reflect the now-recognized robust positive association between fat-free mass and energy intake (23), and the lack of such an association between fat mass and energy intake (50). In this context, BAT could be considered a third component of this body composition model. Certainly, it shares histological characteristics with white adipose tissue (51), as well as an ontogenic origin and thermogenic role with skeletal muscle (52). However, no association was observed between BAT volume or ^{18}F-FDG uptake and energy intake, assessed via either the ad libitum meal or the habitual dietary intake. Fat-free mass is a strong predictor of not only energy intake, but also energy expenditure (53). Therefore, the lack of association between BAT volume or ^{18}F-FDG uptake and energy intake is consistent with previous studies showing that the BAT energy expenditure elicited by mild cold or meal ingestion only accounts for 10–15 kcal/d even when continuously and maximally activated (17, 18, 54). Paradoxically, in these earlier studies, BAT showed a higher energy expenditure, a higher blood flow increase, and a higher fractional substrate uptake per unit volume than any other tissue measured (16, 18, 54). However, the relatively small volume of BAT thought to be present in human adults (39) explains why it accounts for <1% of whole-body energy expenditure (18, 54). Likewise, a scant volume in adults would probably explain why BAT appeared not to be associated with energy intake or appetite-related sensations after the standardized breakfast or ad libitum meal. Nonetheless, new technologies for in vivo BAT quantification are needed to confirm the small volume of BAT observed in previous human studies (13). Moreover, it cannot be ruled out that human BAT has endocrine connections with the appetite regulation system (31, 32), as occurs in mice (29–31). For instance, a recent study has shown that, in these animals, the gut hormone secretin activates BAT thermogenesis, which in turn regulates postprandial thermogenesis and satiety (31). Importantly, serum secretin is also increased in humans under postprandial conditions, and secretin infusions increase BAT ^{18}F-FDG uptake (31). Further studies are needed if we are to fully understand the role of human BAT in appetite regulation.

In the present work, energy intake and appetite-related sensations were measured in a warm environment. It cannot be ruled out, therefore, that BAT is associated with energy intake or appetite sensations in cold environments. Indeed, an earlier study in humans suggested that a cold-induced increase in energy expenditure might not be compensated for by energy intake or appetite-related sensations in the following hours (55).

