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ORIGINAL ARTICLE

Brown adipose tissue transplantation ameliorates male fertility impairment caused by diet-induced obesity

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Male fertility

Summary Populations with obesity or overweight have a high incidence of infertility. We hypothesised that brown adipose tissue (BAT) transplantation can attenuate the impairment of male fertility caused by diet-induced obesity. BATs were transplanted from male donor mice into age and sex matched recipient mice fed high-fat diets (HFD). Sperm motility experiment was conducted after surgical procedure. X-ray computed tomography scanning, biochemical assay, real-time PCR and western blot analysis were performed. BAT transplantation reduced body fat and epididymal fat mass, as well as triglycerides (TG) content in testis and epididymis and total cholesterol (TCHO) contents in epididymis compared with the HFD group. Sperm motility and progressiveness were recovered and mRNA and protein levels of genes related to sperm motility such as cullin 3 (Cul3), peroxisome proliferator activated receptor alpha (PPARα) and its down-stream genes were significantly down-regulated post BAT transplantation. BAT transplantation partially ameliorated impairment of male fertility caused by diet-induced obesity.

Introduction

Obesity has increased dramatically worldwide over the past 20—30 years. According to the report from WHO, approximately 1.6 billion adults were

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classed as being overweight and 400 million adults were obese in worldwide in 2005 [1]. Statisticians had predicted that, by 2015, approximately 2.3 billion adults would be overweight and 700 million would be obese [1]. The worldwide epidemic of obesity has shown numerous negative effects on health; it has increased the risk of developing chronic diseases such as diabetes mellitus, hypertension, cardiovascular diseases and stroke [2]. Until recently, scientists begin to realise that populations with obesity or overweight exhibit a high incidence of infertility [3—7]. There are some reports indicating that paternal obesity may negatively affect basic sperm parameters such as concentration and motility [7—9]. However, the relationship between obesity and male fertility need to be further elucidated.

Recently, studies found that brown adipose tissue (BAT) transplantation can improve whole-body energy metabolism, regulates glucose homeostasis and insulin sensitivity [10—12]. BAT is a mitochondrial rich tissue that uses glucose and fatty acids as a fuel rather than the traditional adipose tissue as a lipid storage tissue. BAT regulates thermogenesis upon environmental stresses to maintain energy balance and protect the organism from hypothermia [13]. BAT thermogenesis is achieved by dissipating heat produced from fatty acid oxidation. Weight loss can be achieved by increasing energy expenditure through activating BAT [14]. The previous study has shown that BAT transplantation can regulate the whole-body energy metabolism, significantly decreased body weight and improved glucose metabolism and insulin sensitivity, in both normal diet-fed and high fat diet-fed mice [15]. In this study, we aim to investigate whether BAT transplantation can also affect the male fertility by diet-induced obesity.

Materials and methods

Animals breeding and diet

Three weeks old male C57BL/6J mice (Vital River Laboratories, Beijing, China) were maintained in a SPF grade facility, on a standard 12-h light/12-h dark cycle. Twenty four mice were randomly divided into two groups according to the original body weight using the software SPSS 17.0 (SPSS, Inc., Chicago, IL). Normal diet group (ND) as control ($n = 8$) were fed with a standard mouse diet (10% kcal from fat) (Research Diets Inc.), high fat diet group ($n = 16$) were fed with a high-fat diet (60% kcal from fat) (Research Diets Inc.). Body weights of mice fed with a high-fat diet for 8 weeks were increased by 24% compared to ND group. Then, these obese mice were equally divided into two subgroups, one group for brown adipose tissue transplantation (BAT + HFD) and another group for HFD sham group (HFD). Two subgroups continued to feed with high-fat diet after operation until the end of experiment. All animal care and experimentation were conducted in accordance with the National Research Council publication Guide for Care and Use of Laboratory Animals [16].

