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 (trBAT) from C57/BL6 mice into the dorsal subcutaneous (SUB) 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, while it reduced the levels of circulating free triiodothyronine (T3) and thyroxine (T4) which regulate thyroid hormone sensitivity in peripheral tissues. BAT transplantation also increased β3 adrenergic receptor and fatty acid oxidation related gene expression in SUB 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.

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 subcutaneous WAT are different not only in anatomical location, but also entirely different in physiological role. For example, size of SUB adipocyte is 24% larger than visceral adipocyte (5); and there are more mitochondria in SUB fat than in visceral fat (6). Multiple lines of evidences indicate that SUB fat has a beneficial effect on metabolism compared with visceral fat which has detrimental effect (7).

Recently, several landmark studies fundamentally altered our understanding of adult human BAT. In those studies, positron-emission tomography (PET)–computed tomography (CT) using radiotracers such as ^{18}F-fluorodeoxyglucose (^{18}F-FDG) indicated the presence of BAT

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Abbreviations:

and its relevance for BMI in adult humans (8–10). Thus, it may suggested that weight loss could be achieved by increasing energy expenditure through activating BAT (11). Additionally, trBAT on the type I diabetes mice reversed the clinical symptoms of type I 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 (HFD) induced obesity and pre-existing 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, since they have several metabolic phenotypes including hyperphagia, glucose intolerance and adipocyte hyperplasia. These mice have 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 weeks old C57B/L6 male mice into the dorsal SUB 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 Ob/Ob mice mouse model.

Materials and Methods

Mice

Six weeks old male C57BL/6J donor mice were purchased from Vital River Laboratory Animal Technology. Co. Ltd, (Beijing, China). Recipient B6, V-Lepob/NJU mice from Nanjing Biomedical Research Institute of Nanjing University were used for transplantation. Mice were housed 4 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 intra-scapular region of 6 weeks old C57BL/6J donor mouse and implanted into the dorsal SUB region of recipient B6, V-Lepob/NJU mice. After cervical dislocation of donor mice, BAT was removed and peripheral white fat was excluded, then the remaining BAT (0.2g) washed with sterile PBS and transplanted into the dorsal subcutaneous region of recipients as quickly as possible. Recipient mice were anesthetized by intra-peritoneal injection with 400 mg/kg body weight avertin, then BAT was transplanted underneath of skin. For the control group, a sham operation was performed with same procedure.

Gene expression analysis

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

Metabolic assessment

For glucose tolerance tests (GTT), animals were fasted for 16 hours (17:00–9:00) with free access to drinking water. Blood glucose levels were determined by using an Accu-Chek glucose monitor (Roche Diagnostics Corp, Pleasanton, CA, USA) immediately before, 15, 30, 60 and 120 minutes after intra-peritoneal glucose injection (1.2g/kg). For insulin tolerance test (ITT), mice were fasted for 4 hours (9:00–13:00) and intra-peritoneal injection with human insulin (0.8 U/kg Humulin R). Blood glucose levels were determined immediately before, 15, 30, 45 and 60 minutes after insulin injection.

Energy intake, digested energy and total movement measurement

Mice were housed one animal per cage, and free access to food and water. Food intake and oxygen consumption were measured for consecutive 3 days after 2 days of acclimation using a TSE lab master system (Chesterfield, MO, USA) 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, Columbus, OH, USA) over 24 hours and expressed as the 24-hour average activity. Rectal temperature was measured before and after 4 hrs cold exposure with thermometer (Shenzhen zhongyidapeng, AT210).

Body composition analysis

After all mice were anesthetized by injection of Avertin intraperitoneally, whole body fat mass was measured using non-radiotracer computerized tomography (Hitachi Aloka Latheta LCT-200, Tokyo, Japan) according to manufacturer's instruction.

