The role of fractalkine – CX3CR1 signaling in the development of obesity

Theses of the Ph.D dissertation

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Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that impairs health. Obesity affects all socioeconomic backgrounds and ethnicities and is a prerequisite for metabolic syndrome. Metabolic syndrome is a clustering of risk factors, such as central obesity, insulin resistance, dyslipidaemia and hypertension that together culminate in the increased risk of type 2 diabetes mellitus and cardiovascular diseases [1].

Obesity and overweight are associated with chronic low grade inflammation. This inflammatory condition plays an important part in the etiology of the metabolic syndrome and largely contributes to the related pathological outcomes [2].

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended [3]. The consumed energy is used for basal metabolism, physical activity and adaptive thermogenesis or stored in adipose tissue. The components of energy homeostasis are shown in Fig. 1.

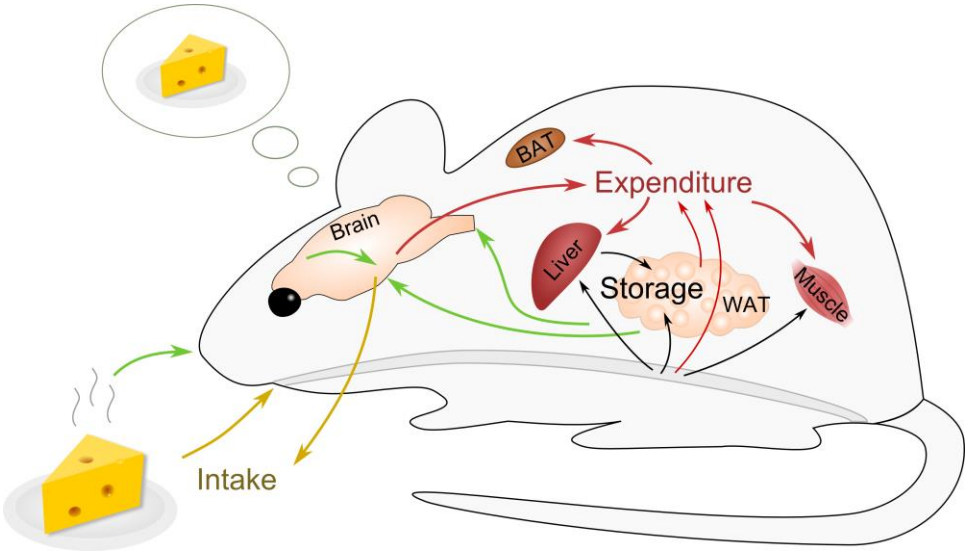

Figure 1. Components of energy homeostasis.

Brain regulates energy intake (yellow arrows) and energy expenditure (red arrows) in response to external and internal signals (green arrows). The fuels used for physical activity, basal metabolism, and adaptive thermogenesis originates from absorbed food. Excess nutrients are stored in adipose tissues, liver or muscles for further use (black arrows).

There are two types of adipose tissue, the white and the brown fat, which have distinct functions. White adipose tissue (WAT) stores excess energy as triglycerides and displays endocrine functions by secreting adipokines and cytokines [4-6]. Brown adipose tissue (BAT) is the major site of cold-, stress- and diet-induced thermogenesis with which BAT significantly affects systemic glucose and lipid metabolism [7-9]. In 2007 Nedergaard et al. published that adult humans possess active BAT (Nedergaard, Bengtsson et al. 2007). The amount of BAT is inversely correlated with body-mass index, especially in older people (Cypess, Lehman et al. 2009). Metabolically active BAT seems to be particularly low in patients with obesity or diabetes (Bartelt and Heeren 2014). 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 (Harms and Seale 2013). 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.

Adipose tissue contains various immune-related cells including resident macrophages (adipose tissue macrophages – ATMs), eosinophils, mast cells and T cells, which significantly contribute to their function via release (adipo)cytokines and transmitters in paracrine or endocrine fashion [10-13]. Expanding WAT in obese subjects attracts various immune cells and release pro-inflammatory cytokines that contribute to the “low grade chronic metabolic inflammation” that represents a significant health burden [14]. Growing evidence implicates that obesity-induced tissue inflammation is not limited to the visceral WAT but also seen in the liver and in the hypothalamus [15]. In either tissue, diet-induced inflammation is always associated with recruitment/proliferation and activation of various immune-competent cells such as monocytes, macrophages and T cells. However the mechanism of macrophage recruitment is not fully elucidated.

Monocyte/macrophage trafficking is a complex process that involves many molecules [16]. One of these molecules is fractalkine, which could play a critical role, as it may act either as an adhesion molecule or/and as a chemokine, thus it could be involved in multiple steps of the trafficking (Fig.2).

