

Transplantation of interscapular brown adipose tissue in rats

Aydin Aynehchi¹, Leila Roshangar^{2*}, Soltanali Mahboob³, Nasser Ahmadiasl⁴, Parisa Habibi⁵, Hadi

Yousefi⁶, Mehdi Fasihi¹, Neda Jourabchi-Ghadim¹, Mehrangiz Ebrahimi-Mameghani^{7*}

¹School of Nutrition & Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran;

²Stem Cell Research Centre, Tabriz University of Medical Sciences, Tabriz, Iran;

³Department of Biochemistry, Higher Education Institute of Rab-Rashid, Tabriz, Iran;

⁴Neurosciences Research Centre (NSRC), Tabriz University of Medical Sciences, Tabriz, Iran;

⁵Department of Physiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran;

⁶Department of Physiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran;

⁷Nutrition Research Centre, Department of Community Nutrition, School of Nutrition & Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

*Corresponding author E-mail: ebrahimimamagani@tbzmed.ac.ir and lroshangar@yahoo.com

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Background: the activity of brown adipose tissue (BAT) – a highly metabolic tissue – has a magnificent role in the management of metabolic diseases such as obesity and diabetes and numerous researchers have attempted to increase BAT via different methods including transplantation of BAT in animal models. Aims: to describe the protocol of the BAT transplantation in rats, as an animal model for studies about elevated BAT. Methods: twenty female Wistar rats were randomly divided in two groups (donor and recipient). Interscapular BAT (iBAT) were carefully removed from donors and transplanted into the interscapular region of the recipient rats, subcutaneously. To approve the successfulness of the operation, expression of the UCP1 mRNA and histological changes were evaluated in the grafts two months after surgery. Results: all operated rats have survived and fifty percent of the operations were successful. Conclusion: transplantation of BAT following this procedure in rats could be used as an acceptable animal model to investigate multiple aspects of elevated BAT.

Key words: rats; transplantation; brown adipose tissue; obesity; diabetes; surgery

Introduction

Increasing Brown and/or Beige Adipose Tissue (BBAT) has been proposed as a promising approach to increase metabolic rate and therefore to the treatment of obesity and diabetes and other related diseases. Brown adipose Tissue (BAT) transplantation in laboratory rodents has been an appropriate research model in which the intrascapular BAT (iBAT) of a donor mouse is transplanted to the same region of the recipient animal. The difference between the BBAT of rodents and human as well as the diversity of different kinds of brown and beige adipocytes in the body, make it difficult to design an accurate research model for simulation of increased BBAT.

Until recently, different animal models have been introduced in which BBAT has increased using pharmaceutical agents (particularly β -adrenergic agonists, PPAR γ activators, etc.), cold, interleukin-6, etc. (Wu et al., 2012; Ohno et al., 2012; Barbatelli et al., 2010; Ma et al., 2015). Due to the systemic effects of IL-6, cold, sympathomimetics, etc. the final response in these models are complicated and not only the effect of increased BAT. Production of the BBAT through tissue engineering and transplanting the engineered tissue to a recipient has an advantage, which makes it possible to investigate the effects of an exact BBAT cell type, but it is expensive and requires so advanced technology (Jimenez et al., 2013). However, transplanting the iBAT in rodents (as called transBATation by Zhu et al. 2014) is economic, convenient and fast, however, there are some limitations such as the difference between the adipocytes of iBAT and human cells which are mostly beige (Park et al., 2014).

Owing to performing all the transplanting studies on mice (Zhu et al., 2014; Gunawardana, Piston, 2012; Liu et al., 2015; Stanford et al., 2011), and some advantages of rats over mice, in the present paper we have described the transplantation process in rats, which has rarely done before (Yuan et al., 2016). Briefly, two rats with the same age, sex, race and preferably siblings, are anesthetized simultaneously. Then, the iBAT of the donor rat is dissected and perfused in sterile ringer lactate serum, additional tissues are removed, iBAT is weighed and transplanted subcutaneously into the intracapsular region of the recipient rat.

