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Diet-induced obesity regulates adipose-resident stromal cell quantity and extracellular matrix gene expression

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ABSTRACT

Adipose tissue expansion during periods of excess nutrient intake requires significant turnover of the extracellular matrix (ECM) to allow for maximal lipid filling. Recent data suggest that stromal cells may be a primary contributor to ECM modifications in visceral adipose. The purpose of this study was to investigate the capacity for high fat diet (HFD)-induced obesity to alter adipose-derived stromal cell (ADSC) relative quantity and ECM gene expression, and determine the extent to which exercise training can mitigate such changes. Male C57BL/6J mice were placed on control or HFD for 8 weeks prior to and following initiation of a 16 week treadmill exercise program. ADSCs (Sca-1$^+$CD45$^-$) were isolated from epididymal adipose tissue and mRNA was evaluated using high throughput qPCR. Stromal cells were also obtained from skeletal muscle (MDSC). HFD decreased the quantity of ADSCs and markedly altered gene expression related to ECM remodeling (Col1α1, MMP2, MMP9, Timp1). Exercise did not reverse these changes. MDSCs were minimally altered by HFD or exercise. Overall, the data from this study suggest that ADSCs decrease in quantity and contribute to adipose ECM remodeling in response to obesity, and exercise training does not significantly impact these outcomes.

1. Introduction

Excess nutrient availability results in deposition of lipid into preexisting adipocytes (adipose hypertrophy) or newly formed adipocytes (adipose hyperplasia). Adipocyte filling or preadipocyte expansion, however, cannot occur without supportive changes to the microenvironment, including extracellular matrix (ECM) and vascular remodeling (Cawthorn et al., 2012; Cinti et al., 2005; Strissel et al., 2007; Sun et al., 2011). The stromal vascular fraction (SVF) is a heterogeneous mixture of non-adipocyte cells in the adipose tissue that allow for tissue plasticity. The SVF includes immune cells, fibroblasts, preadipocytes, and important vascular-associated stem cells, including endothelial progenitor cells (EPCs) (CD45$^-$CD31$^+$CD34$^+$), adipose-derived stromal cells (ADSCs) (predominantly mesenchymal stem cells (MSCs)) (CD45$^-$CD31$^-$CD146$^-$NG2$^-$), and pericytes (CD45$^-$CD31$^-$CD34$^-$CD146$^+$NG2$^+$) (Qin et al., 2014; Sun et al., 2011). The complex cell-cell interactions that occur within the SVF to allow for appropriate tissue remodeling and expansion during conditions of healthy weight gain or obesity are not fully understood.

Stem cell antigen-1 (Sca-1) is a glycosyl phosphatidylinositol-anchored cell surface protein that was originally used as a marker to identify stem cells from bone marrow in the mouse, and is subsequently expressed by a variety of murine stem and progenitor cells in multiple tissues, including MSCs (Sharom & Lehto, 2002; van de Rijn et al., 1989). Recent studies suggest that ADSCs and adipocyte progenitors express Sca-1 (Berry et al., 2014; Ong et al., 2014; Schulz et al., 2011) and that the lineage negative (Lin$^-$) fraction (CD45$^-$CD31$^-$Ter119$^-$) in white adipose tissue specifically represents MSCs. Sca-1^{high} stromal cells extracted from subcutaneous and visceral fat express a gene signature that reflects significant contribution to the synthesis and degradation of ECM molecules, including matrix metalloproteinases (MMPs) (Tokunaga et al., 2014), and widespread downregulation of MSC gene expression is observed in Zucker diabetic fatty rats (Ferrer-Lorente et al., 2014). Interestingly, Sca-1$^{-/-}$ mice display extensive skeletal muscle fibrosis following injury as a result of a deficiency in MMP activity (Kafadar et al., 2009) and develop insulin resistance and elevated blood glucose in response to a HFD (Staszkiewicz et al., 2012). Thus, these studies suggest that ADSCs provide an essential role in adipose tissue remodeling, and engagement of ADSCs in this event may be

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impaired with obesity as a result of long-term high fat feeding. However, to our knowledge, the impact of long-term HFD-induced obesity on ADSC quantity and gene expression has not been evaluated.

Endurance exercise training results in adipose lipolysis and attenuation of fibrosis (Kawanishi et al., 2013a) and inflammation (Linden et al., 2014; Vieira et al., 2009) in adipose and skeletal muscle, independent of weight loss. Thus, participation in an endurance training program is a well-established recommendation for individuals diagnosed with Type 2 diabetes. Currently, the impact of an endurance training on stromal cell quantity and function in both adipose and skeletal muscle has not been investigated, particularly in the context of obesity. We have previously established that muscle-derived MSC (mMSC) relative quantity is increased in skeletal muscle in response to an acute bout of eccentric exercise, and that mMSC transplantation can facilitate improvements in myofiber growth and strength in response to training (Valero et al., n.d.). Thus, we speculate that resident stromal cells may provide the basis for positive changes in the structure and function of a variety of tissues, including both skeletal muscle and adipose, observed as a result of exercise.

