Novel Eicosapentaenoic Acid-derived F3-isoprostanes as Biomarkers of Lipid Peroxidation

Received for publication, March 3, 2009, and in revised form, June 9, 2009. Published, JBC Papers in Press, June 11, 2009, DOI 10.1074/jbc.M109.024075

Wen-Liang Song1, Georgios Paschos‡, Susanne Fries1, Muredach P. Reilly‡, Ying Yu‡, Joshua Rokach§, Chih-Tsung Chang§, Pranav Patel†, John A. Lawson‡, and Garret A. FitzGerald†‡§

From the ‡Institute for Translational Medicine and Therapeutics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104 and the §Claude Pepper Institute and Department of Chemistry, Florida Institute of Technology, Melbourne, Florida 32901

Isoprostanes (iPs),2 a family of prostaglandin (PG) isomers generated by free radical-catalyzed peroxidation of polyunsaturated fatty acids (PUFAs). Urinary F2-iPs, PGF2α isomers derived from arachidonic acid (AA) are used as indices of lipid peroxidation in vivo. We now report the characterization of two major F3-iPs, 5-epi-8,12-iso-iPF3α-VI and 8,12-iso-iPF3α-VI, derived from the ω-3 fatty acid, eicosapentaenoic acid (EPA). Although the potential therapeutic benefits of EPA receive much attention, a shift toward a diet rich in ω-3 PUFAs may also predispose to enhanced lipid peroxidation. Urinary 5-epi-8,12-iso-iPF3α-VI and 8,12-iso-iPF3α-VI are highly correlated and unaltered by cyclooxygenase inhibition in humans. Fish oil-dose-dependently elevates urinary F3-iPs in mice and a shift in dietary ω-3/ω-6 PUFAs is reflected by an increasing slope [m] of the line relating urinary 8, 12-iso-iPF3α-VI and 8,12-iso-iPF2α-VI. Administration of bacterial lipopolysaccharide evokes a reversible increase in both urinary 8,12-iso-iPF3α-VI and 8,12-iso-iPF2α-VI in humans on an ad lib diet. However, while excretion of the iPs is highly correlated (R² median = 0.8), [m] varies by an order of magnitude, reflecting marked inter-individual variability in the relative peroxidation of ω-3 versus ω-6 substrates. Clustered analysis of F2- and F3-iPs refines assessment of the oxidant stress response to an inflammatory stimulus in vivo by integrating variability in dietary intake of ω-3/ω-6 PUFAs.

Isoprostanes (iPs),2 a family of prostaglandin (PG) isomers generated by free radical-catalyzed peroxidation of polyunsaturated fatty acids (PUFAs) in cell membranes. They can be immunodetected and quantified by mass spectrometry (1). They are then cleaved by phospholipases (2), circulate in plasma, and are excreted in urine (3). F2-iPs, isomers of PGF2α (3), derived from peroxidation of arachidonic acid (AA), are the most studied species. F2-iPs can be quantified in normal animal and human biological fluids and tissues, implying ongoing lipid peroxidation under physiological conditions, despite replete and diversified endogenous antioxidant defense systems (4).

The measurement of urinary F2-iPs isoprostanes has been used to reflect lipid peroxidation noninvasively in several human diseases (5–8). In addition to their utility as markers of oxidant stress (OS), high concentrations of some F2-iPs also possess biological activity in vitro, including bronchoconstriction (9), vasoconstriction (10), platelet aggregation (11, 12), and adhesion (13). These effects result from iPs acting as incidental ligands at prostaglandin receptors. It is unknown whether this capacity of individual iPs to ligate prostanoid receptors has relevance to the concentration of the multiple endogenous iP species likely to be formed simultaneously under conditions of oxidant stress in vivo.

iPs analogous to the F2-iPs may be formed from other fatty acid substrates (14–19), including the fish oil constituent, eicosapentaenoic acid (EPA) (20). Potentially beneficial effects of EPA consumption have been supported by a variety of epidemiological and interventional studies. EPA competes with AA for access to the cyclooxygenase enzymes (21), reducing production of AA-derived PGs (22, 23). This effect and/or substituted formation of EPA-derived PGs may explain the anti-inflammatory and cardioprotective effects ascribed to fish oils.