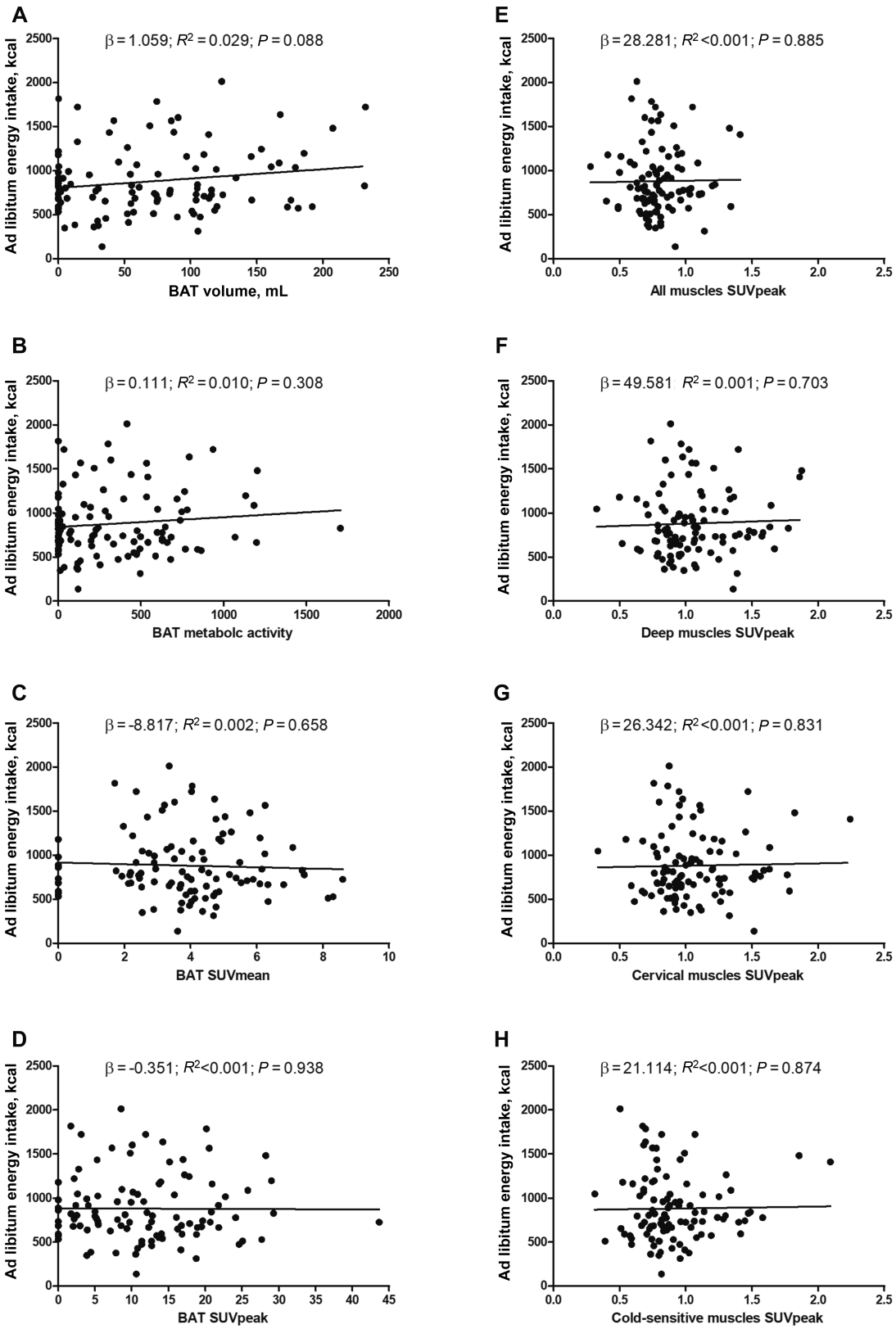

FIGURE 2 Associations of BAT volume and BAT and skeletal muscle ^{18}F-fluorodeoxyglucose uptake with ad libitum energy intake. (A) BAT volume, (B) BAT metabolic activity, (C) BAT SUVmean, (D) BAT SUVpeak, (E) all muscles SUVpeak, (F) deep muscles SUVpeak, (G) cervical muscles SUVpeak, (H) cold-sensitive muscles SUVpeak. Unstandardized β, R^2, and P values are from simple linear regression analyses ($n = 102$). All muscles: paracervical, sternocleidomastoid, scalene, longus colli, trapezius, parathoracic, supraspinatus, subscapular, deltoid, pectoralis major, and triceps brachii. Deep muscles: paracervical, scalene, longus colli, parathoracic, and subscapular. Cervical muscles: paracervical, sternocleidomastoid, scalene, and longus colli. Cold-sensitive muscles: sternocleidomastoid, scalene, longus colli, and pectoralis major. BAT, brown adipose tissue; SUV, standardized uptake value.

TABLE 2 Associations of BAT volume and BAT and skeletal muscle ^{18}F-fluorodeoxyglucose uptake with ad libitum energy intake[1]

	Model 1			Model 2			Model 3		
	β	R^2	P	β	R^2	P	β	R^2	P
BAT volume	1.02	0.029	0.13	0.40	0.264	0.50	0.49	0.283	0.43
BAT metabolic activity	0.09	0.012	0.42	0.07	0.264	0.50	0.09	0.284	0.41
BAT SUVmean	−15.29	0.011	0.47	3.76	0.261	0.84	9.67	0.281	0.61
BAT SUVpeak	−1.76	0.007	0.71	0.50	0.260	0.91	1.73	0.280	0.69
All muscles SUVpeak	−6.56	0.006	0.97	19.39	0.260	0.91	−41.47	0.279	0.82
Deep muscles SUVpeak	26.27	0.006	0.85	36.08	0.261	0.76	12.80	0.279	0.91
Cervical muscles SUVpeak	0.71	0.006	1.00	46.26	0.262	0.68	25.90	0.279	0.82
Cold-sensitive muscles SUVpeak	2.02	0.006	0.99	72.13	0.263	0.54	53.48	0.280	0.66

