Attenuation of niacin-induced prostaglandin D₂ generation by omega-3 fatty acids in THP-1 macrophages and Langerhans dendritic cells

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Abstract: Niacin, also known as nicotinic acid, is an organic compound that has several cardio-beneficial effects. However, its use is limited due to the induction of a variable flushing response in most individuals. Flushing occurs from a niacin receptor mediated generation of prostaglandins from arachidonic acid metabolism. This study examined the ability of docosahexaenoic acid, eicosapentaenoic acid, and omega-3 polyunsaturated fatty acids (PUFAs), to attenuate niacin-induced prostaglandins in THP-1 macrophages. Niacin induced both PGD₂ and PGE₂ generation in a dose-dependent manner. Niacin also caused an increase in cytosolic calcium and activation of cytosolic phospholipase A₂. The increase in PGD₂ and PGE₂ was reduced by both docosahexaenoic acid and eicosapentaenoic acid, but not by oleic acid. Omega-3 PUFAs efficiently incorporated into cellular phospholipids at the expense of arachidonic acid, whereas oleic acid incorporated to a higher extent but had no effect on arachidonic acid levels. Omega-3 PUFAs also reduced surface expression of GPR109A, a human niacin receptor. Furthermore, omega-3 PUFAs also inhibited the niacin-induced increase in cytosolic calcium. Niacin and/or omega-3 PUFAs minimally affected cyclooxygenase-1 activity and had no effect on cyclooxygenase-2 activity. The effects of niacin on PGD₂ generation were further confirmed using Langerhans dendritic cells. Results of the present study indicate that omega-3 PUFAs reduced niacin-induced prostaglandins formation by diminishing the availability of their substrate, as well as reducing the surface expression of niacin receptors. In conclusion, this study suggests that the regular use of omega-3 PUFAs along with niacin can potentially reduce the niacin-induced flushing response in sensitive patients.

Keywords: flushing, prostaglandin E₂, phospholipids, GPR109A, cardiovascular, docosahexaenoic acid, arachidonic acid

Introduction

Nicotinic acid (niacin) is a water soluble vitamin that has been widely used in the prevention of cardiovascular disease.^{1,2} The Coronary Drug Project Study reported that niacin reduced nonfatal myocardial infarction by 24% and stroke by 22%.³ Some of the general cardio-beneficial effects of niacin at a dose of 1.5 g/day include the reduction of total cholesterol, triglycerides, very-low-density lipoprotein, low density lipoprotein (LDL), and lipoprotein(a) by 20%.⁴⁻⁷ Niacin has also been shown to effectively increase high-density lipoprotein (HDL) by approximately 20%.^{8,9} These cardio-beneficial effects of niacin are mediated largely through a receptor-independent mechanism.

Alternative lipid-reducing medications are statins, best known for their LDL-cholesterol lowering effects. Niacin, in contrast, is the most effective, clinically

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available agent for increasing HDL-cholesterol. The combined use of niacin and statins is recommended for improved outcomes of cardiovascular events.^{10–14} For example, an HDL-Atherosclerosis Treatment Study¹⁵ (HATS) reported that a combined treatment of simvastatin plus niacin resulted in significant regression of angiographic coronary atherosclerosis and reductions in the rate of clinical events during 2.5 years of follow-up.¹⁶ However, a very recent Atherothrombosis Intervention in Metabolic Syndrome with low HDL/High Triglyceride: Impact on Global Health Outcomes (AIM-HIGH) study reported no incremental clinical benefits from the addition of niacin to simvastatin therapy during a 36-month follow-up period, despite significant improvements in HDL cholesterol and triglyceride levels.^{17,18} The outcome of this study decreased enthusiasm for the combined use of niacin and statins. In addition to this, niacin use is limited by the majority of patients experiencing a “flushing response” that is characterized by severe reddening of the skin, itching, and tingling, which leads many patients to discontinue use.^{19,20} Several studies have shown that flushing occurs in response to the vasodilatory effects of prostaglandin D₂ (PGD₂) and prostaglandin E₂ (PGE₂), and their metabolites, which are elevated after treatment with niacin.^{21–23} Recently, it has been shown that the epidermal Langerhans cells are one of the cell types responsible for niacin-induced PGD₂ release.^{24,25} The niacin-induced flushing appears to be initiated through a receptor-mediated process.^{26–28} For example, mice deficient in PUMA-G, a murine niacin receptor, failed to show a niacin-induced increase in ear blood flow (a measure of niacin flush).²⁹ These investigators have also demonstrated that mice lacking PGD₂ and PGE₂ receptors had reduced flushing responses.²⁹ Furthermore, other evidence also suggests that PGD₂ acting through the DP1, a subtype of PGD₂ receptor, and PGE₂ acting via type 2 and type 4 PGE₂ receptors, mediates the niacin-induced flushing response.^{29,30} Recent studies have demonstrated that Langerhans dendritic cells respond to niacin with a transient increase in the cytoplasmic Ca²⁺ concentration and have suggested that Gi (GTP binding protein) is activated through the niacin receptor.²⁵ It is well known that an increase in the cytoplasmic Ca²⁺ concentration causes activation of phospholipase A₂ (PLA₂), which liberates arachidonic acid (AA) from membrane phospholipids. AA can be further metabolized to PGD₂ and PGE₂ by the ubiquitously expressed type 1 cyclooxygenase (COX) and both PGD₂ and PGE₂ synthases, which are present in Langerhans dendritic cells. Benyo et al²⁵ suggest that the release of PGD₂ and PGE₂ from niacin-activated cells then

results in vasodilation in the dermal papillae of the upper dermis layer, where the Langerhans cells are localized. This sensitization of epidermis by PGD₂ and PGE₂ results in the characteristic flushing response.

Both epidemiological^{31–33} and prospective randomized clinical trials^{34–36} have reported a decrease in morbidity and mortality from heart disease in patients with diets supplemented with omega-3 polyunsaturated fatty acids (PUFAs). Two particular omega-3 PUFAs are of interest: eicosapentaenoic acid (EPA; 20:5, omega-3) and docosahexaenoic acid (DHA; 22:6, omega-3).

Omega-3 PUFAs improve the plasma lipid profile. Harris³⁷ concluded that omega-3 PUFAs generally lowered triglycerides (TG) by 25%–28% in an analysis of 72 human trials, where normal subjects or hypertriglyceridemic patients were given 7 g or less of omega-3 PUFAs/day for at least a 2 week period. Harris³⁷ further noted that omega-3 PUFAs were able to lower lipoprotein cholesterol in animal studies, but there was only a minor impact on lipoprotein cholesterol levels in human studies. Another study by Mori et al also observed similar findings in mildly hypertriglyceridemic patients. Intake of omega-3 PUFAs (4 g/day for 6 weeks) reduced TG levels by 18%–20% but had a minimal impact on low-density lipoprotein cholesterol or high-density lipoprotein cholesterol (HDL-C).³⁸ In contrast to these studies, long-term treatment of hypertriglyceridemic patients with omega-3 PUFAs (4 g/day for 16 weeks) led to a significant reduction in TG by 47%, while TG levels rose by 16% with a placebo (corn oil). This omega-3 PUFA effect was associated with a decrease in ratios of total cholesterol to HDL (20%) and a modest increase in high-density lipoprotein cholesterol (13%).³⁹ Similar results were also reported in another study where hypertriglyceridemic patients were treated with omega-3 PUFAs (4 g/day) for 6 months.⁴⁰ It appears from different studies⁴¹ that higher levels of omega-3 PUFAs for longer durations have beneficial effects on the plasma lipid profile.