Methods

Twenty adult female Wistar rats, aged eight weeks and weighing from 180 to 200 g (Tabriz University of Medical Sciences, Iran) were used in this study. One week prior to the operation, all rats were kept in the standard situation (22 °C and 12/12 hours of dark and light cycle). Rats were fasted for 8 hours prior to the surgery while they had access to water (*ad libitum*). All the animal experiments presented in the study have been approved by the Ethic Committee of the Tabriz University of Medical Sciences (ethical code: "TBZMED.REC.1394.741" 23.11.2015) and were in accordance with National Institute of Health (NIH) guidelines for the care and use of laboratory animals.

Basic techniques and procedures

In order to minimize the possibility of graft rejection, select the recipient and donor rats from siblings. It is recommended that .i perform the operations with an acceptable order and speed to maintain the graft alive and functional.

All procedures are carried out under sterile condition. .ii

Clean the blood from the surgical area, using sterilized cotton swabs. To keep the surgical area irrigated during the surgery, .iii pour the saline solution on the tissues by a syringe (Qin et al., 2013).

Absorbable chromic sutures (5-0) and non-absorbable silk sutures (5-0) are used for ligating internal (including the fascia and .iv the graft) and external (the skin) tissues, respectively (Khajuria et al., 2012).

Provide oral amoxicillin (500 mg/L of drinking water) 24 hours before the operation (Gs et al., 2014).

