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Adipocyte lipid storage and adipokine production are modulated by lipoxygenase-derived oxylipins generated from 18-carbon fatty acids

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ABSTRACT

Generation of oxylipins (oxygenated metabolites of fatty acids) by lipoxygenases may be responsible for the beneficial effects of 20- and 22-carbon n-3 fatty acids on adipose tissue dysfunction in obesity, but the potential actions of oxylipins derived from 18-carbon fatty acids, which are generally at higher levels in the diet, are unknown. We therefore compared the effects of select lipoxygenase-derived oxylipins produced from α-linolenic acid (ALA, C18:3 n-3), linoleic acid (LA, C18:2 n-6), and arachidonic acid (AA, C20:4 n-6) on key adipocyte functions that are altered in obesity. Individual oxylipins were added to the culture medium of differentiating 3T3-L1 preadipocytes for 6 days. Lipid accumulation was subsequently determined by Oil Red O staining, while Western blotting was used to measure levels of proteins associated with lipid metabolism and characteristics of adipocyte functionality. Addition of all oxylipins at 30 nM was sufficient to significantly decrease triglyceride accumulation in lipid droplets, and higher levels completely blocked lipid production. Our results establish that lipoxygenase-derived oxylipins produced from 18-carbon PUFA differentially affect multiple adipocyte processes associated with lipid storage and adipokine production. However, these effects are not due to the oxylipins blocking adipocyte maturation and thus globally suppressing all adipocyte characteristics. Furthermore, these oxylipin species decrease the lipid content of adipocytes regardless from which precursor fatty acid or lipoxygenase they were derived. Consequently, adipocyte characteristics can be altered through the ability of oxylipins to selectively modulate levels of proteins involved in both lipid metabolism and adipokine production.

1. Introduction

Dietary fat is an important source of energy, however, excess intake is associated with obesity and its associated morbidities, cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). On the other hand, fat provides essential fatty acids (FA) that are required for brain development, blood clotting and other critical physiological and cellular processes. Since fat is composed of a variety of different constituent FAs, the composition of dietary fat has become an issue of considerable debate (Schwab and Uusitupa, 2015). Based on a variety of epidemiological and observational studies, there is a consensus for consuming diets low in saturated fatty acids (SFA) and higher in polyunsaturated fatty acids (PUFA). The recommendation to replace SFAs with PUFAs is largely based on data that suggests consumption of PUFAs can reduce the risk of both CVD and T2DM (Mozaffarian et al., 2010; Schwab et al., 2014).

The biological actions of dietary FAs can be mediated a number of different ways, through changes in membrane dynamics and receptor function, as well as activation of various receptors and enzymes that modulate intracellular signaling and gene expression (Mollace et al., 2013). Additionally, the metabolism of FAs, particularly the PUFAs, by several different enzymes can result in the formation of products that act as receptor ligands and exhibit properties that are quite distinct from the precursor FAs. The two main families of lipid mediators that

Abbreviations: AA, arachidonic acid; ACTB, β-actin; ADIPOQ, adiponectin; ATGL, adipose triglyceride lipase; ALA, alpha-linolenic acid; C/EBP-β, CCAAT/enhancer-binding protein-β; COX, cyclooxygenase; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FABP, fatty acid binding protein; FAS, fatty acid synthase; FBS, fetal bovine serum; HETE, hydroxyicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HOTrE, hydroxyoctadecatrienoic acid; HSL, hormone-sensitive lipase; LA, linoleic acid; LOX, lipoxygenase; MCP-1, monocyte chemoattractant protein-1; NONO, non-POU domain containing, octamer-binding; PGE_2, prostaglandin E2; PREB, prolactin regulatory element binding protein; PLIN1, perilipin-1; PUFA, polyunsaturated fatty acid; RT-PCR, real-time polymerase chain reaction; SFA, saturated fatty acid; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor-α

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originate from PUFAs are endocannabinoids and oxylipins (Poudyal et al., 2011; Wainwright and Michel, 2013). Oxylipins are oxygenated FA metabolites derived through the actions of cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450, and those that originate from marine long-chain omega-3 FAs (eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA) are of significant interest because of their ability to modulate inflammation. However, information regarding the formation and subsequent actions of oxylipins that are derived from the omega-6 linoleic acid (LA) and omega-3 alpha-linolenic acid (ALA) are almost completely unexplored, especially in the context of obesity.

Under conditions of caloric excess, the adipose tissue depots expand through an increase in both adipocyte number and size in order to enhance storage of fat in the form of triglycerides. Although not all adipocytes become enlarged, hypertrophy of these cells is associated with major changes in gene expression, metabolic properties and the profile of secreted cytokines and adipokines. These altered properties are the hallmarks of adipocyte dysfunction, a condition that is linked to the development of a more pro-inflammatory environment due to a decrease in the production of vasodilatory and anti-inflammatory mediators. While oxylipins are also produced by adipose tissue, and diet can modulate both the amount and species of oxylipin that are synthesized (Balvers et al., 2012), the role of these lipid mediators in this tissue has not been explored in detail.