[1]Unstandardized β, R^2, and P values are from multiple linear regression analyses ($n = 102$). Model 1: adjusted for the date when the PET/CT scan was performed. Model 2: adjusted as for Model 1 plus sex. Model 3: adjusted as for Model 2 plus fat mass and lean mass. All muscles: paracervical, sternocleidomastoid, scalene, longus colli, trapezius, parathoracic, supraspinatus, subscapular, deltoid, pectoralis major, and triceps brachii. Deep muscles: paracervical, scalene, longus colli, parathoracic, and subscapular. Cervical muscles: paracervical, sternocleidomastoid, scalene, and longus colli. Cold-sensitive muscles: sternocleidomastoid, scalene, longus colli, and pectoralis major. BAT, brown adipose tissue; SUV, standardized uptake value.

In animal models, however, cold acclimation induces similar increases in energy expenditure and energy intake (27). These findings suggest that cold exposure increases energy intake in the long term, thereby compensating for the increase in energy expenditure, although maybe not in the very short term. Whether this applies to humans remains to be seen.

Muscle thermogenesis seems to be the main contributor to cold-induced thermogenesis, even in mild cold exposures where shivering is minimized (20, 54). An anatomical dimorphism has been reported in several studies, showing that some muscle groups are especially active in response to cold (16, 20, 54). Whether this augmented metabolism is mediated by shivering (20) or nonshivering (56) mechanisms is still unknown. In addition, it has been suggested that this muscle metabolism may be partly mediated by endocrine signals released from the BAT (54, 57). Interestingly, the interaction cold-sensitive skeletal muscle ^{18}F-FDG uptake × time elapsed after meal consumption showed a trend toward having a significant effect on appetite-related sensations after the standardized breakfast and ad libitum meal. Moreover, an association between the AUC for fullness sensations and skeletal muscle ^{18}F-FDG uptake was detected. This raises the possibility that skeletal muscle, rather than BAT, could be the tissue responsible for both energy expenditure and energy intake stimulation in response to cold. Similarly, it seems reasonable that cold exposure is a useful physiological state for studying the mechanistic connections that might explain the link between fat-free mass and energy intake (50).

The present results should be understood with caution because this study is not free from limitations. The study population was made up of young healthy adults, hence it remains unknown whether these findings hold true for older or unhealthy individuals. Although we checked that results remained consistent after adjusting for menstrual cycle phase, we have no record of oral contraceptive use, and therefore could not check its influence on the results. On the other hand, energy intake estimations by dietary recalls can suffer from important bias and imprecision (58), and a single ad libitum meal is less accurate for estimating daily energy intake than a 24-h ad libitum procedure. However, both the ad libitum meal and the dietary recall records were sensitive enough to detect the already known relation between lean mass and energy intake, and they were associated with one another (Supplemental Figure 4). Moreover, both the ad

