Adipose-derived Mesenchymal Stem Cells from Obese Mice Prevent Body Weight Gain and Hyperglycemia

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Short report

Abstract

Disease status always affects mesenchymal stem cells (MSCs) function which limits their therapeutical effects. This study aims to evaluate the effects of adipose-derived MSCs (ADSCs) on body weight and glucose homeostasis in obese mice model. After 10 weeks high-fat diet feeding, mice were injected with either phosphate buffered saline (PBS), ADSCs from normal control mice (N-ADSCs) or ADSCs from obese mice (O-ADSCs). Mice fed with chow-fat diet were injected with PBS served as normal controls. Obese mice received O-ADSCs showed slower body weight gain, better blood glucose levels and prevented glucose intolerance deterioration. The inguinal fat weights were reduced in mice receiving O-ADSCs compared to other obese groups, may as the increased lipolysis of inguinal fat. Moreover, O-ADSCs improved insulin signaling molecule expression in muscle. These data reveal that obesity derived autologous ADSCs infusion is an effective treatment for obesity and hyperglycemia.

Introduction

The worldwide epidemic of obesity poses an enormous global challenge. The increased prevalence of obesity accompanies with the increased prevalence of type 2 diabetes mellitus (T2DM). Obesity is an insulin resistance state, affects both insulin resistance (IR) and impaired insulin secretion which responsible for T2DM. Therapeutic strategy for tackling obesity is the key to prevent T2DM[1].

Mesenchymal stem cells (MSCs) are self-renewable multipotent cells that have been identified in various tissues like bone marrow, fetal annexes, adipose tissue, dental tissue and liver tissue[2]. Adipose-derived MSCs (ADSCs) are considered to have more advantages than other types of MSCs because of lack of ethical concerns, easy accessibility, abundant sources and subcutaneous location[3]. Previous evidences have confirmed the anti-obesity and hyperglycemia benefit of ADSCs. Cao et al. revealed that ADSCs therapy reduced body weight and blood glucose levels in high-fat diet-induced obese mice, and these effects may part from the suppression of inflammation in the liver and the protection of pancreatic β-cell[4]. ADSCs infusion also alleviated hyperglycemia and insulin resistance in T2DM rats via restoring insulin signaling pathway on the cell membrane in skeletal muscle, liver, and adipose tissue[5]. In addition, ADSCs infusion have quick effect of improving blood glucose within 24 h in T2DM rats depending on regulation of glycogen metabolism and gluconeogenesis[6].

The selection of allogeneic or autologous MSCs still is questionable. The allogeneic MSCs derived from donor are "off-the-shelf" cellular therapy. Although MSCs previously identified as immune-privileged, studies also believed that allogeneic MSCs trigger local inflammation by allo-specific T-effector cells. Side effects even accelerated after multiple infusions of allogeneic MSCs by boosting of memory allo-response[7]. The allo-response limits the clinical application of allogeneic MSCs. Autologous MSCs therapy has higher acceptance and lower infectious disease risk, but disease status always affects the function of MSCs. Adipose tissue from obesity is a chronic state with inflammatory environment, hypoxia and metabolic dysfunction. This abnormal state then lead to the dysfunction of ADSCs including

multipotent differentiation ability, metabolism and immunomodulation, which reduce the curative effect of autologous ADSCs[8, 9].

In the present study, we injected ADSCs isolated from normal control mice or obese mice into obese mice, and investigated their therapeutic effects and the underlying mechanisms in maintain body weight and glucose homeostasis. Our aim is to provide the feasible foundation of autologous cell therapy in obese.

Materials And Methods

Animals and experimental design

Four-week-old male C57BL/6 mice were purchased from Shanghai Jihui Laboratory Animal Care. After 1 week adaptation to the environment, rats were randomized to fed with a standard chow-fat diet (10 % of calories from fat) or a high-fat diet (HFD: 60 % of calories from fat). Mice fed with chow-fat diet served as normal controls. 10 weeks later, the HFD mice were then randomized into 3 groups and received three times tail vein injection at d0, d4, d9 with either 1) phosphate buffered saline (PBS)(n=5), 2) 5×10^5 ADSCs from normal control mice (N-ADSCs) (n=5), 3) 5×10^5 ADSCs from obese mice (O-ADSCs) (n=6). Body weight and blood glucose levels were continuous monitored and % change was calculated. At 2 months after cell infusion, intraperitoneal glucose tolerance (IPGTT) and insulin releasing test (IRT) were performed after 12-hours fast. At the end, the mice were sacrificed and tissues of adipose, liver, and skeletal muscle were rapidly removed and stored at -80°C.

