Brown Adipose Tissue Transplantation Reverses Obesity in Ob/Ob Mice

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Increasing evidence indicates that brown adipose tissue (BAT) transplantation enhances whole-body energy metabolism in a mouse model of diet-induced obesity. However, it remains unclear whether BAT also has such beneficial effects on genetically obese mice. To address this issue, we transplanted BAT from C57/BL6 mice into the dorsal subcutaneous region of age- and sex-matched leptin deficient Ob/Ob mice. Interestingly, BAT transplantation led to a significant reduction of body weight gain with increased oxygen consumption and decreased total body fat mass, resulting in improvement of insulin resistance and liver steatosis. In addition, BAT transplantation increased the level of circulating adiponectin, whereas it reduced the levels of circulating free T_3 and T_4, which regulate thyroid hormone sensitivity in peripheral tissues. BAT transplantation also increased β3-adrenergic receptor and fatty acid oxidation related gene expression in subcutaneous and epididymal (EP) white adipose tissue. Accordingly, BAT transplantation increased whole-body thermogenesis. Taken together our results demonstrate that BAT transplantation may reduce obesity and its related diseases by activating endogenous BAT. **(*Endocrinology* 156: 2461–2469, 2015)**

Obesity occurs when energy intake exceeds energy expenditure (1). The excess energy is mostly stored as triglycerides in white adipose tissue (WAT), which represents at least 10% of the body weight of normal healthy adult humans (2). Humans and small mammals have three different kinds of adipose tissues: WAT, brown adipose tissue (BAT), and brown in white (brite or beige) adipose depot (3, 4). Adipose tissues from visceral and sc WAT are different not only in anatomical location but also entirely different in its physiological role. For example, the size of subcutaneous (sc) adipocyte is 24% larger than visceral adipocyte (5), and there are more mitochondria in sc fat than in visceral fat (6). Multiple lines of evidences indicate that sc fat has a beneficial effect on metabolism compared with visceral fat, which has a detrimental effect (7).

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Abbreviations: AUC, area under the curve; BAT, brown adipose tissue; EP, epididymal; FGF21, fibroblast growth factor-21; GTT, glucose tolerance test; ITT, insulin tolerance test; Ob/Ob, B6.V-Lepob/J; PGC1, PPAR-γ coactivator 1; PPAR, peroxisomal proliferator-activated receptor; TG, triglyceride; trBAT, transplanted BAT; UCP1, uncoupling protein 1; WAT, white adipose tissue.

Recently several landmark studies fundamentally altered our understanding of adult human BAT. In those studies, positron emission tomography-computed tomography using radiotracers such as ^{18}F-fluorodeoxyglucose indicated the presence of BAT and its relevance for body mass index in adult humans (8–10). Thus, it may suggested that weight loss could be achieved by increasing energy expenditure through activating BAT (11). Additionally, transplanted BAT (trBAT) on the type 1 diabetes mice reversed the clinical symptoms of type 1 diabetes such as hyperglycemia, loss of adiposity, and polyphagia, suggesting that multiple adipokines from trBAT are involved in glycemic controls (12). Recently we and another group showed that BAT transplantation improved energy expenditure and glucose homeostasis (13, 14). Furthermore, BAT transplantation reversed high-fat diet-induced obesity and preexisting obesity (14). However, whether BAT can reverse Ob/Ob mice is not yet known. Leptin-deficient Ob/Ob mice are a widely used mouse model for studying obesity-induced diabetes because they have several metabolic phenotypes including hyperphagia, glucose intolerance, and adipocyte hyperplasia. These mice have a lower metabolic rate and hypothermia due to a defect in BAT function (15, 16). To explore whether BAT could reverse Ob/Ob mice, we performed BAT transplantations from 6-week-old C57B/L6 male mice into the dorsal sc region of age- and sex-matched Ob/Ob recipient mice. We demonstrate here that BAT transplantation significantly inhibits body weight gain and reduce whole-body fat composition in an Ob/Ob mice mouse model.

Materials and Methods

Mice

Six-week-old male C57BL/6J donor mice were purchased from Vital River Laboratory Animal Technology Co Ltd. Recipient B6, V-Lepob/NJU mice from Nanjing Biomedical Research Institute of Nanjing University were used for transplantation. Mice were housed four per cage in an Office of Laboratory Animal Welfare-certified animal facility with a 12-hour light, 12-hour dark cycle.

Tissue transplantation

BAT was removed from the intrascapular region of 6-week-old C57BL/6J donor mouse and implanted into the dorsal sc region of recipient B6, V-Lepob/NJU mice. After cervical dislocation of donor mice, the BAT was removed and peripheral white fat was excluded, and then the remaining BAT (0.2 g) was washed with sterile PBS and transplanted into the dorsal sc region of recipients as quickly as possible. Recipient mice were anesthetized by ip injection with 400 mg/kg body weight avertin, and then BAT was transplanted underneath the skin. For the control group, a sham operation was performed with the same procedure.