Western blot

Tissues were dissolved in RIPA buffer (150 mM sodium chloride, 1.0% TritonX-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mMTris, protease and phosphatase inhibitor cocktail (Roche Diagnostics Corp, Pleasanton, CA, USA)). Protein concentrations were determined using a BCA assay kit (Pierce Diagnostics Corp, Pleasanton, CA, USA). Protein was separated by 10% SDS-PAGE, transferred to PVDF membrane (Millipore Billerica, MA, USA), blocked in 5% skim milk in TBST (0.02M Tris base, 0.14M Nacl, 0.1% Tween 20, PH 7.4), incubated with primary antibodies for overnight at 4°C and then incubated with secondary antibodies conjugated with HRP. Primary antibodies used in this study are phosphor Ser473, total Akt, phosphor Erk Thr202/Tyr204, Erk (Cell Signaling Technology, Danvers, MA, USA), UCP1, OXPHOS (Abcam, co, Cambridge, MA, USA) and β-actin (Sigma Chemical Co, St. Louis, MO, USA). Signals were detected with Super Signal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA).

Tissue staining and analysis of adipocyte size

Tissues were fixed in4% paraformaldehyde overnight at room temperature and then embedded in paraffin. Sections of 5 μm thickness were stained with hematoxylin and eosin (H&E) then images were taken by microscope (DS-RI1, Nikkon, JP). The standard streptavidin-biotin-peroxides immunostaining procedure was used to the detection of tyrosine hydroxylase (TH). Tissues specimens were blocked with 10% normal goat serum for 30 minutes and then incubated with TH antibody (Pel FreeZ, P40101–150) overnight at 4°C, followed by a 1 hour incubation at room temperature with HRP-conjugated goat antirabbit IgG. To quantify the size of adipocyte, more than five sections were taken from each mouse fat pad then the area of 500 to 600 cells from five fields of each section were measured. Each field was separated at a certain distance to avoid repeated cell measurement with double-blind manner.

TSH and Leptin measurements

Plasma TSH and Leptin were measured using ELISA kits (NanJing JianCheng Bioengineering Institute, NanJing, China), 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, ANCOVA 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 0.2g) was taken from C57BL/6J male mouse and then subcutaneously implanted to the sex and age matched Ob/Ob mice. Since we previously found that subcutaneous transplant either EP fat tissue or muscle did not improve a recipient's metabolic phenotype under high fat diet feeding (14), 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 ± 2.2g vs 50.6 ± 3.1g, $P < .03$) after BAT transplantation and the biggest difference was found at 12 weeks after transplantation (51.6 ± 3.7g vs 60.3 ± 3.9g, $P < .001$). The body composition was analyzed by computerized tomography. The percentage of whole body fat of BAT transplanted mice decreased 11% compared with that of the sham-operated mice (Figure 1b-c). In parallel, the weight of SUB 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 white adipose tissues between groups. Compared with control mice, there was a significant reduction of adipocyte size in SUB fat (Figure 2a-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 circulating IL-6 levels or IL-6 mRNA in EP fat in our study (Figure 2d-e). These result highlight that BAT transplantation blunted adipose tissue hypertrophy without alteration of adipose tissue inflammation.

