



Effects of preadipocytes derived from mice fed with high fat diet on the angiogenic potential of endothelial cells

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KEYWORDS

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Abstract *Background and aims:* Obesity promotes a persistent inflammatory process in the adipose tissue, activating the endothelium and leading to vascular dysfunction. Preadipocytes can interact with endothelial cells in a paracrine way stimulating angiogenesis. However, the potential of preadipocytes from adipose tissue of high fat diet (HFD) fed animal to stimulate angiogenesis has not been evaluated yet. The aim of this study was to investigate the effects of such diet on the angiogenic potential of preadipocytes in a mice model.

Methods and results: We have evaluated body weight gain, fasting glucose levels and insulin resistance, mRNA expression in preadipocytes and endothelial cells after co-culture with preadipocytes, *in vivo* vascular function and *in vitro* endothelial cell migration and tubulogenesis. High fat diet promoted an increase in body weight, glycemic index and insulin resistance in mice. Preadipocytes mRNA expression of factors involved in angiogenesis was higher in these animals. In endothelial tEnd cells mRNA expression of factors involved in vessel growth were higher after co-culture with preadipocytes derived from mice fed with HFD. Although no significant differences were observed in *in vivo* vasodilatation response between control and HFD groups, endothelial tEnd cells showed an increase in migration and tubulogenesis when cultivated with conditioned media from preadipocytes derived from mice fed with HFD.

Conclusion: Hypoxic and growth factors produced by preadipocytes derived from mice fed with HFD have higher capacity than preadipocytes derived from mice fed with standard diet to stimulate the angiogenic potential of endothelial cells, contributing to vascular disorders in obesity.

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Introduction

Obesity is defined by body fat accumulation as result of energy unbalance and it is influenced by behavioral, environmental, cultural and genetic factors [1]. It also contributes to development of acute myocardial infarction [2], insulin resistance [3,4] and type 2 diabetes mellitus [5].

In response to several signals, such as insulin, cortisol and catecholamines, adipocytes secrete adipokines that act both locally and systemically, regulating a lot of biological processes, such as endothelial function, atherogenesis, insulin action and regulation of the energetic balance [6–8].

When energy intake consistently exceeds energy expenditure, the adipose tissue expands due to hypertrophy in particular but also hyperplasia of adipocytes. Hypertrophied adipocytes secrete adipokines that are able to recruit macrophages, inducing an inflamed state in the adipose tissue [9].

The pathophysiological alterations due to expansion of the adipose tissue are deleterious to the circulatory system, activating the endothelium and leading to endothelial dysfunction, eliciting alterations in endothelium-dependent vasodilatation and dysregulation of interactions between endothelium and blood cells, leading to inflammation and severe vascular lesions [10].

Due to expansion of the adipose tissue during obesity, angiogenesis and neovascularization occur, providing the tissue in expansion oxygen and nutrient supply. It is already known that preadipocytes can interact with endothelial cells in a paracrine way stimulating angiogenesis [11]. However, the potential of preadipocytes from the adipose tissue of high fat diet fed animal to stimulate angiogenesis has not been evaluated yet. Therefore, our study investigated the effects of high fat diet on angiogenic potential of preadipocytes in a mice model.

Methods

Detailed Methods are provided in the Online Supplementary material.

Animal care

This study followed the Principles of Laboratory Animal Care published by US National Institute of Health (NIH publication, 1985) and was approved by the State University of Rio de Janeiro Committee for Animal Experimentation (number 23/2016).

Male C57BL/6 mice ($n = 52$) were used in the experiments with chow and water provided *ad libitum*.

Experimental chow

Two types of chows were made according to the following parameters: standard diet with 15.9% of energy derived from fat and high fat diet with 40.6% of energy derived

from fat. Detailed chow composition is provided in *Supplementary material*.

Experimental design

Immediately after the weaning period, animals were divided into two groups and fed different diets: high fat diet group (HF, $n = 29$) and control group with standard chow (C, $n = 23$).

Mice were fed their respective diet for 18 weeks. Body weight and food intake were measured weekly. By the end of this period, mice were used for: (1) insulin resistance tests, (2) extraction of epididymal adipose tissue for molecular analysis or preadipocytes primary cell culture or (3) microcirculatory analysis of epididymal adipose tissue.

Isolation and primary cell culture of preadipocytes

Epididymal adipose tissue was excised from the animal, minced and digested using 0.07% type IV collagenase at 37 °C for 1 h, vortexing every 10 min. Digested tissue was filtered in a 100 μ m-pore cell strainer (BD Biosciences, U.S.A.) and resuspended in 10 ml of Dulbecco's modified essential medium (DMEM) containing 10% fetal bovine serum (FBS) and 5% bovine serum albumin. Resuspension was centrifuged at 500 g for 5 min. Pellet was collected with preadipocyte fraction, resuspended in a solution of 1:4 ammonium chloride/DMEM in order to lyse erythrocytes and centrifuged at 500 g for 5 min. Cells were then washed extensively and plated in 6-well plates (TPP, Switzerland). When they reached confluency, the cells were scraped and total RNA extraction was performed.

Preparation of conditioned media

Primary preadipocytes were seeded at a density of 2×10^5 cells in a 35-mm dish with DMEM with 10% FBS. Once the cells attained 70% confluency, the growth media was replaced with fresh media and incubated at 37 °C with 5% CO₂. After 48 h of incubation, the media was collected and centrifuged at 200 g for 5 min. Supernatant was collected, filtered with 0.2 mm filter and stored at –80 °C.

Cell culture of tEnd cells for migration assay

A murine endothelioma cell line (tEnd) was used as endothelium model [12–14]. The cells were seeded (5×10^4) onto the upper membranes of Transwell® permeable supports (8- μ m pore size filter membranes; Becton Dickinson) and placed in 24-well plates (Falcon). Conditioned medium of preadipocytes from mice fed standard or high fat diet was added in the bottom chamber. tEnd cells were allowed to migrate for 16 h at 37 °C and 5% CO₂.

Matrigel™ tube-formation assay

Basement membrane gels for three-dimensional assays were prepared by polymerizing Matrigel at 37 °C. tEnd

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