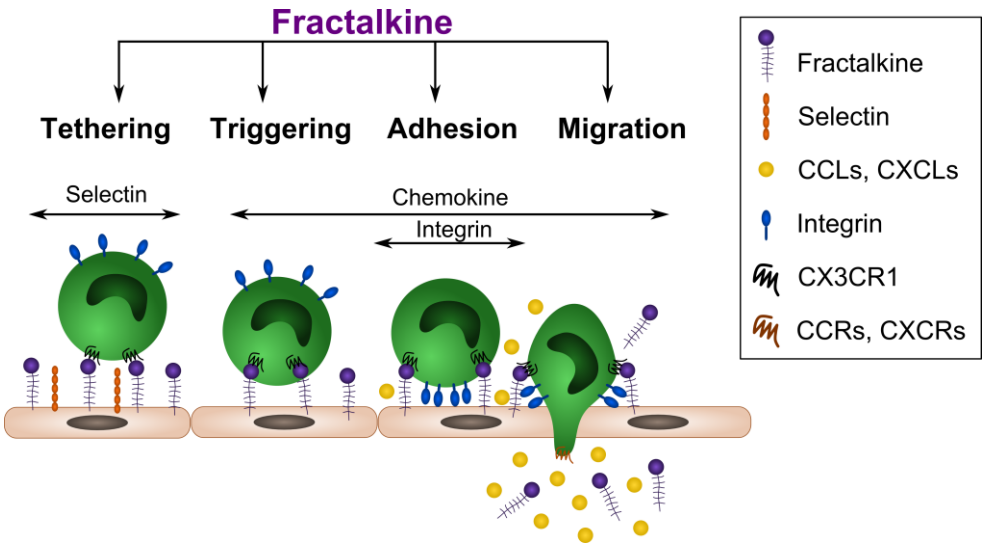

Figure 2. Schematic model of fractalkine-mediated pathways in the adhesion cascade.
Fractalkine is expressed on endothelial cells as the membrane-bound form and captures CX3CR1 expressing leukocytes in a selectin- and integrin-independent manner. Interaction between fractalkine and CX3CR1 can also increase integrin avidity, resulting in firmer adhesion. CX3CR1 expressing leukocytes then extravasate through the vascular wall into the tissue to a chemokine gradient. Fractalkine may facilitate extravasation of circulating CX3CR1 expressing leukocytes by mediating cell adhesion through the initial tethering and final transmigration steps [17].

Fractalkine (CX3CL1) is expressed by endothelial cells, vascular smooth muscle cells, hepatocytes, adipocytes and neurons as, while it's receptor, CX3CR1 is expressed by various leukocytes (monocytes, macrophages, microglia) [18, 19]. The role of fractalkine – CX3CR1 signaling in cardiovascular diseases (such as atherosclerosis), rheumatoid arthritis, other inflammatory diseases and cancer is well described [20, 21], and though fractalkine has been identified in the WAT as a novel adipocytokine with increased expression in obese individuals [22], its function in the development of obesity is not fully known. Furthermore, it is not known whether fractalkine – CX3CR1 signaling has a role in BAT inflammation and/or function.

My aims were to identify the role of fractalkine/CX3CR1 signaling in the recruitment and activation of immune cells in key central (hypothalamus) and peripheral (visceral WAT, BAT and liver) structures in obesity and, to reveal the role of obesity-related, fractalkine – CX3CR1 dependent, local inflammation in regulation of triglyceride- and thermo-metabolism in BAT of obese mice.

Methods

- Experiments were performed in male CX3CR1 +/gfp (+/gfp, control), and CX3CR1 gfp/gfp (gfp/gfp, fractalkine deficient) mice [18]. The background C57Bl/6J strain has been shown to be genetically vulnerable to diet-induced obesity [23]. 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.
- Mice were fed with normal diet (ND) or fat enriched diet (FatED) for 10 weeks, body weight and food consumption was measured weekly. In the 10th week, glucose tolerance test (GTT) was performed after overnight fasting. Two days after the GTT, mice were decapitated, plasma and organs were stored for further use. A separate set of animals underwent cold tolerance test. Body composition was assessed on another set of animals.
- Glucose tolerance was performed after overnight fasting. 2 mg/g of body weight D-glucose was injected intraperitoneally and blood glucose was measured from tail vein by DCont Personal Blood Glucose Meter (77 Elektronika Kft. Hungary) at 0 min (just before glucose injection) and at 15-, 30-, 60-, 90- and 120-min intervals after the glucose load.
- Body composition was determined using EchoMRI™ Body Composition Analyzer (EchoMRI, Houston, TX, USA).
- In cold tolerance test rectal temperature was measured before and 60, 120, 180 and 240 min after cold exposure.
- Plasma adrenocorticotrophic hormone (ACTH) and corticosterone concentrations were measured by radioimmunoassay (RIA).
- Plasma cytokine levels were measured by ELISA using DuoSet ELISA kits for IL1a, IL1b and IL6 (R&DSystems, Minneapolis, MN, USA).
- Histological analysis was performed on paraffin embedded, hematoxylin-eosin stained sections of EWAT, SWAT and BAT.
- Macrophages on paraffin embedded tissues were visualized by DAB-Ni and fluorescence F4/80 immunohistochemistry.
- Gene expression analysis was performed by quantitative real-time PCR.
- UCP1 protein from BAT was determined by western blot analysis.
- WAT immune cell composition was analized by flow cytometer.
- Statistical analysis was performed by factorial ANOVA with Newman–Keuls post-hoc test in Statistica 11 (StatSoft Inc.). Flow cytometric data were analyzed by two-way ANOVA followed by Sidak's multiple comparison test (GraphPad Prism).

