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Exogenous fatty acids and niacin on acute prostaglandin D_2 production in human myeloid cells

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Abstract

Niacin activates HCA_2 receptor that results in the release of PGD_2. However, little is known on PGD_2-producing cells and the role of fatty acids. Notably M-CSF macrophages exhibited a timely dependent PGD_2 production upon niacin challenge. Short pretreatment of M-CSF macrophages with autologous postprandial TRLs induced the down-regulation of *HCA_2* gene and up-regulation of genes encoding COX1 and COX2 enzymes in a fatty acid-dependent manner. These effects were paralleled by a higher PGD_2 production with postprandial TRL-SFAs. The niacin-mediated transcriptional activity of all genes involved in PGD_2 biosynthesis was desensitized in a time-dependent manner by postprandial TRLs, leading to a decreased PGD_2 release. *In vivo*, the net excursions of PGD_2 in plasma followed similar fatty acid-dependent patterns as those found for PGD_2 release *in vitro*. The predominant fatty acid class in the diet acutely modulates PGD_2 biosynthetic pathway both *in vitro* and *in vivo*.

Keywords: Niacin; Exogenous fatty acids; Postprandial state; Prostaglandin D2; Myeloid cells

1. Introduction

Niacin (also commonly known as nicotinic acid or vitamin B3) is well established for the treatment of dyslipidemia [1] and reduction of cardiovascular risk [2]. However, its clinical use has been greatly limited due to an intense skin flush within a few minutes of niacin ingestion [3]. This side effect is mediated by the niacin-induced agonism of hydroxy-carboxylic acid receptor 2 (HCA_2) to produce the vasodilator prostaglandin D2 (PGD_2) in epidermal Langerhans cells and keratinocytes [4]. PGD_2 is also an early-phase mediator in several inflammatory conditions [5] and hypersensitivity reactions such as anaphylaxis [6], with the major contribution from resident mast cells in peripheral tissues [7]. The stimulation of HCA_2 by niacin triggers a cascade of distal but functionally linked enzymes that encompasses the cytosolic phospholipase A2 (PLA_2) for the release of endogenous arachidonic acid from the cell membrane, the constitutive cyclooxygenase isoform 1 (COX1) and the inducible cyclooxygenase isoform 2 (COX2) as the rate-limiting steps for the conversion of arachidonic acid into the unstable cyclic endoperoxide PGH_2, and the PGD synthase (PTGDS) for the isomerization of PGH_2 to yield PGD_2 [8]. PGD_2 can be non-enzymatically dehydrated to form 15-deoxy-$\Delta^{12,14}$ prostaglandin J2 (15d–PGJ_2), the most potent endogenous ligand of peroxisome proliferator-activated receptor γ (PPARγ) that drives the feedback regulation of gene encoding HCA_2 [9]. The PPARs are promiscuous in terms of their interaction with ligands and they also exhibit distinctive affinity for selective fatty acids, including saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and long-chain polyunsaturated fatty acids (PUFAs) of the omega-3 family exogenously supplied by postprandial triglyceride-rich lipoproteins (TRLs) [10]. These observations raise the issue of whether the acute metabolism of dietary fatty acids might be linked to signaling mechanism for niacin-induced PGD_2 production.

The full-length cDNA of HCA_2 was first cloned from a cDNA library of human monocytes [11] but other cells of innate immune system (neutrophils and antigen-presenting cells derived from $CD34^+$ umbilical blood precursor cells) also expressed HCA_2 [12,13]. The physiological role of HCA_2 and its contribution to niacin-induced PGD_2 production in monocytes and neutrophils is unclear. However, primary peritoneal macrophages from patients subjected to paracentesis and macrophage-like THP-1 cells following treatment with phorbol-12-myristate-13-acetate have been shown to produce PGD_2 by a COX-dependent mechanism in response to niacin [14]. These data

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suggest that the niacin-HCA_2 axis is usable for PGD_2 production in the myeloid-differentiated lineage. In different tissue environments, monocyte myeloid cells can mature and differentiate into macrophages and dendritic cells (DCs) [15]; macrophages can further undergo polarization into M1 classical macrophages by Th1 mediators and into M2 alternative macrophages by Th2 mediators [15]. Little is known regarding the niacin-induced PGD_2 production in these cells.