In addition to their effects on the lipid profile, omega-3 PUFAs also exert many cardio-beneficial effects via their involvement in several cellular processes. The incorporation of omega-3 PUFAs into cellular membranes changes the nature of the substrate for phospholipases. PLA₂ is a physiologically important enzyme. Its activity is stimulated by niacin through phospholipase C-mediated calcium mobilization.⁴² Cytosolic PLA₂ (cPLA₂) catalyzes the hydrolysis of fatty acids from the sn-2 position of membrane phospholipids, resulting in the production of proinflammatory AA-derived eicosanoids and platelet-activating factors.⁴³ Not much is

known about the direct regulatory effect of omega-3 PUFAs on cellular PLA₂ activity. However, omega-3 PUFAs are easily incorporated into membrane phospholipids on the sn-2 position, where AA is usually present. Cytosolic PLA₂ hydrolyzes phospholipids containing omega-3 PUFAs, and then generates free DHA or EPA. DHA, EPA, and AA all compete for COX and lipoxygenase enzymes; however, AA is the strongest substrate of the three.^{44,45} It is therefore possible that omega-3 PUFAs can effectively reduce the generation of AA-derived proinflammatory eicosanoids including PGD₂ and PGE₂. Furthermore, DHA is a unique fatty acid, because it significantly alters basic properties of cell membranes, including acyl chain order and fluidity, phase behavior, elastic compressibility, ion permeability, fusion, rapid flip-flop, and resident protein function.⁴⁶ Similarly, recent studies have demonstrated that several signaling proteins, including surface receptors, are enriched in lipid

rafts and can be displaced from membrane rafts by both DHA and EPA.^{47,48} Various possible mechanisms for niacin-induced PGs formation are outlined in Figure 1.

The objective of this study was to investigate if omega-3 PUFAs are capable of inhibiting the niacin-induced PGD₂ and PGE₂ generation. To test the “proof of the principle,” differentiated macrophages were used, as it has been shown that macrophages are capable of responding to niacin in a similar fashion to that of Langerhans cells.⁴⁹ Experiments on human cultured epidermal Langerhans cells were also performed to further confirm the results. Niacin treatment caused generation of both PGD₂ and PGE₂ in macrophages, but induced formation of only PGD₂ in Langerhans cells. Omega-3 PUFAs reduced AA levels in the cellular membranes and also reduced surface expression of niacin receptors. This results in an attenuation of niacin-induced PGD₂ and PGE₂ synthesis.

Figure 1 Proposed cellular mechanism for Niacin-induced prostaglandins formation.

Notes: Niacin binding to its receptor, GPR109A, causes influx of calcium (Ca²⁺) through voltage gated channels. Ca²⁺ binds to cPLA₂, which subsequently phosphorylated by cellular kinases and translocated to plasma membrane. Phospholipids containing AA, EPA, or DHA are degraded into free fatty acids by the action of cPLA₂. COX-1 and -2 catalyze oxygenation of AA into 2-series PG and TXA and oxygenation of EPA into 3-series PGs and TXA. DHA is not a substrate for COX-1; however, in endothelial cells COX-2 enzymes convert EPA and DHA into hydroxy fatty acids in the presence of aspirin. These are released from the endothelium and are rapidly converted by 5-LOX in adherent leukocytes into bioactive compounds termed as resolvins (resolution phase interaction product). Resolvins derived from EPA are designated as E series (RvE) and those derived from DHA are termed as D series.

Abbreviations: 5-lipoxygenase, 5-LOX; AA, arachidonic acid; COX-1 and -2, Cyclooxygenase-1 and -2; cPLA₂, cytosolic phospholipase A2; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PG, prostaglandins; TXA, thromboxanes.

Materials and methods

Materials

Niacin was obtained from Sigma-Aldrich (St Louis, MO). PGD₂ and PGE₂ monoclonal enzyme immunoassay (EIA) kits came from the Cayman Chemical Company (Ann Arbor, MI). COX Fluorescent Activity Assay Kits were also purchased from Cayman Chemical. Phycoerythrin-conjugated rat monoclonal anti-human HM74A/GPR109A and Rat IgG Isotype Control-PE were obtained from R&D Systems (Minneapolis, MN). THP-1 or Human Acute Monocytic Leukemia cells were purchased from American Type Culture Collection (Manassas, VA). Langerhans dendritic cells expressing CD1a, MHC class II, and Birbeck granules, were obtained from MatTek Corporation (Ashland, MA). Diisopropyl fluorophosphate and sodium fluoride were from Sigma Chemical Co (St Louis, MO). Phorbol-12-myristate-13-acetate was acquired from Calbiochem (San Diego, CA).

Cell culture and differentiation of THP-1 macrophages

THP-1 human monocytic cell line was maintained in RPMI 1640 media supplemented with L-glutamine, 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin-antimycotic (100X; GIBCO, Grand Island, NY). Cells were maintained at 37°C and 5% CO₂. To initiate differentiation, the cells were plated at near 90% confluence in serum-free RPMI 1640 (GIBCO) with 25 nM phorbol-12-myristate-13-acetate for 48 hours at 37°C and 5% CO₂. Differentiation was verified via bright-field microscopy as well as Western blot analysis of CD36 expression.

Culturing of Langerhans cells

Freshly isolated human dendritic/Langerhans cells were obtained from MatTek Corporation (Ashland, MA) in a T-10 flask. Cell suspension was centrifuged at 800 xg; cells were re-suspended, and then plated at a density of 40,000 cells per well in a 96-well plate in dendritic-cells maintenance media (DC-MM) supplemented with 5% FBS and a proprietary mixture of cytokines to maintain the dendritic cell phenotype as per the supplier's instructions. Cells were allowed to attach overnight in an incubator maintained in 37°C and 5% CO₂. Cells were then treated with niacin and/or fatty acids as described in the following sections.

Cell growth assay

Stock solutions (1 mM) of DHA, EPA, and oleic acids (OLA) were prepared by complexing with fatty acid-free bovine serum albumin (BSA).⁴ Varying concentrations (0, 12.5, 25, 50, 75, and 100 μM) of fatty acids were used to treat the THP-1 derived macrophages. Cell viability was tested using the WST-1 assay. The assay was performed according to the manufacturer's instructions (Roche, Indianapolis, IN).