It has been reported that resolvins and protectins, oxylipins generated from EPA and DHA, inhibit macrophage recruitment and increase adiponectin secretion in adipose explants from obese mice (Borgeson and Godson, 2012). Such observations have generated considerable interest in these novel oxylipins in the context of obesity since alterations in the dietary intake of certain PUFAs could influence adipose tissue functionality in the obese state thus leading to a decrease in comorbidities such as insulin resistance and cardiovascular disease. On the other hand, oxylipins generated from other PUFAs may produce similar or opposite results. However, there is very little data on the effects of oxylipins derived from C18 PUFAs such as LA on specific functions of adipose tissue (Goto et al., 2015), and none with respect to the oxylipins originating from ALA, despite the fact that these 18-carbon-derived oxylipins are present at higher levels in tissues than the more well-characterized oxylipins derived from longer-chain fatty acids such as arachidonic acid (AA) and EPA (Gabbs et al., 2015). Therefore, the effects of select LA and ALA oxylipins on lipid metabolism, specifically lipid storage in lipid droplets, and functional characteristics such as adipokine secretion were examined for the first time with differentiated 3T3-L1 cells, an established cell line derived from mouse that exhibits the metabolic characteristics of mature adipocytes.

2. Materials and methods

2.1. 3T3-L1 cell culture

Preadipocytes were propagated in DMEM containing 10% fetal bovine serum (FBS). Differentiation was initiated by adding a cocktail of dexamethasone, insulin and methylisobutylxanthine to cells that had been confluent for 2 days, as described by Yeganeh et al. (Yeganeh et al., 2012). Oxylipins (Cayman Chemical, Ann Arbor, MI), all prepared in 100% ethanol which was used as the vehicle control, were added in conjunction with the differentiation cocktail, and all analyses were performed 6 days later at which point the cells exhibited the properties typically associated with mature adipocytes. The medium and treatments were refreshed every second day. Preadipocytes harvested prior to reaching confluence were used as undifferentiated controls.

2.2. Lipid droplet visualization

Cells grown in 96-well dishes were stained with Oil Red O as previously described (Stringer et al., 2010) and photographs taken on day 6. The amount of Oil Red O stain in the cells was quantified by absorbance at 550 nm after extraction of the dye with isopropanol.

2.3. Western blotting

Immunoblotting was performed as described by Zahradka et al. (Zahradka et al., 2009), with 5 μg of total protein loaded per well. All primary antibodies (adiponectin, adipose triglyceride lipase (ATGL), CCAAT/enhancer-binding protein-β (C/EBPβ), fatty acid binding protein (FABP), fatty acid synthase (FAS), hormone-sensitive lipase (HSL), monocyte chemoattractant protein-1 (MCP-1), perilipin-1, prolactin regulatory element binding protein (PREB), tumor necrosis factor-α (TNF-α)) were obtained from Cell Signaling (Danvers, MA). All data were generated from the same blots by sequentially probing with the indicated antibodies, which were removed before application of the subsequent antibody.

2.4. qRT-PCR

Total RNA was extracted from cells using spin column-based RNA extraction kits (E.Z.N.A. Total RNA kits, Omega Bio-Tek, Norcross, GA). On-column DNase digestion was performed as specified by the manufacturer. RNA concentration and quality were assessed using the Experion™ RNA StdSens Analysis Kit (Bio-Rad Laboratories, Mississauga, ON) for the Experion™ electrophoresis system. One μg of RNA was reverse transcribed to complementary DNA using qScript cDNA Supermix (Quanta Biosciences, Gaithersburg, MD). Reactions were carried out in a PTC-100 Programmable Thermal Controller (MJ Research, Inc., St. Bruno, QC, Canada) using the following program: 5 min at 25 °C; 30 min at 42 °C; 5 min at 85 °C. Primers for qPCR were designed using Primer Blast software (NCBI, Bethesda, MD) and were synthesized by Integrated DNA Technologies (San Diego, CA). Specific sequences were for perilipin-1: PLIN1–R 5′-TGCACGTGGAGA GTAAGGATG-3′, PLIN1-L 5′-TTCTGGAAGCACTCACAGGTC-3′; adiponectin: ADIPOQ-R 5′-CCCAGTCATGCCGAAGATGA-3′, ADIPOQ-L 5′-CACAAGTTCCCTTGGGTGGA-3′; β-actin: ACTB-R 5′-AGCAAGCA GGAGTACGATGAG-3′, ACTB-L 5′-GGGTGTAAAACGCAGCTCAGTA-3′, Non-POU domain containing, Octamer-binding: NONO-R 5′TGCTCCT GTGCCACCTGGTACTC-3′, NONO-L 5′-CCGGAGCTGGACGGTTGAAT GC-3′. Optimal annealing temperature and template quantity for each primer pair were determined by performing gradient qPCR and standard curve analyses. qPCR was carried out on a Mastercycler Gradient Realplex (Eppendorf, Hamburg, Germany) using the following program: 2 min at 95 °C; 40 cycles of 15 s at 95 °C, 15 s at the primer-specific annealing temperature, and 20 s at 68 °C. Melt curve analysis was used to determine the homogeneity of the PCR amplicon, thereby establishing the specificity of the primers for their intended product. ΔΔCt values, which were obtained relative to the reference gene NONO, were used to normalize perilipin-1 (PLIN1) and adiponectin (ADIPOQ) relative to the housekeeping gene β-actin (ACTB).

2.5. Statistics

Data were analyzed by one-way ANOVA followed by post-hoc testing using Duncan's multiple range test (SAS version 9.3). $P < 0.05$ was considered significant.

3. Results

3.1. Effect of oxylipins on lipid content

A panel of 4 oxylipins was selected as being representative of the products originating from the 18-carbon PUFAs LA (hydroxyoctadecadienoic acid [HODE]) and ALA (hydroxyoctadecatrienoic acid [HOTrE]) via LOX and these were compared to 3 oxylipins (hydroxyicosatetraenoic acid [HETE]) generated from AA via lipoxygenase