Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Omega-3 fatty acids promote fatty acid utilization and production of pro-resolving lipid mediators in alternatively activated adipose tissue macrophages

Martina Rombaldova [a, b], Petra Janovska [a], Jan Kopecky [a], Ondrej Kuda [a, *]

[a] Department of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 14220 Praha 4, Czech Republic
[b] Charles University in Prague, Faculty of Science, Department of Analytical Chemistry, Albertov 2030, 128 43 Prague, Czech Republic

ARTICLE INFO

Article history:
Received 26 June 2017
Accepted 27 June 2017
Available online xxx

Keywords:
Adipose tissue macrophages
Omega-3 PUFA
Fatty acid re-esterification
Lipolysis
Lipid mediators

ABSTRACT

It is becoming increasingly apparent that mutual interactions between adipocytes and immune cells are key to the integrated control of adipose tissue inflammation and lipid metabolism in obesity, but little is known about the non-inflammatory functions of adipose tissue macrophages (ATMs) and how they might be impacted by neighboring adipocytes. In the current study we used metabolipidomic analysis to examine the adaptations to lipid overload of M1 or M2 polarized macrophages co-incubated with adipocytes and explored potential benefits of omega-3 polyunsaturated fatty acids (PUFA). Macrophages adjust their metabolism to process excess lipids and M2 macrophages in turn modulate lipolysis and fatty acids (FA) re-esterification of adipocytes. While M1 macrophages tend to store surplus FA as triacylglycerols and cholesteryl esters in lipid droplets, M2 macrophages channel FA toward re-esterification and β-oxidation. Dietary omega-3 PUFA enhance β-oxidation in both M1 and M2. Our data document that ATMs contribute to lipid trafficking in adipose tissue and that omega-3 PUFA could modulate FA metabolism of ATMs.

1. Introduction

Obesity-associated changes in white adipose tissue (WAT) are consistent with the immunometabolic concept that the immune and metabolic systems are interconnected [1–4] and that immune cells affect tissue and systemic metabolism to support the adaptation to environmental challenges [5,6]. WAT stores excess energy, serves as an endocrine organ and also contains a high number of almost all types of immune cells [1,7,8].

Macrophages are the most frequently studied immune cells in the WAT since the discovery that they contribute to low-grade WAT inflammation and insulin resistance [9–11]. In addition to their immune functions, adipose tissue macrophages play a role in WAT lipid metabolism [12–15]. Lipid storage in ATMs helps to relieve adipocytes of excess lipids and improves systemic insulin sensitivity [16,17], but sustained lipid accumulation in macrophages leads to WAT insulin resistance [14] and the formation of ATMs syncytia in WAT, known as “crown-like structures” [18]. One part of the lipid processing mechanism – the lysosomal lipid-degradation program [9] – has been described, but other lipid processing pathways in ATMs, including FA re-esterification, are poorly understood.

The content of ATMs is impacted by changes in metabolic state of the tissue as they come, leave or proliferate locally [7,12,19–22]. Simultaneously, the macrophages express different functional programs to adapt to microenvironmental challenges and they polarize into a spectrum of functional states from M1 (pro-inflammatory) to M2 (anti-inflammatory) phenotypes [1,8]. M2 ATMs produce anti-inflammatory cytokines and lipid mediators, promote WAT remodeling and support systemic insulin sensitivity (reviewed in Refs. [4,23,24]) while M1 secrete pro-inflammatory cytokines and negatively affect WAT functions [10,11]. The ATMs

Abbreviations: 17-HDHA, 17-hydroxy docosahexaenoic acid; ATMs, adipose tissue macrophages; BMM, bone marrow-derive macrophages; CE, cholesteryl ester; DAG, diacylglycerol; DHA, docosahexaenoic acid; DHEA, N-docosahexaenoylethanolamine; FA, fatty acid; glycerol-3P, glycerol-3-phosphate; HF, high fat (diet); HFA, adipocytes from HF diet-fed mice; HFF, high fat diet with omega-3 PUFA; HFFA, adipocytes from HFF diet-fed mice; MAG, monoacylglycerol; PA, phosphatidic acid; PUFA, polyunsaturated fatty acids; TAG, triacylglycerol; WAT, white adipose tissue.

* Corresponding author. Department of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 142 20 Prague, Czech Republic.
E-mail address: ondrej.kuda@fgu.cas.cz (O. Kuda).

http://dx.doi.org/10.1016/j.bbrc.2017.06.170
0006-291X/

polarization can be influenced by dietary lipid composition [1,15] and long-chain omega-3 PUFA (omega-3 PUFA) via PUFA-derived anti-inflammatory lipid mediators drive the polarization toward M2 phenotype, decrease ATMs content, and improve insulin sensitivity in obese mice [23,25–27].

We hypothesized that the beneficial effects of omega-3 PUFA on WAT metabolism in obesity could be partially mediated by stimulation of M2 macrophage lipid processing. To test this, mice were fed a corn oil-based high-fat diet (HF), or HF diet in which 15% of lipids were replaced by omega-3 PUFA (HFF), and collagenase-liberated adipocytes from WAT were co-incubated ex-vivo with in-vitro polarized macrophages. Lipolysis, FA re-esterification and production of anti-inflammatory lipid mediators were explored.

2. Materials and methods

2.1. Materials

Standards for analysis of oxylipins, phospholipids, acylglycerols, various FA and the deuterated standards were purchased from Cayman (Neratovice, Czech Republic), Sigma-Aldrich (Prague, Czech Republic) or Avanti Polar Lipids (Alabaster, Alabama, USA). All other chemicals were purchased from Sigma. Mass spectrometry-grade solvents were purchased from Acros (Lach-Ner, Czech Republic).

