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Increase in Brown Adipose Tissue Activity after Weight Loss in Morbidly Obese Subjects

G. H. E. J. Vijgen, N. D. Bouvy, G. J. J. Teule, B. Brans, J. Hoeks, P. Schrauwen, and W. D. van Marken Lichtenbelt

Department of Human Biology (G.H.E.J.V., J.H., P.S., W.D.v.M.L.), School for Nutrition, Toxicology, and Metabolism, NUTRIM, and Departments of General Surgery (G.H.E.J.V., N.D.B.) and Nuclear Medicine (G.J.J.T., B.B.), Maastricht University Medical Centre, 6200 MD Maastricht, The Netherlands

Context: Stimulation of thermogenesis in brown adipose tissue (BAT) is a potential target to treat obesity. We earlier demonstrated that BAT activity is relatively low in obese subjects. It is unknown whether BAT can be recruited in adult humans.

Objective: To study the dynamics of BAT, we observed BAT activity in morbidly obese subjects before and after weight loss induced by bariatric surgery.

Design: This was an observational prospective cohort study.

Setting: The study was conducted at a referral center.

Patients: Ten morbidly obese subjects eligible for laparoscopic adjustable gastric banding surgery were studied before and 1 yr after bariatric surgery.

Main Outcome Measure: The main outcome measure was BAT activity, as determined after acute cold stimulation using ^{18}F-fluorodeoxyglucose positron emission tomography and computed tomography.

Results: Before surgery, only two of 10 subjects showed active BAT. One year after surgery, the number of subjects with active BAT was increased to five. After weight loss, BAT-positive subjects had significantly higher nonshivering thermogenesis compared with BAT-negative subjects ($P < 0.05$).

Conclusions: The results show that in humans BAT can be recruited in the regions in which it was also reported in lean subjects before. These results for the first time show recruitment of BAT in humans and may open the door for BAT-targeted treatments of obesity. **(*J Clin Endocrinol Metab* 97: E1229–E1233, 2012)**

Until very recently, the role of brown adipose tissue (BAT) in human physiology was considered to be of importance only during the first years of life. However, metabolic imaging techniques [^{18}-fluoro-deoxy-glucose (^{18}F-FDG), positron emission tomography (PET), and computed tomography (CT)] showed functionally active BAT upon cold exposure in adult man (1–4).

We previously found that BAT activity is inversely related to body mass index (BMI) and body fat percentage (BF%) (2). This could suggest that a low level of BAT activity may predispose to obesity, when assumed that BAT activity is an intrinsic characteristic of an individual. Alternatively, low BAT activity may be an adaptive trait of obesity, for example, due to improved insulation, which would predict that functional BAT could be re-recruited when body composition would change, for example, after significant weight loss. Postobese subjects indeed report an increased cold sensation during weight loss by bariatric surgery (5) (Bouvy, N.D., and G.H.E.J. Vijgen, personal observations). It is still unknown whether BAT can be recruited in man. However, the principle of recruitable BAT has been observed after prolonged cold exposure in

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Abbreviations: BAT, Brown adipose tissue; BF%, body fat percentage; BMI, body mass index; BMR, basal metabolic rate; CT, computed tomography; ^{18}F-FDG, ^{18}-fluoro-deoxy-glucose; FFM, fat free mass; LAGB, laparoscopic adjustable gastric banding; NST, nonshivering thermogenesis; PET, positron emission tomography; SUV, standard uptake value; SUV^{Mean}, average SUV uptake; SUV^{Total}, SUV^{Mean} times the volume of the region; t, time; WL, weight loss.

TABLE 1. Subject characteristics in 10 patients (two males, eight females) with measurements before and after weight loss

	Before	After	*P* value
Age (yr)	40 ± 9	41 ± 9	
BMI (kg/m^2)	41.7 ± 4.4	29.8 ± 4.2	<0.001
Body mass (kg)	127.0 ± 17.7	90.8 ± 16.7	<0.001
BF%	48.6 ± 5.2	34.8 ± 7.9	<0.001
Fat mass (kg)	62.1 ± 10.9	32.4 ± 10.5	<0.001
FFM (kg)	63.5 ± 11.7	57.7 ± 12.2	<0.001

rodents and primates (6). In man, seasonal variation influences BAT activity within subjects (3, 6). *In vitro* stimulation of white adipose tissue with peroxisome proliferator-activated receptor-γ induced the development of thermogenic so-called brite adipocytes (7). Moreover, treating preadipocytes isolated from human sc adipose tissue with bone morphogenic protein-7 showed brown adipogenesis (8). Based on these observations, BAT recruitment in man seems feasible but has not yet been shown. Therefore, we investigated BAT activity in 10 morbidly obese patients before and 1 yr after laparoscopic adjustable gastric banding (LAGB) surgery as a model for caloric restriction.