The relatively unsaturated EPA and docosahexaenoic acid (DHA) in fatty fish are likely to be more susceptible to lipid peroxidation than AA, although it is unknown whether this might constrain beneficial effects derived from a shift in substrate-dependent enzymatic product formation. While F2-iPs and F3-iPs are excreted into urine in their original form, the F3-iPs formed from DHA (Fig. 1) are at least partly metabolized to F2-iPs before excretion (20, 24). Given the potential utility of noninvasive biomarkers of EPA and DHA peroxidation and our previous work with F2-iPs (25, 26), we sought to determine whether members of group VI (Fig. 1B), 5-epi-8,12-iso-iPF3α-VI and 8,12-iso-iPF3α-VI (Fig. 1C) might be detectable in urine.

**EXPERIMENTAL PROCEDURES**

Standards—Synthetic 5-epi-8,12-iso-iPF3α-VI and 8,12-iso-iPF3α-VI were synthesized as previously described (20, 27). Stereospecific synthesis of deuterated standards, d5-5-epi-8,12-iso-iPF3α-VI and d4-8,12-iso-iPF3α-VI will be described elsewhere. iPF2α-III and iPF3α-III were purchased from Cay-

---

1 The Robert L. McNeil, Jr. Professor in Translational Medicine and Therapeutics. To whom correspondence should be addressed: 153 Johnson Pavilion, School of Medicine, Hamilton Walk, Philadelphia, PA 19104. Tel.: 215-898-1184; Fax: 215-573-9135; E-mail: garret@spirit.gcrc.upenn.edu.
2 The abbreviations used are: Ip, isoprostane; PFA, polyunsaturated fatty acids; EPA, 5,8,11,14,17-eicosapentaenoic acid; SPE, solid phase extraction; SRM, selected reaction monitoring; MAP, mean systemic arterial pressure; LPS, lipopolysaccharide; Cre, creatinine; CID, collision-induced dissociation; AA, arachidonic acid; COX, cyclooxygenase.
3 The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S5.
4 © 2009 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.

23636 JOURNAL OF BIOLOGICAL CHEMISTRY VOLUME 284 • NUMBER 35 • AUGUST 28, 2009
Chemical Co. (Ann Arbor, MI). We utilized purified deionized water (Milli-Q water purification system, Millipore) in the preparation of all aqueous solutions and mobile phases. HPLC-grade acetonitrile and ethyl acetate were purchased from J. T. Baker. Reagent grade acetic acid was purchased from Fisher. HPLC-grade ammonium hydroxide was purchased from Mallinckrodt.

Urine Analysis—Internal standards were added to 1 ml of human urine or 0.3 ml of mouse urine and allowed to equilibrate for 15 min. The pH was then adjusted with formic acid to ≈2.5. The samples were purified by solid phase extraction (SPE) using StrataX cartridges (Phenomenex, Torrance, CA). The SPE cartridge was conditioned with 1 ml of acetonitrile and equilibrated with 1 ml of water. The sample was applied to the cartridge, which was then washed with 1 ml of 5% acetonitrile in water and dried with a vacuum for 15 min. The analyte and internal standards were eluted from the cartridge using 1 ml of 5% acetonitrile in ethyl acetate. The eluate was collected and dried under a gentle stream of nitrogen. The resulting residue was then reconstituted in 200 μl of 20% acetonitrile in water and filtered by centrifugation. The 0.2-μm Nylon microspin filters were purchased from Alltech Associates.

High Performance Liquid Chromatography—The HPLC included an Accela solvent delivery system (Thermo, Waltham, MA) and a Hypersil GOLD C18 (2), 200 mm × 2.1 mm, 1.9 μm particle size column (Thermo). The mobile phase consisted of water (solvent A) and acetonitrile: methanol (95:5, solvent B), both with 0.005% acetic acid adjusted to pH 5.7 with ammonium hydroxide. The flow rate was 350 μl/min. The separations involved various linear solvent gradient programs.

Mass Spectrometry—A TSQ Quantum Ultra instrument (Thermo) equipped with a heated electrospray source, and a triple quadrupole analyzer was used in these studies. The ESI source used nitrogen for both sheath and auxiliary gas, set to 70 and 5 arbitrary units, respectively. The mass spectrometer was operated in the negative ion mode with a capillary temperature of 350 °C and a spray voltage of 2.0 kV. The source offset was 6 V. The analyzer was operated in the selected reaction monitoring (SRM) mode for the analysis of urinary iPs. The transitions m/z 351 > 115 for the endogenous material and m/z 355 > 115 for the tetradeuterated internal standards were monitored. The collision energy was 23 eV. 8,12-iso-iPF3VI was measured as previously described (28).