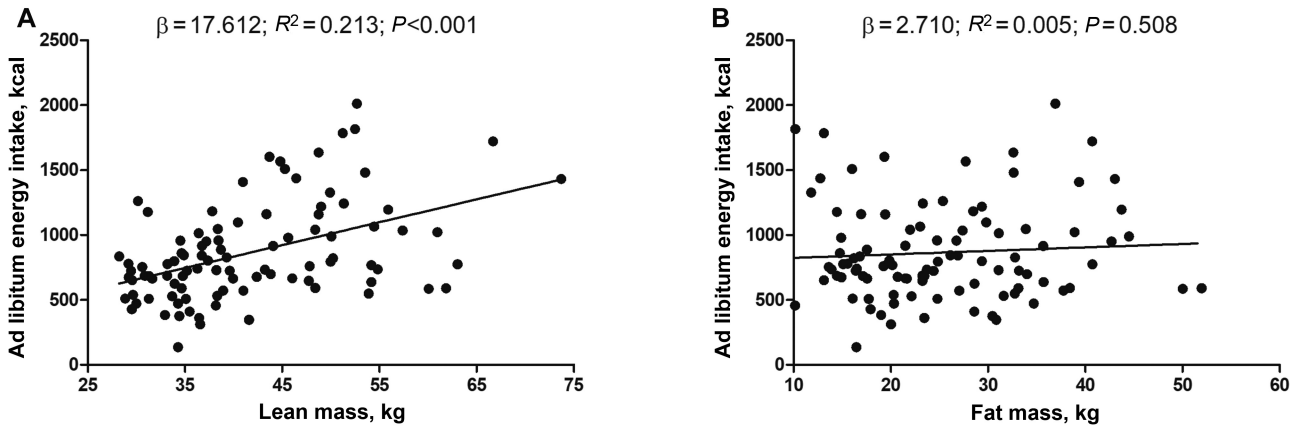

FIGURE 3 Associations between body composition and ad libitum energy intake. (A) Lean mass, (B) fat mass. Unstandardized β, R^2, and P values are from simple linear regression analyses ($n = 96$).