The increase in energy expenditure that is observed upon mild cold exposure in absence of shivering in skeletal muscle is defined as nonshivering thermogenesis (NST) (9). Active BAT in adult man is suggested to contribute to NST. Indeed, subjects with active BAT show significantly higher NST than subjects without BAT activity (10, 11). Therefore, we also studied NST in our subjects.

Materials and Methods

Approval was obtained from the local ethical commission. Written informed consent was received from two male and eight female morbidly obese subjects, with a mean BMI of 41.7 ± 4.4 kg/m and a body mass of 127.0 ± 17.7 kg. One female subject used levothyroxine for hypothyroidism and was euthyroid for several years. Diabetes mellitus and use of β-blockers were strict exclusion criteria.

Study protocol

All measurements took place several weeks before surgery between 0800 and 1300 h under overnight fasted conditions. In the morning, subjects put on light standardized clothing [socks 0.02 clo, shirt 0.09 clo, sweatpants 0.28 clo, underwear 0.04 clo, total clo factor 0.43 clo (12)]. Body composition [BF%, fat mass, fat free mass (FFM)] was determined by dual x-ray absorptiometry (type Discovery A; Hologic, Bedford, MA). To determine body core temperature, a telemetric pill was orally ingested (CoreTemp, HQ Inc., Palmetto, FL USA). Skin temperature was measured by applying iButtons at 14 ISO-defined skin sites (13). Next, subjects were placed in a specially adapted climate controlled tent (Colorado Altitude Training, Louisville, CO). The temperature inside the tent was regulated by an air conditioner (accuracy of tent air temperature ±0.5 C). Energy expenditure was measured by indirect calorimetry using a ventilated hood system (Oxycon, Jaeger, Germany).

Personal cooling protocol and ^{18}F-FDG-PET-CT

In all subjects we performed the personal cooling protocol described earlier (10). In short, subjects were placed in the climate tent. They rested semisupine on a nephrodialysis chair, covered with a water-perfused mattress. To ensure maximal NST, every subject was cooled until they reported shivering. Shivering was confirmed by electromyography of the musculus pectoralis major. Upon the first signs of shivering, the temperature was raised slightly (on average 1.8 ± 0.6 C) to ensure only NST.

One hour of measuring under thermoneutral conditions [room temperature before weight loss: 22.8 ± 0.6 C, after weight loss: 23.7 ± 0.9 C, $P = 0.079$, time (t) 0-t60] was followed by 2 h of individual mild cold (t60-t180). Laser Doppler flowmetry (PF5000; Perimed, Jarfalla, Sweden) was used to determine whether a subject was in its thermoneutral zone (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). At the end of the first hour of cooling (t120), 10 ml FDG was injected. After the second hour of cooling, ^{18}F-FDG-PET-CT imaging was performed to quantify metabolically active BAT. The scanning protocol, FDG activity (74 MBq), and data analysis were identical with our previous study (2).

Follow-up

All subjects underwent LAGB without any perioperative or postoperative complications. One year after the LAGB procedure, all measurements were repeated.

Statistical analysis

Total BAT activity was expressed in kilobequerels and standard uptake value [SUV; as calculated by uptake (kilobequerels

TABLE 2. Total BAT activity before and after weight loss

	Before		After		*P* value	
	Group	BAT+	Group	BAT+	Group	BAT+
BAT activity (kBq)	7.8 ± 18.0	15.6 ± 24.0	51.5 ± 87.8	103.0 ± 103.6	0.147	0.153
BAT volume (cm^3)	7.1 ± 16.2	14.2 ± 21.6	42.5 ± 71.6	85.0 ± 83.9	0.148	0.154
BAT activity (kBq/cm^3)	0.2 ± 0.5	0.4 ± 0.6	0.6 ± 0.6	1.1 ± 0.1	0.074	0.062
SUV^{Total}	30.3 ± 72.4	60.6 ± 97.5	118.0 ± 210.6	236.0 ± 255.0	0.238	0.259
SUV^{Max}	1.4 ± 3.0	2.8 ± 3.9	3.1 ± 3.9	6.3 ± 3.2	0.197	0.212