In the present study, we sought first to determine the competence of primary monocytes, neutrophils, and four monocyte/macrophage-derived subsets (M-CSF macrophages as referred to only differentiated in M-CSF but not polarized macrophages, M1, M2 macrophages, and DCs) to produce PGD_2 upon niacin challenge. In M-CSF macrophages, which were the major niacin-induced PGD_2-producing myeloid subset, the influence of niacin and postprandial TRLs with different class of predominant fatty acids (SFAs, MUFAs or omega-3 long-chain PUFAs) on the gene expression level and function of HCA_2 and downstream signaling enzymes for PGD_2 synthesis was also investigated.

2. Materials and methods

This study was conducted according to Good Clinical Practice Guidelines and in line with the principles outlined in the Helsinki Declaration of the World Medical Association. Informed consent for the study was obtained (University of Seville, AGL2011–29,008).

2.1. Postprandial TRL isolation

Oral fat emulsions were prepared according to the method described by our Patent WO/2014/191,597. They consist of water, sucrose, fat (50 g/m^2 body surface area), emulsifier, and flavoring. Six volunteers, aged 25 to 35 years, non-smokers, with no medical history of disease known, abnormality of hematological or biochemical parameters were recruited. After an overnight fasting period of 12 h, all of them were given, over three different occasions, oral fat emulsions containing cow's milk cream (meal rich in SFAs), refined olive oil (meal rich in MUFAs) or refined olive plus a dose of omega-3 long-chain PUFAs, which consisted of 920 mg of eicosapentaenoic acid (EPA) and 760 mg of docosahexaenoic acid (DHA) (meal rich in MUFAs + omega-3 long-chain PUFAs). At the postprandial lipemic peak, *i.e.* 2–3 h following the ingestion of the oral fat emulsion, venous blood was collected into K_3EDTA-containing Vacutainer tubes (Becton Dickinson, NJ, USA). TRLs were isolated, pooled, and dialyzed against cold PBS [16]. TRLs were then immediately stored at −80 °C. Lipid oxidizability of postprandial TRL was checked (TBARS level) during isolation and storage, but oxidation of lipids was not detected. TRLs were tested for LPS contamination using the Pierce LAL Chromogenic Endotoxin Quantification kit (Thermo Scientific, Madrid, Spain). LPS contamination was always<0.2 EU/mL. Triglyceride concentration in postprandial TRLs was determined by colorimetric assay kit TG GPO-POD (Bioscience Medical, Madrid, Spain).

2.2. Fat and TRL fatty acid composition

The fatty acid composition of cow's milk cream, refined olive oil, and refined olive oil plus omega-3 long-chain PUFAs was determined, in triplicate from the same lot, by the method described in EEC/796/2002 [17] using a gas chromatography system (HP-5890, Hewlett-Packard) equipped with flame ionization detector and a SP-2380 capillary column (Supelco, 30 m×0.32 mm) packed with cyanopropyl siloxane (0.25 μm) (Table 1). The initial column temperature was 165 °C, which was held for 10 min, then programmed from 165 °C to 200 °C at 1.5 °C/min. Injector and detector temperature were 250 °C, with the carrier gas H_2. For fatty acid composition in postprandial TRLs (named TRL-SFAs from cow's milk cream, TRL-MUFAs from refined olive oil, and TRL-MUFAs+ω3 from refined olive oil plus omega-3 long-chain PUFAs), aliquots of 100 μL were lyophilized. A solution composed of 2.64 mL of methanol:toluene: dimethoxypropane:sulphuric acid (16.5:5:1:1) and heptane was added on the lyophilized residue. After shaking, and incubating the mixture at 80 °C for 1 h, the upper phase was transferred to another vial and dried with a stream of N_2 gas. The resulting extract was dissolved in heptane and the fatty acid methyl esters were analyzed into a gas chromatography system as described above (Table 2).

2.3. Monocyte and neutrophil isolation

The same six volunteers who took part as donors of postprandial TRLs participated as donors of leukocytes. After an overnight fasting period of 12 h, peripheral blood samples were drawn from a large antecubital vein and collected into K_3EDTA-containing tubes (BD). Peripheral blood mononuclear cells (MNCs) were isolated by centrifugation over a Ficoll-Histopaque (Sigma, Madrid, Spain) gradient. Monocytes were isolated from peripheral blood MNCs using CD14 microbeads and LS columns on a midiMACS system (Miltenyi Biotec, Madrid, Spain). Neutrophils were isolated by dextran sedimentation (2% dextran/0.9% NaCl) (Sigma) from the fraction of peripheral blood polymorphonuclear cells (PMNCs). Residual erythrocytes were removed using hypotonic lysis with 0.2% and 1.6% saline solutions. Monocyte ($CD14^+$) and neutrophil ($CD16^+$) purity was routinely>90% by flow cytometry analysis (FACScanto II flow cytometer and FACSDiva software, BD) and cell viability>95% by trypan blue exclusion (Sigma). The monocytes and neutrophils were seeded at a density of 5×10^5 cells/mL and 3×10^6 cells/mL, respectively, and cultured in ultra low attachment flasks in RPMI 1640 medium supplemented with L-glutamine, penicillin, streptomycin, and 10% heat-inactivated fetal bovine serum (complete culture medium). PGD_2 was determined in the medium of monocytes and neutrophils after 30 min.