Isolation of donor tissue and transplantation

The transplanted brown adipose tissue (BAT) was obtained from intrascapular region from the donor mice sacrificed by cervical dislocation, and rinsed in pre-warmed 37 °C PBS before transplantation. All the operations should be manipulated in a sterile laminar flow hood. Transplantation surgeries were performed under general anesthesia with avertin (250 mg/kg i.p.). For age and sex matched recipient mouse, about 0.15 g donor BAT from intrascapular region was transplanted into 8 week old C57BL/6J mice, respectively. A small (1—2 mm) incision were made by scissors underneath the skin of the dorsal body surface, a subcutaneous pocket was made by blunt tweezers. Donor tissue was introduced into the pocket with Dumont forceps and the incision was closed by wound closure. The others mice were operated a sham operation. The post-operation mice were warmed up under a warming lamp until recovery, and post-operation anti-inflammation was provided with sodium penicillin (100,000 U/kg/d i.m.) for about one week. After the operation, all of mice were bred with corresponding diet for each group for another 14 weeks.

X-ray computed tomography scanning

After 14 weeks, the body fat composition was measured by an X-ray computed tomography (CT) system for small experimental animals with a mouse mode (Latheta LCT-200, Hitachi Aloka Medical, Ltd., Tokyo, Japan). The visceral and subcutaneous fat volumes computed automatically were compared with those after the radiologist's adjustments. Energy from −140.0 to 350.0 was defined as lean, and from −550.0 to 140.0 was defined as fat.

Sperm motility and progressive analysis

After X-ray CT scanning, the males were sacrificed for sperm motility and progressive analysis and samples collection for biochemistry analysis, real time PCR and western blot. The parameters of sperm motility and progressive were measured by computer assisted sperm analysis (CASA) system

(Version.12 CEROS, Hamilton Thorne Research), as described elsewhere [17]. Semen from the caudal epididymis were incubated in 1.0 ml of 0.9% NaCl solution at 37 °C for 15 min and loaded into CASA assay chambers (Hamilton Thorne Research, Beverly, MA). The settings of the instrument are as follows: for sperm cell detection: minimal contrast, 50; minimal cell size, 4 pixels; and 60 frames were acquired at a frame rate of 60 Hz. At least 200 tracks were measured for each specimen at 37 °C with a Slide Warmer (#720230, Hamilton Thorne Research).

Histological sections studies

The brown adipose tissues were fixed overnight at 4 °C in 4% (w/v) PFA (paraformaldehyde) in PBS (phosphate-buffered saline) (pH 7.3–7.4). Then, they were embedded in paraffin, sectioned at 5 μm, and stained with HE (hematoxylin and eosin) for light-microscopic examination.

Biochemical assays

Triglycerides (TG) and total cholesterol (TCHO) in testis and epididymis were measured with a commercial TG kit and a TCHO kit (Applygen Technologies Inc., Beijing, China). TG and TCHO was quantified by absorption (550 nm), using a Gen5 Microplate reader (BioTek® Instruments, Inc., USA).

Transcriptional levels of genes related sperm motility in testis

Total RNA were isolated from left testis collected from individual mouse each group, using Trizol reagent (ambion, Life technologies, USA) according to the manufactures' instructions. The concentration and purity were measured by NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA). 2 μg of total RNA was used for subsequent 20 μL system reverse transcription reaction. The reaction systems are as follows: 2 μg total RNA, 2 μL dNTPs (2.5 mM), 0.2 μL (500 μg/μL) Oligo-dT (18 mer), adds up RNAse free water to 10 μL. The procedure is 70 °C for 5 min, and holds at 4 °C. Then add 10 μL mixture of 4 μL 5× M-MLV buffer, 0.5 μL (200 U/μL) M-MLV reverse transcriptase (Promega, USA), 0.5 μL (40 U/μL) RNAse inhibitor (Promega, USA) and 5 μL RNAse free water. The procedure is 42 °C for 60 min, 95 °C for 5 min and finished by hold at 4 °C.