The product ion scan mode was used for spectral analysis of 5-epi-8,12-iso-iPF3VI and 8,12-iso-iPF3VI. Precursor ions (m/z 351 for endogenous 5-epi-8,12-iso-iPF3VI and 8,12-iso-iPF3VI and m/z 355 for the internal standards) were collisionally activated at 23 eV under 1.5 mT argon gas producing the CID spectra.

Studies in Mice—All studies were performed following protocol review and approval by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania.

FIGURE 1. F3-iPs derived from EPA. A, formation and metabolism of isoprostanes. AA is more abundant than EPA and DHA in cell membranes obtained from individuals consuming a Western diet. Isoprostanes are formed from the corresponding PUFA substrate in situ following free radical attack. F2-iPs and F3-iPs are excreted into urine in their original form, but F4-iPs are at least partly metabolized to F3-iPs before excretion. (F2-iPs: F2-isoprostanes; F3-iPs: F3-isoprostanes; F4-iPs: F4-isoprostanes). B, six types of F3-iPs. C, 5-epi-8,12-iso-iPF3VI and 8,12-iso-iPF3VI.
Fish Oil Diet—Eight-week-old female C57/BL6 mice (n = 5) were fed with a 0, 1, or 5% fish oil-rich diet (Harlan Teklad, Madison, WI). Urine was collected for 24 h in metabolic cages before, 6 weeks after, and 11 weeks after initiation of the various diets. Total lipids were extracted using a modified Folch procedure (1). EPA and AA were measured by LC/MS/MS (29).

Systemic Blood Pressure Response—Procedures were performed as previously described (30). Briefly, 12-week-old male C57/BL6 mice (n = 3) were anesthetized with pentobarbital (Nembutal, Abbott Laboratories, Abbott Park, IL). The carotid artery was isolated from the surrounding tissues and cannulated by a catheter. The catheter was tied in place and connected to a pressure transducer. Mean systemic arterial pressure (MAP) values were recorded continuously for the duration of each experiment. Another catheter was inserted into the jugular vein for drug or vehicle infusions. Experiments were started 20–30 min after completion of surgical procedures. Mice received bolus injections of vehicle (0.9% sodium chloride solution), iPF2\(_{20}\)-III and 8,12-iso-iPF3\(_{20}\)-VI at 10, 25, or 50 \(\mu\)g/kg. Vehicle or drugs were injected as a 1-ml/kg solution in saline.

Platelet Aggregation Studies—Twelve-week-old male C57/BL6 mice (n = 3) were anesthetized with pentobarbital, and blood was obtained through the inferior vena cava. Samples were centrifuged to obtain platelet-rich plasma, and platelet counts were determined with a Coulter counter. Platelet-poor plasma supernatant was obtained and used to adjust volumes for aggregation assays. Aggregation studies were performed under constant stirring at 37 °C, and light transmittance was measured with a dual-channel aggregometer. ADP was used as platelet agonist (10 \(\mu\)M).

Clinical Studies—Two clinical studies were performed. The study protocols were approved by the Institutional Review Board of the University of Pennsylvania and by the Advisory Council of the Clinical and Translational Research Center (CTRC) of the University of Pennsylvania. All volunteers were apparently healthy on physical examination, were nonsmokers, and refrained from all medications for 2 weeks before and during the course of the studies. Volunteers with a history of coagulation disorders, a bleeding tendency, drug allergy, or gastrointestinal disorders were excluded from participation in the studies.
In the first study, 10 volunteers (5 male and 5 female) received a bolus injection of bacterial lipopolysaccharide (LPS), 3 ng/kg, under controlled conditions, as we have previously described (31). Subjects were admitted to the CTRC the evening before the study, and an intravenous infusion of saline was commenced. The study involved a 60-h inpatient stay in the CTRC of the University of Pennsylvania comprising an overnight acclimatization phase, a 24-h saline administration control phase, and a 24-h post-LPS study phase. Urinary iPs were assessed in urines collected at the following time intervals before (0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 18, 18 to 24 h) LPS administration. Data are plotted at the midpoint of each corresponding urine collection.

In the second study, a single dose of aspirin (325 mg) was administered to 18 healthy volunteers (9 males and 9 females). Urinary iPs were measured in spot urines at time 0 and 4 h after dosing.

