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Cell type-specific modulation of lipid mediator's formation in murine adipose tissue by omega-3 fatty acids

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

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

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ABSTRACT

Mutual interactions between adipocytes and immune cells in white adipose tissue (WAT) are involved in modulation of lipid metabolism in the tissue and also in response to omega-3 polyunsaturated fatty acids (PUFA), which counteract adverse effects of obesity. This complex interplay depends in part on *in situ* formed anti- as well as pro-inflammatory lipid mediators, but cell types engaged in the synthesis of the specific mediators need to be better characterized. We used tissue fractionation and metabolipidomic analysis to identify cells producing lipid mediators in epididymal WAT of mice fed for 5 weeks obesogenic high-fat diet (lipid content 35% wt/wt), which was supplemented or not by omega-3 PUFA (4.3 mg eicosapentaenoic acid and 14.7 mg docosahexaenoic acid per g of diet).

Our results demonstrate selective increase in levels of anti-inflammatory lipid mediators in WAT in response to omega-3, reflecting either their association with adipocytes (endocannabinoid-related N-docosahexaenoylethanolamine) or with stromal vascular cells (pro-resolving lipid mediator protectin D1). In parallel, tissue levels of obesity-associated pro-inflammatory endocannabinoids were suppressed. Moreover, we show that adipose tissue macrophages (ATMs), which could be isolated using magnetic force from the stromal vascular fraction, are not the major producers of protectin D1 and that omega-3 PUFA lowered lipid load in ATMs while promoting their less-inflammatory phenotype. Taken together, these results further document specific roles of various cell types in WAT in control of WAT inflammation and metabolism and they suggest that also other cells but ATMs are engaged in production of pro-resolving lipid mediators in response to omega-3 PUFA.

1. Introduction

Obesity-associated changes in white adipose tissue (WAT) are consistent with the emerging concept that immune and metabolic systems are interconnected [1–4]. Aside from adipocytes, pre-adipocytes and endothelial cells, almost all types of immune cells are present in WAT [5], but it is mainly the content of adipose tissue macrophages (ATMs) that is impacted by changes in metabolic state (lean versus obese) of the tissue [6–9]. ATMs can comprise up to 40% of the cells in obese WAT, their number correlates with the degree of obesity [10,11] and represent the strongest predictor of type 2 diabetes development in obese patients [12].

The “macrophage phenotype M2 → M1 switch” model of obesity [6] describes the immunological extremes of lean and obese states. WAT in lean subjects contains so called “alternatively activated” ATMs (M2 form) that produce anti-inflammatory cytokines, promote WAT remodeling and support systemic insulin sensitivity [reviewed in Refs. [4,13,14]] while obesity is accompanied by low-grade inflammation and macrophage repolarization to the pro-inflammatory (classically activated) M1 state [6,7], which secrete pro-inflammatory cytokines, activate NO and ROS production, and negatively affect WAT functions [10,11].

In addition to their immune functions, macrophages play a role in WAT lipid metabolism [15–17]. Lipid storage in ATMs helps to relieve adipocytes of excess lipids and improves systemic glucose

Abbreviations: 2-AG, 2-arachidonoylglycerol; anandamide, N-arachidonoylethanolamine; 17-HDHA, 17-hydroxy docosahexaenoic acid; ATMs, adipose tissue macrophages; CD11b, integrin αM chain; DHA, docosahexaenoic acid; DHEA, N-docosahexaenoylethanolamine; FA, fatty acid; cHF, high-fat (diet); cHF + F, high-fat diet with omega-3 PUFA; FACS, fluorescence-activated cell sorting; PD1, protectin D1; PDX, protectin DX; PUFA, polyunsaturated fatty acids; SVF, stromal vascular fraction; WAT, white adipose tissue.

* Corresponding author.
E-mail address: kopecky@biomed.cas.cz (J. Kopecky).

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tolerance [18,19], but sustained lipid accumulation in macrophages leads to M1 polarization and WAT insulin resistance [16]. ATMs also scavenge residual lipid particles from dead adipocytes, forming syncytia known as "crown-like structures" [20] and finally foam-like cells [16,20].

Although the M2 and M1 forms are currently well defined macrophage states based on in-vitro studies, macrophage polarization is more dynamic in-vivo and is influenced by dietary lipid composition [1,17]. For example, in dietary obese mice, ATMs are polarized toward M1 whereas treating obese mice with long-chain omega-3 PUFA (omega-3 PUFA) drives the polarization toward M2 [14]. In addition, docosahexaenoic acid (C22:6n-3, DHA)-derived anti-inflammatory lipid mediators such as resolvin D1 or protectin D1 (PD1) have been reported to decrease ATM accumulation, to shift macrophage polarization toward M2 and to improve insulin sensitivity in obese mice [21–24]. Yet another class of lipid mediators – endocannabinoids – is important for WAT inflammation and insulin sensitivity (reviewed in Ref. [1]). The anti-inflammatory properties of omega-3 PUFA derived endocannabinoid-related molecules, namely N-eicosapentaenoylethanolamine and N-docosahexaenoylethanolamine (DHEA), were documented in mouse WAT, where levels of pro-inflammatory 2-arachidonoylglycerol (2-AG) and anandamide were decreased while levels of those from omega-3 PUFA increased [25].