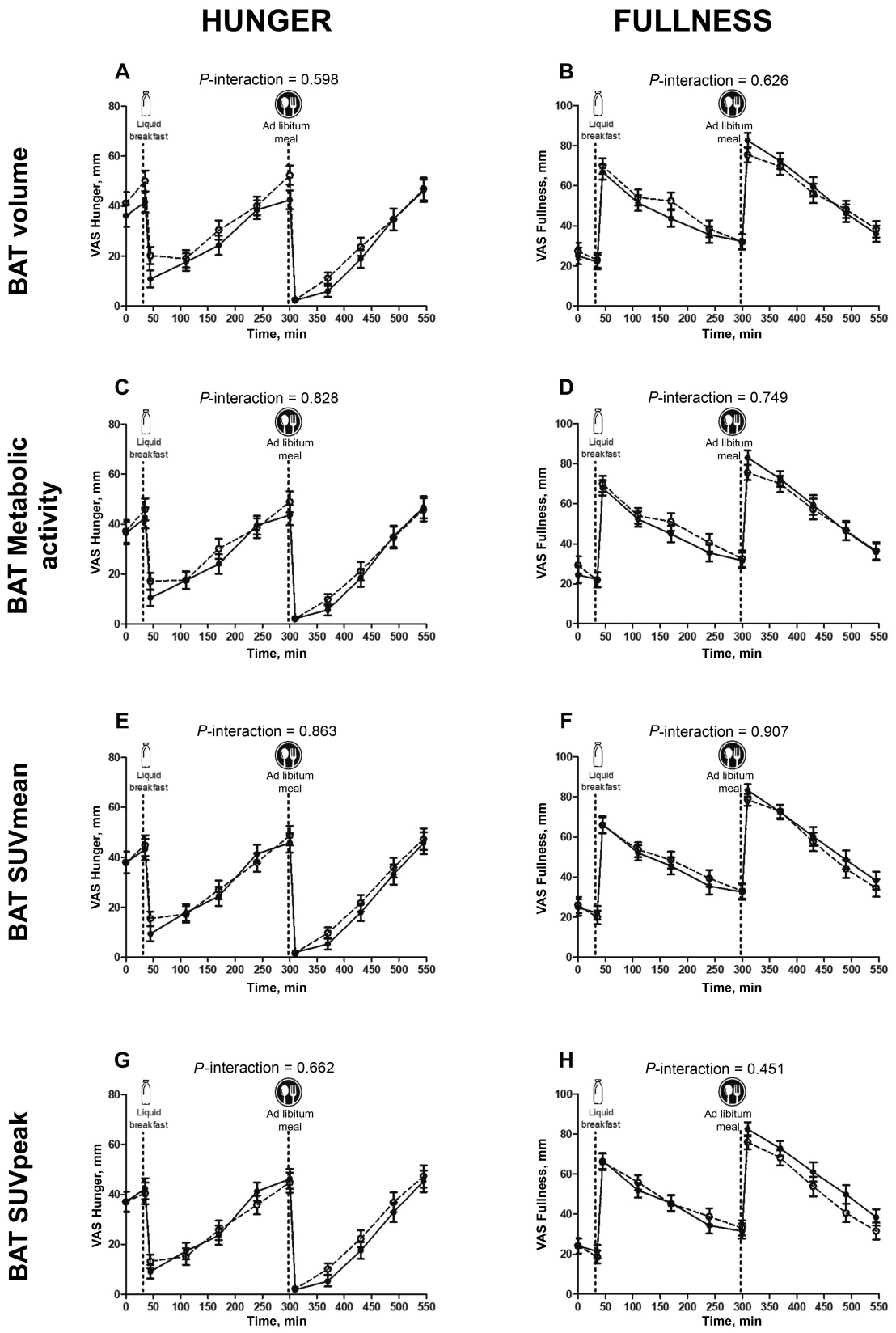

FIGURE 4 Changes in hunger (A–D) and fullness (E–H) sensations over time elapsed since meal consumption, with respect to BAT variables. (A, B) BAT volume, (C, D) BAT metabolic activity, (E, F) BAT SUVmean, (G, H) BAT SUVpeak. Dashed lines represent the high-BAT group ($n = 28$) and solid lines represent the low-BAT group ($n = 26$). The "high" and "low" BAT groupings refer to the highest and lowest tertiles for the BAT variables. The *P* values are for 2-factor (tertile and time elapsed since meal consumption) mixed ANOVAs. BAT, brown adipose tissue; SUV, standardized uptake value; VAS, visual analog scale.