Lipid extraction analysis

THP-1 monocytes (5×10^6) were differentiated in a T-75 flask for 48 hours. The resulting macrophages were treated with 0, 12.5, 25, 50, 75, and 100 μM DHA, EPA, or OLA for 24 hours in RPMI 1640 supplemented with L-glutamine, 25 mM HEPES, 5% FBS, and 1% penicillin-streptomycin-antimycotic at 37°C and 5% CO₂. The cells were harvested by trypsinization and centrifuged at 800 xg for 5 minutes and then washed in phosphate buffered saline (PBS) containing 1% fatty-acid free BSA. The cells were re-suspended and then lysed in PBS by sonicating on ice. An internal standard (C23:0) was added to 500 μl of cell lysate and a portion of the remaining 100 μl was used to establish a protein concentration in order to normalize fatty acid content to the amount of protein present. Lipids were extracted with chloroform: methanol (2:1) using the Folch method⁵⁰ and fatty acids were converted into methyl esters at room temperature for 24 hours as described previously.⁵¹ The fatty acids were separated on a gas chromatography system equipped with an auto sampler, flame ionizing detector (GC2010; Shimadzu Corporation, Kyoto, Japan), and a Zebron ZB-WAX plus column (100 m, 0.25 mm ID, 0.25 m; Phenomenex, Torrance CA). The oven temperature increased from 30°C (2 minute hold) to 180°C at 20°C/minute (2 minute hold) to 207°C at 4°C/minute (3 minute hold) to 220°C at 2°C/minute (2 minute hold) to 240°C at 2°C/minute (2 minute hold). The flame ionization detector was used at 250°C to detect the resolved fatty acids peaks, which were identified using authentic standards (Restek Corp, Bellefonte, PA). Data was analyzed with Shimadzu's GC solutions software (v2.30.00).

Prostaglandin D₂ and E₂ EIA assay

Prostaglandin D₂ and E₂ content in the culture media were measured using the competitive EIA kit from Cayman Chemical (Ann Arbor, MI). The assay was carried out according to the manufacturer's protocol. Briefly, THP-1 differentiated macrophages (3×10^4) or Langerhans cells (4×10^4) were

treated with DHA, EPA, and OLA in 96 well plates in duplicate for 24 hours in RPMI-1640 containing 5% FBS and 1% penicillin-streptomycin-antimycotic (100X) at 37°C and 5% CO₂. After washing with PBS containing 0.1% bovine serum albumin, the cells were treated with varying concentrations of niacin for 30 minutes. The supernatant (50 µl) was then analyzed for the presence of prostaglandins.

Niacin receptor expression

THP-1 differentiated macrophages (1×10^6) were treated with DHA, EPA, or OLA for 24 hours. The cells were scraped with a rubber policeman and washed in PBS containing 0.5% bovine serum albumin and finally resuspended in 50 µl of this labeling buffer. Cells were then labeled with 0.5 µg phycoerythrin-conjugated GPR109A antibody for 45 minutes. The cells were washed twice with PBS containing 0.5% bovine serum albumin. To ensure specificity of the antibody, an isotype control was established for each sample. Analysis was performed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) at a 488 nm wavelength. The results indicate the mean fluorescent intensity of the THP-1 cells.

Determination of calcium mobilization

THP-1 macrophages, treated with fatty acids for 24 hours, were loaded with fura-2 acetoxymethyl ester (fura 2-AM). Briefly, cells (1×10^7) were incubated in Hanks' balanced salt solution (HBSS) in the presence of 5 µM fura-2 acetoxymethyl ester for 15 minutes at 37°C. After incubation, cells were washed twice with modified (Ca²⁺ and Mg²⁺ free) HEPES-buffered (1 mg/ml) HBSS (pH 7.2) and finally resuspended in the same buffer (prewarmed at 37°C). Cells (1×10^6) were preincubated for 1 minute in modified HEPES-buffered HBSS in a temperature-controlled Perkin-Elmer LS50B luminescence spectrometer (Perkin-Elmer Ltd, Beaconsfield, England) to determine baseline excitation fluorescence 340/380 nm ratios (R) at 510 nm emission. Cells were stimulated with niacin (3 mM) to determine relative alterations in R due to intracellular release of calcium by continuously monitoring (5 minutes) at 340 and 380 nm excitation.

Western blot analysis of phosphorylated-cPLA₂

Lysates of THP-1 macrophages treated with varying concentrations of niacin were prepared in radioimmuno-

precipitation assay lysis buffer (Millipore, Temecula, CA) containing 2.5 mM diisopropyl fluorophosphate, 100 mM sodium fluoride and protease inhibitors cocktail (Roche, Indianapolis, IN). Proteins in the samples were separated by sodium dodecyl sulfate page electrophoresis and transferred onto nitrocellulose membranes. The membranes were blocked with 10% western blocking reagent (Roche, Indianapolis, IN) in 1× Tris-Buffered Saline-Tween at room temperature for 1 hour. The membranes were then incubated overnight at 4°C with the phospho-cPLA₂ primary antibody (Cell Signaling Technologies, Danvers, MA) at 1:1000 in 1× Tris-Buffered Saline-Tween. Blots were developed using biotinylated secondary antibodies linked to horse radish peroxidase (GE Healthcare, Little Chalfont, UK) and the signal was detected using enhanced chemi-luminescence plus western blocking detection reagents (Amersham, Little Chalfont, UK).

COX activity EIA assay

The total COX activity and COX-1 and COX-2 content in the culture media were measured using the fluorescent activity assay kit from Cayman Chemical. THP-1 differentiated macrophages (1.5×10^6) were treated with 50 µM DHA, EPA, or OLA for 24 hours at 37°C and 5% CO₂. Cells were scraped using a rubber policeman. The cells were sonicated in 100 mM Tris HCl (pH 7.5) containing protease inhibitors (Sigma). The lysate was centrifuged at 10,000 ×g for 15 minutes at 4°C and the supernatant was transferred to another micro-centrifuge tube. 10 µl of each sample was transferred to a 96 well plate and the assay was carried out according to the manufacturer's protocol. The plate was analyzed using a fluorescent well plate reader (Perkin-Elmer). The total COX, COX-1 and COX-2 activities were determined against a standard curve and using the COX-1 inhibitor SC-560 and the COX-2 inhibitor DuP-697.

Results

Fatty acid treatment and cell growth

To ensure cell survival during experiments, THP-1 macrophages were exposed to 0–100 µM DHA, EPA, and OLA. DHA treatment at concentrations below 50 µM did not induce any substantial effect on cell viability; however, concentrations at 75–100 µM showed a reduction in cellular viability by 20%–25% ($P > 0.05$) when compared to the non-treated control (data not shown). EPA-treatments showed less impact on THP-1 viability, with less than a 10% decrease (non-significant) at 100 µM. The OLA-treatment resulted in only

<2% decrease in cell viability at the highest concentration of 100 μM . Based on these results, the authors performed most subsequent experiments at 50 and 75 μM fatty acids.