**Data Analysis**—Urinary iPs are expressed after correction for urinary creatinine (Cre) concentration and are reported as ng/mg Cre. Results are expressed as mean ± S.E. Statistical comparisons were performed initially using a two way analysis of variance, with subsequent two-tailed comparisons as appropriate. Differences were judged significant if p < 0.05.

**RESULTS**

**Identification of 5-epi-8,12-iso-iPF_{3a}-VI and 8,12-iso-iPF_{3a}-VI in Urine**—We have synthesized two deuterated standards, d_{4}-5-epi-8,12-iso-iPF_{3a}-VI and d_{4}-8,12-iso-iPF_{3a}-VI to facilitate detection of the endogenous iPs in urine. Endogenous 5-epi-8,12-iso-iPF_{3a}-VI and 8,12-iso-iPF_{3a}-VI (Fig. 2, A, panel b and B, panel b) were identified, co-eluting with their respective deuterated internal standards in both human (Fig. 2A) and mouse urine (Fig. 2B). Co-injection of exogenous synthetic 5-epi-8,12-iso-iPF_{3a}-VI and 8,12-iso-iPF_{3a}-VI increased peak height but not width, confirming the identification and homogeneity of the compounds (Fig. 2, A, panels c and d, and B, panels c and d). Their relative abundance is much higher than other classes of F_{3}-iPs (data not shown). Levels of 5-epi-8,12-iso-iPF_{3a}-VI and 8,12-iso-iPF_{3a}-VI are highly correlated in human urine (supplemental Fig. S1). Thus, we usually quantified only 8,12-iso-iPF_{3a}-VI as a representative F_{3}-iP. Assay reproducibility and variability was assessed on a single sample stored at three different temperatures and assayed three times on 3 separate days (supplemental Fig. S2A). The assay was linear over a range of 0.01–100 ng of added authentic material (supplemental Fig. S2B). A single dose of 325 mg of aspirin failed to alter urinary levels of 8,12-iso-iPF_{3a}-VI in healthy volunteers, confirming the cyclooxygenase-independent derivation of this F_{3}-iP (supplemental Fig. S3). Data from mice in which COX expression has been genetically manipulated afford further evidence of their COX-independent origin. (supplemental Fig. S4).

**Mass Spectrometric Analysis of 5-epi-8,12-iso-iPF_{3a}-VI and 8,12-iso-iPF_{3a}-VI**—Collision-induced dissociation (CID) of d_{4}-5-epi-8,12-iso-iPF_{3a}-VI and d_{4}-8,12-iso-iPF_{3a}-VI at m/z 355 gave rise to a series of major fragment ions with m/z values of 115, 177, 221, 275, 311, 337, and a base peak of 355 (Fig. 3, A and C), which was virtually identical to the mass spectrum obtained from endogenous 5-epi-8,12-iso-iPF_{3a}-VI and 8,12-iso-iPF_{3a}-VI, which included m/z values of 115, 173, 217, 271, 307, 333 and a base peak of 351 (Fig. 3, B and D). The differences in m/z values between these two groups were either 0 or 4 mass units reflecting fragments with or without deuterium. (supplemental Fig. S5).

**Blood Pressure and Platelet Response to F_{3}-isoprostanes**—A prototypic F_{2}-iP, iPF_{2α,III} (8-iso-PGF_{2α}) increases blood pres-
Figure 4 shows the bioactivity of F2 versus F3-iPs. A, intravenous injection of iPF2-III (50 µg/kg body weight) caused a rapid and substantial increase in MAP in mice. B, intravenous injection of iPF2-III (50 µg/kg body weight) failed to elevate MAP significantly. C, dose-dependent increase in MAP by iPF2-III but not by iPF3-III. D and E, pretreatment with iPF2-III, but not iPF3-III, augments ADP-induced platelet aggregation.

