Evidence of Brown Fat Activity in Constitutional Leanness

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Background: Brown adipose tissue (BAT) was considered essentially nonexistent in adults until recent evidence obtained using 18-fluorodeoxyglucose (18-FDG) positron emission tomography/ computed tomography. It seems to play a role in whole body metabolism, but it has not been evaluated in underweight conditions, such as in young females with constitutional leanness (CL) or anorexia nervosa (AN).

Subjects and Methods: Thirty-eight subjects were evaluated from October 2011 to March 2012 : 7 CL (21.7 ± 3.6 y, body mass index [BMI] 16.2 ± 1.0 kg/m^2), 7 AN (23.4 ± 4.5 y, BMI 15.5 ± 0.8), 3 of the 7 AN after stable refeeding (R-AN, 21.3 ± 1.5 y, BMI 18.8 ± 1.1), and 24 normal weight (NW) women (25.6 ± 3.9 y, BMI 22.2 ± 1.5). Fasting resting metabolic rate and respiratory quotient were measured by indirect calorimetry, body composition by bioimpedentiometry (only in CL, AN, and refed AN), and BAT activity by 18-FDG positron emission tomography/computed tomography scan, all in standardized conditions.

Results: All CL (100%), none of the AN and refed AN (0%), and 3 of the 24 NW (12%) subjects showed FDG uptake. Average FDG maximum standardized uptake value was $11.4 + 6.7$ g/mL in CL and 5.5 ± 1.2 g/mL (min 3.7, max 8.3) in the 3 NW subjects. In CL, the maximum standardized uptake value was directly correlated to resting metabolic rate, corrected for fat-free mass, and inversely correlated with respiratory quotient.

Conclusion: BAT activity has been shown in CL in resting thermoneutral conditions and may exert a role against adipose tissue deposition. (*J Clin Endocrinol Metab* 98: 0000–0000, 2013)

Constitutional leanness (CL) represents a peculiar physiological condition whereby the body is resistant to fat storage, even in overfeeding conditions. Therefore, it may be a useful model for studying biological factors regulating energy balance, in particular energy expenditure. This topic currently is of great interest, given the epidemic of obesity, overall reduced physical exercise/exposure to cold, and continuous search for antiobesity drugs able to affect energy balance or expenditure, and so forth. To the best of our knowledge, only a few studies have been carried out on energy regulation in CL. In a previous study (1), we evaluated resting metabolic rate (RMR), nonexercise activity thermogenesis (NEAT), and respiratory quotient (RQ), as index of preferential substrate oxidation, in CL, anorexia nervosa (AN), and obesity. NEAT is a facultative or regulatory component of adaptive thermogenesis with a specific role in energy balance regulation (ie, the prevention of long-term fat accumulation) (2); it is

Abbreviations: AN, anorexia nervosa; BAT, brown adipose tissue; BIA, bioimpedentiometry; BMI, body mass index; CL, constitutional leanness; CT, computed tomography; 18F-FDG, 18-fluorodeoxyglucose; FFM, fat-free mass; NEAT, nonexercise activity thermogenesis; NW, normal weight; PET, positron emission tomography; REE, resting energy expenditure; RMR, resting metabolic rate; R-AN, after stable refeeding; RQ, respiratory quotient; SUVmax, maximum standardized uptake value.

part of adaptive thermogenesis and is supposedly controlled by the autonomic nervous system, with sympathetic activity playing a stimulatory role. NEAT includes all minor activities other than volitional physical activity, including repeated muscle contractions (or fidgeting). The role of NEAT in overall energy balance is becoming significant because of the increasing lack of physical activity in affluent societies. In this study (1), we reported significant increased NEAT in CL but not in the other groups studied: AN and obese patients and control normal weight (NW) subjects. In the CL group, RQ also showed a negative correlation with NEAT (evaluated as fidgeting), suggesting a potential correlation between NEAT and fat oxidation.

In recent years, the combination of 18-fluorodeoxyglucose (18F-FDG) positron emission tomography (PET) with computed tomography (CT) has given clear evidence of brown adipose tissue (BAT) activity in adult humans, and several specific metabolic roles have been identified (3–10). A well-documented action of BAT in regulatory (cold or diet-induced, etc.) thermogenesis is also present. Furthermore, BAT activity is regulated by the autonomic nervous system and, among other things, requires free fatty acids and glucose, which recently appeared to perhaps play a role as mitochondrial fuel (8–11).