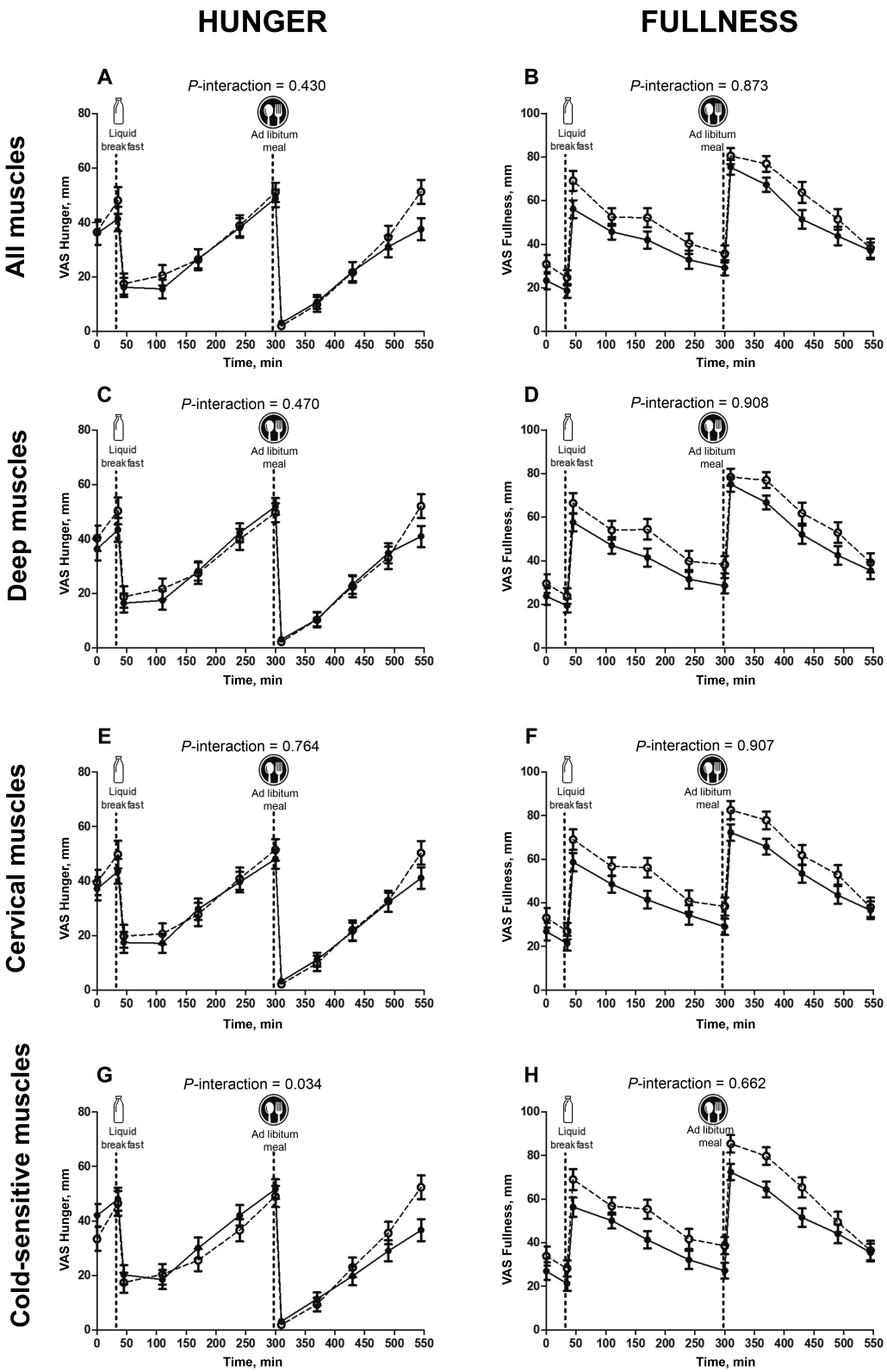

FIGURE 5 Changes in hunger (A–D) and fullness (E–H) sensations compared with skeletal muscle ^{18}F-FDG uptake. (A, B) All muscles, (C, D) deep muscles, (E, F) cervical muscles, (G, H) cold-sensitive muscles. Dashed lines represent high–muscle ^{18}F-FDG group ($n = 24$), and solid lines represent low–muscle ^{18}F-FDG group ($n = 29$). The high and low skeletal muscle ^{18}F-FDG uptake groups are the highest and lowest ^{18}F-FDG uptake tertiles. The *P* values are for 2-factor (tertile and time elapsed since meal consumption) mixed ANOVAs. All muscles: paracervical, sternocleidomastoid, scalene, longus colli, trapezius, parathoracic, supraspinatus, subscapular, deltoid, pectoralis major, and triceps brachii. Deep muscles: paracervical, scalene, longus colli, parathoracic, and subscapular. Cervical muscles: paracervical, sternocleidomastoid, scalene, and longus colli. Cold-sensitive muscles: sternocleidomastoid, scalene, longus colli, and pectoralis major. VAS, visual analog scale; ^{18}F-FDG, ^{18}F-fluordeoxyglucose.

libitum meal and VASs are reliable, valid methods for assessing appetite regulation (41, 42). It should be noted that although ^{18}F-FDG is currently the best available method for assessing human BAT volume, it suffers serious limitations as a method for assessing BAT metabolic activity (13). Finally, it would have been desirable to have known the concentrations of several appetite-related peptides and hormones under fasting and postprandial conditions.

In summary, BAT volume and ^{18}F-FDG uptake after a personalized cold exposure appear not to be associated with energy intake or meal-induced appetite-related sensations in young, healthy adults. These results suggest that BAT plays no important role in the regulation of energy intake in humans.

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The authors' responsibilities were as follows—GS-D, GF, CG, IL, AG, JEB, and JRR: designed the research; GS-D, FMA, BM-T, JML-E, and JRR: conducted the experiments; GS-D, FMA, and BM-T: analyzed the data; GS-D, JEB, and JRR: wrote the manuscript; GS-D and JRR: were primarily responsible for the final content; and all authors: discussed the results and read and approved the final manuscript. The authors report no conflicts of interest.

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