ADSCs isolation and culture

Fresh inguinal fats were isolated from normal chow or obese mice and were washed with Dulbecco's phosphate buffered saline (D-PBS, Gibico). The adipose tissues were cut into small fragments and then digested with 2mg/ml type Ⅰ collagenase (Sigma-Aldrich) for 30 minutes at 37°C on a shaker. Cells were resuspended after mixtures centrifuged at 1500 rpm for 5 min. Next the cell suspension was filtered through a 40-mm nylon filter mesh (BD Falcon) to remove tissue residues. After twice washes, cells were resuspended with alpha-MEM medium (Gibico) plus 10% fetal bovine serum (Gibico) and seeded in 75-cm^2 tissue-culture flasks (BD Falcon). Cells were cultured in a humidified 5% CO2 incubator at 37°C. Floating cells were removed at 24 hours and mediums were changed per 3 days. At 90% confluence, cells were detached with 0.25% trypsin-EDTA for passage.

Intraperitoneal glucose tolerance and insulin releasing test

For IPGTT, all mice received intraperitoneal injection with 2 g/kg of glucose after an overnight fast. At 0, 15, 30, 60, 90, and 120 minutes after glucose load, blood glucose levels were measured with a glucometer (Roche). At 0, 15 and 30 minutes, 0.1mL blood samples were collected from the orbital venous plexus. The samples were centrifuged immediately and plasma were stored at -80°C. Subsequent insulin assay

was performed by ELISA (Alpco) according to the manufacturer's protocol. HOMA-IR index was calculated by the equation: HOMA-IR index = (FBG [in mmol/L] × FINS [in units/L])/22.5.

RNA extraction and quantitative RT-PCR

Total RNA was extracted from liver, muscle and adipose, then converted into first-strand cDNA with the first-strand cDNA synthesis kit (Takara). Quantitative RT-PCR was performed using SYBR Master Mix (Takara) and a LightCycler 480 System (Roche). The quantity of mRNA was normalized to β-actin in liver and muscle and to 36B4 in adipose.

Statistical analyses

Statistical calculations were performed using SPSS version 25 and Graph Pad Prism 8. Data were expressed as mean±SD. Student's t test or ANOVA was performed to analyze the differences. The area under the curve (AUC) of the IPGTT and IRT were calculated. $P<0.05$ was considered statistically significant.

Results

Generation of obese mice

The obese mice model was established by HFD feeding. Compared with chow group, both body weight and fasting blood glucose (FBG) were significantly increased after 10 weeks HFD feeding. Obese mice showed impaired glucose tolerance by the IPGTT. HFD induced insulin resistance with higher insulin levels in obese mice (Fig. 1).

ADSCs infusion reduced body weight

We then injected ADSCs into the obese mice. Interestingly, O-ADSCs significantly slowed down the body weight gain of mice, while the effect of N-ADSCs was not obvious (Fig. 2A). To identified the cause of body weight improvement, we measured epididymal fat and inguinal fat weights at the end of the experiment. There was no significant difference in epididymal fat weights between obese mice injected with PBS and mice receiving ADSCs. Compared to obese controls, the inguinal fat weights were significantly reduced in mice receiving O-ADSCs, but not N-ADSCs (Fig. 2B). Next, we found that ADSCs infusion reduced adipogenesis gene stearoyl-CoA desaturase 1 (Scd-1) expression in epididymal fat, while no effect on inguinal fat (Fig. 2C, D). What is more, beta3-adrenergic receptor (Adr-β3) which is a lipolysis gene was higher in mice receiving O-ADSCs than obese controls in inguinal fat. There was no

significant difference in lipolysis gene expression of epididymal fat between obese controls and mice receiving ADSCs (Fig. 2E, F).