Effect of Diet on the Formation of the F3-iPs in Mice—Mice were administered isocaloric high fat diets (HFDs) enriched with 0, 1, or 5% fish oil for six or eleven weeks and levels of 8,12-iso-iPF3-6 and 8,12-iso-iPF2-6 were measured in urine. In mice fed the diet without fish oil, urinary 8,12-iso-iPF3-6-VI significantly increased from 1.15 ng/mg Cre at baseline to 41.61 ng/mg Cre at 6 weeks and to 104.31 ng/mg Cre at 11 weeks (p < 0.001). While much less abundant, urinary 8,12-iso-iPF2-6-VI levels also significantly increased from 1.19 ng/mg Cre at baseline to 4.83 ng/mg Cre at 6 weeks to 7.71 ng/mg Cre at 11 weeks (p < 0.001) (Fig. 5C). Substitution of ω-3 for ω-6 PUFAs in the diet dose-dependently increases the slope of the relationship between urinary 8,12-iso-iPF3-6-VI and 8,12-iso-iPF2-6-VI (Fig. 5, D and E). Reflective of diet-induced tissue accumulation of the lipid substrate, increased levels of EPA, as measured by LC/MS/MS, were observed in mouse heart after ingestion of the EPA-enriched diets (supplemental Fig. S6).

Interindividual Variation in Lipid Peroxidation of ω-3 versus ω-6 PUFAs in Response to an Inflammatory Stimulus—LPS evokes a systemic inflammatory response in humans. Previously we reported that a time-dependent increase in urinary excretion of three distinct F2-iPs: iPF2-6, iPF3-6, and 8,12-iso-iPF2-6-VI coincides with the maximal constitutional response to LPS in healthy volunteers (34).

Here, LPS also induced a mean increase in 8,12-iso-iPF3-6-VI from 1.81 ng/mg Cre at baseline to 2.57 ng/mg Cre at 2–4 h and 2.92 ng/mg Cre at 4–6 h after administration (Fig. 6A). After the inflammatory response, it returned to basal levels during the clinical resolution phase (12–18 h after LPS) (30). 8,12-iso-iPF3-6-VI and 8,12-iso-iPF2-6-VI were weakly related for the group as a whole (Fig. 6B). However, within each individual, 8,12-iso-iPF3-6-VI and 8,12-iso-iPF2-6-VI are highly correlated (R2 median 0.8; range 0.54–0.97). The slope [m] of the relationship between the two products, reflecting peroxidation products of ω-3 versus ω-6 substrates, varied by approximately an order of magnitude (0.02–0.19) among the individuals in the study (Fig. 6C).

Discussion

EPA is metabolized by cyclooxygenases (COXs) to series 3 prostaglandins (35) and is transformed non-enzymatically by free radicals to series 3 iPs, that include F3-iPs (15). Whereas EPA is utilized less efficiently as a substrate by COXs than AA, some of its analogous products, such as TxB3, but not PGI3, also possess less biological activity (20). Thus, a reduction in formation of TxA2, formation of the less potent TxA3, and retained bioactivity of PGI1 compensating to some degree for depressed
PGI2 may contribute to a cardioprotective effect of fish oils (21–23).

Here we identify a noninvasive approach to quantifying lipid peroxidation of EPA and to some degree, DHA. While observational studies have associated high fish consumption with a reduction in cardiovascular risk, there has been concern that this may reflect the influence of lifestyle covariates. Indeed, some of the studies that have reported such benefit have been conducted in populations where the fish consumed are lean and relatively deficient in /H9275-3 PUFAs (36, 37). Interventional prospective trials of fish oil supplements have given encouragement that there might be benefit (38–40), although the results have varied (41–44). Because EPA and DHA possess more double bonds than AA, they may be more susceptible to oxidation by free radicals than is AA. This might adversely influence cell membrane function or constrain beneficial effects of fish oils via autacoidal effects of F3- and F4-iPs. Alternatively, EPA and DHA may serve as a buffer or “sink” for free radicals, thereby protecting AA from reactive oxygen species and the consequent formation of biologically active F2-iPs.

We have previously reported that F3-iPs can function as incidental agonists at receptors for TxA2 and PGF2a (30, 45). Here we show that iPF3-III, the series 3 analog of one of these F2-iPs, does not share the capability of the AA-derived product to activate platelets or cause vasoconstriction. Thus, iPs derived from fish oils, like enzymatically derived eicosanoids, may exhibit diminished biological activity compared with those formed from AA. However, despite this difference it is unknown whether a shift in biological properties of high concentrations (1–10 M) of individual iPs in vitro is of relevance to the likely much lower (fM-pM) concentrations attained in vivo. Moreover, multiple iPs are likely to be formed together under conditions of oxidative stress, rendering assumptions based on concentrations or comparative bioactivities of single species difficult to relate to conditions in vivo.