Omega-3 PUFAs reduced niacin-induced PGD_2 and PGE_2 production

To test the effect of the omega-3 PUFAs on niacin induced PGD_2 and PGE_2 release in macrophages, THP-1 cells were treated with DHA, EPA, and OLA prior to exposure to increasing concentrations of niacin. Niacin increased both

PGD_2 and PGE_2 formation in a dose dependent manner. PGD_2 formation was greatly increased by 2–7-fold in a dose dependent manner on niacin-treatment (Figure 2), whereas the effect of niacin on PGE_2 formation resulted in only a moderate 1.5–2 fold increase (Figure 3). Both DHA and EPA reduced basal as well as niacin-induced increases in PGD_2 and PGE_2 formation (Figure 2A and B and Figure 3A and B,

Figure 2 Effect of fatty acids on niacin induced PGD_2 secretion in THP-1 macrophages. **Notes:** THP-1 macrophages were incubated with 50 and 75 μM (A) DHA, (B) EPA, or (C) OLA for 24 hours before being exposed to varying concentrations of niacin for 30 minutes. Concentration of PGD_2 in the medium was determined using an EIA kit as per manufacturer's instructions (Cayman Chemical, Ann Arbor, MI). Values are the means $\pm$ the standard deviations of duplicate experiments.

Abbreviations: DHA, docosahexaenoic acid; EIA, enzyme immunoassay; EPA, eicosapentaenoic acid; PGD_2 , prostaglandin D_2 ; OLA, oleic acid.

Figure 3 Effect of fatty acids on niacin induced PGE_2 secretion in THP-1 macrophages. **Notes:** THP-1 macrophages were incubated with 50 and 75 μM (A) DHA, (B) EPA, and (C) OLA for 24 hours before being exposed to varying concentrations of niacin for 30 minutes. Concentration of PGE_2 in the medium was determined using an EIA kit as per manufacturer's instructions (Cayman Chemical, Ann Arbor, MI). Values are the means $\pm$ the standard deviations of duplicate experiments.

Abbreviations: DHA, docosahexaenoic acid; EIA, enzyme immunoassay; EPA, eicosapentaenoic acid; PGD_2 , prostaglandin D_2 ; OLA, oleic acid.

respectively). In contrast, OLA treatment resulted in further enhancement of basal, as well as niacin-induced PGD_2 and PGE_2 formation (Figures 2C–3C).

Omega-3 PUFAs alter FA profile

The authors next examined the incorporation of fatty acid in THP-1 cells. An analysis of membrane fatty acid composition suggested that DHA treatment increased the incorporation of DHA in the phospholipids in a dose dependent manner (2 to 168 $\mu\text{g}/\text{mg}$ protein). Furthermore, the increased DHA levels in phospholipids occurred at the expense of AA,

whose levels decreased from 24 to 13 μg AA/mg protein (Figure 4A). EPA-treatment subsequently increased EPA incorporation in phospholipids in a dose dependent manner (2 to 190 $\mu\text{g}/\text{mg}$ protein) (Figure 4B). EPA incorporation into membrane phospholipids also occurred at the expense of AA and resulted in its reduction from 25 to 9 μg AA/mg protein (Figure 5). Although basal levels of OLA were substantially greater than DHA or EPA, OLA amounts significantly increased in a concentration dependent manner (51 to 391 $\mu\text{g}/\text{mg}$ protein); however, OLA incorporation had a minimal effect on AA displacement (Figure 4C).

Figure 4 Fatty acid incorporation into phospholipids of THP-1 macrophages. **Notes:** THP-1 macrophages were treated with increasing concentrations of (A) DHA, (B) EPA or (C) OLA for 24 hours. Lipids were isolated and fatty acid concentrations were determined by gas chromatography as described in the text. **Notes:** Values are the mean $\pm$ the standard deviation of triplicates. Results are analyzed using Student's *t* test. *P < 0.01 compared to non-supplemented cells. **Abbreviations:** AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PGD_2 , prostaglandin D_2 ; OLA, oleic acid.

Omega-3 PUFAs down regulate the GPR109A receptor

The authors analyzed the effects of DHA, EPA, and OLA on the expression of the niacin receptor, GPR109A, using flow cytometry. Data in Figure 5 indicate that DHA-treatment at 50 μM showed a 60% decrease in GPR109A expression, while 75 μM DHA further decreased the expression to 75% fewer receptors than compared to the control. 50 μM EPA reduced GPR109A receptor expression by 67%; however, 75 μM EPA did not further reduce its expression. In contrast, OLA treatment did not result in a significant reduction of niacin receptor expression.

Effect of omega-3 PUFAs on cellular calcium release

The authors further analyzed the effect of niacin on downstream calcium mobilization (Figure 6). In untreated cells niacin increased intracellular calcium (red line) in a time dependent manner. However, niacin-induced intracellular calcium increases were not observed when cells were pretreated with DHA (blue line), EPA (green line), or OLA (pink line).

Niacin induces cPLA_2 activation

One of the downstream targets for Ca^{2+} is cPLA_2 , whose activity causes the release of AA, a substrate for PGD_2 and PGE_2 synthesis. Therefore, the effect of niacin treatment on cPLA_2 activity was measured. Earlier experiments (Figure 4) indicate that DHA and EPA incorporated into phospholipids at the expense of AA. As a result, it was realized that measuring AA release by niacin in DHA and EPA-treated cells is not an appropriate assay for PLA_2 activity. Hence, the phosphorylation of PLA_2 by niacin was used as a measure of PLA_2 activity. Results shown in Figure 7 indicate that niacin at 1.5 mM caused a maximal 2-fold increase in cPLA_2 phosphorylation at 30 minutes of incubation. Higher

Figure 5 Effect of fatty acids on GPR109A niacin receptor expression in THP-1 macrophages.

Notes: Macrophages were treated with 50 or 75 μ M DHA, EPA, and OLA for 24 hours prior to stimulation with niacin (30 minutes). Cells were labeled with phycoerythrin-conjugated GPR109A and expression of GPR109A was determined using a FACSCalibur flow cytometer as described in the text. **(A)** Filled histograms represent the isotype control and open histograms represent GPR109A. Data is representative of the 75 μ M treatments. **(B)** Data were quantified as the percent change of mean fluorescent intensity. Values are the means $\pm$ the standard deviations of three independent duplicate experiments. Results are analyzed using ANOVA, followed by pair-wise comparisons with the Bonferroni adjustment. * $P < 0.01$ compared to niacin treatment controls.

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PGD₂, prostaglandin D₂; OLA, oleic acid.

concentration of niacin at 3 mM also induced a 2-fold increase in phosphorylation of cPLA₂ at 10 minutes of incubation which did not change on a longer incubation (30 minutes).

Effect of omega-3 PUFAs on COX activity

The authors observed that niacin itself has no effect on COX-1 activity (Table 1). DHA treatment alone slightly reduced

COX-1 activity, which was further decreased when DHA-treated cells were stimulated with niacin. EPA treatment alone had no effect COX-1 activity; however, activity was decreased when EPA-treated cells were stimulated with niacin. OLA also had no effect on COX-1 activities, and this activity was also decreased when OLA-treated cells were stimulated with niacin. THP-1 cells also showed very little COX-2 activity that was not affected by niacin or fatty acid treatment (Table 1).

Figure 6 Effect of fatty acids on calcium mobilization in THP-1 macrophages.

Notes: Macrophages were treated with 50 μ M DHA, EPA, and OLA for 24 hours. Cells were labeled with Fura 2-AM, and the change in ratio of fluorescence intensity at 340/380 (R) was monitored as described in the text. Results show a representation from three experiments.