Gene expression analysis

Total RNA was isolated using the RNeasy minikit (QIAGEN). The cDNA was synthesized using random hexamers (Invitrogen) for subsequent real-time quantitative PCR analysis (ABI Prism VIIA7; Applied Biosystems Inc). PCR products were detected using Sybr Green and normalized by cyclophilin expression. Primers were designed using Primer Quest (Integrated DNA Technologies, Inc).

Metabolic assessment

For glucose tolerance tests (GTTs), animals were fasted for 16 hours (5:00 PM to 9:00 AM) with free access to drinking water. Blood glucose levels were determined by using an Accu-Chek glucose monitor (Roche Diagnostics Corp) immediately before and 15, 30, 60, and 120 minutes after an ip glucose injection (1.2 g/kg). For the insulin tolerance test (ITT), mice were fasted for 4 hours (9:00 AM to 1:00 PM) and an ip injection with human insulin (0.8 U/kg Humulin R; Novo Nordisk). Blood glucose levels were determined immediately before and 15, 30, 45, and 60 minutes after the insulin injection.

Energy intake, digested energy, and total movement measurement

Mice were housed one animal per cage with free access to food and water. Food intake and oxygen consumption were measured for 3consecutive days after 2 days of acclimation using a TSE laboratory master system as described previously (17). Digested energy was analyzed as described previously (18). Ambulatory activity of each mouse was measured using the optical beam technique (Opto-M3; Columbus Instruments) over 24 hours and expressed as the 24-hour average activity. Rectal temperature was measured before and after 4 hours of cold exposure with a thermometer (Shenzhen Zhongyidapeng; AT210).

Body composition analysis

After all mice were anesthetized by an injection of Avertin ip, whole-body fat mass was measured using non-radio tracer computerized tomography (LCT-200; Hitachi Aloka Latheta) according to the manufacturer's instruction.

Western blot

Tissues were dissolved in a cell lysis buffer [150 mM sodium chloride, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM Tris, and protease and phosphatase inhibitor cocktail (Roche Diagnostics Corp)]. Protein concentrations were determined using a bicinchoninic assay kit (Pierce Diagnostics Corp). Protein was separated by 10% SDS-PAGE, transferred to a polyvinyl difluoride membrane (Millipore), blocked in 5% skim milk in a buffer of 0.02 M Tris base, 0.14 M NaCl, and 0.1% Tween 20 (pH 7.4), incubated with primary antibodies overnight at 4°C, and then incubated with secondary antibodies conjugated with horseradish peroxidase. Primary antibodies used in this study are phosphor-Ser473, total Akt, phosphor-ERK Thr202/Tyr204, and ERK (Cell Signaling Technology), uncoupling protein 1 (UCP1), oxphos (Abcam Co), and β-actin (Sigma Chemical Co). Signals were detected with the Super Signal West Pico chemiluminescent substrate (Pierce).

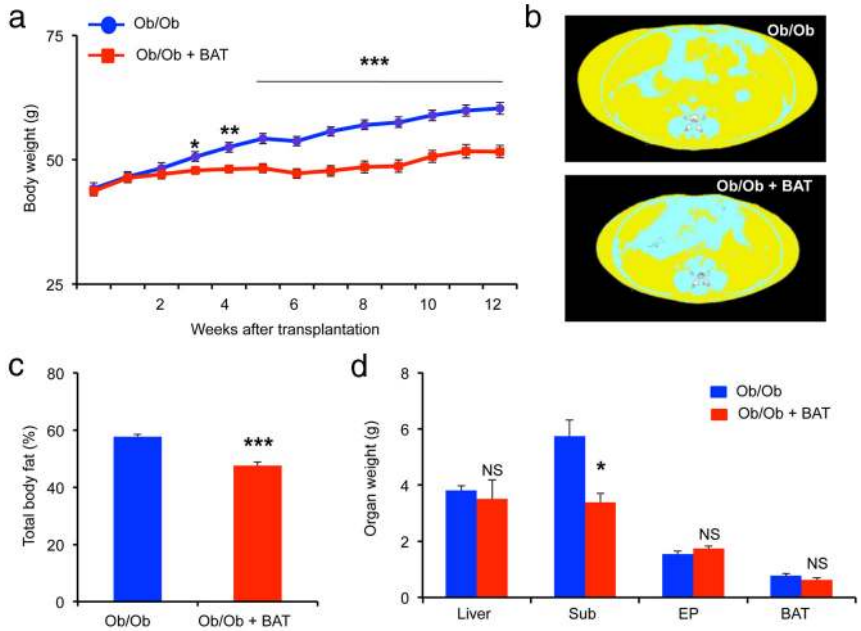

Figure 1. BAT transplantation inhibits body weight gain. To test whether BAT has beneficial effect on genetic obesity, BAT transplantation was performed on Ob/Ob mice. Results show that BAT transplantation could significantly inhibit body weight gain (a), decrease total body fat mass (b and c), and significantly reduce subcutaneous (Sub) fat mass (d) in Ob/Ob mice. Data are mean ± SEM (n = 9–10/group). *, $P < .05$; **, $P < .01$; ***, $P < .001$. NS, not significant.