ADSCs infusion improved glucose homeostasis and insulin sensitivity

As shown in Fig. 3A, obese mice received normal or obese derived ADSCs both showed lower blood glucose levels than control. O-ADSCs even more effective than N-ADSCs at day 2 after the first injection. IPGTT performed at 2 months after first injection. The glucose tolerance further impaired in obese mice as the HFD feeding lasts (Fig. 3B). However, the ADSCs infusion decelerated the deterioration of glucose tolerance, especially with O-ADSCs at 15 min (Fig. 3C-D). The insulin response after glucose load was declined in control group, while maintain well in ADSCs groups (Fig. 3E). Obese mice receiving ADSCs also tended to improve insulin sensitivity, but no significantly difference (Fig. 3F). We next measured the mRNA expression of INSR and IRS-1 in the adipose, liver and muscle (Fig. 3G-I). INSR expression was only higher in muscle from mice receiving O-ADSCs than obese controls and mice receiving N-ADSCs.

Discussion

This study indicates, for the first time, O-ADSCs have better curative effect on obesity and glucose homeostasis than N-ADSCs in HFD mice. The body weight improvement was accompanied by an increase of lipolysis gene expression after O-ADSCs treatment. O-ADSCs also tended to alleviate insulin resistance after glucose loading, coincided with a restoration of INSR in muscle.

Animal studies have demonstrated that infusion of ADSCs reduced blood glucose levels via multiple mechanisms[10]. The effect of ADSCs on body weight is associated with disease progression. ADSCs reduce the body weight in obesity and neutral impact on T2DM, while gradually increase the body weight in the late phase of diabetes[4, 5, 11]. We delivered ADSCs to obese mice at 10 weeks after HFD as an early intervention, and our results reinforced the above-mentioned effects of ADSCs. Although previous studies suggested that the detrimental microenvironment in obese tissue impaired the functionality of ADSCs, we found that O-ADSCs better control of body weight and blood glucose. In this study, we chose inguinal fat, a kind of subcutaneous adipose tissue (SAT), as the source of MSCs. Research indicated that SAT less influenced by obesity and T2DM than visceral adipose tissue (VAT)[12]. More importantly, MSCs are often quiescent and their immunosuppressive properties are induced by inflammatory cytokines such as IFNγ, IL-17 and TNF in the microenvironment[13]. Hypoxic state also enhances the functions of MSCs via increasing the expression of IL-6, VEGF and chemokines[14]. These findings suggest that a certain level of changes in the microenvironment are required to activation of MSCs and the selection of appropriate location and time for MSCs isolation is an important consideration in MSCs therapy.

Obesity is characterized by excessive fat accumulation and weight loss is the main management. Consistent with previous study, there were no differences in liver (data not show) and epididymal fat

weights among control, N-ADSCs and O-ADSCs groups[4]. Yet we found O-ADSCs infusion decreased inguinal fat weight compared to PBS and N-ADSCs infusion. Another study revealed that the anti-obesity effect of ADSCs is due to decreased lipogenesis and increased lipolysis through hormone-sensitive lipase activation and acetyl-CoA carboxylase1 suppression[15]. In the present study, we indicated both N-ADSCs and O-ADSCs inhibited lipogenesis by reducing Scd-1 expression of epididymal fat, while only O-ADSCs activated lipolysis by enhancing Adr-β3 expression in inguinal fat.

We also found that ADSCs prevented the decline of insulin response after glucose load in HFD mice. Further analysis suggested that O-ADSCs restored INSR expression in muscle, but not liver and adipose tissues. Muscle is the main target organ of insulin action and is central to systemic insulin resistance. Considering glucose uptake of muscle account for approximately 80% insulin-mediated glucose utilization, muscle play a key role in whole-body glucose homeostasis[16]. These data may partly explain the slight advantage of O-ADSCs over N-ADSCs in glycemic control.

In conclusion, our results provided evidence that O-ADSCs better improved body weight and blood glucose than N-ADSCs. Further mechanism exploration found that O-ADSCs increased lipolysis of subcutaneous fat and improved insulin resistance of muscle. These findings revealed O-ADSCs infusion was an effective treatment in controlling obesity and hyperglycemia, and provided a theoretical basis for autologous ADSCs therapy in obesity and diabetes.