Quantitative real-time PCRs were performed on the LightCycler®480 qPCR system (Roche Diagnostics GmbH, Switzerland) using SYBR Green Real Master Mix without Rox (Tiangen, China). Primers for 21 genes involved the sperm motility (supplementary information Table S1) are designed to investigate mRNA expression variations. The housekeeping gene Cyclophilin A was used as an internal control. The real-time PCR amplification procedure was pre-denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s and elongation at 72 °C for 20 s. Before finished, melting curve analysis was performed to verify the specificity of the amplification, which was displayed as a single peak. The relative quantification of target genes was calculated based on $2^{-\Delta\Delta CT}$ method [18,19]. The equation is as the following form:

$$2^{-\Delta\Delta CT} = [(C_T \text{ gene of interest} - C_T \text{ gene of internal control}) \text{ sample A} - (C_T \text{ gene of interest} - C_T \text{ gene of internal control}) \text{ sample B}]$$

Cyclophilin A was previously validated as a suitable internal control in the conditions of this study [20].

Western blot

Frozen testis tissue samples were homogenised using a super fine homogeniser (Fluko, Germany) for 1–2 min on ice in RIPA (Thermo scientific, USA) containing 1% PMSF (Sigma–Aldrich, USA) and 1% phosphatase inhibitor (Sigma–Aldrich, USA). The protein concentration was determined by using a BCA kit (Pierce, Thermo scientific, USA). Total protein were mixed with 5× loading buffer and denatured by boiling for 5–10 min, then cooled on ice immediately. Approximately 40 μg of total protein was loaded on 10% sodium dodecyl sulfate (SDS)-polyacrylamide-gels and then transferred to polyvinylidene fluoride (PVDF) membranes (Amersham Biosciences, Piscataway, NJ). The blotted membranes were blocked for 1 h in TBS containing 0.1% Tween-20 (TBST) and 5% skim milk powder and then incubated with primary antibody anti-Cul3 (Abcam, UK) and anti-Pparα (Abcam, UK), respectively, dissolved by TBST at a dilution of 1:1000, overnight at 4 °C. After washing with TBST, the membranes were then incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G as the secondary antibody for 1 h at room temperature. The immunoreactive bands were detected with an ECL reagent (Tiangen, China) on the Image Quant LAS 4000 instrument (GE, USA).