In this study, we aimed to detect BAT activity in different groups of women (CL, AN, NW) and possibly correlate its activity with the values obtained for RMR, NEAT, and RQ.

Subjects and Methods

The study group consisted of 38 young women, evaluated between October 2011 and March 2012: 7 CL (21.7 ± 3.6 y; body mass index [BMI] 16.2 ± 1.0 kg/m^2); 7 restricted AN patients (AN: 23.4 ± 4.5 y, BMI 15.5 ± 0.8 kg/m^2), 3 of whom were studied again after stable refeeding (R-AN: 21.3 ± 1.5 y; BMI 18.8 ± 1.1 kg/m^2); and 24 NW women who acted as controls (NW: 25.6 ± 3.9 y; BMI 22.2 ± 1.5 kg/m^2). CL and AN patients were recruited in the outpatient clinic for malnutrition secondary to eating disorders of the Clinical Nutrition Unit of the Federico II University Hospital. Age-matched control subjects were selected among female patients in the BMI range of 20 to 25 kg/m^2, who underwent total body 18F-FDG PET/CT for routine ordinary follow-up: none of them were smokers, all had stable body weight and were free of neoplastic (or other) diseases for at least 1 year.

Diagnosis of AN was made according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria. CL definition was supported by the presence of menses, normal thyroid and cardiac function, normal insulin-resistance, and so forth (for additional details see Ref. 1). AN patients and CL women were free from any endocrine, metabolic, or other relevant diseases. Three of the 7 AN patients had completed the refeeding therapeutic protocol and were studied after at least 2 months of stable regain of 10% initial body weight.

All PET/CT studies were performed between October and March to minimize seasonal influences on BAT activity. Air temperature was controlled in both waiting and injection rooms, as well as in the scan room, and kept constant at 20° to 22°C and 22° and 24°C, respectively. All subjects stayed in the waiting room 15 minutes before tracer administration. The 18F-FDG PET/CT scans were acquired after 8 hours fasting and 60 to 90 minutes after intravenous administration of 18F-FDG (350–370 MBq). None of the patients reported uneasiness or cold perception or shivering throughout the procedure. Blood glucose level, measured just before tracer administration, was <120 mg/dL in all patients. The 18F-FDG PET/CT scans were obtained using a combined PET/CT Discovery LS8 (GE Medical Systems). All scans were performed in two-dimensional mode. Emission scan was carried out in the caudo-cranial direction, from the upper thigh to the base of the skull (4 min/each bed position). Iterative image reconstruction was completed with an ordered subset-expectation maximization algorithm (2 iterations, 28 subsets). A CT with a 4-slice multidetector helical scanner was used (detector row configuration 4 × 5 mm, pitch 1.5, gantry rotation speed of 0.8 seconds per revolution, table speed of 30 mm per gantry rotation, 140 kV and 80 mA). Attenuation-corrected emission data were obtained using filtered back projection CT reconstructed images (Gaussian filter with 8 mm full width at half maximum) to match PET resolution. Transaxial, sagittal, and coronal images and coregistered images were examined using Xeleris software (GE Healthcare).

All images were reviewed at a workstation using PET/CT fusion software (Volumetrix for PET; GE Healthcare). Each set of PET/CT studies was interpreted by two experienced (LP and RF) operators by consensus. The value of 18F-FDG uptake in BAT was considered present when the uptake in the characteristic areas of brown fat localization, having the CT density of adipose tissue (−250 to 50 Hounsfield units), was greater than background soft-tissue activity. Otherwise, 18F-FDG BAT was considered absent. In addition, when 18F-FDG BAT uptake was present, the site of uptake was determined as: 1) neck and supraclavicular, 2) mediastinum and paravertebral, 3) prevertebral and intercostals, 4) paracardiac, or 5) infradiaphragmatic (perirenal and perihepatic). Thereafter, maximum standardized uptake values (SUVmax) were determined by the vendor-provided software (Volumetrix for PET-CT; GE Healthcare) on PET scans. Region of interest diameter was set at 1 cm; SUVmax was body weight-corrected. For each patient showing 18F-FDG BAT uptake, the maximum SUVmax was recorded. SUVmax normalized to body weight is given by the following equation:

$$SUVmax = (ACvoi\ (kBq/mL))/(FDGdose\ (MBq)/BW\ (kg))$$

BAT activity was evaluated by recording the number of typical anatomical areas of BAT showing FDG uptake, and SUVmax for each area. The sum of SUV$_{max}$ measurements was used for statistical analysis (12, 13).