Tissue staining and analysis of adipocyte size

Tissues were fixed in 4% paraformaldehyde overnight at room temperature and then embedded in paraffin. Sections of 5 μm thickness were stained with hematoxylin and eosin, and then images were taken by microscope (DS-RI1; Nikon). The standard streptavidin-biotin-peroxides immunostaining procedure was used to the detection of tyrosine hydroxylase. Tissues specimens were blocked with 10% normal goat serum for 30 minutes and then incubated with the tyrosine hydroxylase antibody (P40101–150; Pel FreeZ) overnight at 4°C, followed by a 1-hour incubation at room temperature with horseradish peroxidase-conjugated goat antirabbit IgG. To quantify the size of adipocyte, more than five sections were taken from each mouse fat pad, and then the area of 500–600 cells from five fields of each section were measured. Each field was separated at a certain distance to avoid the repeated cell measurement in a double-blind manner.

TSH and leptin measurements

Plasma TSH and leptin were measured using ELISA kits (NanJing JianCheng Bioengineering Institute), according to the manufacturer's instructions. The lower detection limit for TSH is 1 pg/mL and that for leptin is 0.1 ng/mL.

Statistics

Comparisons between groups were made by ANOVA, analysis of covariance, or Student's *t* tests. A difference between groups of $P < .05$ was considered significant.

Results

BAT transplantation reduces body weight gain

To investigate whether increased BAT mass reverses Ob/Ob mice, we increased BAT mass using transplantation. BAT (average 0.2 g) was taken from C57BL/6J male mouse and then sc implanted to the sex- and age-matched Ob/Ob mice. Because we previously found that sc transplant of either epididymal (EP) fat tissue or muscle did not improve a recipient's metabolic phenotype under high-fat diet feeding (14), the sham-operated Ob/Ob mouse was used as the sole control mouse in this study. The Ob/Ob mice with BAT transplantation did not gain as much weight as the sham-operated control Ob/Ob mice (Figure 1A). This difference in the body weight gain emerged as early as 3 weeks (47.8 ± 2.2 g vs 50.6 ± 3.1 g, $P < .03$) after BAT transplantation, and the biggest difference was found at 12 weeks after transplantation (51.6 ± 3.7 g vs 60.3 ± 3.9 g, $P < .001$). The body composition was analyzed by computerized tomography. The percentage of whole-body fat of the BAT-transplanted mice decreased 11% compared with that of the sham-operated mice (Figure 1, B and C). In parallel, the weight of sc adipose tissue, but not EP adipose tissue, endogenous BAT or liver tissue was dramatically decreased in BAT transplanted Ob/Ob mice (Figure 1D). These results indicate that BAT transplantation significantly reduces the gain of body weight and adiposity in Ob/Ob mice. Next, we analyzed the adipocyte size in WATs between groups. Compared with control mice, there was a significant reduction of adipocyte size in sc fat (Figure 2, A and B) but not in EP fat (Figure 2C) after BAT transplantation. It has been reported that BAT transplantation significantly increased circulating IL-6 (13). There, however, was no change either in the circulating IL-6 levels or IL-6 mRNA in EP fat in our study (Figure 2, D and E). These results highlight that BAT transplantation blunted adipose tissue hypertrophy without alteration of adipose tissue inflammation.

Hepatic steatosis is reversed by BAT transplantation

Severe hepatic steatosis was found in our control Ob/Ob mice as described previously (Figure 3A, upper panel). Surprisingly, hepatic steatosis was completely reversed in BAT-transplanted Ob/Ob mouse (Figure 3A, lower panel). In parallel, the expression levels of peroxisomal proliferator-activated receptor (PPAR)-γ2 and TNFα in liver were significantly down-regulated after