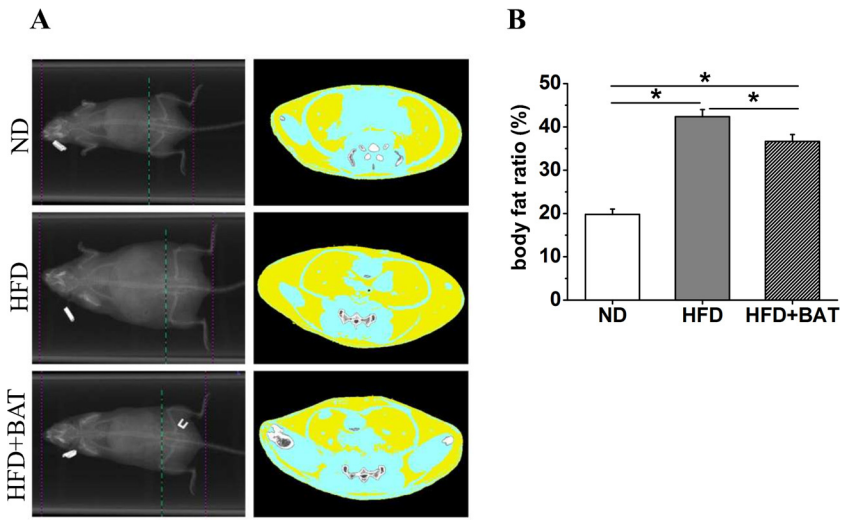

Figure 1 X-ray CT scanning of the ND, HFD and HB groups. (A) Representative images in X-ray CT scanning of the whole bodies of mice. For body fat ratio measurements, tomographic images were acquired at 1.5-mm intervals in the measurement area presented as two red lines. The right part of panel A is cross-sectional appearance of mice in the ND, HFD and HB groups. Tomographic X-ray CT images of the same quartered lumbar vertebral regions shown with a green line in the left part of panel A. The area indicated in yellow is fat, cyan is lean. *Note*: ND: sham-operated mice fed with normal diet, HFD: sham-operated mice fed with high fat diet, HB: brown adipose tissue transplanted mice fed with high fat diet. (B) The percentage of body fat ratio of ND, HFD and HB groups' mice were analyzed and recorded by an X-ray CT scanning. * $p < 0.05$ (ND vs HFD); # $p < 0.05$ (HB vs HFD). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Statistical analysis

Data were analyzed using SPSS for Windows 17.0 Software (SPSS, Inc., Chicago, IL) and presented as means with standard errors (mean ± SE). Differences between the control and the treatment groups were determined using *t*-tests. A *p* value of <0.05 was considered statistically significant. The graphs were developed by the software Origin 8.5.0 SR1 (Origin Lab Corporation, USA).

Results

The effect of BAT transplantation on body fat composition

To investigate whether the transplanted BATs are still alive after transplantation surgery, histological sections of BATs were analyzed by HE staining. Results showed that transplanted BATs are still well shaped (supplementary information Figure S1). In addition, in the previous study, the BAT-specific gene, such as uncoupling protein 1 (UCP1), PR domain containing 16 (PRDM16) and cell death-inducing DFFA-like effector a (CIDEA) were also detected by real time PCR in the transplanted BAT, which further validates its effect [15]. Among which, one transplantation surgery found failure due to the tissue necrosis, was excluded from next experiment.

A significant difference in body weight was observed in male C57BL/6J mice consuming the high-fat diet compared with fed with normal diet group, meanwhile, BAT transplanted mice did not show significant body weight gain, and the mean body weight was significantly lower than the littermates consuming the same high-fat diet [15].

Body fat ratios were 19.8%, 42.4%, and 36.6% for ND, HFD, and HFD + BAT, respectively. It was significantly higher in HFD groups compared with ND group, while decrease in HFD + BAT group (Fig. 1).

Sperm motility assayed by CASA system

We next analyzed the sperm motility of male mice in each group. Compared with ND groups, HFD group mice exhibited significant decrease in motile and progressive sperms. HFD group males exhibited a 69% decrease in sperm motility, and 82% decrease in progressiveness (Fig. 2A), an indicator of sperm viability and potential for fertilisation. In contrast, sperm collected from the HFD + BAT group showed 83% improvement in motility and 86% of progressive movement, which implied the attenuation effect of BAT transplantation on male fertility.