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OLA, oleic acid.

Omega-3 PUFAs also regulate niacin-induced PGD_2 and PGE_2 production in Langerhans cells

After the initial completion of this study of THP-1 cells, the authors evaluated the effects of omega-3 PUFAs in the more disease relevant epidermal Langerhans cells. Data in Figure 8 show that untreated Langerhans cells exhibited a dose-dependent increase in PGD_2 production with increasing concentrations of niacin. When treated with 50 μ M DHA, the PGD_2 production decreased to 71% and 42% at 1.5 and 3 mM concentrations of niacin, respectively. EPA decreased PGD_2 production to 53% and 85% at 1.5 and 3 mM concentrations of niacin, respectively. PGE_2 production showed very little change with both concentrations of niacin (data not shown).

Discussion

Several studies have reported that niacin's receptor-mediated enhanced release of PGD_2 from tissue macrophages causes flushing, particularly by epidermal Langerhans dendritic cells.^{22,24,25,30,49,52} Several mechanisms have been proposed to reduce flushing in patients on niacin treatment, including reducing absorption of niacin, blocking DP1 receptors, and preventing production of prostaglandins.⁵³ Initial attempts to make slow release niacin, which effectively reduced flushing, failed because of its hepatotoxicity.^{54,55} Conversely, an extended-release niacin (rates between immediate release and slow release niacin) formulation (Niaspan, Abbott Park, IL) improved CHD outcome and had no hepatotoxic effects, but continued to cause flushing in patients when given higher doses (1–2 g/day) in combination with statins.^{56–58} A selective DP1 antagonist, laropiprant, was developed to

further mitigate the niacin-induced flushing and has shown promise to allow 1–2 g/day dosing regimen of niacin.⁵⁹ Although laropiprant is a potent inhibitor of DP1, it does not eliminate flushing in all patients,⁹ suggesting that other pathways may be involved in the residual flushing. Inhibition of prostaglandin synthesis by blocking the activities of COX enzymes with aspirin,^{60–62} indomethacin,⁶³ ibuprofen,⁵³ and naproxen²³ has also been shown to decrease niacin-induced flushing. In the present study, another approach was used to prevent production of prostaglandins. Since prostaglandins are synthesized directly from AA, an omega-6 PUFA, it was hypothesized that replacing cellular content of AA by omega-3 PUFAs (DHA and EPA) would reduce niacin-induced PGD_2 synthesis. The effects of omega-3 PUFAs were tested in differentiated THP-1 macrophages, as these were easy to maintain in culture and also reported to respond in a similar fashion as that of the epidermal Langerhans cells.⁴⁹ OLA, an omega-9 monounsaturated fatty acid, was also included to demonstrate the specificity of omega-3 PUFA effects.

This study's data has shown that EPA and OLA did not significantly impact THP-1 macrophage growth; however, DHA did exhibit modest growth inhibition of these cells at higher concentrations ($\geq 75 \mu\text{M}$). As previously reported by several investigators, the data also clearly demonstrated that niacin induced PGD_2 synthesis in a dose-dependent manner. Furthermore, both DHA and EPA effectively reduced basal, as well as niacin-induced PGD_2 synthesis. In contrast, OLA alone stimulated PGD_2 release, which was further enhanced in the presence of niacin. The effect of niacin on PGE_2 synthesis was also measured. The extent of PGE_2 synthesis by niacin was substantially lower than that of PGD_2 synthesis. However, both DHA and EPA were able to reduce niacin-induced PGE_2 synthesis, whereas

Figure 7 Niacin induces cPLA₂ activation in THP-1 macrophages.

Notes: Macrophages were treated with 1.5 or 3 mM niacin at three time points. **(A)** Western blot analysis was used to determine expression of phosphorylated cPLA₂ as described in the text. **(B)** Data is the densitometry analysis of bands and reported as the mean $\pm$ SD of ratios of cPLA₂/GAPDH of at least three determinations. Results are analyzed using ANOVA, followed by pair-wise comparisons with the Bonferroni adjustment. * $P < 0.01$ compared to niacin treatment controls.

Abbreviations: cPLA₂ cytosolic phospholipase A₂.

OLA had a stimulatory effect. Production of eicosanoids can be regulated by the availability of their substrate, AA, in phospholipids. Therefore the effect of fatty acids on AA content in membrane phospholipids was measured. The data clearly indicated that both DHA and EPA incorporated into cellular phospholipids at the expense of AA. This result is consistent with the authors⁴⁶ previous reports that omega-3 PUFAs effectively decrease cellular AA content. However, it is notable that OLA, despite its substantial incorporation into phospholipids, had no effect on AA levels and exhibited

a stimulatory effect on PGD₂ and PGE₂ synthesis. The data indicates (not shown) that OLA mostly incorporated at the expense of stearic and palmitic acid (18:0 and 16:0, respectively), and therefore has no impact over AA incorporation. Furthermore, OLA is not a substrate for the COX pathway and, therefore, it does not compete with AA for eicosanoid generation. It is not clear from the present investigation why OLA had a stimulatory effect on PGD₂ and PGE₂ synthesis. It is possible that the released stearic and/or palmitic acid induced COX-1 or -2 activities. Clearly, further investigation

Table 1 Effect of fatty acids on COX-I activity in THP-1 macrophages

	COX-I activity (nM/min/mg protein)		COX-2 activity (nM/min/mg protein)	
	Niacin		Niacin	
	0	3 mM	0	3 mM
Vehicle	15.5 ± 2.1	16.1 ± 4.2	16.7 ± 6.7	6.9 ± 1.7
DHA	12.7 ± 2.9	9.7 ± 3.8	8.5 ± 5.1	5.3 ± 3.5
EPA	16.0 ± 3.8	10.2 ± 0.6	7.9 ± 4.5	11.8 ± 1.0
OLA	14.9 ± 2.9	11.6 ± 0.8	11.9 ± 2.2	10.3 ± 5.0

Notes: Macrophages were treated with 75 μ M DHA, EPA, and OLA for 24 hours prior to a 30 minute exposure to 3 mM niacin. COX-I and -2 activities were measured according to the kit manufacturer's specifications. Values are the mean $\pm$ the standard deviation of triplicates. Results are analyzed using Student's *t*-test. No statistical difference is found in any treatment group compared to niacin-treated controls.

Abbreviations: COX-I and -2, cyclooxygenase-I and -2; cPLA₂, cytosolic phospholipase A2; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OLA, oleic acid.

is needed to understand the effect of OLA on PGD₂ and PGE₂, which was beyond the scope of this study.