Abbreviations

MSCs: Mesenchymal stem cells; ADSCs: Adipose-derived MSCs; PBS: Phosphate buffered saline; N-ADSCs: ADSCs from normal control mice; O-ADSCs: ADSCs from obese mice; T2DM: Type 2 diabetes mellitus; IR: Insulin resistance; HFD: High-fat diet; IPGTT: Intraperitoneal glucose tolerance; IRT: Insulin releasing test; D-PBS: Dulbecco's phosphate buffered saline; AUC: Area under the curve; FBG: Fasting blood glucose; Scd-1: Adipogenesis gene stearoyl-CoA desaturase 1; Adr-β3: Beta3-adrenergic receptor; SAT: Subcutaneous adipose tissue; VAT: Visceral adipose tissue.

Declarations

Acknowledgements

Authors' contributions

Shengxian Li, Wenjie Zhang, Liu Wei and Liu Wei drafted the study design. Yicheng Qi, Wen Liu and Xiangsheng Wang conducted the experiments and collected the data. Jing Ma provided technical assistance. Yicheng Qi wrote the first draft of the manuscript. Shengxian Li and Wenjie Zhang was responsible for critical revision of the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experiments were performed according to standard protocols, in compliance with the Guide of the Animal Ethics Committee of Shanghai Jiao Tong University School of Medicine.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no conflicts of interests.

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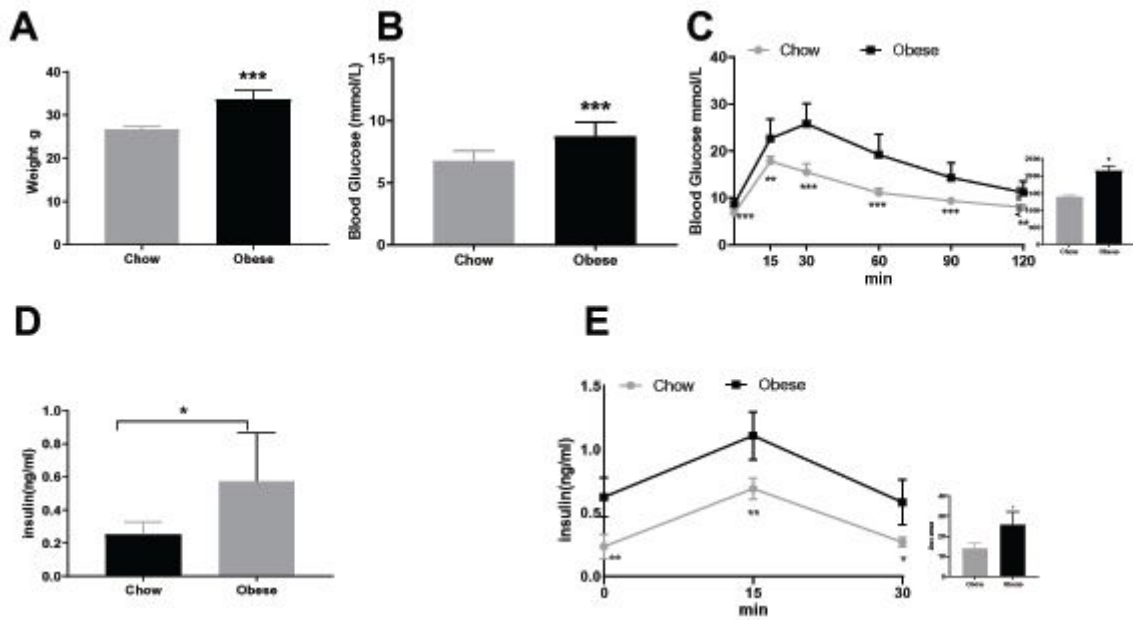
Figures