In the present study, we hypothesized that ADSC quantity would be reduced (Ferrer-Lorente et al., 2014), yet expression of genes related to ECM would be enhanced with HFD-induced obesity to sustain adipocyte growth. We also predicted that a 16 week endurance training program would prevent adipocyte growth and concomitant changes in ADSC quantity and gene expression. Using the same rationale, we hypothesized that muscle-derived stromal cell (MDSC) ECM gene expression would be elevated in skeletal muscle following endurance training to facilitate structural remodeling.

2. Materials and methods

2.1. Animals

Three month old male C57BL/6J mice (n = 20) were purchased from Jackson Laboratories (Bar Harbor, ME) and were group-housed (3–4 mice per cage). Mice were kept on a 12 h dark/light cycle (lights on 07:00 to 19:00 h) in a pathogen free, temperature-controlled facility and fed ad libitum. For the in vitro experiment, three male mice (mixed genetic background, SJL x C57BL/6) were used from our breeding colony. These mice were fed standard chow (Harlan-Teklad, 13% calories from fat). National Institutes of Health guidelines for the care and use of laboratory animals were strictly followed, and all experiments were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign.

2.2. Design

Mice were randomly assigned to one of four groups: control diet-sedentary (no exercise) (Con-Sed, n = 5), control diet-exercise (Ex) (Con-Ex, n = 5), high fat diet-sedentary (HFD-Sed, n = 5) or HFD-exercise (HFD-Ex, n = 3). Five animals were originally assigned to HFD-Ex, but two died immediately before study completion and were omitted from the analysis. All diets were purchased from Research Diets Inc. (New Brunswick, NJ) and consisted of 10% or 60% of the calories from fat (OpenSource Diets D12450B or D12492, for Con or HFD respectively). Nutrient composition of these diets is matched and reported on the Research Diets website. Mice were fed specialized diets for 8 weeks, and then either remained sedentary or were subjected to a 16 week progressive aerobic exercise program. The original diets (Con or HFD) were maintained throughout the study. At the end of 24 weeks, 36 h after the last exercise bout, mice were euthanized by CO_2 asphyxiation, tissues were harvested, and stem cells were isolated from epididymal fat pads and gastrocnemius-soleus muscle complexes. A graphical illustration of study design is presented in Fig. 1.

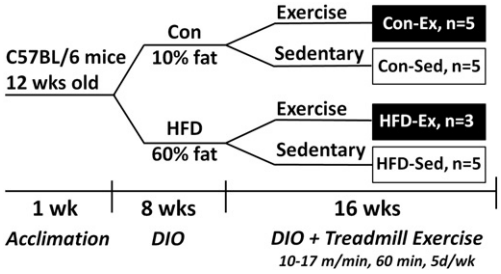

Fig. 1. Study design. Mice were randomly assigned to one of four groups and were fed CON or HFD diets for 8 weeks. Mice then remained sedentary or were subjected to a 16 week progressive aerobic exercise program while maintaining their diets. Con, control diet; HFD, high fat diet; Sed, sedentary (no exercise); Ex, exercise; DIO, diet-induced obesity.

2.3. Progressive aerobic exercise training

Exercise training was conducted on a motorized treadmill (Jog-a-Dog, Ottawa Lake, MI) for 60 min/day at 12–17 m/min, 5% grade, 5 days/week, for 16 weeks. Mice were introduced to treadmill exercise for a week, gradually running for 10–60 min at 10–12 m/min. Running speed was then increased every few weeks until the last two weeks of the study when running speed was 17 m/min. All animals complied with the exercise protocol. To control for stress associated with the training protocol, non-exercised control animals were exposed to similar noise and handling.

2.4. Hindlimb grip strength measurement

Grip strength was measured using a hindlimb pull bar on a 1027DM grip strength meter (Columbus instruments, Columbus, OH). Measurements were taken three weeks before the end of the study (week 21). All mice were habituated to the procedure for 3 consecutive days followed by a day of rest before being subjected to grip strength measurement (on the fifth day). To assess strength, mice were secured by their scruff and tail and allowed to grip the pull bar using their hindlimbs. The tester then gradually pulled the mouse back horizontally until the mouse grip was released. After 10 successful trials were performed only the 5 middle trials were used to record grip force in grams. The highest of these five values was used as peak force and the average of the five values was used as average force. Body weight was measured before each grip strength test and was used to express grip strength relative to weight (grip strength (g) divided by body weight (g)). All measurements were performed by the same evaluator.

2.5. Plasma analyses

Fasting plasma was collected in lithium-heparin coated conical tubes (Microvette CB300, Sarstedt, Nümbrecht, Germany) from the submandibular vein after 6 h fasting, 5 days before the end of the study. Samples were centrifuged for 15 min (2000*g* at 4 °C) and were stored in −80 °C until analysis. Plasma glucose was measured using a colorimetric assay (Cayman chemical, Ann Arbor, MI) and insulin was measured using an Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem, Downers Grove, IL), both according to the manufacturer instructions. The homeostasis model assessment method (HOMA-IR) was used to evaluate insulin resistance utilizing the following formula: Fasting glucose (mmol/l) ∗ Fasting insulin (μU/ml) / 22.5 (Lee et al., 2008). Systemic inflammation was evaluated using a Mouse Serum Amyloid-A ELISA kit (Alpco diagnostics, Salem, NH). Colorimetric and ELISA assays were analyzed using a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek, Winooski, VT).