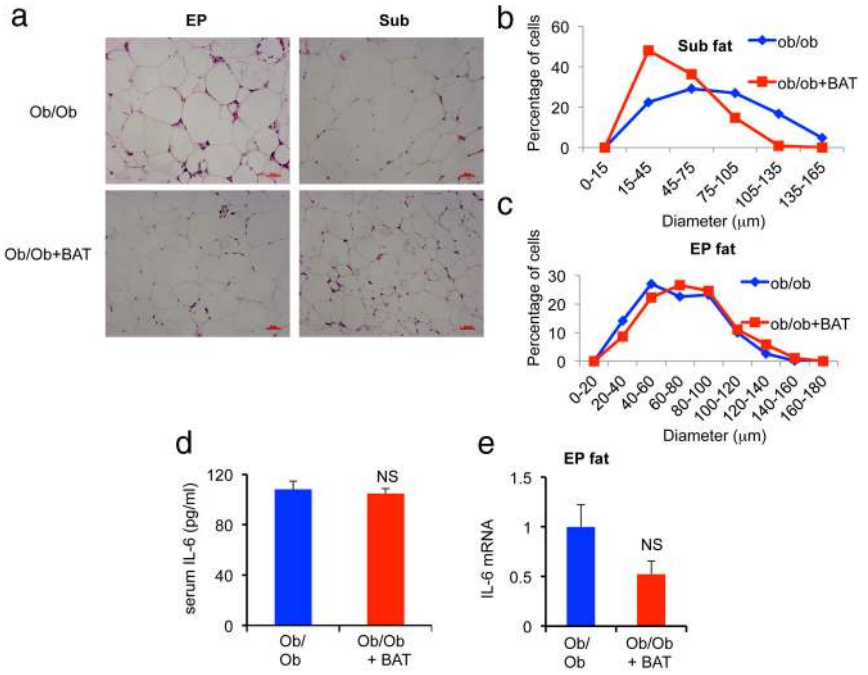

Figure 2. BAT transplantation decreases the adipose tissue hypertrophy. There were decreases in the size of the adipocyte in EP fat (a–c), whereas there were no changes in the circulating IL-6 and EP fat IL-6 mRNA level (d and e) after BAT transplantation. There is no significant change in IL-6 mRNA in EP fat (e). Data are mean ± SE (n = 9–10/group). *, $P < .05$; **, $P < .01$; ***, $P < .001$. NS, not significant.

BAT transplantation (Figure 3B). Interestingly, PPAR-γ coactivator 1 (PGC1)-α, which is known to induce the expression of genes that regulate hepatic fatty acid metabolism (19), was significantly increased after BAT transplantation (Figure 3B). Although it is well known that BAT consumes large amounts of fatty acid and glucose (20), there was no difference in the expression of other fatty acid metabolism-related genes such as *CPT1β*, *PGC1β*, and *PPARα* (Figure 3B). Sterol regulatory element-binding protein-1c mRNA expression and hepatic triglyceride (TG) contents were significantly decreased in BAT-transplanted mouse liver (Figure 3, C and D). In parallel, circulating TGs, cholesterol, and low-density lipoprotein levels were significantly decreased after BAT transplantation (Supplemental Table 1). These results demonstrated that BAT transplantation significantly improved hepatic steatosis in Ob/Ob mice.

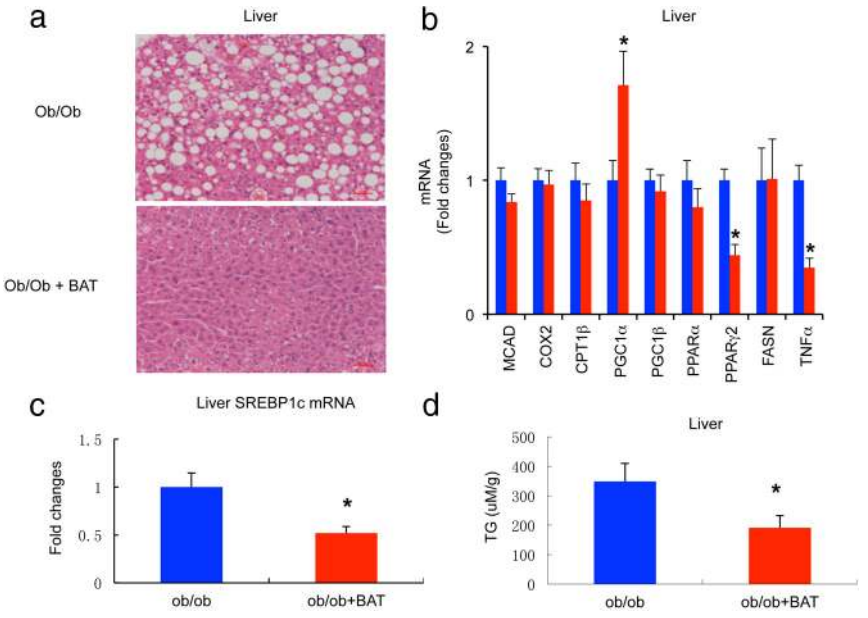

Figure 3. Hepatic steatosis reversed post-BAT transplantation. BAT transplantation could totally reverse hepatic steatosis (a), significantly decrease the liver gene expression of *pparγ2* and TNFα and increase PGC1α expression (b), and significantly decrease the SREBP1c mRNA expression and TG level (c and d) in liver tissue. Data are mean ± SEM (n = 9–10/group). *, $P < .05$. SREBP1c, sterol regulatory element-binding protein-1c.

BAT transplantation improves insulin sensitivity

The amelioration of plasma lipid profiles after BAT transplantation suggests that trBAT might affect whole-body insulin sensitivity. To test whether BAT transplantation improved glucose metabolism, a GTT was performed. BAT transplantation markedly improved insulin sensitivity (Figure 4, A and B). ITTs and the value of the area under the curve (AUC) further supported the significant improvement of insulin sensitivity after BAT transplantation (Figure 4, C and D). Concomitantly, Akt phosphorylation of EP fat was notably increased after BAT transplantation (Figure 4E). These results demonstrated that the BAT transplantation into Ob/Ob mice significantly improved glucose homeostasis.