Figure 1

Generation of obese mice. A, Body weights, B, Fasting blood glucose, C, IPGTT, D, Insulin levels and E, IRT in C57BL/6 mice fed with standard chow-fat diet or a high-fat diet. Data are mean±SD. *p<0.05, **p<0.01, ***p<0.001.
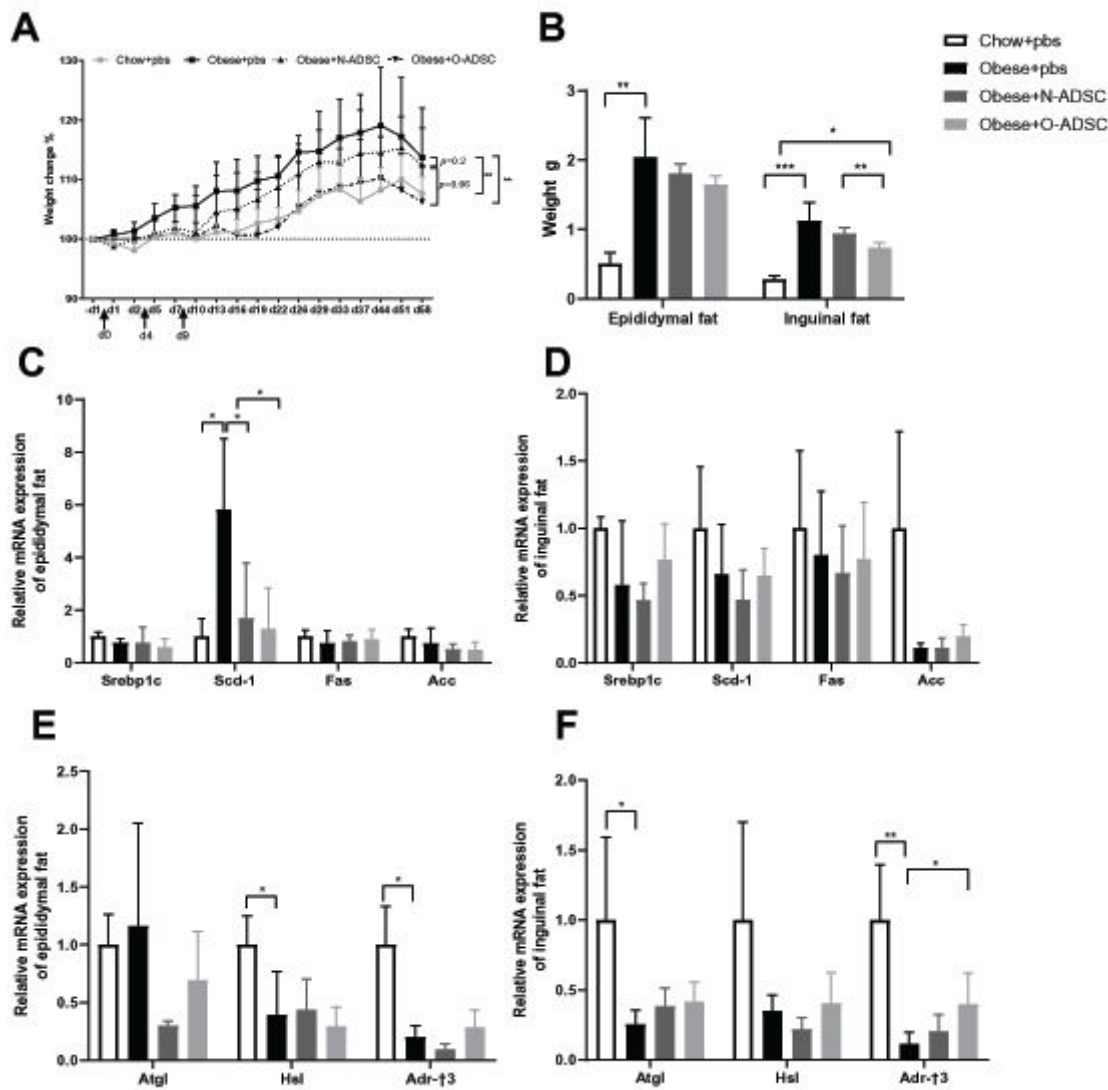
Figure 2