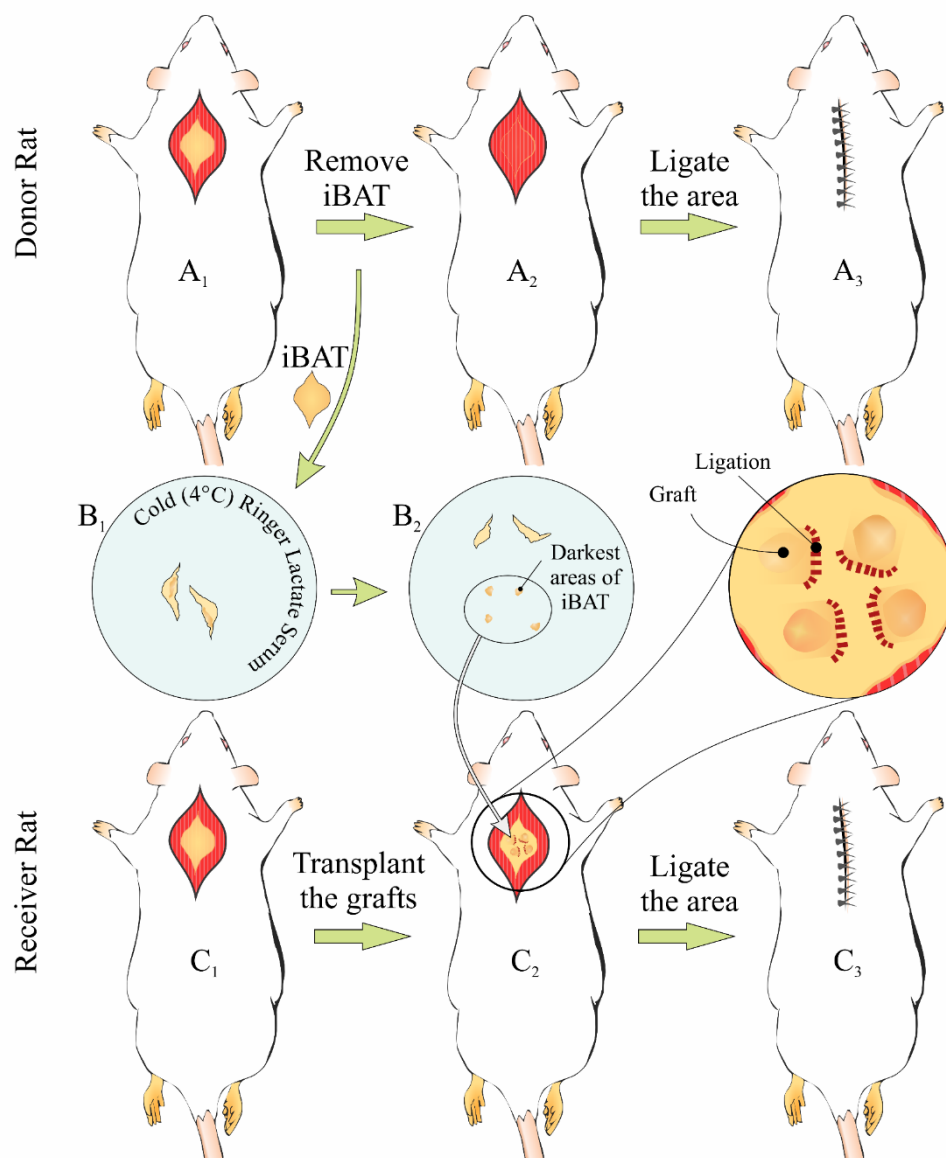


Figure 1. Different Stages of the iBAT transplantation in rats. The different stages of transplantation of iBAT is illustrated in the picture. A₁ to A₃: the operation of the donor rat. B₁ and B₂: Ex-vivo preparation of the graft. C₁ to C₃: the operation of the receiver rat.

Donor Operation

The operation of dissecting iBAT from the donor rat has been illustrated in Fig. 1 (A₁-A₃).

This process takes eight minutes in average. Anesthetize rats with peritoneal injection of xylazine and ketamine (10 and 100 mg per Kg, respectively) (Wickham et al., 2015). Then pinch the tail and hind feet to control possible reflexes and ascertain that the rat is adequately anesthetized. Place the rat over a thermal pad and fix the rat in prone position.

Prevent the rat's eyes from dryness through anesthesia, using eye ointment (Calik et al., 2014).

Inject paracetamol (100 mg/kg BW) subcutaneously to provide analgesia and repeat for three days (Waite et al., 2015).

Shave the dorsomedial side up to middle of the rat's neck. Then sterilize the corresponding skin three times via povidone iodine and 76% ethanol.

Open the interscapular area by a longitudinal incision in dorsal midline (extending for about 4-5 cm caudally). IBAT occurs at the interscapular region of the rat. Carefully, dissect the whole adipose tissue using scissors and do not damage the surrounding tissues. Perfuse the dissected tissue in cold (0-4 °C) sterile ringer lactate serum (Oldani et al., 2012).

Wash the surgical area with pre-warmed (37 °C) saline solution and clean the blood and other liquids from the region using sterile cotton swabs. Ligate the fascia and the skin using aforementioned 5-0 sutures (chromic and silk, respectively).

Ex vivo Graft Preparation

All the procedures for the ex vivo preparation of the graft, would be in a plate full of cold (0-4 °C) sterile ringer lactate serum. The entire process takes 3 minutes, approximately.

Dissect all the other tissues attaching the iBAT. IBAT could be distinguished by its darker color in comparison with surrounding tissues (Fig. 1, B₁-B₂).

Weigh the final tissue in the plate and choose a certain amount (e.g. 300 mg) for transplantation.

Recipient Operation

The entire procedures of the transplantation of the graft to the recipient rat are illustrated in Fig. 1 (C₁-C₃) and takes approximately 10 minutes.

Preparation of the recipient rats would be exactly as the donor groups (steps I to IV).

Make a longitudinal incision in the interscapular area to the beginning of the neck (for about 4-5 cm). Make some tiny incisions and scratches on the interscapular adipose tissue of the recipient rat and put the graft on them. This is done to make the preparation of the blood supply for the graft easier.

Close the incision by 5-0 sutures exactly as the donor rats.