New scientific results

Thesis I.

I have shown that FatED induced obesity and metabolic inflammation is associated with elevated fractalkine expression in white (WAT) and brown adipose tissue (BAT)

Related publications: [J1], [J2], [C1], [C2], [C3], [C4].

I have found that

- 10 weeks of fat enriched diet (FatED) results in increased body weight gain in mice.
- Body weight gain/obesity is due to increased fat deposition
- Obese mice display glucose and cold intolerance.
- FatED results in morphological rearrangements and recruitment of leukocytes/macrophages in white and brown adipose tissue depots.
- Obese mice have metabolic inflammation with
    - increased plasma levels of proinflammatory cytokine IL1b;
    - increased mRNA levels of chemokines in white and brown adipose tissue;
    - elevated local expression of proinflammatory cytokines in the WAT and BAT.
- I have shown for the first time that FatED results in fractalkine mRNA induction in the BAT;
- FatED-induced inflammation was localized in the adipose tissues but not seen in the liver or in the hypothalamus.

Thesis II.

I have shown that fractalkine – CX3CR1 signaling contributes to the development of obesity

Related publications: [J1], [J2], [C1], [C2], [C3], [C4].

Because fractalkine-fractalkine receptor system is involved in the signaling, trafficking, recruitment and activation of various leukocytes at the site of inflammation, I have investigated its involvement in metabolic inflammation.

I have provided new evidence that mice with targeted disruption of the fractalkine receptor (CX3CR1) gene (CX3CR1 gfp/gfp)

- are less sensitive to obesogenic effect of FatED;
- gain less weight on FatED and do not develop glucose intolerance during the diet;
- their adipocytes are smaller and display significantly less crown like structures in the epididymal WAT and BAT;
- have equally increased expression of fractalkine, but decreased *Ccl2* expression;
- recruit much less GFP+ and F4/80+ macrophages in the WAT and BAT when kept on FatED;
- display attenuated expression of proinflammatory cytokines in the epididymal WAT and BAT;
- do not develop serious metabolic inflammation –

- compared to CX3CR1 heterozygotes, which have intact fractalkine signaling.

Thesis III.

I have shown that obesity associated fractalkine – CX3CR1 dependent macrophage infiltration and inflammation in BAT affects thermogenesis

Related publications: [J1].

I have found that the obesity resistant phenotype of fractalkine receptor deficient mice is mechanistically associated with

- increased lipolysis due to diet-induced expression of lipolytic - and unchanged lipogenic enzymes;
- and increased expression of uncoupling protein (UCP1) in the BAT –
- which was not seen in obese mice with normal fractalkine signaling.

Changes in the brown adipocyte of mice with normal and impaired fractalkine signaling in response to FatED are shown in Fig.3.