BAT transplantation increases energy expenditure

Decreased energy expenditure is a predominant phenotype of Ob/Ob mice. To investigate whether BAT transplantation increased energy expenditure in Ob/Ob mice, we assessed whole-body energy metabolism by using indirect calorimetry

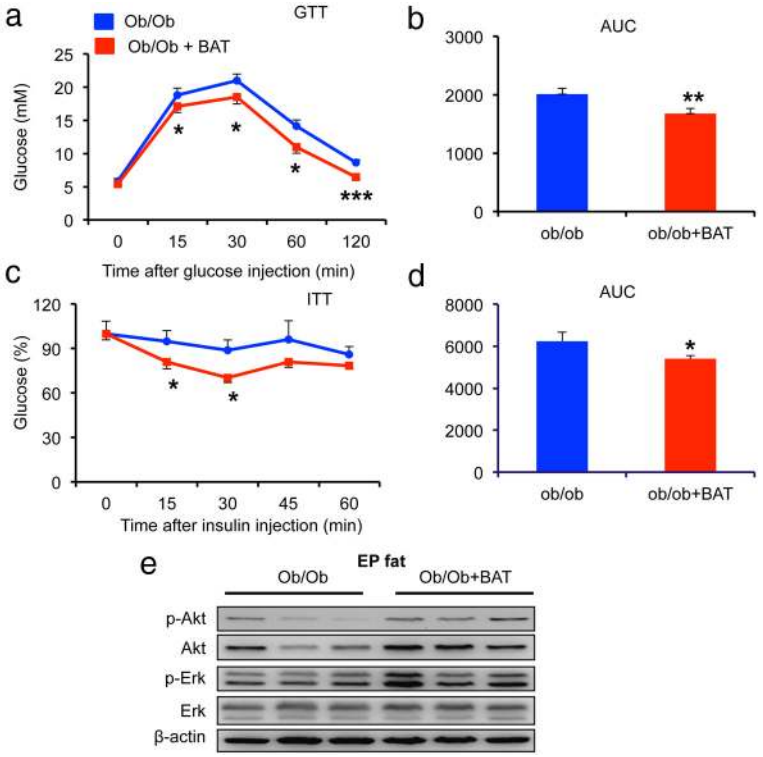

Figure 4. BAT transplantation improves insulin sensitivity in Ob/Ob mice. Ob/Ob mice with BAT transplantation improves insulin sensitivity as evidenced by the GTT (a), the AUC of the GTT (b), the ITT (c), and the AUC of the ITT (d) and improves the AKT phosphorylation in EP adipose tissue (E). Data are mean ± SEM (n = 9–10/group). *, $P < .05$; **, $P < .01$.

(TSE Labmaster system). Oxygen consumption was significantly increased (Figure 5, A and B) in the BAT transplanted group, whereas energy intake was unaltered after BAT transplantation (Figure 5C). In addition, there was no change in the respiratory quotient (Supplemental Figure 2C), which indicates the flexibility of the body to switch the energy source from oxidizing fat to glucose in response to the energy homeostasis (Figure 5D). Generally, the obese mouse has lower physical activity (21); however, BAT transplantation led to a significant increase of physical activity in the Ob/Ob mouse (Figure 5E). Notably, fatty acid oxidation-related genes, *COX7a*, *CPT1b*, and *PPARa*, were up-regulated in the muscle of the BAT-transplanted group compared with the sham-operated group (Supplemental Figure 1A). The muscle fiber type, however, did not show any change between groups (Supplemental Figure 2A). It was previously reported that a sc leptin injection induces body weight reduction in Ob/Ob mice (22). We wondered whether BAT transplantation alters the plasma leptin level. Interestingly, there was no change in the circulating leptin level after BAT transplantation (Figure 5F). All together, these results indicated that BAT transplantation increased whole-body energy metabolism without alteration in energy intake.