Table 1
Fatty acid composition of dietary fats

	Cow's milk cream	Refined olive oil	Refined olive oil+ omega-3 long-chain PUFAs
Fatty acid	g/100 g of fatty acid		
4:0, butyric	0.83±0.16	-	-
6:0, caproic	0.25±0.02	-	-
8:0, caprylic	0.61±0.07	-	-
10:0, capric	2.47±0.13	-	-
12:0, lauric	3.09±0.42	-	-
14:0, myristic	10.9±0.91	-	-
16:0, palmitic	35.5±0.82	20.4±0.89	20.5±0.64
16:1(ω-7), palmitoleic	3.60±0.32	0.97±0.17	0.82±0.12
18:0, stearic	11.5±0.75	5.70±0.11	4.49±0.36
18:1(ω-9), oleic	25.3±0.71	61.9±1.23	61.5±0.97
18:2(ω-6), linoleic	4.27±0.82	7.97±0.65	8.04±0.53
18:3(ω-3), α-linolenic	0.39±0.05	1.04±0.13	0.94±0.03
20:5(ω-3), eicosapentaenoic	-	-	0.92±0.09
22:6(ω-3), docosahexaenoic	-	-	0.72±0.10
Others	0.96±0.42	2.05±1.08	2.01±0.88

Data are means ± SD (n=3).

2.4. Monocyte differentiation and polarization

Monocytes were induced to differentiate for 6 days in the presence of human recombinant M-CSF (50 ng/mL) to obtain naïve (M-CSF) macrophages. Complete culture medium was replaced every 2 days with fresh medium and the cytokine. Degree of differentiation of the resulting population was determined for CD68 antigen using anti-human CD68 monoclonal antibody (Miltenyi Biotec) by flow cytometry analysis (more than 95% of cells were positive for CD68). A similar procedure but using human recombinant GM-CSF (50 ng/mL) and IL-4 (20 ng/mL) was follows to obtain DC-like cells from monocytes. For M1 or M2 polarization, M-CSF macrophages were exposed to LPS (100 ng/mL) and IFNγ (20 ng/mL) or to IL-4 (20 ng/mL), respectively, for additional 24 h. Gene expression levels of markers for DC (CD209 and CD1a), M1 (CD80 and CD64), and M2 (MRC1 and CD200R) were determined by RT-qPCR.

Table 2
Fatty acid composition of postprandial triglyceride-rich lipoproteins

	TRL-SFAs	TRL-MUFAs	TRL-MUFAs+ω3
Fatty acid	g/100 g of fatty acid		
4:0, butyric	0.22±0.09	—	—
6:0, caproic	0.13±0.06	—	—
8:0, caprylic	0.36±0.12	—	—
10:0, capric	1.42±0.41	—	—
12:0, lauric	3.77±1.06	—	—
14:0, myristic	9.04±1.55	—	—
16:0, palmitic	36.3±2.31	11.8±1.97	12.1±1.34
16:1(ω-7), palmitoleic	1.59±0.08	0.88±0.32	1.46±0.43
18:0, stearic	17.1±1.54	5.98±0.93	5.62±0.83
18:1(ω-9), oleic	22.8±2.03	66.4±3.27	60.7±2.18
18:2(ω-6), linoleic	4.24±1.06	8.93±1.27	10.1±1.44
18:3(ω-3), α-linolenic	2.00±0.61	3.21±1.12	3.17±1.08
20:4(ω-4), arachidonic	0.53±0.38	1.07±0.21	1.82±0.34
20:5(ω-3), eicosapentaenoic	—	0.82±0.28	2.51±0.38
22:6(ω-3), docosahexaenoic	—	0.74±0.32	2.14±0.03
Others	0.53±0.26	0.23±0.12	0.36±0.24

Data are means ± SD (n=18).