Enrichment of omega-3 PUFAs in cellular membranes often results in changes in the surface expression of membrane proteins, including receptors.^{46,48} Therefore, the surface expression of GPR109A, a niacin receptor, was examined. The authors found that both DHA and EPA reduced surface expression of the niacin receptor, whereas OLA was ineffective. Diminished GPR109A expression could result in a reduction in niacin-induced flushing. The present study did not investigate if the reduced expression of GPR109A is due to reduced synthesis at the transcriptional or translational

level, or if it is due to reduced translocation to the surface from intracellular storage. It is likely that omega-3 PUFAs reorganize lipid rafts⁴⁸ and therefore affected the translocation of the receptor. The downstream events to niacin receptor were then examined. As previously reported,²⁵ niacin induced an increase in cytosolic calcium, which was effectively blocked by both DHA and EPA as well as by OLA. Stimulation of cells with niacin caused a gradual rise in calcium in the absence of a rapid initial rise. This suggests that the rise in calcium was due to influx from extracellular sources rather than release from intracellular stores. L-type voltage gated calcium channels that regulate influx of calcium from extracellular sources have previously been shown to be inhibited by omega-3 PUFAs.⁶⁴⁻⁶⁶ It is therefore likely that omega-3 PUFAs inhibited calcium influx through inhibiting L-type calcium channels. Other unsaturated fatty acids have been shown to act on these channels⁶⁷ and it is therefore possible that OLA also inhibited L-type calcium channels in the present study. One of the targets for rising cytosolic calcium is activation of cPLA₂, which releases AA for PGD₂ and PGE₂ synthesis. The effect of niacin on cPLA₂ activity was accessed by measuring its phosphorylation. The results clearly indicate that niacin-treatment of THP-1 cells resulted in cPLA₂ phosphorylation, indicating that niacin induces PGD₂ and PGE₂ formation by liberating AA from phospholipids. However, when AA is replaced by DHA or EPA, niacin-induced cPLA₂ activity releases

Figure 8 Effect of fatty acids on niacin-induced PGD₂ secretion in Langerhans cells.

Notes: Langerhans cells were treated with 50 μ M fatty acids for 24 hours before a 30 minute exposure to 1.5 or 3 mM niacin. Concentrations of PGD₂ in the medium were determined using an EIA kit as per manufacturer's instructions (Cayman Chemical, Ann Arbor, MI). Values are the means $\pm$ the standard deviations of triplicate experiments from one preparation of Langerhans cells.

Abbreviations: EIA, enzyme immunoassay.

DHA or EPA. Both DHA and EPA compete with AA for downstream COX-1 activity.^{68–71} During the present investigation, none of the fatty acids had any significant effect on COX-1 activity. It is therefore possible that inhibition of PGD₂ and PGE₂ synthesis by DHA or EPA is, to some extent, due to a reduction of their substrate, AA. However, the effect of EPA and DHA on GPR109A expression, and inhibition of niacin-induced calcium release also play a role in the reduction of niacin-induced flushing response by omega-3 PUFA. Furthermore, it is also possible that DHA and EPA directly induced anti-inflammatory effects through GPR120, a specific omega-3 fatty acids receptor expressed on macrophages;⁷² however, it is not known if EPA and DHA mediated effects through GPR120 regulate pro-inflammatory prostaglandin synthesis.

In order to correlate these studies to relevant primary cell line, the effect of DHA, EPA, and OLA on niacin-induced PGD₂ and PGE₂ synthesis was also measured in Langerhans dendritic cells. Both DHA and EPA were able to inhibit niacin-induced PGD₂ synthesis, whereas OLA was not effective. The authors also noticed that EPA and OLA caused a basal increase in PGD₂ synthesis. The reason for this increase is not clear. One possibility is variation in the incorporation and metabolism of EPA in the different cell types. In contrast, niacin did not increase PGE₂ synthesis and fatty acid-treatments had variable responses. However, this study's results are consistent with other studies where niacin enhanced only PGD₂ production in Langerhans dendritic cells.^{25,26} These data suggest that PGD₂ plays key a role in mediating niacin-induced flushing responses. It is unclear why PGE₂ production is stimulated by niacin in THP-1 cells but not in Langerhans cells. Perhaps a second co-factor is required for niacin stimulation that is not present in the Langerhans cells. Further investigation is required to determine this.

It is important to note that the outcome from the recent AIM HIGH study reduces enthusiasm for the use of niacin and statin combination therapy; however, it is possible that use of omega-3 fatty acids with a combination therapy of niacin and statins may be advantageous in improving cardiac events. As mentioned above, long term use of omega-3 fatty acids would have added benefits on improving LDL/HDL ratios and reducing triglycerides. Several pharmaceutical companies are aggressively marketing omega-3 fatty acids preparations for cardiac benefits. For example, preparations such as Crystal EPA-TG (Equateq, Scotland), Omevital TG, Omevital TG-Gold, Omevital –TG-ultra and Omevital-90%TG (Cognis, UK), Incromea (Croda, Goole, UK), and algal-DHA (Martek, Columbia, MD) have been

developed that have substantially improved contents of omega-3 fatty acids than that of commercially available menhaden fish oils. In addition to this prescription grade omega-3, supplements containing ethyl esters of DHA + EPA (Lovaza, GlaxoSmithKline, London, UK) are also available. More recently pure ethyl ester preparations of EPA (AMR101, Amarin Corporation, Bedminster, NJ) were developed to treat patients for high or very high triglyceride levels. These preparations supply enriched quantities of omega-3 fatty acids higher than typically present in over-the-counter fish oil supplements.

Conclusions

The data indicated that omega-3 PUFAs can effectively inhibit niacin-induced synthesis of PGD₂ and PGE₂, the key mediators of flushing caused by niacin intake. The possible inhibitory mechanisms appear to be diminished niacin receptor expression and reduced availability of AA, a substrate for PGD₂ and PGE₂ synthesis. These effects are mediated within cellular membranes and are based on the enhanced incorporation of omega-3 PUFAs into membrane phospholipids. Therefore, this study suggests that regular intake of fish, fish oil supplements, prescription grade DHA and EPA formulation (GlaxoSmithKline) or other commercially available DHA-, and EPA-enriched preparations, will not only be beneficial in reducing the flushing response of niacin, but also provide several known cardio-beneficial effects.⁷³

Acknowledgments

The research was supported by a research grant from Abbott Laboratories, Abbott Park, IL 60064.

Disclosure

The authors report no conflict of interest.

References

1. Meyers CD, Kamanna VS, Kashyap ML. Niacin therapy in atherosclerosis. *Curr Opin Lipidol*. 2004;15(6):659–665.
2. Carlson LA. Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review. *J Intern Med*. 2005;258(2):94–114.
3. The Coronary Drug Project Research Group. Clofibrate and niacin in coronary heart disease. *JAMA*. 1975;231(4): 360–381.
4. van Greevenbroek MM, Voorhout WF, Erkelens DW, van Meer G, de Bruin TW. Palmitic acid and linoleic acid metabolism in Caco-2 cells: different triglyceride synthesis and lipoprotein secretion. *J Lipid Res*. 1995;36(1):13–24.
5. Wahlberg G, Walldius G, Olsson AG, Kirstein P. Effects of nicotinic acid on serum cholesterol concentrations of high density lipoprotein subfractions HDL2 and HDL3 in hyperlipoproteinaemia. *J Intern Med*. 1990;228(2):151–157.
6. Ganji SH, Zhang L-H, Kamanna VS, Kashyap ML. Effect of niacin on lipoproteins and atherosclerosis. *Future Lipidol*. 2006;1(5):549.

