Biochimica et Biophysica Acta 1861 (2016) 1614–1622

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Brown adipose tissue in obesity: Fractalkine-receptor dependent immune cell recruitment affects metabolic-related gene expression

Ágnes Polyák [a,b], Zsuzsanna Winkler [a,c], Dániel Kuti [a,c], Szilamér Ferenczi [a], Krisztina J. Kovács [a,*]

[a] Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Budapest, Hungary
[b] Tamás Roska Doctoral School of Sciences and Technology, Pázmány Péter Catholic University, Budapest, Hungary
[c] János Szentágothai Doctoral School of Neurosciences, Semmelweis University Budapest, Hungary

ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 5 July 2016
Accepted 9 July 2016
Available online 12 July 2016

Keywords:
Fractalkine
Macrophage
Inflammation
Triglyceride metabolism
Thermogenesis
Obesity
BAT

ABSTRACT

Brown adipose tissue (BAT) plays essential role in metabolic- and thermoregulation and displays morphological and functional plasticity in response to environmental and metabolic challenges. BAT is a heterogeneous tissue containing adipocytes and various immune-related cells, however, their interaction in regulation of BAT function is not fully elucidated. Fractalkine is a chemokine synthesized by adipocytes, which recruits fractalkine receptor (CX3CR1)-expressing leukocytes into the adipose tissue. Using transgenic mice, in which the fractalkine receptor, *Cx3cr1* gene was replaced by *Gfp*, we evaluated whether deficiency in fractalkine signaling affects BAT remodeling and function in high-fat-diet - induced obesity. Homo- and heterozygote male CX3CR1-GFP mice were fed with normal or fat enriched (FatED) diet for 10 weeks. Interscapular BAT was collected for molecular biological analysis. Heterozygous animals in which fractalkine signaling remains intact, gain more weight during FatED than CX3CR1 deficient gfp/gfp homozygotes. FatED in controls resulted in macrophage recruitment to the BAT with increased expression of proinflammatory mediators (*Il1a, b, Tnfa and Ccl2*). Local BAT inflammation was accompanied by increased expression of lipogenic enzymes and resulted in BAT "whitening". By contrast, fractalkine receptor deficiency prevented accumulation of tissue macrophages, selectively attenuated the expression of *Tnfa, Il1a and Ccl2*, increased BAT expression of lipolytic enzymes (*Atgl, Hsl and Mgtl*) and upregulated genes involved thermo-metabolism (*Ucp1, Pparg Pgc1a*) in response to FatED. These results highlight the importance of fractalkine-CX3CR1 interaction in recruitment of macrophages into the BAT of obese mice which might contribute to local tissue inflammation, adipose tissue remodeling and regulation of metabolic-related genes.

1. Introduction

Obesity and diabetes are worldwide epidemics driven by the disruption in energy balance [1]. Brown adipose tissue (BAT) is the major site for cold- and diet-induced thermogenesis with which BAT significantly affects systemic glucose and lipid metabolism [2–4]. In 2007 Nedergaard et al. published that adult humans possess active BAT [5]. The amount of BAT is inversely correlated with body-mass index, especially in older people [6]. Metabolically active BAT seems to be particularly low in patients with obesity or diabetes [7]. These results suggest a significant role of brown adipose tissue in adult human metabolism and opens new opportunities to develop therapeutic interventions to treat obesity.

Brown adipocytes and inducible brown-in-white (brite, beige) adipocytes are multilocular and contain significantly higher number of mitochondria than other adipocytes in the body [8]. These cells are specialized to dissipate energy in the form of heat by uncoupled thermogenesis, mediated by the dissociation of mitochondrial respiratory chain electron transport from ATP synthesis via the action of uncoupling protein UCP1. In addition to adipocytes, adipose tissues contains various immune-related cells including resident macrophages, eosinophils, mast cells and T cells, which significantly contribute to their function via release (adipo)cytokines and transmitters in paracrine or endocrine fashion. [9–12]. Both types of adipose tissues (BAT and WAT) are