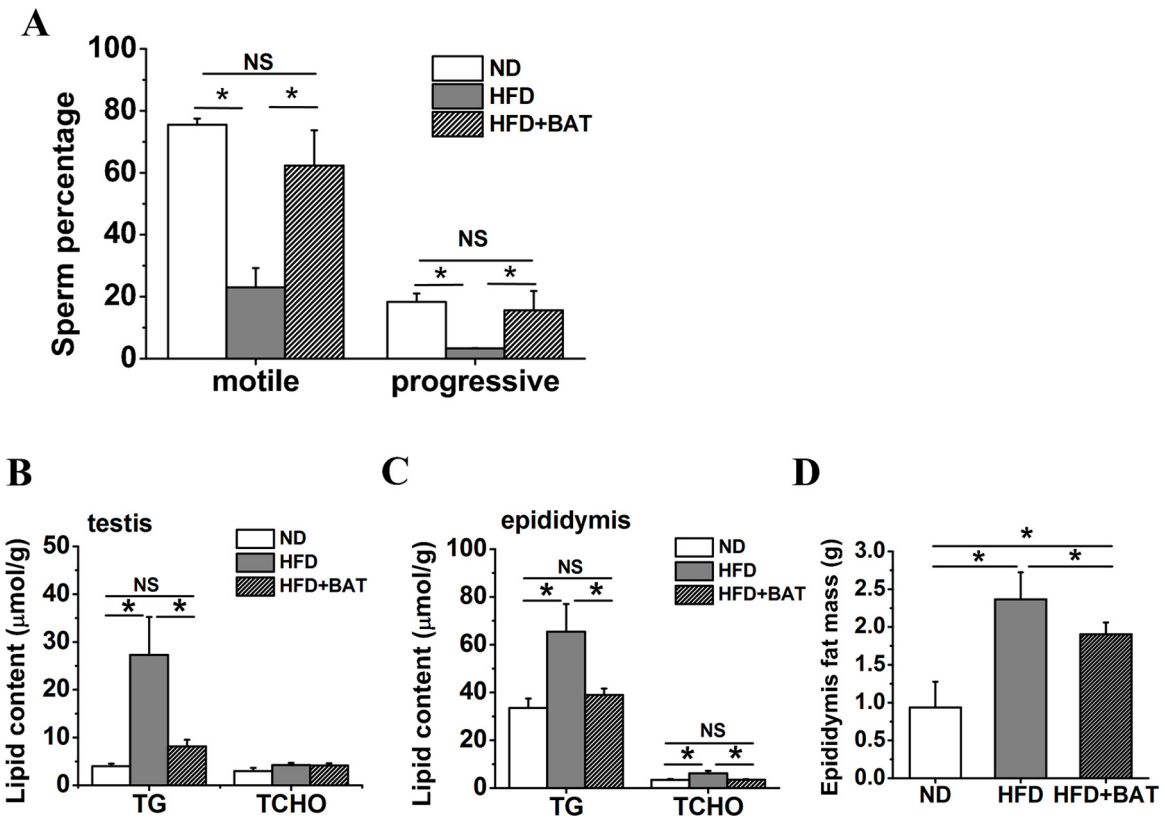

Figure 2 Sperm motility parameters of ND, HFD and HFD + BAT mice were analyzed. (A) All data represented were obtained 10 min after release into NaCl solution. *Note*: motile: total motile sperm; progressive: progressively motile sperm; (B, C) Triglycerides (TG) and total cholesterol (TCHO) levels in epididymis and testis from ND, HFD and HFD + BAT mice. Values are means ± SEM ($n = 7—8$ mice/group). (D) Mass of epididymal fat from HFD group increased significantly compared with ND group, while HFD + BAT group showed a significant decrease compared with HFD group. * $p < 0.05$; NS: non-significant.

TG and TCHO levels

The concentration of total triglycerides (TG) and total cholesterol (TCHO) in testis and epididymis show that excessive lipid was accumulated in the testis and epididymis of HFD group compared with ND and HB group (Fig. 2B and C). Also, the mass of epididymal fat from HFD group increased significantly compared with ND group, while HB group showed significant decrease compared with HFD group (Fig. 2D).

Transcriptional levels of testicular genes related male fertility and western blot analysis

We surveyed the transcriptional levels of 21 genes related male fertility in testis after BAT transplantation. Cullin 3 (Cul3) and laminin alpha 5 (Lama5) were found significantly upregulated in HFD group compared with ND and HFD + BAT groups (Fig. 3A). In addition, Cul3 protein expression increased 3.02-fold ($p < 0.05$) in HFD group mice compared with ND group (Fig. 3B). In contrast, the transcriptional and protein levels of Clu3 in the testis of HFD + BAT group were not significant change compared with ND group.