RMR was measured by indirect calorimetry using a canopy system (Vmax29, Sensor Medics, Anaheim, California) at a room temperature of 23° to 25°C and according to standard procedures (see Ref. Marra). NEAT was evaluated according to Kousta et al (14) as the SD from the average of 30 energy expenditure measurements in the resting condition (ie, every minute for 30 min) based on the assumption that, in the steady state, increased fidgeting will increase resting energy expenditure (REE) variability. After RMR measurements, single-frequency bioimpedentiometry (BIA) was also performed at 50 KHz (STA/BIA; Akern, Firenze, Italy). The determined BIA variables were resistance and reactance: the resistance index

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Table 1. Body Composition, RMR, Fidgeting, and RQ

	CL (n = 7)	AN (n = 7)	R-AN (n = 3)	NW[a] (n = 20)
Weight, kg	41.1 ± 4.7	39.6 ± 3.9	46.2 ± 2.4	56.0 ± 6.2
BMI, kg/m^2	16.2 ± 0.9	15.3 ± 0.8	18.8 ± 1.1	21.7 ± 2.4
FFM, kg	36.2 ± 4.1	35.5 ± 3.5	38.8 ± 2.5	39.6 ± 3.9
FAT, %	11.9 ± 1.51	10.2 ± 1.79	15.6 ± 0.69[a]	28.9 ± 6.5
Phase angle, degree	6.75 ± 1.04[b]	5.39 ± 0.40	5.76 ± 0.86	6.24 ± 1.12
RMR, kcal/die	1267 ± 221[c]	1012 ± 125	1078 ± 72	1192 ± 173
RMR/FFM, kcal/kg	35.9 ± 9.6[c]	28.7 ± 4.0	27.9 ± 3.5	30.2 ± 4.3
Fidgeting/RMR, %	2.5 ± 0.2[c]	1.8 ± 0.3	1.9 ± 0.1	2.10 ± 0.70
RQ	0.89 ± 0.06	0.92 ± 0.08	0.85 ± 0.15	0.84 ± 0.05

Abbreviation: FAT, ◆ ◆ ◆ ◆ ◆.

[a] Data of our laboratory reference group (1).

[b] $P < .05$ R-AN vs CL and AN.

[c] $P < .05$ CL vs R-AN and AN.

was calculated as the ratio height^2/Rx and the result used to obtain fat-free mass (FFM) was calculated according to Kushner and Schoeller (15).

Informed consent was obtained from all patients and volunteers before participation.

The protocol of the study was approved by Ethics Committee of the Federico II University Hospital, Naples, Italy.

Statistics

Results are expressed as mean and SD. Two-way statistical analysis (SPSS-WIN version 10; SPSS, Chicago, Illinois) was performed when appropriate to compare data between different groups (post hoc analysis Tukey honestly significant difference). Wilcoxon rank sum test has been performed for nonparametric data. Significant differences between groups were confirmed with Mann-Whitney test and two-sample Kolmogorov-Smirnov test. Simple linear correlation was used to assess the associations between variables. Differences were considered statistically significant when $P < .05$.

Results

The 4 groups of young women studied (CL, AN, R-AN, and controls) did not differ in age or height; AN and CL had body weight and BMI significantly lower than did NW subjects; BMI and body weight values in R-AN patients were intermediate between those of AN and NW participants. In Table 1, CL, AN, R-AN and, as reference, NW values of our internal control group (1), BIA, and measured RMR parameters are reported. The 3 groups examined did not differ significantly for the FFM calcu-

lated; the phase angle was significantly higher ($P < .05$) in CL than in AN and R-AN. REE was higher in CL than in AN and R-AN patients and, when expressed per kilogram FFM, it was significantly higher ($P < .05$) in CL than in AN and R-AN. RQ did not differ between CL and AN patients. Fidgeting, corrected for REE, was significantly higher ($P < .05$) in CL than in the other two groups.