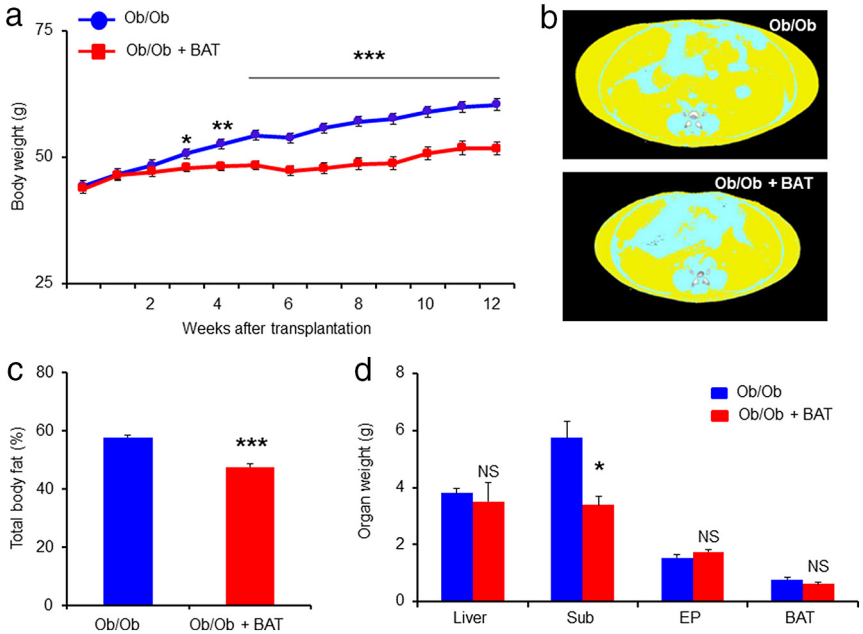

Figure 1. BAT transplantation inhibits body weight gain To test whether BAT has beneficial effect on genetic obesity, BAT transplantation was performed on Ob/Ob mice. Results show that BAT transplantation could a) significantly inhibit body weight gain, b-c) decrease total body fat mass and d) significantly reduce SUB fat mass in Ob/Ob mice. Data are mean ± SEM. n = 9–10/group. *$P < .05$, ** $P < .01$, *** $P < .001$.

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 (Fig-

ure 3a lower panel). In parallel, the expression levels of PPARγ2 and TNFα in liver were significantly down-regulated after BAT transplantation (Figure 3b). Interestingly, PGC1α 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, PPARα (Figure 3b). SREBP1c mRNA expression and hepatic TG contents were significantly decreased in BAT transplanted mouse liver (Figure 3c-d). In parallel, circulating triglycerides (TG), cholesterol (CHO), low-density lipoprotein (LDL) levels were significant decreased after BAT transplantation (Supplemental Table 1). These results demonstrated that BAT transplantation significantly improved hepatic steatosis in Ob/Ob mice.

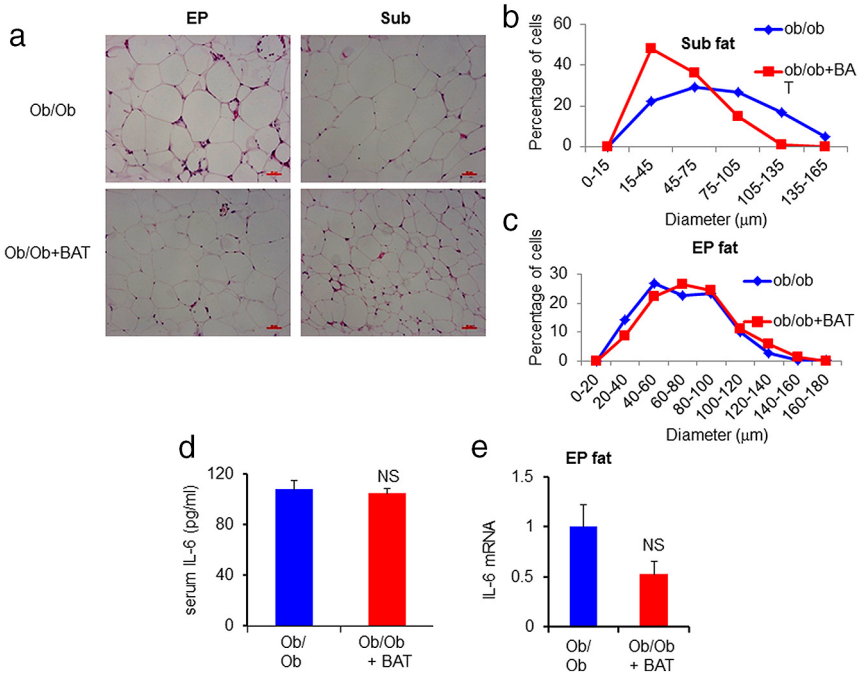

Figure 2. BAT transplantation decreases the adipose tissue hypertrophy. a-c) decreases the size of the adipocyte in EP fat, whereas no changes in d-e) circulating IL-6 and EP fat IL-6 mRNA level after BAT transplantation. There is no significant change in e) IL-6 mRNA in EP fat. Data are mean ± SEM. n = 9–10/group. * $P < .05$, ** $P < .01$, *** $P < .001$.