7. Capuzzi DM, Guyton JR, Morgan JM, et al. Efficacy and safety of an extended-release niacin (Niaspan): a long-term study. *Am J Cardiol*. 1998;82(12A):74U–81U; discussion 85U–86U.
8. Backes JM, Gibson CA. Effect of lipid-lowering drug therapy on small-dense low-density lipoprotein. *Ann Pharmacother*. 2005;39(3):523–526.
9. Parhofer KG. Review of extended-release niacin/laropiprant fixed combination in the treatment of mixed dyslipidemia and primary hypercholesterolemia. *Vasc Health Risk Manag*. 2009;5:901–908.
10. Vogt A, Kassner U, Hostalek U, Steinhagen-Thiessen E. Evaluation of the safety and tolerability of prolonged-release nicotinic acid in a usual care setting: the NAUTILUS study. *Curr Med Res Opin*. 2006;22(2):417–425.
11. Ballantyne CM, Davidson MH, McKenney JM, Keller LH, Bajorunas DR, Karas RH. Comparison of the efficacy and safety of a combination tablet of niacin extended-release and simvastatin with simvastatin 80 mg monotherapy: the SEACOAST II (high-dose) study. *J Clin Lipidol*. 2008;2(2):79–90.
12. Ballantyne CM, Davidson MH, McKenney J, Keller LH, Bajorunas DR, Karas RH. Comparison of the safety and efficacy of a combination tablet of niacin extended release and simvastatin vs simvastatin monotherapy in patients with increased non-HDL cholesterol (from the SEACOAST I study). *Am J Cardiol*. 2008;101(10):1428–1436.
13. Knopp RH. Drug treatment of lipid disorders. *N Engl J Med*. 1999;341(7):498–511.
14. Shepherd J, Packard CJ, Patsch JR, Gotto AM Jr, Taunton OD. Effects of nicotinic acid therapy on plasma high density lipoprotein subfraction distribution and composition and on apolipoprotein A metabolism. *J Clin Invest*. 1979;63(5):858–867.
15. National Heart, Lung, and Blood Institute. HDL-atherosclerosis treatment study (HATS). In: ClinicalTrials.gov [website on the Internet]. Bethesda, MD: US National Library of Medicine; 1999 [updated June 23, 2005]. Available from: <http://clinicaltrials.gov/ct2/show/NCT00000553?term=NCT00000553&rank=1>. NLM identifier: NCT00000553. Accessed February 10, 2012.
16. Brown BG, Zhao XQ, Chait A, et al. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med*. 2001;345(22):1583–1592.
17. Boden WE, Probstfield JL, Anderson T, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med*. 2011;365(24):2255–2267.
18. National Heart, Lung, and Blood Institute. Niacin plus statin to prevent vascular events. In: ClinicalTrials.gov [website on the Internet]. Bethesda, MD: US National Library of Medicine; 2005 [updated June 3, 2011]. Available from: <http://clinicaltrials.gov/ct2/show?term=NCT00120289&rank=1>. NLM identifier: NCT00120289. Accessed February 12, 2012.
19. Gray DR, Morgan J, Chretien SD, Kashyap ML. Efficacy and safety of controlled-release niacin in dyslipoproteinemic veterans. *Ann Intern Med*. 1994;121(4):252–258.
20. Stern RH, Spence JD, Freeman DJ, Parbtani A. Tolerance to nicotinic acid flushing. *Clin Pharmacol Ther*. 1991;50(1):66–70.
21. Nozaki S, Kihara S, Kubo M, Kameda K, Matsuzawa Y, Tarui S. Increased compliance of nicotinic treatment by addition of aspirin: relationship between changes in prostaglandins and skin flushing. *Int J Clin Pharmacol Ther Toxicol*. 1987;25(12):643–647.
22. Morrow JD, Parsons WG III, Roberts LJ II. Release of markedly increased quantities of prostaglandin D2 in vivo in humans following the administration of nicotinic acid. *Prostaglandins*. 1989;38(2):263–274.
23. Eklund B, Kaijser L, Nowak J, Wennmalm A. Prostaglandins contribute to the vasodilation induced by nicotinic acid. *Prostaglandins*. 1979;17(6):821–830.
24. Maciejewski-Lenoir D, Richman JG, Hakak Y, Gaidarov I, Behan DP, Connolly DT. Langerhans cells release prostaglandin D2 in response to nicotinic acid. *J Invest Dermatol*. 2006;126(12):2637–2646.
25. Benyo Z, Gille A, Bennett CL, Clausen BE, Offermanns S. Nicotinic acid-induced flushing is mediated by activation of epidermal langerhans cells. *Mol Pharmacol*. 2006;70(6):1844–1849.
26. Lorenzen A, Stannek C, Burmeister A, Kalvinsh I, Schwabe U. G protein-coupled receptor for nicotinic acid in mouse macrophages. *Biochem Pharmacol*. 2002;64(4):645–648.
27. Soga T, Kamohara M, Takasaki J, et al. Molecular identification of nicotinic acid receptor. *Biochem Biophys Res Commun*. 2003;303(1):364–369.
28. Wise A, Foord SM, Fraser NJ, et al. Molecular identification of high and low affinity receptors for nicotinic acid. *J Biol Chem*. 2003;278(11):9869–9874.
29. Benyo Z, Gille A, Kero J, et al. GPR109A (PUMA-G/HM74A) mediates nicotinic acid-induced flushing. *J Clin Invest*. 2005;115(12):3634–3640.
30. Cheng K, Wu T-J, Wu KK, et al. Antagonism of the prostaglandin D2 receptor 1 suppresses nicotinic acid-induced vasodilation in mice and humans. *Proc Natl Acad Sci U S A*. 2006;103(17):6682–6687.
31. Daviglus ML, Stamler J, Orenca AJ, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. *N Engl J Med*. 1997;336(15):1046–1053.
32. Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and risk of sudden cardiac death. *JAMA*. 1998;279(1):23–28.
33. Bang HO, Dyerberg J, Hjoorne N. The composition of food consumed by Greenland Eskimos. *Acta Med Scand*. 1976;200(1–2):69–73.
34. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet*. 1999;354(9177):447–455.
35. de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet*. 1994;343(8911):1454–1459.
36. Leaf A, Albert CM, Josephson M, et al. Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. *Circulation*. 2005;112(18):2762–2768.
37. Harris WS. n-3 fatty acids and lipoproteins: comparison of results from human and animal studies. *Lipids*. 1996;31(3):243–252.
38. Mori TA, Burke V, Puddey IB, et al. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr*. 2000;71(5):1085–1094.
39. Harris WS, Ginsberg HN, Arunakul N, et al. Safety and efficacy of Omacor in severe hypertriglyceridemia. *J Cardiovasc Risk*. 1997;4(5–6):385–391.
40. Abe Y, El-Masri B, Kimball KT, et al. Soluble cell adhesion molecules in hypertriglyceridemia and potential significance on monocyte adhesion. *Arterioscler Thromb Vasc Biol*. 1998;18(5):723–731.
41. McKenney JM, Sica D. Prescription omega-3 fatty acids for the treatment of hypertriglyceridemia. *Am J Health Syst Pharm*. 2007;64(6):595–605.
42. Rhee SG. Regulation of phosphoinositide-specific phospholipase C. *Ann Rev Biochem*. 2001;70:281–312.
43. Liberty IF, Raichel L, Hazan-Eitan Z, et al. Cytosolic phospholipase A2 is responsible for prostaglandin E2 and leukotriene B4 formation in phagocyte-like PLB-985 cells: studies of differentiated cPLA2-deficient PLB-985 cells. *J Leukoc Biol*. 2004;76(1):176–184.
44. Capdevila JH, Falck JR, Harris RC. Cytochrome P450 and arachidonic acid bioactivation. Molecular and functional properties of the arachidonate monooxygenase. *J Lipid Res*. 2000;41(2):163–181.
45. Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem*. 1996;271(52):33157–33160.
46. Stillwell W, Shaikh SR, Zerouga M, Siddiqui R, Wassall SR. Docosahexaenoic acid affects cell signaling by altering lipid rafts. *Reprod Nutr Dev*. 2005;45(5):559–579.