We hypothesized that the beneficial effects of omega-3 PUFA on WAT metabolism in obesity reflect in part modulation of the tissue levels of both pro- and anti-inflammatory lipid mediators produced by various cell types within the tissue. To test this, WAT was separated into adipocytes and stromal vascular fraction (SVF), containing also immune cells, and lipid mediators were measured. Moreover, an effect of omega-3 PUFA, namely DHA-rich omega-3 concentrate, on ATMs was explored using metabolipidomics.

2. Materials and methods

2.1. Materials

Standards for analysis of oxylipins and the deuterated standards were purchased from Cayman (Neratovice, Czech Republic). All other chemicals were purchased from Sigma. Mass spectrometry-grade solvents were purchased from Acros (Lach-Ner, Czech Republic). PD1 standard was kindly provided by Prof. Charles N. Serhan, Harvard Medical School, Boston.

2.2. Animals

Male mice (C57BL/6J; Jackson Laboratory, ME, USA) fed low-fat Chow diet (extruded R/M-H diet, Ssniff Spezialdiäten, Germany) till 3 months of age were habituated to a corn oil-based high-fat diet (cHF; lipid content 35%, wt/wt) for 2 weeks and then randomly assigned to various dietary treatments for 5 weeks. 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 (cHF + F diet) was conducted as before [23,25]. Some Chow diet-fed mice were also used as controls. In accordance with our previous experiments [23,26], feeding cHF diet accelerated body weight gain reflecting increase in adiposity as compared with the Chow-diet feeding, while this effect were ameliorated in the cHF + F mice (final body weight 48.5 g, 43.9 g, 32.0 g; weight of epididymal WAT 2770 mg, 2185 mg, 795 mg; cHF, cHF + F and Chow, respectively, $n = 8$).

2.3. Isolation of adipocytes and stromal vascular fraction (SVF) of WAT

Adipocytes were isolated from epididymal WAT according to published method [23,27] using Krebs-Ringer bicarbonate medium with 5 mM glucose and 4% BSA (fraction V, FA free, Sigma–Aldrich). Collagenase-digested samples were passed through a sterile 250 μm nylon mesh and the suspension was centrifuged at 4 g for 5 min. The pellet was collected as SVF and the top buoyant layer was washed twice and collected as floating adipocytes. From the SVF, CD11b-positive cells were isolated using magnetic force [6,7] and eventually incubated in DPBS with 5 μM A23187 for 30 min [28]. Schematic representation of the experiment using the cHF and cHF + F mice is summarized in Fig. 1.

2.4. Metabolipidomics

Targeted analysis of oxylipins, N-acylethanolamines and monoacylglycerols was performed according to published methods [29,30]. Briefly, WAT, adipocytes, SVF cells or subfractions were frozen in liquid nitrogen after each experiment, lipids were extracted using Strata-X SPE columns and analyzed with LC-MS/MS (Ultimate 3000 Binary RSLC System, Thermo, coupled to Qtrap 5500, AB-Sciex, CA, USA) using scheduled multiple reaction monitoring (sMRM) method. The residual pellets were used for DNA measurement [23]. Targeted metabolipidomics profiling was performed using LC-MS/MS according to previously published methods [31–33] in both positive and negative modes with sMRM.

2.5. Statistics

Statistical analysis was performed with SigmaStat and $^{*}p < 0.05$ was considered significant. R and VANTED software was used to analyze omics data [33].

3. Results

3.1. Sources of lipid mediators in WAT

We previously showed high levels of pro-resolving lipid mediator PD1 in WAT of mice fed high-fat diet supplemented by omega-3 PUFA concentrate rich in DHA, especially under the condition of 10% calorie restrictions. However, the cellular origin of PD1 was unknown [23]. Therefore, mature adipocytes and SVF were isolated from epididymal WAT of mice fed obesogenic cHF diet, cHF + F diets, as well as the Chow diet. The obesogenic effect of the cHF diet was counteracted by omega-3 PUFA (see Materials and methods), in parallel with amelioration of low-grade inflammation of WAT induced by cHF-feeding (as revealed by the number of ATMs detected in the tissue, not shown; see our previous publications [23,26]). Levels of lipid mediators were measured in both cell fractions, as well as the whole WAT (Figs. 1 and 2). In line with the previous findings [23], PD1 and its precursor 17-HDHA were elevated in WAT from mice fed cHF + F diet (and also in the cHF + F mice under calorie restriction, not shown). Measurement of DNA content indicated that ~85% of cells in WAT were recovered as adipocytes (~72%) and SVF (~13%), respectively. However, comparable amounts of 17-HDHA per gram of WAT were detected in the SVF cells pellet and the adipocyte fractions suggesting that SVF cells are responsible for the majority of 17-HDHA, precursor of PD1. Although absolute PD1 levels could be affected by collagenase digestion, the distribution pattern followed levels measured in intact tissue. Importantly, PD1 was not detected in isolated adipocytes. Next, we tried to isolate and characterize immune cells, especially macrophages, from SVF using fluorescence-activated cell