Abbreviations: BAT, brown adipose tissue; WAT, white adipose tissue; CX3CL1, fractalkine; CX3CR1, fractalkine receptor; GFP, green fluorescent protein; HFD, high fat diet; FatED, fat enriched diet; ND, normal diet; PND, postnatal day; qPCR, quantitative real time polymerase chain reaction; IL1A, interleukin 1 alpha; IL1B, interleukin 1 beta; IL6, interleukin 6; TNFa, tumor necrosis factor alpha; CCL2 (MCP1), chemokine (C—C motif) ligand 2; UCP1, uncoupling protein 1; PPARG, peroxisome proliferator-activated receptor gamma; PGC1A (PPARGC1A), peroxisome proliferator-activated receptor gammacoactivator 1 alpha; TH, tyrosine hydroxylase; ADRB3, adrenoceptor beta 3; DIO2, Type 2 Iodothyronine Deiodinase; GLUT4, Glucose transporter type 4; DGAT1, diacylglycerol O-acyltransferase 1; MGAT, mannosyl (alpha-1,3-)-glycoprotein beta-1,2-*N*-acetylglucosaminyltransferase; GPAT, glycerol-3-phosphate acyltransferase; ATGL (PNPLA2), adipose triglyceride lipase; HSL (LIPE), lipase hormone-sensitive; MGL, monoglyceride lipase; FA, fatty acid; KPBS, potassium phosphate buffered saline; RIPA, radioimmunoprecipitation assay buffer; HRP, horse radish peroxidase; TBST, tris buffered saline with Tween.

* Corresponding author at: Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Szigony utca 43, Budapest, Hungary.
E-mail address: kovacs@koki.hu (K.J. Kovács).

http://dx.doi.org/10.1016/j.bbalip.2016.07.002
1388-1981/

sensitive to environmental (temperature) - hormonal (T3, leptin, insulin, corticosteroid) - and metabolic (high fat diet) cues and display significant cellular and functional remodeling in response to these challenges. For instance, high fat diet results in hypertrophy and hyperplasia of white adipocytes and recruitment of monocytes into the WAT [13]. Furthermore, in obese animals and humans there is a shift from alternatively (anti-inflammatory) polarized macrophages to those that produce predominantly proinflammatory mediators [14,15]. However, the accumulation of macrophages to BAT, the mechanisms that recruit and activate them, and their effect on thermometabolic genes has not been fully elucidated. Because these changes contribute to insulin resistance and low grade systemic metabolic inflammation which is seen in a subset of obese patients with metabolic X [16], it is important to understand the mechanisms that recruit and activate adipose tissue macrophages and the means with which local inflammation affects lipid metabolism and thermoregulation.

Fractalkine (CX3CL1) is a chemokine expressed in endothelial cells, vascular smooth muscle cells, hepatocytes, adipocytes and neurons as a transmembrane protein and involved in trafficking and capturing various leukocytes (monocytes, macrophages, microglia) expressing its'cognate receptor, CX3CR1 [17,18]. Fractalkine -released from the cell surface by proteolytic cleavage- acts in paracrine and endocrine manner and has been identified in the WAT as a novel adipocytokine with increased expression in obese individuals [19]. It has been shown previously that lack of CX3CL1-CX3CR1 signaling results in reduced macrophage accumulation into white adipose tissue and reduced body weight gain during the development of obesity [20].

The aim of the present study was to identify the role of fractalkine/CX3CR1 signaling in the recruitment of monocytes into the brown adipose tissue and to reveal the role of local inflammation in regulation of genes involved in triglyceride- and thermo-metabolism in obese mice.

2. Materials and methods

2.1. Animals and diet

Experiments were performed in male CX3CR1 +/gfp (+/gfp), and CX3CR1 gfp/gfp (gfp/gfp) mice [17]. Animals were obtained from the European Mouse Mutant Archive (EMMA Cx3cr1^{tm1Litt} MGI:2670351). The background C57Bl/6J strain has been shown to be genetically vulnerable to diet-induced obesity [21]. In these mice, the *Cx3cr1* gene was replaced by a *Gfp* reporter gene such that heterozygote CX3CR1 +/gfp mice express GFP in cells of the myeloid linage and retain receptor function, whereas monocytes in homozygote CX3CR1 gfp/gfp mice are labeled with GFP and lack functional CX3CR1. Genotype of the animals has been verified by PCR using combination of three different primers as described by Jung et al. [17].