As for 18F-FDG PET/CT, all CL subjects showed FDG uptake in the regions investigated, with the following frequency: neck and supraclavicular area in 7 subjects (100%), thoracic and paravertebral in 6 (86%), mediastinal in 5 (71%), prevertebral and intercostal in 3 (43%), and paracardiac and sovrarenal in two (28%). Alternatively, FDG uptake could be described as present in 6 subjects in all the sites investigated and in 1 patient in 2 of the 5 sites investigated. None of the AN subjects, even after refeeding, showed evidence of FDG uptake in the area investigated. Moreover only 3 (12%) of the 24 NW females showed some FDG uptake, limited to the neck and supraclavicular regions. In the 7 CL and 3 NW subjects in whom BAT activity was detected, the highest SUVmax values were observed in the supraclavicular region; mean data and ranges are reported in Table 2.

In CL subjects, RMR, corrected RMR/FFM, RQ, and fidgeting could be correlated to the sum of SUVmax values recorded in the active anatomical sites presenting BAT. In CL subjects, the sum of SUVmax had a positive linear correlation with RMR (r = 0.71, $P < .07$), RMR/FFM

Table 2. SUVmax and Mean SUVmax in CL and NW

	CL (n = 7)			NW (n = 3/24)		
	Mean	SD	Min-Max	Mean	SD	Min-Max
SUVmax, g/mL	48.6[a]	33.4	3.2–93.2	2.9	8.7	0–38.2
mean SUVmax, g/mL	5.4[a]	3.7	0.36–10.36	0.32	0.96	0–4.24

AN data not reported (SUV always absent).

[a] $P < .05$ vs NW.

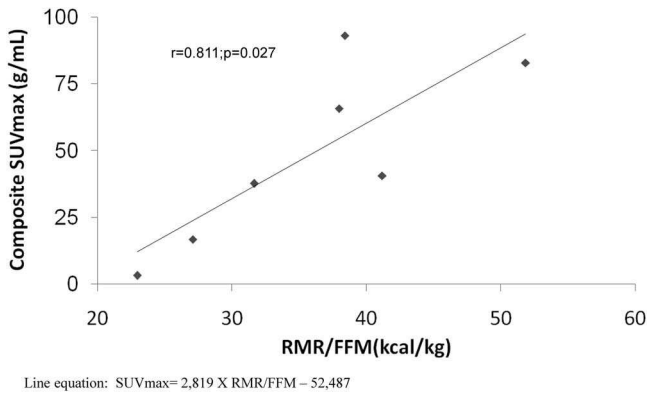Line equation: SUVmax= 2,819 X RMR/FFM – 52,487

Figure 1. Linear correlation between composite SUVmax and RMR/FFM in 7 constitutionally lean young women.

F1
F2

(0.81, $P < .03$) (Figure 1) and a negative correlation with fasting RQ ($r = -0.74$, $P < .05$) (Figure 2).

Discussion

Basic research on the physiological role of BAT started in the late 1950s and developed in the 1970s (16–20). BAT was first described in newborns, whereas in adults, except for some clinical observations in patients with end-stage pheochromocytoma, BAT was considered absent or without a significant physiological role (21). A possible role in the pathogenesis of human obesity was considered questionable (22, 23). In recent years, with the advent of the 18F-FDG PET-CT technique, which was developed primarily to identify secondary neoplasms, clear evidence of BAT activity in adult humans emerged (3, 24–27), leading to identification of some roles for BAT malfunctioning in the development of currently common diseases (8, 28). In the current study we confirm that REE is higher in CL than in AN, even after refeeding of those with AN; this finding is associated with a higher phase angle in CL, which is an index of improved intracellular or extracellular water distribution and consequently of better FFM anatomy (1). We also provide evidence, for the first time to the best of our knowledge, that BAT activity is well represented in
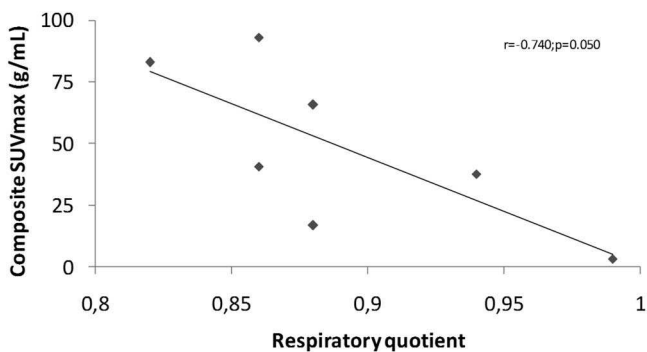

Figure 2. Linear correlation between composite SUV max and respiratory quotient in 7 constitutionally lean young women.