Aside from their biological activity, the quantitation of F3-iPs reflects peroxidation of EPA and DHA. Clustered analysis of iPs derived from both ω-6 with ω-3 PUFAs is likely to refine their utility as indices of oxidant stress in vivo. Thus, we show that dietary supplementation with fish oils dose-dependently elevates urinary F3-iPs, reflected by an increasing slope of the line relating urinary 8,12-iso-iPFa-VI and 8,12-iso-iPFa-VI. (11 weeks on special diet). *, p < 0.05; **, p < 0.01; ***, p < 0.001.

FIGURE 5. Biosynthesis of F3-iPs in mice fed with fish oil. A, no significant change in levels of 8,12-iso-iPFa-VI and 8,12-iso-iPFa-VI in mice on a high fat diet with 0% EPA (n = 5). B, in mice fed with a diet of 1% fish oil, urinary 8,12-iso-iPFa-VI tended to increase but did not attain significance. A decline in urinary 8,12-iso-iPFa-VI did attain significance. Five mice were studied in each group. C, in mice fed with a diet containing 5% fish oil, urinary 8,12-iso-iPFa-VI significantly increased at 6 and 11 weeks. Urinary 8,12-iso-iPFa-VI levels also significantly increased. Five mice were studied in each group. D and E, fish oil administration dose-dependently elevates urinary F3-iPs, reflected by an increasing slope of the line relating urinary 8,12-iso-iPFa-VI and 8,12-iso-iPFa-VI. (11 weeks on special diet). *, p < 0.05; **, p < 0.01; ***, p < 0.001.
levels of both 8,12-iso-iPF3-VI and 8,12-iso-iPF2-VI were present (~1 × 10^{-5} % of total AA and EPA). However, these quantities and the dose-dependent responses of the two iPs to EPA-enriched diets and LPS administration indicate that the predominant source of iPs is the in vivo oxidation of AA and EPA in the cell membranes.

Clustered analysis of the F2- and F3-iPs is also informative in the oxidant stress response to an acute inflammatory stimulus in humans. Acute administration of LPS augments excretion of both urinary 8,12-iso-iPF2-VI and 8,12-iso-iPF3-VI coincident with the systemic response to LPS in volunteers (34). Here excretion of both F2- and F3-iPs is highly correlated within individuals, but the slope describing this relationship exhibits substantial (approximately an order of magnitude) heterogeneity. These individuals were on ad lib diets, the constituents of which were not characterized. However, our data from mice suggest strongly that the slope variation reflects differences among the volunteers in dietary intake of ω-3 versus ω-6 PUFAs.

In summary, we have identified and characterized two major F3-iPs that are abundant and readily detectable in urine of mice and humans. These afford a quantitative index of the peroxidation of ω-3 PUFAs enriched in fish oils. Differential formation of F3-iPs rather than F2-iPs may contribute to the health benefits of a diet rich in ω-3 PUFAs. Furthermore, clustered analysis of F3- and F2-iPs promises to refine analysis of oxidant stress in inflammation by accounting for interindividual variation in the intake of dietary PUFAs.

Acknowledgments—We thank Xue Liang, Wenxuan Li, Helen Zou, and Weili Yan for technical help and Molly Reagan and Wilma Cohen for administrative help. Dr. Rokach also wishes to acknowledge the National Science Foundation for the AMX-360(CHE90-13145) and Bruker 400 MHz(CHE-03-42251) NMR instruments.

REFERENCES
1. Praticò, D., Iuliano, L., Mauriello, A., Spagnoli, L., Lawson, J. A., Rokach, J., Maclouf, J., Violi, F., and FitzGerald, G. A. (1997) J. Clin. Invest. 100, 2028–2034
2. Stafforini, D. M., Sheller, J. R., Blackwell, T. S., Sapirstein, A., Yull, F. E., McIntyre, T. M., Bonventre, J. V., Prescott, S. M., and Roberts, L. J., 2nd. (2006) J. Biol. Chem. 281, 4616–4623
3. Morrow, J. D., Awad, J. A., Boss, H. J., Blair, I. A., and Roberts, L. J., 2nd. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 10721–10725
4. Lawson, J. A., Rokach, J., and FitzGerald, G. A. (1999) J. Biol. Chem. 274, 24441–24444
5. Reilly, M. P., Praticò, D., Delanty, N., D’Mino, G., Tremoli, E., Rader, D., Kapoor, S., Rokach, J., Lawson, J., and FitzGerald, G. A. (1998) Circulation 98, 2822–2828