2.2. Animals

Male mice (C57BL/6J; Jackson Laboratory, ME, USA) were habituated to a corn oil-based high-fat diet (HF; lipid content 35%, wt/wt) for 2 weeks starting at 2 months of age and then randomly assigned for 5 weeks to various dietary treatments. Experiment with omega-3 PUFA concentrate (46% wt/wt DHA, 14% wt/wt EPA; product EPAX 1050 TG; EPAX, Alesund, Norway) replacing 15% wt/wt of dietary lipids (HFF diet) was conducted as before [28,29].

2.3. Isolation of adipocytes and macrophages

Adipocytes were isolated from epididymal fat according to published methods [28,30]. Bone marrow-derived macrophages were prepared by harvesting bone marrow from the femurs and tibias of 3-month-old C57BL/6J mice [12,31]. Murine macrophage cell line RAW 264.7 was maintained in culture and used as described before [32]. Macrophages were polarized in-vitro using either IL-4 or lipopolysaccharide with IFN-γ for 24 h before the experiments [31]. To promote macrophage state more similar to the microenvironment in WAT, cells were co-incubated with explants of epididymal WAT in hanging inserts for 24 h [12] and explant viability was confirmed by metabolomics analysis (not shown).

2.4. In-vitro lipolysis

Isolated adipocytes were incubated in 24-well plates with or without polarized RAW cells for 2 h at the incubator while shaking [30]. Free glycerol (Glycerol reagent, Sigma-Aldrich) and non-esterified FA (WAKO-C, Wako chemicals, Germany) in media were measured after the experiment and cells were processed for metabolomics or lipidomics.

2.5. Metabolipidomics

Adipocytes or macrophages were frozen in liquid nitrogen after each experiment, stored at −80 °C or immediately extracted with 0.3 ml of chilled solvent (75% acetonitrile in water). Samples were homogenized using pulsed bath sonication (Ultrasonic-cleaner US-10, Lab. Companion, USA) for 10 s and centrifuged at 12,000*g* at 4 °C for 10 min. The supernatants were used for LC-MS analysis. The residual pellets were used for DNA measurement [26]. Targeted metabolomics for profiling of polar metabolites and lipidomics were performed using an UPLC system (UltiMate 3000 Binary RSLC System, Thermo) coupled to Qtrap 5500 (Sciex, CA, USA) mass spectrometer based on previously published methods [28,29,33–35].

2.6. Statistics

Statistical analysis was performed with SigmaStat and $p < 0.05$ was considered significant. R and VANTED software was used to analyze omics data [34].

3. Results

3.1. M2 macrophages modulate adipocyte lipolysis

To examine the interaction between adipocytes and macrophages and test the functional consequences of the paradigm of ATMs polarization in obesity [19,20], we developed an in-vitro system, where we co-incubated primary adipocytes and RAW 264.7 macrophages polarized into M1 or M2 states [31]. Adipocytes freshly isolated from epididymal WAT of mice fed either HF or HFF diet and RAW 264.7 macrophages were either co-incubated or incubated alone for 2 h in the absence (Fig. 1) or presence of 10 μM isoproterenol and the medium concentrations of glycerol and FA were measured. Unstimulated adipocytes (Fig. 1) from HF animals released free glycerol and FA at a ratio of 1:1, suggesting that 2 out of 3 FA molecules were re-esterified back into triacylglycerol (TAG). When co-incubated with M1 macrophages, production of glycerol and FA to media was not altered. Surprisingly, co-incubation with M2 macrophages increased production of glycerol 1.4-fold and that of FA 2.2-fold, suggesting modulation of lipolysis and FA re-esterification in adipocytes by M2 macrophages. Adipocytes isolated from HFF animals responded to macrophages similarly as HF adipocytes, but when co-incubated with M2 cells, production of FA was significantly lower as compared to HF adipocytes, suggesting more FA were re-esterified (Fig. 1). The interaction between adipocytes and M2 macrophages was visible only in the unstimulated state. The addition of isoproterenol or BRL3744, a β3-specific adrenergic agonist, induced a strong lipolytic response, which overrided the effect of macrophages on lipolysis (not shown). No catecholamines were detected in the media and blocking prostanoid production with cyclooxygenase inhibitors didn't alter the lipolytical response (not shown). These results documented modulation of FA metabolism in adipocytes by macrophages, dependent on their polarization, as well as in response to dietary omega-3 PUFA.

3.2. M1 and M2 macrophages differ in the metabolic adaptation to excess FA

We performed global metabolite profiling using mass spectrometry on both adipocytes and RAW 264.7 macrophages to look for changes reflecting the interactions between the two cell types. The metabolic profile (glycolysis, TCA cycle, etc.) of adipocytes was not significantly altered by co-incubation with macrophages when compared with macrophages, except for activation of lipolysis (Fig. 1D) documented by increased monoacylglycerol (MAG) levels. In contrast, macrophages, polarized into M1 and M2 metabotype [31,36,37] (Figs. S1 and S2), responded dynamically to the presence of adipocytes by enhancing metabolic activity.

Macrophages co-incubated with adipocytes were exposed to

Please cite this article in press as: M. Rombaldova, et al., Omega-3 fatty acids promote fatty acid utilization and production of pro-resolving lipid mediators in alternatively activated adipose tissue macrophages, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.06.170