The effect of ADSCs infusion on body weights and fat mass. A, Percentages of body weight changes after ADSCs injection. B, Epididymal fat weights and inguinal fat weights after ADSCs injection. C. Effect of ADSCs infusion on adipogenesis genes levels in epididymal fat. D. Effect of ADSCs infusion on adipogenesis genes levels in inguinal fat. E. Effect of ADSCs infusion on lipolysis genes levels in epididymal fat. F. Effects of ADSCs infusion on lipolysis genes levels in inguinal fat. Data are mean±SD. *p<0.05, **p<0.01, ***p<0.001.
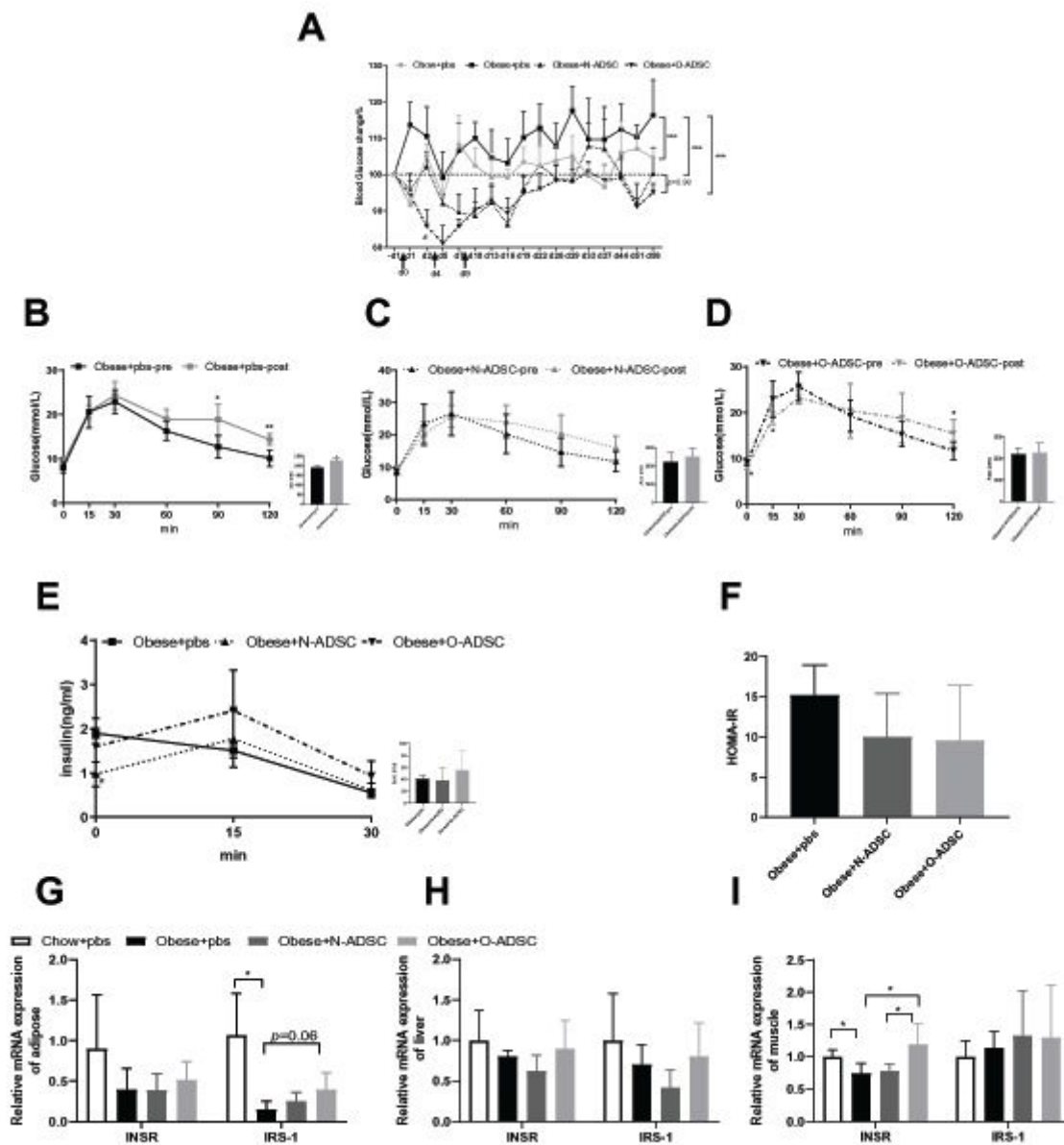
Figure 3

The effect of ADSCs infusion on blood glucose levels and insulin sensitivity. A, percentages of blood glucose changes after ADSCs injection. B-D, IPGTT and area under the curve before and after PBS, N-ADSCs or O-ADSCs injection. E. Insulin releasing test before and after PBS, N-ADSCs or O-ADSCs injection. F. HOMA-IR after ADSCs injection. G-I. Effect of ADSCs infusion on the mRNA expression of INSR and IRS-1 in insulin target tissues. Data are mean±SD. *p<0.05, **p<0.01, ***p<0.001.