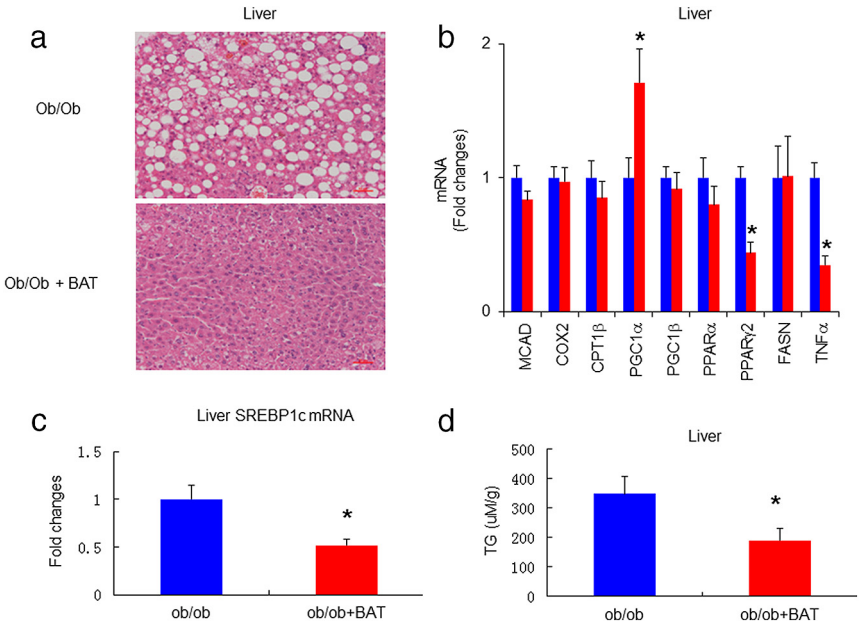

Figure 3. Hepatic steatosisis reversed post BAT transplantation BAT transplantation could a) totally reverse hepatic steatosis, b) significantly decrease the liver gene expression of pparγ2 and TNFα and increase PGC1α expression, c-d) significantly decrease the SREBP1c mRNA expression and TG level in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

BAT transplantation improves insulin sensitivity

The amelioration of plasma lipid profiles after BAT transplantation, suggest that transplant BAT might affect whole body insulin sensitivity. To test whether BAT transplantation improved glucose metabolism, a glucose tolerance test (GTT) was performed. BAT transplantation markedly improved insulin sensitivity (Figure 4a-b). Insulin tolerance tests (ITTs) and the value of area under the curve (AUC) further supported the significant improvement of insulin sensitivity after BAT transplantation (Figure 4c-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 if BAT transplantation increased energy expenditure in Ob/Ob mice, we assessed whole body energy metabolism by using indirect calorimetry (TSE labmaster system). Oxygen consumption was significantly increased (Fig-

ure 5a-b) in the BAT transplanted group, whereas energy intake was unaltered post BAT transplantation (Figure 5c). In addition, there was no change in respiratory quotient (RQ, Supplemental data Figure 2c), which indicates the flexibility of body to switch 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 ob/ob mouse (Figure 5e). Notably, fatty acid oxidation related genes, COX7a, CPT1b and PPARa, were upregulated in muscle of BAT transplanted group compare with 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 subcutaneous leptin injection induces body weight reduction in Ob/Ob mice (22). We wonder whether BAT transplantation alters plasma leptin level. Interestingly, there was no change in 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.