We next surveyed the transcriptional levels of 39 genes involved in lipid homeostasis such as β-oxidation in testis after BAT transplantation. The transcriptional expressed level of peroxisome proliferator activated receptor alpha (PPARα) was increased significantly in HFD group compared with ND and HFD + BAT groups, respectively (Fig. 3C). However, the transcriptional and protein levels of PPARα in the testis of HFD + BAT group were not change compared with ND group (Fig. 3D).

The transcriptional expressed levels of 39 PPARα target genes were also measured (supplementary information Table S2). Acyl-Coenzyme A oxidase 1 (Acox1), hydroxyacyl-Coenzyme A dehydrogenase (Hadha and Hadhb), very low density lipoprotein receptor (Vldlr) and fatty acid elongase 6 (Elovl6) were found significantly up-regulated in HFD group

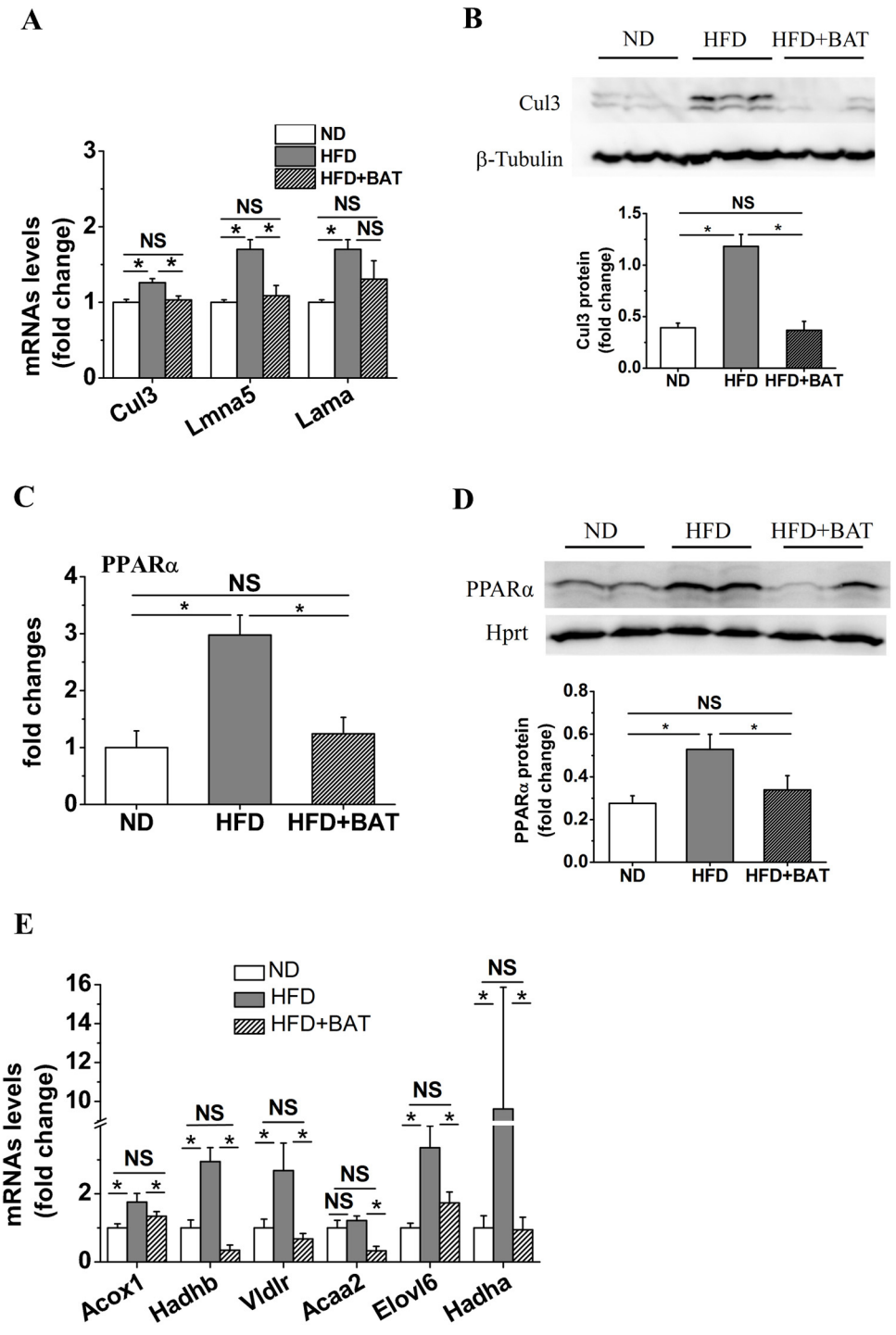

Figure 3 (A) Quantitative RT-PCR analysis of testicular tissue mRNA levels of mice of ND, HFD and HFD + BAT groups. Abbreviations: Cul3, cullin 3; Lmna, lamin a; Lama5, laminin, alpha 5. (B) Western blot analysis of testicular protein Cul3 extracted from mice of ND, HFD and HFD + BAT group. (C) Quantitative RT-PCR analysis of testicular tissue PPARα mRNA levels of mice of ND, HFD and HFD + BAT groups. Abbreviations: PPARα, peroxisome proliferator activated receptor a; (D) Western blot analysis of testicular protein PPARα extracted from mice of ND, HFD and HFD + BAT group. (E) Quantitative RT-PCR analysis of PPARα target genes in testicular tissues of mice of ND, HFD and HB groups. Abbreviations: Acox1: acyl-Coenzyme A oxidase 1; Hadha and Hadhb: hydroxyacyl-CoA dehydrogenase, alpha and beta subunit; Vldlr: very low density lipoprotein receptor; Acaa2: acetyl-CoA acyltransferase 2; Elovl6: ELOVL fatty acid elongase 6 (mean ± SEM; $n = 6$). * $p < 0.05$; NS: non-significant.

compared with ND and HFD + BAT groups, respectively (Fig. 3E). However, the transcriptional levels of those genes in the testis of HFD + BAT group were not change compared with ND group. The transcriptional levels of other genes did not show any significance among these groups (supplementary information Figure S2 and S3).