47. Horejsi V, Drbal K, Cebecauer M, et al. GPI-microdomains: a role in signalling via immunoreceptors. *Immunol Today*. 1999;20(8):356–361.
48. Siddiqui RA, Harvey KA, Zaloga GP, Stillwell W. Modulation of lipid rafts by omega-3 fatty acids in inflammation and cancer: implications for use of lipids during nutrition support. *Nutr Clin Pract*. 2007;22(1):74–88.
49. Meyers CD, Liu P, Kamanna VS, Kashyap ML. Nicotinic acid induces secretion of prostaglandin D2 in human macrophages: an in vitro model of the niacin flush. *Atherosclerosis*. 2007; 192(2):253–258.
50. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem*. 1957;226:497–509.
51. Xu Z, Harvey KA, Pavlina T, Dutot G, Zaloga GP, Siddiqui RA. An improved method for determining medium- and long-chain FAMES using gas chromatography. *Lipids*. 2010;45:199–208.
52. Papalioudis D, Boucher W, Kempuraj D, et al. Niacin-induced “flush” involves release of prostaglandin D2 from mast cells and serotonin from platelets: evidence from human cells in vitro and an animal model. *J Pharmacol Exp Ther*. 2008;327(3):665–672.
53. Kamanna VS, Ganji SH, Kashyap ML. The mechanism and mitigation of niacin-induced flushing. *Int J Clin Pract*. 2009;63(9):1369–1377.
54. Christensen NA, Achor RW, Berge KG, Mason HL. Nicotinic acid treatment of hypercholesterolemia. Comparison of plain and sustained-action preparations and report of two cases of jaundice. *JAMA*. 1961;177:546–550.
55. McKenney JM, Proctor JD, Harris S, Chinchili VM. A comparison of the efficacy and toxic effects of sustained- vs immediate-release niacin in hypercholesterolemic patients. *JAMA*. 1994;271(9):672–677.
56. McKenney JM, Jones PH, Bays HE, et al. Comparative effects on lipid levels of combination therapy with a statin and extended-release niacin or ezetimibe versus a statin alone (the COMPELL study). *Atherosclerosis*. 2007;192(2):432–437.
57. Kashyap ML, McGovern ME, Berra K, et al. Long-term safety and efficacy of a once-daily niacin/lovastatin formulation for patients with dyslipidemia. *Am J Cardiol*. 2002;89(6):672–678.
58. Hunninghake DB, McGovern ME, Koren M, et al. A dose-ranging study of a new, once-daily, dual-component drug product containing niacin extended-release and lovastatin. *Clin Cardiol*. 2003;26(3):112–118.
59. Bays HE, Ballantyne C. What's the deal with niacin development: is laropiprant add-on therapy a winning strategy to beat a straight flush? *Curr Opin Lipidol*. 2009;20(6):467–476.
60. Cefali EA, Simmons PD, Stanek EJ, McGovern ME, Kissling CJ. Aspirin reduces cutaneous flushing after administration of an optimized extended-release niacin formulation. *Int J Clin Pharmacol Ther*. 2007;45(2):78–88.
61. Dunn RT, Ford MA, Rindone JP, Kwiecinski FA. Low-dose aspirin and ibuprofen reduce the cutaneous reactions following niacin administration. *Am J Ther*. 1995;2(7):478–480.
62. Thakkar RB, Kashyap ML, Lewin AJ, Krause SL, Jiang P, Padley RJ. Acetylsalicylic acid reduces niacin extended-release-induced flushing in patients with dyslipidemia. *Am J Cardiovasc Drugs*. 2009;9(2): 69–79.
63. Kaijser L, Eklund B, Olsson AG, Carlson LA. Dissociation of the effects of nicotinic acid on vasodilatation and lipolysis by a prostaglandin synthesis inhibitor, indomethacin, in man. *Med Biol*. 1979;57(2): 114–117.
64. Hirafuji M, Ebihara T, Kawahara F, Hamaue N, Endo T, Minami M. Inhibition by docosahexaenoic acid of receptor-mediated Ca(2+) influx in rat vascular smooth muscle cells stimulated with 5-hydroxytryptamine. *Eur J Pharmacol*. 2001;427(3):195–201.
65. Rinaldi B, Di Pierro P, Vitelli MR, et al. Effects of docosahexaenoic acid on calcium pathway in adult rat cardiomyocytes. *Life Sci*. 2002;71(9): 993–1004.
66. Hallaq H, Smith TW, Leaf A. Modulation of dihydropyridine-sensitive calcium channels in heart cells by fish oil fatty acids. *Proc Natl Acad Sci U S A*. 1992;89(5):1760–1764.
67. Xiao YF, Ke Q, Wang SY, et al. Single point mutations affect fatty acid block of human myocardial sodium channel alpha subunit Na+ channels. *Proc Natl Acad Sci U S A*. 2001;98(6):3606–3611.
68. Smith WL. Omega-3 and omega-6 essential fatty acids and cyclooxygenase pathways. Paper presented at: Experimental Biology; Apr 2006; San Francisco, CA.
69. Laneuville O, Breuer DK, Xu N, et al. Fatty acid substrate specificities of human prostaglandin-endoperoxide H synthase-1 and -2. Formation of 12-hydroxy-(9Z, 13E/Z, 15Z)-octadecatrienoic acids from alpha-linolenic acid. *J Biol Chem*. 1995;270(33):19330–19336.
70. Hwang D. Fatty acids and immune responses – a new perspective in searching for clues to mechanism. *Annu Rev Nutr*. 2000;20:431–456.
71. Corey EJ, Shih C, Cashman JR. Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis. *Proc Natl Acad Sci U S A*. 1983;80(12):3581–3584.
72. Oh DY, Talukdar S, Bae EJ, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell*. 2011;142(5):687–698.
73. Siddiqui RA, Harvey KA, Zaloga GP. Modulation of enzymatic activities by n-3 polyunsaturated fatty acids to support cardiovascular health. *J Nutr Biochem*. 2008;19:417–443.

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