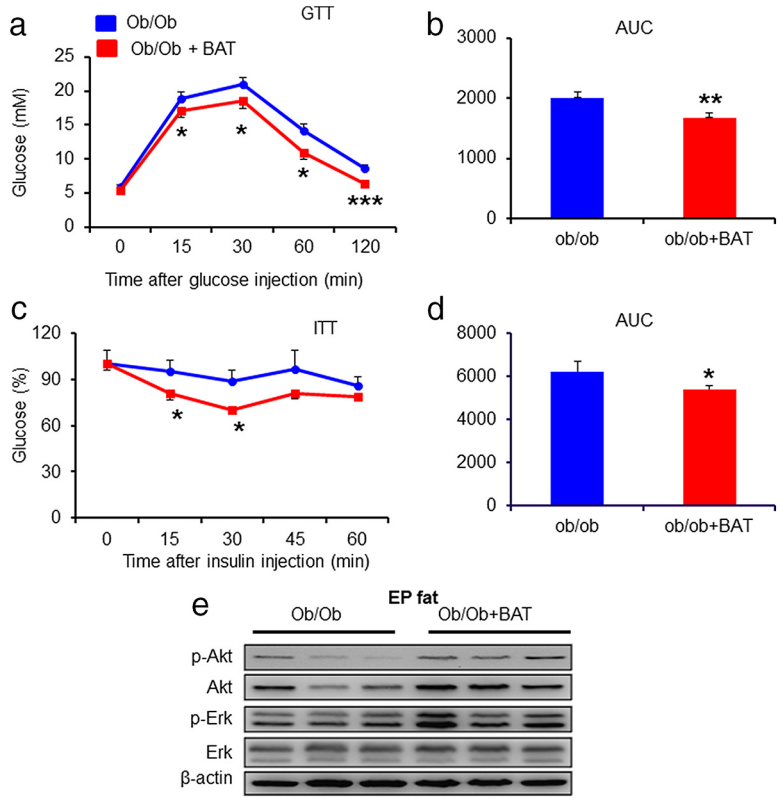

Figure 4. BAT transplantation improves insulin sensitivity in Ob/Ob mice Ob/Ob mice with BAT transplantation improves insulin sensitivity as evidenced by a) glucose tolerance test (GTT), b) the AUC of GTT, c) insulin tolerance test (ITT), d) AUC of ITT, e) and improves AKT phosphorylation in EP adipose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

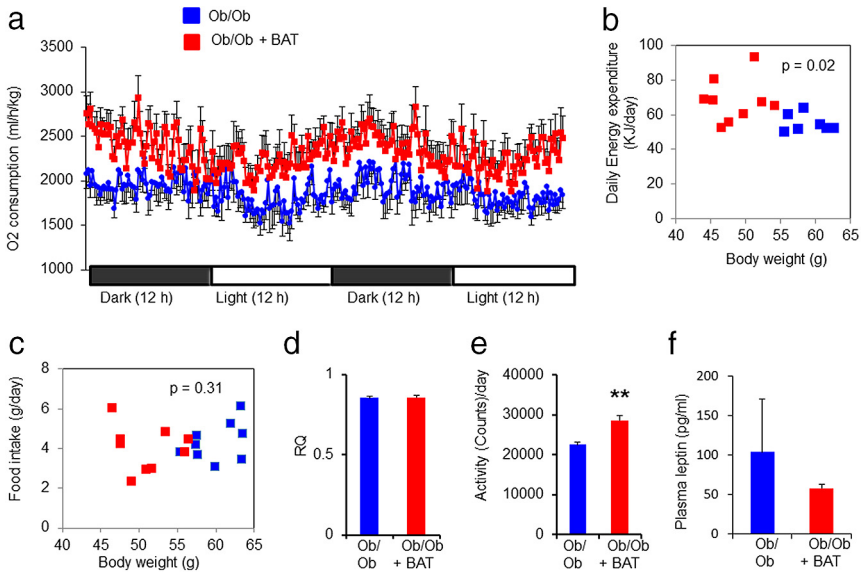

Figure 5. BAT transplantation increases whole-body energy expenditure BAT transplantation in Ob/Ob mice results in increase of a) oxygen consumption, b) energy expenditure and e) physical activity, c-d, f) without significant alterations in food intake, RQ and plasma leptin level. Data are mean ± SEM.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