Group values (n = 10) of BAT activity (kilobequerels), BAT volume (cubic per cubic centimeters), average BAT activity (kilobequerels per cubic centimeters), and total and maximal SUV (SUV^{Total}, maximum SUV). In addition, values are shown for the group of five subjects that were indicated as BAT positive (BAT+) based on their BAT presence after weight loss.

per milliliter) per injected dose (kilobequerels) per patient weight (grams)]. BAT activity of each region is determined by the average SUV uptake (SUV^{Mean}) times the volume of the region (cubic centimeters), expressed as SUV^{Total}. Basal metabolic rate (BMR) correlated significantly to FFM before and after weight loss (respectively, $r = 0.912$, $P = 0.001$, and $r = 0.948$, $P < 0.001$). Therefore, we corrected the BMR for FFM using linear regression (14). Percentage of weight loss is expressed as [(beginning weight − follow-up weight)/beginning weight) × 100]. Repeated measurements were compared using paired Student's *t* tests. Based on BAT presence after weight loss, subjects were categorized as BAT positive or BAT negative, which was compared using unpaired Student's *t* tests.

Results and Discussion

One year after LAGB, the mean body composition decreased significantly by 36.1 ± 8.3 kg with a mean percentage of weight loss of 28.65 ± 6.23% (Table 1).

BAT activity was measured after applying an individualized cooling protocol. Thus, maximal BAT activity is