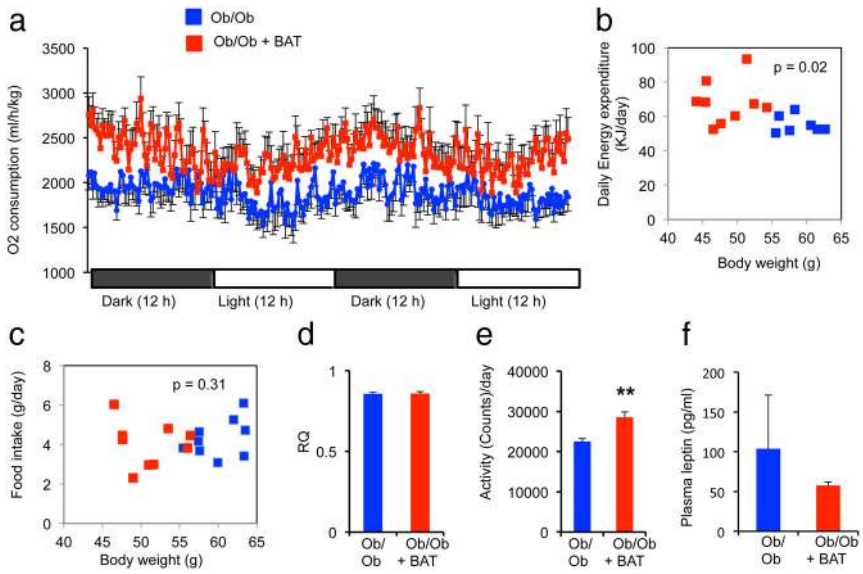

Figure 5. BAT transplantation increases whole-body energy expenditure. BAT transplantation in Ob/Ob mice results in increase of oxygen consumption (a), energy expenditure (b) and physical activity (e) without significant alterations in food intake, respiratory quotient, and plasma leptin level (c, d, and f). Data are mean ± SEM (n = 9–10/group). *, $P < .05$; **, $P < .01$; ***, $P < .001$.

BAT transplantation enhances endogenous BAT activity

We tested whether BAT transplantation might have a beneficial impact on thermogenesis. Core body temperature was significantly increased only when mice were challenged by cold (4°C for 4 h), not by a thermoneutral condition (Figure 6A). The gene expression levels of PGC1β and UCP1, which are mitochondrial biogenesis- and thermogenesis-related genes, were remarkably increased in endogenous BAT but were not elevated in either EP fat or sc fat after BAT transplantation (Figure 6B and Supplemental Figure 1, B and C). BAT could use large amounts of fatty acid to produce heat; therefore, we examined the expression of gene-related fatty acid

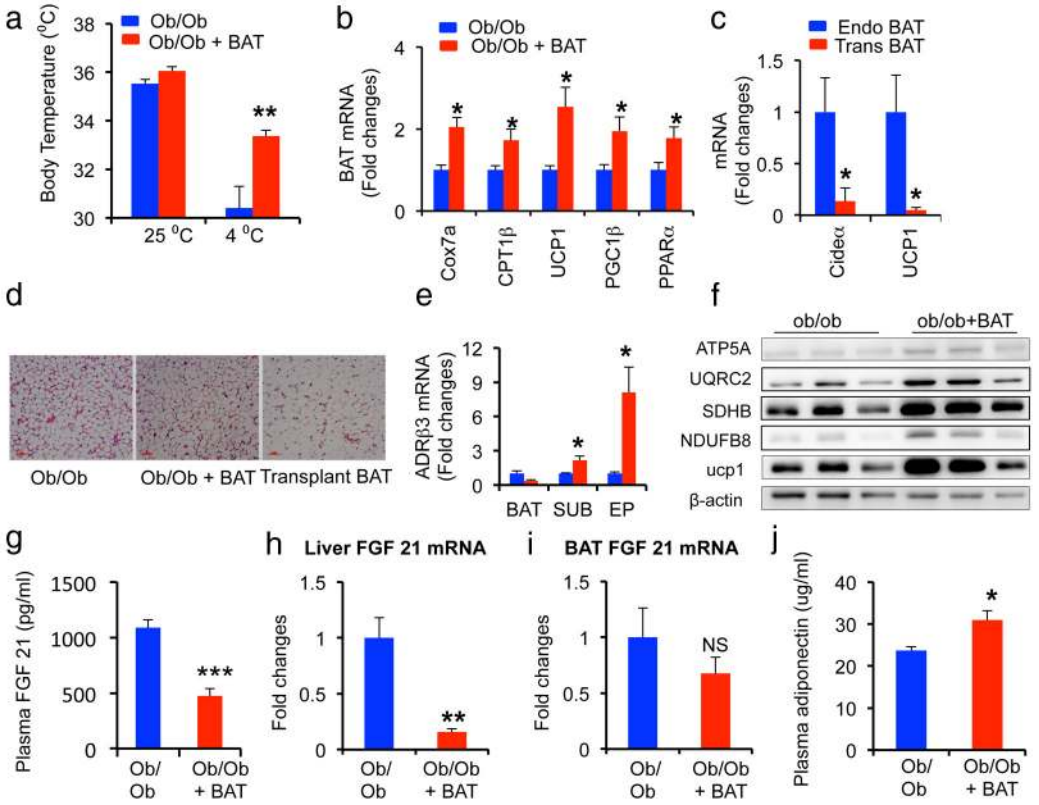

Figure 6. Endogenous BAT activity is enhanced after BAT transplantation. Ob/Ob mice with BAT transplantation increases core body temperature by cold challenge (a) and increases in fatty acid oxidation related gene expression in endogenous BAT (b). c, Compared with the endogenous BAT (endo-BAT), BAT-specific gene expressions were significantly reduced in trBAT. d, BAT histology data were shown. e, Significant increase of β3-adrenergic receptor expression in both sc and EP adipose tissue after BAT transplantation. f, BAT transplantation significantly increases mitochondrial oxphos protein and UCP1 protein expression in endogenous BAT. There are significant decreases in circulating FGF21 (g) and liver FGF21 mRNA expression (i); however, BAT FGF21 mRNA expression is not changed after BAT transplantation (h). j, There is a significant increase in the circulating adiponectin level. *, $P < .05$; **, $P < .01$; ***, $P < .001$.