BAT transplantation enhances endogenous BAT activity

We tested whether BAT transplantation might have beneficial impact on thermogenesis. Core body temperature was significant increased only when mice were challenged by cold (4°C for 4 hours), not by thermo-neutral 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 not elevated in either EP fat or SUB fat after BAT transplantation (Figure 6b and Supplemental Figure 1b-c). BAT could utilize large amounts of fatty acid to produce heat, therefore we examined the expression of genes related fatty acid 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). Previous study showed that Ob/Ob mice were generally resistant to the thyroid hormone responses (23). We did not observed significant alteration in thyroid stimulating hormone (TSH) level after BAT transplantation (Supplemental Table 1). Interestingly, circulating free T3 and T4 were significantly reduced after BAT transplantation (Supplemental Table 1). Consistently, the expression of beta 3 adrenergic receptor was significantly increased both in both SUB and EP adipose tissue after BAT transplantation (Figure 6e). There, however, was no alteration in the expression of deiodinase 2 (DIO2) in SUB, EP and BAT (data not shown). The mRNA and protein expression of TH were also comparable between endogenous BAT and trBAT (Supplemental Figure 3 a-b). These results suggest that BAT transplantation enhances 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 ATP5A, UQCRC2, SDHB, NDUFB8 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 increase circulating adiponectin levels

BAT might maintain glucose homeostasis through releasing 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 FGF 21 mRNA expression in the liver (Figure 6g), but not endogenous BAT

