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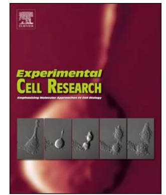

Extracellular matrix remodeling and matrix metalloproteinase inhibition in visceral adipose during weight cycling in mice

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ABSTRACT

Extracellular matrix (ECM) remodeling is necessary for a health adipose tissue (AT) expansion and also has a role during weight loss. We investigate the ECM alteration during weight cycling (WC) in mice and the role of matrix metalloproteinases (MMPs) was assessed using GM6001, an MMP inhibitor, during weight loss (WL). Obesity was induced in mice by a high-fat diet. Obese mice were subject to caloric restriction for WL followed by reintroduction to high-fat diet for weight regain (WR), resulting in a WC protocol. In addition, mice were treated with GM6001 during WL period and the effects were observed after WR. Activity and expression of MMPs was intense during WL. MMP inhibition during WL results in inflammation and collagen content reduction. MMP inhibition during WL period interferes with the period of subsequent expansion of AT resulting in improvements in local inflammation and systemic metabolic alterations induced by obesity. Our results suggest that MMPs inhibition could be an interesting target to improve adipose tissue inflammation during WL and to support weight cyclers.

1. Introduction

A temporary storage of fat is one of the main roles of adipose tissue. During a positive energy balance, preadipocytes become mature adipocytes and their cell size increase, as well as in a negative energy balance, a reduction of the cells size is registered. Not only adipocyte cell shape but also the initial step of adipocyte differentiation and other process is necessary to adipose tissue development, such as angiogenesis, which depends on extracellular matrix (ECM) remodeling [1,2]. Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidase, play an essential role in regulating ECM remodeling, its deregulation was described in obese humans and in experimental models. An elevated expression of MMP-2 was initially described in adipose tissue of obese mice [3], as well as, higher MMP-2 and -9 expression in subcutaneous adipose tissue of overweight patients [4] or elevated serum MMP-2 and MMP-9 [5] in obese patients. From the initial reports, several works investigated the role of different MMPs in adipose tissue development (for review see Lin et al. [6]). Not much is known about the dynamics of the ECM during a negative energy balance. Weight loss (WL), after bariatric surgery, provided improvements in glucose homeostasis and lipid metabolism, but serum levels of MMP-2, MMP-3 and specific inhibitors of metalloproteinases (TIMPs) were not affected, instead MMP-7 serum levels were increased [7]. In another study that followed patients after bariatric surgery, MMP-2 and -9 activity and picrosirius-red-stained collagen accumulation was observed in parallel of collagen I and III degradation in subcutaneous adipose tissue [8]. In visceral adipose tissue of mice after WL, protein level of MMP-2 was maintained, and a higher activity was observed in zymography assay [9]. If little is known about ECM remodeling in a negative energy balance period, there are no reports about this issue during weight cycling (WC), the repeated loss and regain of body weight [10]. In the modern society, "yo-yo dieting" is a controversial topic of debate, whether WC could lead to adverse health consequences providing more weight gain and increased metabolic risk [10,11].

The present study aimed to investigate the effects of WC on MMPs expression and activity. Adipose tissue inflammation and metabolic parameters were also evaluated. In addition, mice were treated with an MMP inhibitor, GM6001 (ilomastat) during WL period and the effects of ECM remodeling intervention were observed after WR.

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2. Methods

2.1. Animals and experimental design

Specific pathogen-free, 5-week-old male Swiss mice were obtained from Centro Multidisciplinar de Investigação Biológica (CEMIB, State University of Campinas, Campinas, São Paulo, Brazil). All experiments were performed in accordance to the principles outlined by the National Council for Animal Experimentation Control (CONCEA, Brazil) and it received approval from Ethics Committee of São Francisco University, Bragança Paulista, SP, Brazil (Protocol CEA/USF 001.05.2014). The mice were initially divided into two groups that received AIN-93 diet (lean group) or high-fat diet (obese group) *ad libitum* for 8 weeks. Pelletized AIN-93G was purchase from Rhoster (Cat RH 19521), Brazil. HFD was prepared in our lab as described before [12]. The obese group was later subdivided into 3 groups, one group was maintained with HFD *ad libitum* and two groups were subjected to moderate caloric restriction by providing daily controlled amounts of AIN-93 (the equivalent amount consumed by lean group in the day before) for the subsequent 8 weeks (weight loss groups, WL). One of WL groups was treated with an MMP inhibitor – GM6001 (a broad spectrum MMP inhibitor, 7.5 mg/kg/day, ip.) throughout the period and it was denominated WLGM. At the end of 16 weeks, the animals were sacrificed resulting in the following experimental groups Lean16, Obese16, WL and WLGM (n = 5 each group). The protocol was repeated and WLs groups were reintroduced to HFD *ad libitum* for additional 8 weeks (weight regain groups, WR). At the end of 24 weeks, Lean24, Obese24, WR and WRGM groups were obtained (n = 5 each group). The GM6001 (Tocris bioscience, Bristol, UK) dosage employed in this study was based in previous literature reports [13–15] and in both protocols (16 and 24 weeks) GM6001 was administered to mice only during WL period. The food intake was recorded in lean16, obese16, lean24, obese24, WR and WRGM groups only in the last two weeks of the protocol (fed *ad libitum)*.