Discussion

Dyslipidemia is an important feature of obesity, which has an impact on semen quality and fertility. A lot of studies suggest a relationship between lipid abnormalities and infertility, but, the detailed mechanism is unclear [21,22]. In the present study, the contents of TG and TCHO in the testis and epididymis were recovered to normal level after BAT transplantation. Meanwhile, the reduction in sperm motility and progressiveness induced by high fat diet was obviously improved after BAT transplantation.

BAT transplantation may improve whole-body energy metabolism, regulates glucose and lipid homeostasis and insulin sensitivity [12]. In present study, BAT transplantation has attenuated the impair effect of sperm caused by high fat diet. The most probably explanation for these recovery effects of BAT transplantation is through the modulation of lipid metabolism. PPARα, a member of the steroid hormone receptor superfamily, has been linked to lipid homeostasis. The action of PPARs in the testis is unclear. However, PPARα may play a role in steroidogenesis and may influence the fertility of spermatozoa. Indeed, PPARα regulates the beta-oxidation of lipids and may also regulate the fatty acid composition of phospholipids in germ cells. The lipid composition of spermatozoa is known to modulate their mobility and its viability [23]. In this study, significant upregulation of testicular PPARα in HFD group were observed compared with ND and HB groups, in contrast, the transcriptional levels of PPARα and its down-stream genes in the testis of HFD + BAT group were recovered to level of ND group. We speculated that the upregulation of PPARα and its down-stream genes involved in β-oxidation may produce excess of oxygen radical such H_2O_2, which may damage the sperm motility. However, the BAT transplantation might balance the lipid homeostasis via modulation of expression of PPARα and its downstream genes.