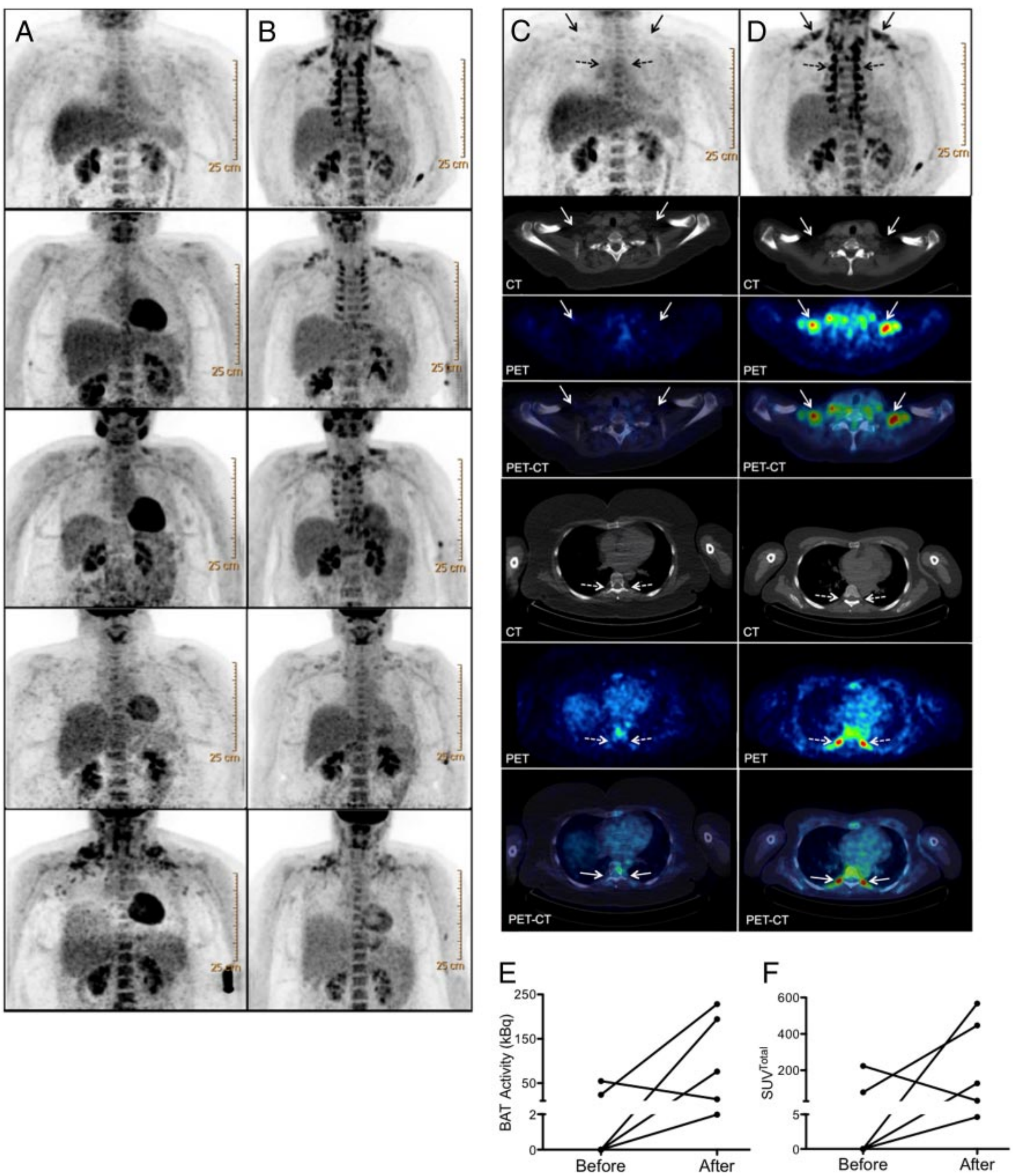

FIG. 1. BAT activity before and after WL. A, PET images of five morbidly obese subjects before bariatric surgery (A) and after WL (B). C, PET image, CT image and PET-CT-fusion image of the subject who showed the largest increase in BAT activity before (C) and after WL (D). BAT activity was recruited in both supraclavicular and paravertebral areas, indicated by *solid* and *dashed arrows*, respectively. *Solid arrows* indicate bilateral supraclavicular regions in which BAT activity was observed after WL. *Dashed arrows* indicate bilateral paravertebral regions in which BAT activity was observed after WL. E, BAT activity in kilobequerels and total SUV (F) before and after WL for the five subjects depicted in A and B. Baseline characteristics of subjects shown are from a report published elsewhere (10).

achieved by low environmental temperatures just above the temperature that causes shivering (10). Mean room temperatures during maximal NST were significantly higher after weight loss (WL) [before WL: 14.2 C (range 12–17), after WL: 15.7 C (range 12–21), $P = 0.026$]. Before surgery, we observed, albeit minimal, cold-induced BAT activity in supraclavicular depots in two female subjects. Their mean BAT activity (39.0 kBq; range 23.6, 54.5 kBq) and volume (35.6 cm^3; range 22.5, 48.6 cm^3) were low [for group results see Table 2, baseline characteristics based on another report (10)]. After WL, we observed BAT in five of 10 subjects, among whom were the two above-mentioned female subjects. Of these five BAT-positive subjects (all female; the two male subjects did not show BAT activity), one subject increased her BAT activity (23.6 kBq to 228.3 kBq), three previously BAT-negative subjects were BAT positive after WL, and one female subject decreased her BAT activity (54.5 kBq to 13.8 kBq). In addition to supraclavicular BAT activity, the depots of active BAT also included paravertebral depots in three patients (Fig. 1). There were no significant differences in body composition between subjects characterized as BAT positive and BAT negative after WL, neither before nor after WL.

The BMR showed a significant decrease after WL (95.3 ± 4.5 *vs.* 88.8 ± 4.8 W, $P = 0.028$). BAT-positive subjects (after WL) showed significantly higher NST compared with BAT-negative subjects (13.7 ± 7.7 *vs.* 2.4 ± 7.1%, $P = 0.042$, Supplemental Fig. 2), in line with previous noninterventional studies on BAT and NST (10, 11).

We show that the presence and activity of BAT in adult humans is dynamic and can increase after weight reduction. This important finding shows for the first time using functional tools (^{18}F-FDG-PET-CT and indirect calorimetry) that BAT recruitment in humans is possible. Reduced activity of BAT could potentially impair total daily energy expenditure and be of importance in the development of obesity.

LAGB induces a caloric restriction that caused significant WL. It does not seem likely LAGB could be responsible for other mechanisms that could have increased BAT because the digestive tract is not altered. For example, gut hormones do not change after LAGB (15).