2.2. Basal glucose, total cholesterol and triglycerides levels and insulin tolerant test

Animals were fasted for 6 h, and glucose homeostasis was evaluated by glucose blood level and insulin tolerance test (ITT) as previously described [16]. The rate constant for glucose disappearance during an insulin tolerance test (kITT) was calculated using the formula 0.693/t1/2. The glucose t1/2 was calculated from the slope of the least square analysis of the plasma glucose concentrations during the linear decay phase [17]. Drops of blood were employed for total cholesterol and triglycerides measurement using Accutrend Plus (Roche Diagnostics, Mannheim, Germany).

2.3. Analysis of energy expenditure by indirect calorimetry

Indirect calorimetry was carried out using the Oxylet/Physiocage system (Panlab, Barcelona, Spain). Animals were individually placed in respiratory chambers (temperature: 22–23 °C, humidity: 45–55% light cycle dark 12/12 h) with an air flow of 0.5 L/min. During the 24 h of analysis, O_2 (%) and CO_2 (%) were measured every 9 min. The Software Metabolism (Panlab) calculated the O_2 consumption, CO_2 and energy expenditure (kCal/h/kg0.75).

2.4. Necropsy and sample collection

Mice were fasted for 6 h and anesthetized by xylasine/ketamine overdose (0.1 mL/30 g body weight of 1:1 v/v of 2% xylasine and 10% ketamine); blood samples were collected by cardiac puncture in tubes without anticoagulant and centrifuged to isolate the serum. Adipose tissue depots (epididymal, subcutaneous, perirenal and mesenteric) and liver were carefully dissected, weighted and expressed as a percentage of body weight (b.w.). Epididymal adipose tissue samples were collected and stored at − 80 °C for further analyses or employed immediately for stromal vascular fraction isolation.

2.5. Stromal vascular fraction isolation and flow cytometry analysis

Epididymal adipose tissue samples were digested by collagenase method [18]. The stromal vascular fraction (SVF) was used for cell analysis. Cells (10^6 cells) were incubated with anti-CD45PerCP (BD Biosciences, CA, USA) for total leukocyte count or with anti-CD14FITC/anti-F4/80PE/anti-CD11bPerCP for macrophage identification (BD Biosciences). For each sample, 10,000 events were collected on a Guava Easy-Cyte HT (Millipore, Hayward, CA, USA) cytometer, defining FSC, SSC on a linear scale and FL1, FL2, and FL3 on a logarithmic scale. Light scatter profiles were obtained for each candidate population using In-Cyte software (Millipore).

2.6. MMPs quantification and activity in adipose tissue

Epididymal adipose tissue biopsies were homogenized in solubilization buffer as described [16]. Total protein extracts were obtained and used in Multiplex Assay kit for MMPs quantification (Milliplex Mouse MMP3MAG-79K, Merck Millipore, MA, USA). MMP activity was determined by gelatin zymography as described [9]. Gels were stained with 0.25% Coomassie brilliant blue R-250 and then destained with 10% acetic acid in 40% methanol. Gels were photographed using Gel Documentation system (Gel Doc™ XR+ v. 5.0, Bio-Rad, CA, USA) and bands with MMP activity identified by the Image Lab™ Software (Bio-Rad).

2.7. Insulin, adipokines, HDL and LDL cholesterol measurement

Insulin was measured in serum using Multiplex kit (Mouse Adipokine, Millipore). HDL and LDL cholesterol serum levels were determined using a commercial kit (LABORLAB, Sao Paulo, Brazil). Adiponectin, leptin, IL-6 and MCP-1 levels in adipose tissue were measured using Milliplex kit (Mouse Adipocyte, Millipore). IL-10 and TNF-α was measured by EIA kit (Quantikine Elisa mouse IL-10, R & D Systems, MN, USA; TNF alpha Mouse Elisa Kit, Abcam, Cambridge, UK).

2.8. Histological analyses

Epididymal and subcutaneous adipose tissue samples were fixed with paraformaldehyde. Subsequently, the specimens were processed, and embedded in paraffin. Histological sections of 5.0 µm were stained with hematoxylin–eosin to determine the size of adipocytes. The area of each intact cell on each image (500 cells per group) was measured by drawing the circumference and the adipocyte area was then extrapolated using ImageJ software (http://rsbweb.nih.gov/ij/). Additional sections were stained with Picro-Sirius Red for collagen analysis (mainly collagen I and III) and it was also analyzed using Image J software.

2.9. Hydroxyproline quantification

Hydroxyproline was measured using a hydroxyproline colorimetric assay as described [19]. Epididymal adipose samples were weighted, dehydrated in acetone, degreased in petroleum ether and dried. Samples were hydrolyzed in 6 N HCl for 18 h at 110 °C and neutralized with 6 N NaOH. Twenty microliters of supernatant was treated with of chloramine T solution and Erlich's solution (4-dimethylamino-benzaldehyde in perchloric acid) and incubated for 20 min at 65 °C. The absorbance was measured at 540 nm and the concentration was determined using the standard curve created with hydroxyproline (0.2–6 µg/mL).

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