Postoperative Treatment and Follow-up

After ligation, disinfect the surgical area twice, using povidone iodine.

Finally, treat the rats (both donor and the recipient) with a subcutaneous injection of ringer lactate serum (1.5 ml).

Keep the rats in a warm (30-35 °C) place for about 60 min to prevent hypothermia.

Add amoxicillin (500 mg/L of water) to the drinking water for a week post transplantation.

Results

In the present study, 20 rats were operated which all the rats have survived.

To ensure about the surveillance of the graft in the recipient animal, the expression of the mRNA for uncoupling protein (the specific gene of the BAT) were measured via Realtime-PCR which was not significantly different from the expression of UCP-1 mRNA in the original iBAT of recipient rats (Fig. 2). Fifty percent of operations were successful.

Different stages of the surgery have been illustrated in Fig. 1.

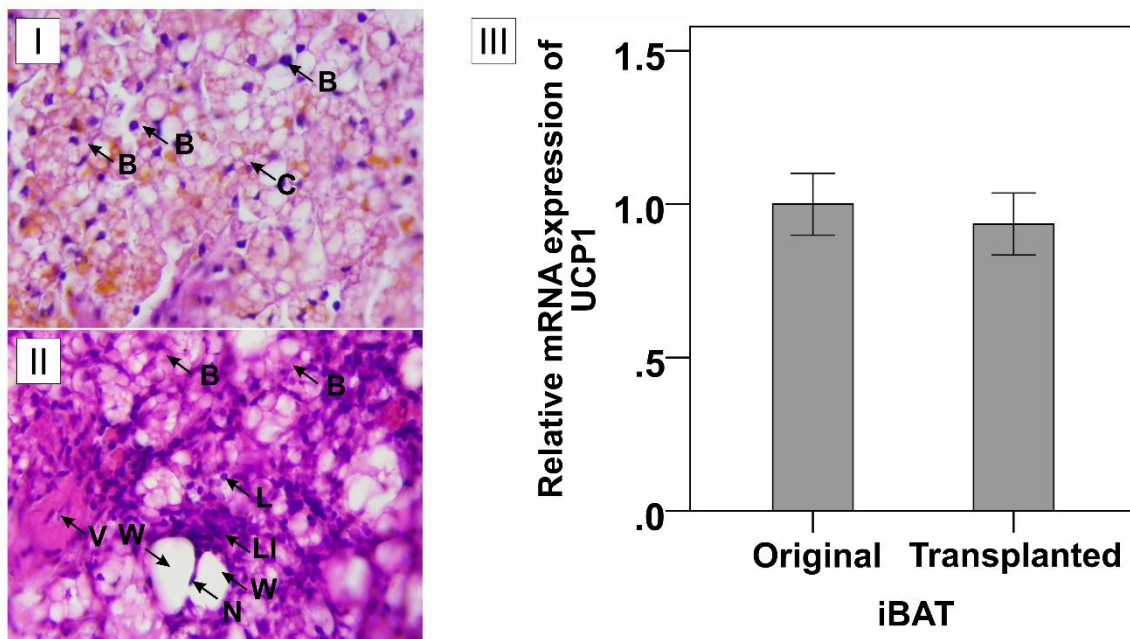


Figure 2. Expression of UCP1 mRNA and histological sections of transplanted iBAT in rats 2 months post-transplant.

I: Histological section of a successfully transplanted iBAT. **II:** Histological section of a rejected graft. iBAT transplantation failure in II was associated with inflammatory response in the graft with infiltration of the lymphocytes. **III:** There is no significant

difference in UCP1 mRNA expression between original and transplanted iBAT. B: brown adipocytes, C: capillary, L: lymphocytes, LI: infiltration of lymphocytes, N: nucleus, V: vessel, W: white adipocyte.