resting, thermoneutral conditions in healthy young adult females with CL and is correlated with the RMR, in particular when corrected for FFM. Thus, the results of our study suggest that having active BAT contributes to constitutional leanness. BAT activity is also inversely correlated with RQ. These observations support the hypothesis that BAT has a protective role toward fat mass accumulation, mostly because of the preferential use of lipids as substrate in resting, thermoneutral conditions, corresponding to our experimental observation conditions. This finding appears to be of some speculative interest if we consider that in some groups of adult premenopausal women, fat gain over time may be predicted by high RQ, index of reduced fat oxidation as preferential fuel (29–31). On the other hand, there seems to be no BAT activity in AN, a marasma-like type of protein energy malnutrition typically characterized by an adaptive reduction of RMR to compensate chronic fuel deficiency caused by chronic restrictive eating behavior. Thus, our results confirm previous observations that anorectic women have no detectable BAT (32). Furthermore, BAT activity could not be detected even in the 3 R-AN patients studied in stable refeeding conditions. In experimental models, BAT activity contributes to both cold- and diet-induced thermogenesis; we hypothesize that in our AN patients, refeeding cannot be considered a real overfeeding stimulus able to activate thermogenic mechanisms (33, 34). As to the sample representative of NW individuals, the group was recruited among age-matched healthy women attending the PET-CT radiology unit in the same period of the year. Only 3 (12%) of the 24 subjects presented some BAT activity, detectable in a small number of anatomical areas, and the SUVmax was consistently lower than that seen in CL. This finding suggests that in a thermoneutral environment, NW subjects have limited BAT activity, whereas CL females present persistent enhanced BAT activity. These preliminary observations require additional investigations in larger groups of subjects of both sexes.

In conclusion, this study for the first time provides evidence of active BAT in adult females with CL. Studies on brown fat usually are carried out in cold conditions to enhance BAT activity (32). In the current protocol, we performed experimental thermoneutrality, which may represent a limitation of the study, as may the lack of evaluation of plasma and urine catecholamine, as has been performed recently by other authors (35). Another limitation of this study is the small simple size, which limits the interpretation of BAT activity and its correlation with measurable parameters.

In this small, selected group of CL females BAT activity was positively correlated with RMR corrected for FFM and inversely correlated with RQ. These findings are suggestive of a protective role of BAT, at least in CL, toward white fat

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deposition, possibly through a preferential free fatty acid utilization. Efforts to stimulate brown fat activity either physiologically or pharmacologically to prevent excess white fat accumulation are encouraged by this observation.