metabolism in endogenous BAT. Notably, fatty acid metabolism-related genes such as *Cox7a*, *CPT1β*, and *PPARα* were significantly increased in endogenous BAT (Figure 6B) after BAT transplantation. In parallel, muscle fatty acid oxidation-related gene expressions were significantly increased after BAT transplantation as well (Supplemental Figure 1A). A previous study showed that Ob/Ob mice were generally resistant to the thyroid hormone responses (23). We did not observe significant alteration in the TSH level after BAT transplantation (Supplemental Table 1). Interestingly, circulating free T_3 and T_4 were significantly reduced after BAT transplantation (Supplemental Table 1). Consistently, the expression of the β3-adrenergic receptor was significantly increased in both sc and EP adipose tissue after BAT transplantation (Figure 6E). There, however, was no alteration in the expression of deiodinase 2 in sc tissue, EP tissue, and BAT (data not shown). The mRNA and protein expression of thyroid hormone were also comparable between endogenous BAT and trBAT (Supplemental Figure 3, A and B). These results suggest that BAT transplantation enhances the sensitivity of peripheral tissues to the thyroid hormone. It is well known that mitochondria are enriched in BAT. To test whether BAT transplantation has any effect on mitochondrial protein expression, the mitochondrial-specific oxphos proteins were quantified using Western blotting. Interestingly, the protein levels of ATP Synthase Alpha Chain, ubiquinol-cytochrome c reductase core protein II, succinate dehydrogenase complex, subunit B, NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, and UCP1were significantly increased in endogenous BAT after BAT transplantation (Figure 6E). Taken together, these results indicate that BAT transplantation significantly enhances endogenous BAT activity.

BAT transplantation increases circulating adiponectin levels

BAT might maintain glucose homeostasis through releasing fibroblast growth factor-21 (FGF21) stimulated by thermogenic activation (24). In contrast, the BAT-transplanted group showed significantly reduced circulating FGF21 levels compared with control mice (Figure 6F). Considering that BAT and liver are two major sources of circulating FGF21 (25), we examined FGF21 mRNA in

these two tissues. Unexpectedly, there was a significant reduction of FGF21 mRNA expression in the liver (Figure 6G) but not endogenous BAT after BAT transplantation (Figure 6, H and I). These data suggested that FGF21 is not a major regulator of the increased energy metabolism after BAT transplantation in the current study. Next we investigated whether these beneficial effects were mediated by implanted BAT, which could directly mobilize fat. At 12 weeks after BAT transplantation, trBAT was still morphologically intact and comparable with the endogenous BAT, but the size of the adipocytes became larger (Figure 6D). However, our quantitative real-time PCR results showed that key thermogenic gene *UCP1* and other BAT-specific genes such as Cidea expressions were dramatically reduced compared with that of endogenous BAT (Figure 6C). These results strongly indicate that trBAT lost some of its molecular characteristics. Therefore, the activation of endogenous BAT rather than the activity of the trBAT itself is more likely involved in whole-body energy metabolism.

Adiponectin, another major hormone secreted from adipose tissue (26), could enhance lipid oxidation and improve insulin action (27). We therefore investigated whether there is any change in the circulating adiponectin level after BAT transplantation. Interestingly, we found that serum adiponectin levels were significantly up-regulated after BAT transplantation (Figure 6J). However, the mRNA expression of adiponectin was not changed in all three adipose tissues as well as endogenous BAT and trBAT (Supplemental Figure 2, B and D). Adiponectin treatment of Ob/Ob mice increased thermogenesis and prompted weight loss and a reduction in serum glucose and lipid levels (28), which supports our results.

WAT transplantation is known to improve insulin sensitivity and obesity by increasing circulating leptin levels in Ob/Ob mice (29). To investigate whether the BAT transplantation increased circulating leptin, we measured circulating leptin between two groups. However, the circulating leptin level was not altered by BAT transplantation, even though there was no change in energy intake (Figure 5C and Supplemental Table 1).

Discussion

A series of studies revealed a negative relationship between BAT mass and body weight (8–10). As an endocrine organ, BAT could serve as a fascinating new potential therapeutic target for obesity and its related diseases (24). A scientific goal of the brown fat research field is to stimulate the activity of BAT and/or increase the amount of BAT for the prevention and treatment of obesity and obesity-related metabolic syndrome. Recently we have demonstrated that BAT transplantation has a beneficial effect on the prevention and treatment of obesity in the high-fat diet-induced obese mouse model (14). The next question we had was whether BAT transplantation might reverse Ob/Ob mice as well. To test this hypothesis, we performed BAT transplantation in the leptin-deficient obese mice model (Ob/Ob).