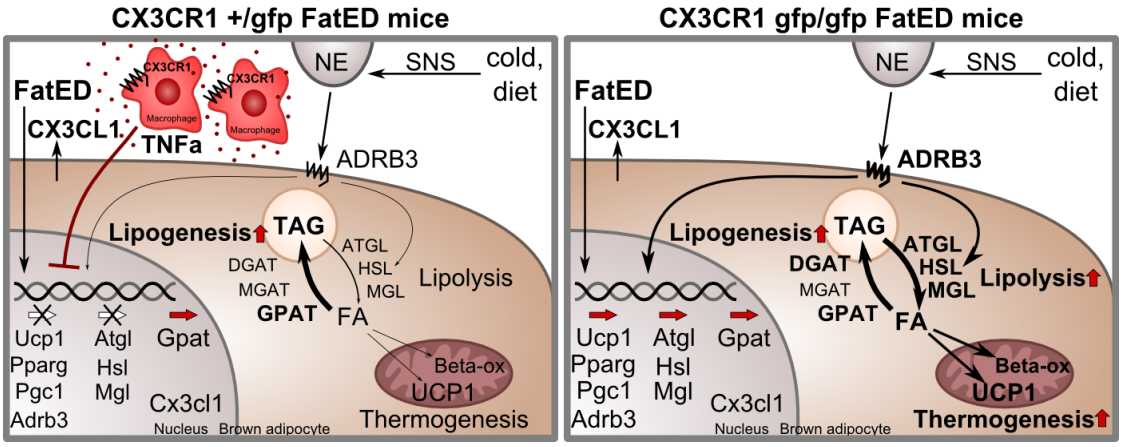

Figure 3. Fractalkine and CX3CR1 contributes to the accumulation of macrophages into BAT during the development of obesity. *The presence of macrophages leads to elevated expression of proinflammatory cytokines, which attenuate the thermogenic capacity of BAT.*

Possible applications

My new scientific results contribute to the understanding of the development of obesity, metabolic inflammation and control of thermogenesis in BAT. Using this knowledge, new potential therapeutic targets can be identified. First of all, pharmacological inhibition of fractalkine receptor by CX3CR1 antagonist could reduce macrophage accumulation to adipose tissues, therefore reduce adipose tissue inflammation and the development of obesity. Treatment with F1, a CX3CR1 antagonist, has been shown to reduce macrophage accumulation in the aortic sinus in mouse models of atherosclerosis [24], thus its use in obesity is promising. Based on my research, though a CX3CR1 antagonist could prevent the development of obesity if it is administered from onset, it requires further investigation whether it could reduce obesity when it is administered in advanced state. Second, BAT, as an important contributor of energy expenditure by non-shivering thermogenesis, could be a potential therapeutic target. Preventing macrophage accumulation thus preventing proinflammatory cytokine expression in BAT, or blocking the proinflammatory cytokine expression in BAT could upregulate the thermogenic machinery therefore the elevated thermogenic activity could modulate energy intake/energy expenditure balance leading to the utilization of excess fat. In an experiment in mice it has been shown that cold exposure rapidly promotes alternative activation of adipose tissue macrophages, which secrete catecholamines to induce thermogenic gene expression in BAT and lipolysis in WAT [25]. My results suggest that the proinflammatory environment is responsible for the impaired

thermogenesis in obese animals. Accordingly setting a cold therapy protocol in humans, which could induce switching of macrophages from proinflammatory to alternatively activated state, could activate BAT thermogenesis in obese people potentially leading to weight loss.

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List of Publications

Journal Publications

[J1] Polyák Á, Winkler Z, Kuti D, Ferenczi S, Kovács KJ.
Brown adipose tissue in obesity: Fractalkine-receptor dependent immune cell recruitment affects metabolic-related gene expression.
Biochim Biophys Acta. 2016 Jul 12;1861(11):1614-1622. doi: 10.1016/j.bbalip.2016.07.002.

[J2] Polyák A, Ferenczi S, Dénes A, Winkler Z, Kriszt R, Pintér-Kübler B, Kovács KJ.
The fractalkine/Cx3CR1 system is implicated in the development of metabolic visceral adipose tissue inflammation in obesity.
Brain Behav Immun. 2014 Jan 21. pii: S0889-1591(14)00011-7. doi: 10.1016/j.bbi.2014.01.010

Conference Publications

[C1] Ágnes Polyák, Zsuzsanna Winkler, Szilamér Ferenczi, Dóra Kővári, Dániel Kuti, Krisztina J. Kovács
Role of fractalkine/Cx3CR1 signaling in macrophage trafficing into adipose tissues and in the development of obesity
Central European Congress on Obesity, 1-3 October 2015, Budapest , Hungary

[C2] Ágnes Polyák, Zsuzsanna Winkler, Szilamér Ferenczi, Dóra Kővári, Dániel Kuti, Krisztina Kovács
Regulation of energy homeostasis in high fat diet fed Cx3CR1 deficient and control mice
MITT, Budapest, Hungary, January 22-23, 2015

[C3] Ágnes Polyák, Zsuzsanna Winkler, Szilamér Ferenczi, Krisztina J. Kovács
Fractalkine/Cx3CR1 signaling in brown adipose tissue and diet induced obesity
From Medicine to Bionics 2014, 2nd European Ph.D. Conference, Budapest, May 9-10, 2014

[C4] Ágnes Polyák, Szilamér Ferenczi, Zsuzsanna Winkler, Ádám Dénes, Rókus Kriszt, Bernadett Pintér-Kübler and Krisztina J. Kovács
The Fractalkine/Cx3CR1 System is Implicated in the Development of Metabolic Visceral Adipose Tissue Inflammation in Obesity
IBRO, january 16-17. Debrecen, Hungary, 2014

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Other Publications

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