Acknowledgments

References

1. **Marra M, Pasanisi F, Montagnese C, et al.** BMR variability in women of different weight. *Clin Nutr*. 2007;26:567–572.
2. **Levine JA, Lanningham-Foster LM, McCrady SK, et al.** Interindividual variation in posture allocation: possible role in human obesity. *Science*. 2005;307:584–586.
3. **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.
4. **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.
5. **Enerback S.** Brown adipose tissue in humans. *Int J Obes*. 2010;34:S43–S46.
6. **Lee P, Zhao JT, Swarbrick MM, et al.** High prevalence of brown adipose tissue in adult humans. *J Clin Endocrinol Metab*. 2011;96:2450–2455.
7. **Nedergaard J, Bengtsson T, Cannon B.** Three years with adult human brown adipose tissue. *Ann N Y Acad Sci*. 2010;1212:E20–E36.
8. **Bartelt A, Bruns OT, Reimer R, et al.** Brown adipose tissue activity controls triglyceride clearance. *Nature Med*. 2011;17:200–205.
9. **Yoneshiro T, Aita S, Matsushita M, et al.** Brown adipose tissue, whole body energy expenditure, and thermogenesis in healthy adult men. *Obesity*. 2011;19:13–16.
10. **Pace L, Nicolai E, D'Amico D, et al.** Determinants of physiologic 18F-FDG uptake in brown adipose tissue in sequential PET/TC examinations. *Mol Imaging Biol*. 2011;13:1029–1035.
11. **Nedergaard J, Bengtsson T, Cannon B.** New powers of brown fat: fighting the metabolic syndrome. *Cell Metabolism*. 2011;13:238–240.
12. **Hicks RJ.** Role of 18F-FDG PET in assessment of response in non-small cell lung cancer. *J Nucl Med*. 2009;50:31S–42S.
13. **Thie JA, Hubner KF, Smith GT.** The diagnostic utility of the log-normal behavior of PET standardized uptake values in tumors. *J Nucl Med*. 2000;41:1664–1672.
14. **Kousta E, Parker KH, Lawrence NJ, et al.** Delayed metabolic and thermogenic response to a mixed meal in normoglycemic European women with previous gestational diabetes. *J Clin Endocrinol Metab*. 2002;87:3407–3412.
15. **Kushner RF, Schoeller DA.** Estimation of total body water by bioelectrical impedance analysis. *Am J Clin Nutr*. 1986;44:417–424.
16. **Sulkin SE, Krutzsch PH, Allen R, Wallis C.** Studies on the pathogenesis of rabies in insectivorous bats. I. Role of brown adipose tissue. *J Exp Med*. 1959;110:369–388.
17. **Himms-Hagen J, Desautels M.** A mitochondrial defect in brown adipose tissue of obese (ob/ob) mouse: reduced binding of purine nucleotides and a failure to respond to cold by an increase in binding. *Biochem Biophys Res Commun*. 1978;88:628–634.
18. **Foster DO, Frydman ML.** Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can J Physiol Pharmacol*. 1979;57:257–270.
19. **Rothwell NJ, Stock MJ.** A role for brown adipose tissue in diet-induced thermogenesis. *Nature*. 1979;281:31–35.
20. **Trayhurn P, James WP.** Thermoregulation and non-shivering thermogenesis in the genetically obese (ob/ob) mouse. *Pflugers Arch*. 1978;373:189–193.
21. **Garruti G, Ricquier D.** Analysis of uncoupling protein and its mRNA in adipose tissue deposits of adult humans. *Int J Obes Relat Metab Disord*. 1992;16:383–390.
22. **Contaldo F, Presta E, Di Biase G.** Preliminary evidence for brown fat defect in human obesity. In: Cioffi LA, James WPT, Van Itallie TB, eds. *The Body Weight Regulatory System: Normal and Disturbed Mechanisms*. New York, NY: Raven Press; 1981:143–146.
23. **Astrup A, Bülow J, Madsen J, Christensen NJ.** Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *Am J Physiol*. 1985;248:E507–E15.
24. **Saito M, Okamatsu-Ogura Y, Matsushita M, et al.** High incidence of metabolically active brown adipose tissue in healthy human adults: effects of cold exposure and adiposity. *Diabetes*. 2009;58:1526–1531.
25. **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.
26. **Virtanen KA, Lidell ME, Orava J, et al.** Functional brown adipose tissue in healthy adults. *N Engl J Med*. 2009;360:1518–1525.
27. **Zingaretti MC, Crosta F, Vitali A, et al.** The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J*. 2009;23:3113–3120.
28. **Yilmaz Y, Ones T, Purnak T, et al.** Association between the presence of brown adipose tissue and non-alcoholic fatty liver disease in adult humans. *Aliment Pharmacol Ther*. 2011;34:318–323.
29. **Ellis AC, Hyatt TC, Hunter GR, Gower BA.** Respiratory quotient predicts fat mass gain in premenopausal women. *Obesity*. 2010;18:2255–2269.
30. **Marra M, Scalfi L, Covino A, Esposito-Del Puente A, Contaldo F.** Fasting respiratory quotient as a predictor of weight hangs in nonobese women. *Int J Obes Relat Metab Disord*. 1998;22:601–603.
31. **Marra M, Scalfi L, Contaldo F, Pasanisi F.** Fasting respiratory quotient as a predictor of long-term weight changes in on obese women. *Ann Nutr Metab*. 2004;48:189–192.
32. **Bredella MA, Fazeli PK, Freedman LM, et al.** Young women with cold-activated brown adipose tissue have higher bone mineral density and lower Pref-1 than women without brown adipose tissue: a study in women with anorexia nervosa, women recovered from anorexia nervosa, and normal-weight women. *J Clin Endocrinol Metab*. 2012;97:E584–E590.
33. **Cannon B, Nedergaard J.** Metabolic consequences of the presence or absence of the thermogenic capacity of brown adipose tissue in mice (and probably in humans). *Int J Obes (Lond)*. 2010;34:S7–S16.
34. **Tan DX, Manchester LC, Fuentes-Broto L, Paredes SD, Reiter RJ.** Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. *Obes Rev*. 2011;12:167–188.
35. **Carey L, Formosa MF, Van Every B, et al.** Ephedrine activates brown adipose tissue in lean but not obese humans. *Diabetologia*. 2013;56:147–155.