In the present study, we demonstrated that BAT transplantation ameliorated the body weight and body fat gain in Ob/Ob mice. This is the first study showing that BAT transplantation enhances the activity of endogenous BAT, eventually leading to the improvement of whole-body energy metabolism and glucose homeostasis. trBAT was morphologically comparable with the endogenous BAT at the end of the experiment (total 20 wk), but the size of the adipocytes tended to be larger than that of endogenous BAT. In addition, trBAT lost its multilocular lipid droplet morphology (Figure 6D), and BAT-specific gene expression was also reduced (Figure 6C). Consistent with our current results, Gunawardana et al also demonstrated that UCP1-positive staining was progressively lost in the trBAT during the experimental periods and was completely lost at the end of an experiment in trBAT (12). However, Stanford et al demonstrated that trBAT still actively uptakes glucose after 12 weeks (13). This difference may be caused by the location of transplantation (sc vs visceral cavity), the age of the mice, and the experimental period. In the current study, we applied different strategies from the previous study (13) in terms of location of transplantation (sc vs visceral cavity), the age of the donor and the recipients (6 wk vs 12 wk). Nonetheless, similar beneficial effects of the BAT transplant were observed in both studies. Therefore, we hypothesized that these beneficial effects might be from the activated endogenous BAT.

We discovered that there was a significant up-regulation of endogenous BAT activity after BAT transplantation, as evidenced by the improvement of the energy expenditure and thermogenic capacity together with an increase in fatty acid oxidation-related gene expression (Figure 6). On the other hand, the serum adiponectin level and β3-adrenergic receptor expression levels in both sc and EP fat were significantly increased after BAT transplantation. Increase of plasma adiponectin after BAT transplantation might enhance the activity of endogenous BAT to consume more TGs as consistent with a previous report (27, 28). To identify the role of adiponectin in the beneficial effects after BAT transplantation, further investigations should be performed. Therefore, we speculate that the activation of endogenous BAT (and WAT) rather than the metabolic activity of the trBAT itself might play the predominant role in this particular experimental set-

ting. In addition, the fact that trBAT touched sc fat and burned adjacent fat might explain the reduction of sc fat mass rather than epididymal fat mass after BAT transplantation (30).

In this study, we did not detect any difference in energy intake after BAT transplantation. In parallel, there was no change in circulating leptin levels. Thus, the improvement of energy metabolism was not simply dependent on circulating leptin. It is interesting to know whether these beneficial effects were mediated by a BAT-secreted adipokine. Increasing evidence suggests that BAT might serve as a secretory organ. Similar to WAT, BAT could synthesize and secret numerous hormones, such as FGF21, to regulate the whole-body energy metabolism (24). However, we observed that there was a significant reduction in circulating FGF21 after BAT transplantation (Figure 6G). trBAT from IL-6 knockout mice could not recapitulate the beneficial effects of the transplant of BAT from wild-type mice; therefore, it has been speculated that the beneficial effect of BAT transplantation is mediated by IL-6 secreted from BAT (13). On the other hand, Gunawardana and Piston (12) also demonstrated that diabetes-induced WAT inflammation was significantly reduced by BAT transplantation by lowering proinflammatory cytokines IL-6 and TNFα. In parallel, our result indicates that circulating IL-6 was not altered after BAT transplantation. These results suggest that there are additional inflammatory and/or other factors that might be involved in whole-body energy metabolism.

BAT transplantation into streptozotocin-induced type 1 diabetic mice completely reversed most of the diabetic symptoms without exogenous insulin treatment (12), and it reversed diet-induced obesity in the mouse model as well. These results highlight that BAT probably secretes adipokine(s) that may exert systemic effects on energy metabolism and those molecules might work through insulin-independent pathways. In the current study, BAT transplantation notably improved liver steatosis, which strongly supports the hypothesis for the systemic effect of BAT transplantation.

In conclusion, BAT transplantation has beneficial effects on obesity and diabetes; however, the underling mechanisms are poorly understood. The results of the current study show that transplantation of BAT reduced adiposity and improved glucose homeostasis in the Ob/Ob mouse by significantly increasing energy expenditure. These beneficial effects were most likely mediated by the enhancement of endogenous BAT activity. These results may open up a new avenue to develop a novel treatment option to target obesity and its related disease such as diabetes.

Acknowledgments

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Author contributions include the following: W.J. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. W.Z.J., X.M.L., S.P.W., H.J.L., J.S.R., and Y.L.Y. wrote the manuscript; MHM performed the histology, imaging studies and Western blot; Z.J.Z. and X.M.L. performed the energy metabolism assays and the gene expression analysis; M.D., J.L., Q.W.Z., C.H.Z., X.X.Y., T.H., and L.Q.L. performed the animal surgery and the GTT and ITT studies; and L.Z., D.H.W., and J.C.Z. contributed to the discussion and editing of the manuscript.

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