Discussion

There are several animal models for investigating different aspects of BBAT. In a review of the literature, researchers have used different approaches such as using pharmaceutical agents, cold exposure and surgery. Increasing the BAT activity by stimulating the sympathetic nervous system has several systemic effects (e.g. on cardiovascular system) that could distort the effects of BAT. Cold exposure can also have many systemic effects. For example, Harri et al. (1975) reported the hypertrophy of the heart along with iBAT in cold exposed rats (Harri, Valtola, 1975). Changing of the gastrointestinal microbiota during cold exposure has been reported (Ziętak et al., 2016). Therefore, increasing the BBAT using cold exposure could not be an appropriate model to study BBAT function. Among all of these methods, increasing the BAT using transplantation could directly increase the BAT without affecting other main systems of the body.

Although BAT transplantation could be more acceptable than other models, it has some limitations. Actually, there are different kinds of adipocytes in the body including different kinds of white, brown and the newly introduced beige adipocytes. Each has a specific gene expression, secretion and function (Park et al., 2014). Beige adipocytes act as brown adipocytes when they are stimulated and after elimination of the stimulus, they stop thermogenesis and act like white adipocytes (Wu et al., 2013). The population of these cell types are different among species, therefore, the brown adipocytes of rodent iBAT is not exactly as the same as human brown adipocytes. Wu et al. (2012) reported that BAT cells in human body are actually beige cells and their gene expression are different from rodent's iBAT cells. Notwithstanding, other studies have reported that brown cells exist with beige adipocytes in human body (Lidell et al., 2013). Moreover, increasing in human BAT may have different effects than BeAT that requires further investigation. Therefore, although increasing the BAT through transplanting iBAT in rodents is not considered as a gold standard approach, it is an applicable model, which compared with the other models appears to have reasonable advantages.

So far, a few studies have employed iBAT transplantation to study the role of BAT, which have mostly performed on mice (Gunawardana et al., 2015; Liu et al., 2013). Due to some practical advantages of studies on rats rather than mice, the present article focused on transplanting the iBAT on rats. To our knowledge, BAT transplantation was firstly done by Roberts et al. (1986) in which iBAT of mice was transplanted to underneath of the kidney capsule of the recipients. It has been concluded that iBAT transplantation could improve metabolic indices and obesity. A recent study has been shown it's benefit in the treatment of Polycystic Ovarian Syndrome in rats, which opens a new area for further investigation. Tran et al. (2008) investigated transplanting of subcutaneous adipose tissue (which is more likely to beige adipose tissue) instead of iBAT and reported positive effects on body weight and metabolism (Tran et al., 2008).

In the present study, the transplanted tissue was the iBAT. There are different adipose tissues in rodents, which have different adipocytes and physiological functions (Tran et al., 2008). Some are brown and white, while others act such as BeAT (please refer to Giralt et al. (2013) and Tran et al. (2008, 2013). IBAT is the main BAT depot in rodents. Moreover, the anatomical position of iBAT makes it a great choice for transplantation because it is located directly underneath of the skin and it reduces the extent of injury during surgery. Other fat depots of rodents compared with iBAT are less active in thermogenesis and mostly act as BeAT. They also locate in internal parts and in turn, their transplantation requires larger incisions in the animal body (Frontini, Cinti, 2010). Furthermore, the graft is transplanted to the interscapular area, adjacent to the original iBAT to simulate the anatomical location of the iBAT. For the surgery, a longitudinal incision versus a cross-sectional one was used because when the rat bends its head, a longitudinal incision could resist more.

This study has some strength such as using tissue transplantation instead of different pharmaceutical agents or cold as well as performing the transplantation on rats rather than mice to increase the amount of BAT. However, there are some limitations as well such as using Real-time PCR instead of PET/CT for approving viability of the graft.

Conclusions

This protocol describes transplantation of BAT in rats, which is an animal model that is used in different studies about BAT function.

Conflict of interest

The authors indicate no conflicts of interest.

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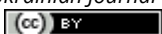
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