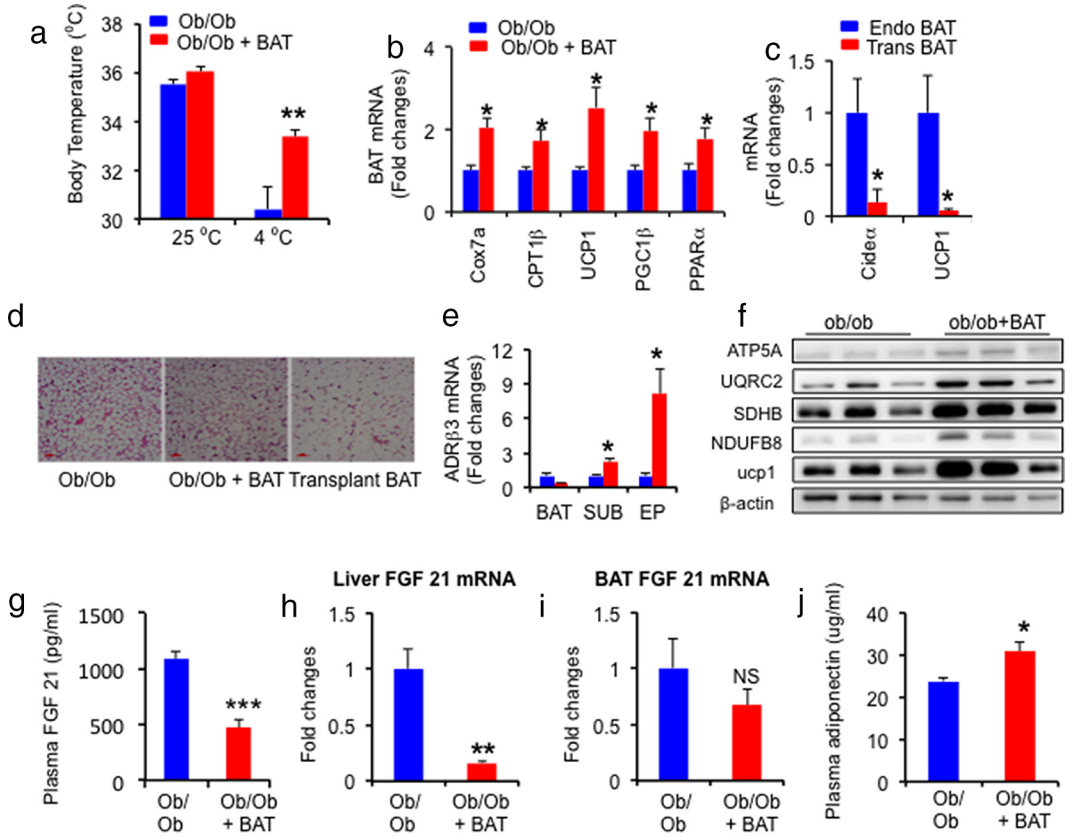

Figure 6. Endogenous BAT activity is enhanced post BAT transplantation Ob/Ob mice with BAT transplantation a) increases core body temperature by cold challenge, b) increases in fatty acid oxidation related gene expression in endogenous BAT. c) Compared with the endogenous BAT (endo-BAT), BAT specific gene expressions were significantly reduced in transplanted BAT (trBAT). d) BAT histology data were shown. e) Significant increase of beta 3 adrenergic receptor expression in both SUB 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 g) circulating FGF-21 and i) liver FGF-21 mRNA expression, however, h) BAT FGF-21 mRNA expression is not changed after BAT transplantation. j) There is significant increase in circulating adiponectin level. * $P < .05$, ** $P < .01$, *** $P < .001$.

after BAT transplantation (Figure 6h-i). These data suggested that FGF21 is not a major regulator of the increased energy metabolism after BAT transplantation in 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 to the endogenous BAT, but size of 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 in endogenous BAT (Figure 6c). These results strongly indicate that trBAT lost some of its molecular characteristics. Therefore, activation of endogenous BAT rather than 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 if there is any change in circulating adiponectin level after BAT transplantation. Interestingly we found that serum adiponectin levels were significantly up-regulated after BAT transplantation (Figure 6j). However, mRAN expression of adiponectin was not changed in all three adipose tissues as well as endoBAT and tranBAT (Supplemental Figure 2b and 2d). Adiponectin treatment to Ob/Ob mice increased thermogenesis, prompted weight loss and reduction in serum glucose and lipid levels (28), which supports our results.

White adipose tissue transplantation is known to improve insulin sensitivity and obesity by increasing circulating leptin levels in ob/ob mice (29). To investigate that whether the BAT transplantation increased circulating leptin, we measured circulating leptin between two groups. However, 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 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 HFD-induced obese mouse model (14). 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 to the endogenous BAT at the end of experiment (total 20 weeks), 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 (18) also demonstrated that UCP1 positive staining was progressively lost in the trBAT during experimental periods, and completely lost at the end of an experiment in trBAT (12). However, Stanford et al (19) demonstrated that trBAT still actively uptake glucose after 12 weeks (13). This difference may be caused by the location of transplantation (subcutaneous vs visceral cavity), age of mice and experimental period. In current study, we applied different strategies from the previous study (13) in terms of location of transplantation (subcutaneous vs visceral cavity), age of donor and recipients (6 weeks vs 12 weeks). Nonetheless, similar beneficial effects of BAT transplant were observed in both studies. Therefore, we hypothesized that these beneficial effects might be from activated endogenous BAT.

We discovered that there was significant up-regulation of endogenous BAT activity after BAT transplantation, as evidenced by improvement of the energy expenditure and thermogenic capacity together with increase in fatty acid oxidation related gene expression (Figure 6). On the other hand, serum adiponectin level and beta 3 adrenergic receptor expression levels in both SUB and EP fat were significantly increased after BAT transplantation. Increase of plasma adiponectin after BAT transplantation might enhance activity of endogenous BAT to consume more triglyceride as consistent with previous report (27, 28). To identify the role of adiponectin in the beneficial effects after BAT transplantation, further investigations should be needed. Therefore, we speculate that activation of endogenous BAT (and WAT) rather than metabolic activity of the trBAT itself might play the predominant role in this particular experimental setting. In addition, trBAT touched subcutaneous fat and burned adjacent fat might explain the reduction of subcutaneous fat mass rather than epididymal fat mass after BAT transplantation (30).

In this study, we could 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 white adipose tissue, BAT could synthesize and secret numerous hormones, such as FGF21, to regulate the whole body energy metabolism (24). However, we observed that there was significant reduction in circulating FGF21 after BAT transplantation (Figure 6g). TrBAT from IL-6 KO mice could not recapitulate the beneficial effects of 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 et al also demonstrated that diabetes induced WAT inflammation was significantly reduced by BAT transplantation by lowering proinflammatory cytokines IL-6 and TNFα (12). 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 (STZ) induced type I diabetic mice completely reversed most of diabetic symptoms without exogenous insulin treatment (12) and it reversed diet-induced obesity in 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 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.

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Supplementary information is available at the journal's website.

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