The increase in BAT presence after WL could be a direct effect of a decreased insulation due to loss of sc adipose tissue. WL significantly changed the thermal distribution, as demonstrated by the observed skin and core temperatures (Supplemental Table 1). Obese subjects show a low gradient between proximal and distal skin temperature during cold exposure, indicating a low level of vasoconstriction (14). This gradient increased after WL (baseline: 0.2 ± 1.9 C before WL and −4.0 ± 2.4 C after WL, $P = 0.001$; cold exposure: −3.1 ± 1.9 C before WL and −6.4 ± 2.2 C after WL, $P = 0.001$), suggesting increased skin vasoconstriction. Insulation of the body's core, as measured by the gradient between core and mean skin temperature, decreased significantly after WL (before WL: 5.6 ± 0.6 C; after WL: 3.8 ± 0.7 C, $P < 0.001$). This indicates the insulation of the body's core decreased, despite increased vasoconstriction of the skin. Compared with BAT-negative subjects, BAT-positive subjects showed a significantly smaller increase in core temperature during cold exposure, accompanied by a smaller decrease in mean skin temperature (Supplemental Table 1), suggesting less vasoconstriction (14). Hence, increased thermogenesis in BAT could be responsible for this difference.

It should be noted that the BAT activity we observed in this study is low in comparison with the levels observed in lean young men [51.5 ± 87.8 kBq *vs.* 428 ± 394 kBq, P = 0.008 (2)]. BAT activity is suggested to be lower in older subjects (3), which could possibly affect the energy balance. Therefore, further studies are needed to investigate the impact of BAT recruitment at this level for the long-term treatment of obesity. Nevertheless, this study provides the first evidence that BAT can be recruited in adults.

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Address all correspondence and requests for reprints to: Guy H. E. J. Vijgen, Department of Human Biology, Maastricht University, P.O. Box 616, 6200 MD, Maastricht, The Netherlands. E-mail: g.vijgen@maastrichtuniversity.nl; or markenlichtenbelt@maastrichtuniversity.nl.

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References

1. **Nedergaard J, Bengtsson T, Cannon B** 2007 Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 293:E444–E452
2. **van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ** 2009 Cold-activated brown adipose tissue in healthy men. N Engl J Med 360:1500–1508
3. **Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M** 2009 High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58:1526–1531

4. **Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S, Nuutila P** 2009 Functional brown adipose tissue in healthy adults. N Engl J Med 360:1518–1525
5. **Isacsson A, Frederiksen SG, Nilsson P, Hedenbro JL** 1997 Quality of life after gastroplasty is normal: a controlled study. Eur J Surg 163:181–186
6. **Cannon B, Nedergaard J** 2004 Brown adipose tissue: function and physiological significance. Physiol Rev 84:277–359
7. **Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J** 2010 Chronic peroxisome proliferator-activated receptor γ (PPARγ) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem 285:7153–7164
8. **Schulz TJ, Huang TL, Tran TT, Zhang H, Townsend KL, Shadrach JL, Cerletti M, McDougall LE, Giorgadze N, Tchkonia T, Schrier D, Falb D, Kirkland JL, Wagers AJ, Tseng YH** 2011 Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. Proc Natl Acad Sci USA 108:143–148
9. **van Marken Lichtenbelt WD, Schrauwen P** 2011 Implications of non-shivering thermogenesis for energy balance regulation in humans. Am J Physiol Regul Integr Comp Physiol 301:R285–R296
10. **Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD** 2011 Brown adipose tissue in morbidly obese subjects. Plos One 6:e17247
11. **Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y, Saito M** 2011 Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. Obesity (Silver Spring) 19:13–16
12. **Bligh J, Johnson KG** 1973 Glossary of terms for thermal physiology. J Appl Physiol 35:941–961
13. **van Marken Lichtenbelt WD, Daanen HA, Wouters L, Fronczek R, Raymann RJ, Severens NM, Van Someren EJ** 2006 Evaluation of wireless determination of skin temperature using iButtons. Physiol Behav 88:489–497
14. **Wijers SL, Saris WH, van Marken Lichtenbelt WD** 2010 Cold-induced adaptive thermogenesis in lean and obese. Obesity (Silver Spring) 18:1092–1099
15. **Bose M, Machineni S, Oliván B, Teixeira J, McGinty JJ, Bawa B, Koshy N, Colarusso A, Laferrère B** 2010 Superior appetite hormone profile after equivalent weight loss by gastric bypass compared to gastric banding. Obesity (Silver Spring) 18:1085–1091