In summary, this study, for the first time, reported that brown adipose tissue transplantation could attenuate impairment of male fertility by diet-induced obesity probably via modulating lipid homeostasis in body. The mechanism needs to be elucidated in the future.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orcp.2016.06.001.

References

[1] Preventing chronic diseases: a vital investment. Geneva: World Health Organization; 2005.
[2] Proietto J, Baur LA. 10: Management of obesity. Med J Aust 2004;180:474—80.
[3] Cabler S, Agarwal A, Flint M, du Plessis SS. Obesity: modern man's fertility nemesis. Asian J Androl 2010;12:480—9.
[4] Parihar M. Obesity and infertility. Rev Gynaecol Pract 2003;3:120—6.
[5] Palmer NO, Bakos HW, Fullston T, Lane M. Impact of obesity on male fertility, sperm function and molecular composition. Spermatogenesis 2012;2:253—63.
[6] Hammoud AO, Gibson M, Peterson CM, Meikle AW, Carrell DT. Impact of male obesity on infertility: a critical review of the current literature. Fertil Steril 2008;90:897—904.
[7] Hammoud AO, Wilde N, Gibson M, Parks A, Carrell DT, Meikle AW. Male obesity and alteration in sperm parameters. Fertil Steril 2008;90:2222—5.
[8] Fejes I, Koloszár S, Szollosi J, Zavaczki Z, Pai A. Is semen quality affected by male body fat distribution? Andrologia 2005;37:155—9.
[9] Magnusdottir EV, Thorsteinsson T, Thorsteinsdottir S, Heimisdottir M, Olafsdottir K. Persistent organochlorines, sedentary occupation, obesity and human male subfertility. Hum Reprod 2005;20:208—15.
[10] van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JMAFL, Kemerink GJ, Bouvy ND, et al. Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009;360:1500—8.
[11] Gunawardana SC, Piston DW. Reversal of type 1 diabetes in mice by brown adipose tissue transplant. Diabetes 2012;61:674—82.
[12] Stanford KI, Middelbeek RJW, Townsend KL, An D, Nygaard EB, Hitchcox KM, et al. Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest 2012.
[13] Nedergaard J, Bengtsson T, Cannon B. New powers of brown fat: fighting the metabolic syndrome. Cell Metab 2011;13:238—40.
[14] Rothwell NJ, Stock MJ. Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. Clin Sci (Lond) 1983;64:19—23.

[15] Liu X, Zheng Z, Zhu X, Meng M, Li L, Shen Y, et al. Brown adipose tissue transplantation improves whole-body energy metabolism. Cell Res 2013;23:851—4.
[16] National Research Council Committee for the Update of the Guide for the C, Use of Laboratory A. The National Academies Collection: Reports funded by National Institutes of Health. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academies Press (US) National Academy of Sciences; 2011.
[17] Palacín I, Vicente-Fiel S, Santolaria P, Yániz JL. Standardization of CASA sperm motility assessment in the ram. Small Rumin Res 2013;112:128—35.
[18] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(—Delta Delta C(T)) method. Methods 2001;25:402—8.
[19] Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nat Protocols 2008;3:1101—8.
[20] Zhong H, Simons JW. Direct comparison of GAPDH, beta-actin, cyclophilin, and 28S rRNA as internal standards for quantifying RNA levels under hypoxia. Biochem Biophys Res Commun 1999;259:523—6.
[21] Ramirez-Torres MA, Carrera A, Zambrana M. High incidence of hyperestrogenemia and dyslipidemia in a group of infertile men. Ginecol Obstet Mexico 2000;68:224—9.
[22] Shalaby MA, el-Zorba HY, Kamel GM. Effect of alpha-tocopherol and simvastatin on male fertility in hypercholesterolemic rats. Pharmacol Res 2004;50:137—42.
[23] Froment P, Gizard F, Defever D, Staels B, Dupont J, Monget P. Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition. J Endocrinol 2006;189:199—209.