Animals were housed in groups of 4–5/cage at the minimal disease (MD) level of the Medical Gene Technology Unit of our Institute, had free access to food and water and were maintained under controlled conditions: temperature, 21 °C ± 1 °C; humidity, 65%; light-dark cycle, 12-h light/12-h dark cycle, lights on at 07:00. At 35 days of age, both CX3CR1 +/gfp (n = 25) and CX3CR1 gfp/gfp (n = 25) mice were randomly distributed into two groups. The first group, normal diet (ND), received standard chow (VRF1 (P), Special Diets Services (SDS), Witham, Essex, UK.). The second group received fat-enriched diet (FatED), by providing a 2:1 mixture of standard chow and lard (Spar Budget, Budapest, Hungary). The energy content and macronutrient compositions of the two diets are given in Table 1. All procedures were conducted in accordance with the guidelines set by the European Communities Council Directive (86/609 EEC) and approved by the Institutional Animal Care and Use Committee of the Institute of Experimental Medicine (permit number: 22.1/3347/003/2007).

Table 1
Energy content and macronutrient composition of diets.

	ND - standard chow		FatED - mixed chow	
	g%	kcal%	g%	kcal%
Protein	19,1	22,5	12,7	9,7
Carbohydrate	55,3	65,0	36,9	28,0
Fat	4,8	12,6	36,5	62,3
kcal/g	3,40		5,27	

2.2. Experimental design

Mice were fed with normal diet (ND) or fat enriched diet (FatED) starting at age of 35 days. Mice were decapitated ten weeks later, interscapular brown adipose tissues were collected, sampled and stored at −70 °C for qPCR, tissue samples were also obtained for histology. A separate set of animals underwent cold tolerance test.

2.3. Histology

BAT samples were immersion fixed in 4% w/v paraformaldehyde in 0.1 mol l^{-1} phosphate buffer, pH 7.4 (PB) for 3 days and stored in 1% w/v paraformaldehyde in 0.1 mol l^{-1} PB at 4 °C then were embedded in paraffin, sectioned and stained with hematoxylin-eosin (H&E). Microscopic slides were digitalized with Pannoramic Digital Slide Scanner (3DHISTECH Kft., Hungary). Lipid droplet number and size of brown adipose cells were counted under 40× magnification in one field of view with ImageJ software (NIH, USA).

2.4. Immunohistochemistry

F4/80 (murine macrophage marker) staining on paraffin-embedded tissue sections was performed by standard immunohistochemical protocol. Slides were deparaffinized and rehydrated then antigen retrieval was performed with proteinase K (10 mg/ml; diluted to 1:25 in 1 M Tris buffer pH = 8.0). Endogenous peroxidase was blocked by 0.3% H_2O_2. After washes in KPBS, nonspecific binding was blocked by 2% normal rabbit serum for 1 h. The sections were incubated with anti-mouse F4/80 antibody made in rat (BMA Biomedicals, 1:50) overnight at 4 °C. Following KPBS (0.01 M potassium phosphate buffer, 0.154 M NaCl pH 7.4) washes (4 × 5 min), slides were incubated for 1 h using biotinylated rabbit anti-rat antibody (Vector Laboratories; 1:250). After rinsing in KPBS, avidin biotin amplification was performed with a Vectastain Elite ABC kit (Vector Laboratories) and immunoreactivity was visualized with nickel-enhanced diaminobenzidine (DAB-Ni) substrate. Sections were analyzed with Nikon Eclipse E600 microscope under 20× magnification in 5 fields of view per section.

Fluorescent F4/80 labeling was performed on 1 mm^3 BAT blocks fixed in 4% w/v paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and stored in cryoprotectant solution at −20 °C. Tissue blocks were blocked with 2% normal rabbit serum then incubated in rat anti-mouse F4/80 antibody (BMA Biomedicals; 1:50) overnight at 4 °C. The antigens were then visualized by biotinylated rabbit anti-rat IgG (Vector Laboratories; 1:500) for 100 min followed by streptavidin Alexa 594 (Molecular Probes; 1:500) for 100 min. Images were taken using a Nikon C2 confocal microscope, at 60× magnification.

2.5. Core body temperature measurement and cold challenge

Rectal temperature was measured with Multithermo thermometer (Seiwa Me Laboratories Inc., Tokyo, Japan). To assess cold tolerance, set of animals (n = 30) from both genotypes were fasted for 5 h, then placed into new individual cages with minimal bedding and transferred to cold room (4 °C). Rectal temperature was measured before and 60, 120, 180 and 240 min after cold exposure.