

Tetranor PGDM, an Abundant Urinary Metabolite Reflects Biosynthesis of Prostaglandin D₂ in Mice and Humans^{*[5]}

Received for publication, August 16, 2007, and in revised form, November 7, 2007 Published, JBC Papers in Press, November 8, 2007, DOI 10.1074/jbc.M706839200

Wen-Liang Song, Miao Wang, Emanuela Ricciotti, Susanne Fries, Ying Yu, Tilo Grosser, Muredach Reilly, John A. Lawson, and Garret A. FitzGerald¹

From the Institute for Translational Medicine and Therapeutics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Prostaglandin D₂ (PGD₂) is a cyclooxygenase (COX) product of arachidonic acid that activates D prostanoid receptors to modulate vascular, platelet, and leukocyte function *in vitro*. However, little is known about its enzymatic origin or its formation *in vivo* in cardiovascular or inflammatory disease. 11,15-Dioxo-9 α -hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid (tetranor PGDM) was identified by mass spectrometry as a metabolite of infused PGD₂ that is detectable in mouse and human urine. Using liquid chromatography-tandem mass spectrometry, tetranor PGDM was much more abundant than the PGD₂ metabolites, 11 β -PGF_{2 α} and 2,3-dinor-11 β -PGF_{2 α} , in human urine and was the only endogenous metabolite detectable in mouse urine. Infusion of PGD₂ dose dependently increased urinary tetranor PGDM > 2,3-dinor-11 β -PGF_{2 α} > 11 β -PGF_{2 α} in mice. Deletion of either lipocalin-type or hemopoietic PGD synthase enzymes decreased urinary tetranor PGDM. Deletion or knockdown of COX-1, but not deletion of COX-2, decreased urinary tetranor PGDM in mice. Correspondingly, both PGDM and 2,3-dinor-11 β -PGF_{2 α} were suppressed by inhibition of COX-1 and COX-2, but not by selective inhibition of COX-2 in humans. PGD₂ has been implicated in both the development and resolution of inflammation. Administration of bacterial lipopolysaccharide coordinately elevated tetranor PGDM and 2,3-dinor-11 β -PGF_{2 α} in volunteers, coincident with a pyrexial and systemic inflammatory response, but both metabolites fell during the resolution phase. Niacin increased tetranor PGDM and 2,3-dinor-11 β -PGF_{2 α} in humans coincident with facial flushing. Tetranor PGDM is an abundant metabolite in urine that reflects modulated biosynthesis of PGD₂ in humans and mice.

Prostaglandin (PGD₂)² is formed from PGH₂, a cyclooxygenase (COX) product of arachidonic acid by the action of either a lipocalin (L)-like or hemopoietic (H) PGD synthase (1). Both enzymes may form PGD₂ *in vitro*, but it is unclear which PGDS enzyme predominates under varied conditions *in vivo*. Suppression of PGD₂ has been implicated in the bronchoconstriction of aspirin-evoked asthma (2, 3), and release of PGD₂ mediates the facial flushing and vascular instability of systemic mastocytosis (4). PGD₂ relaxes vascular smooth muscle cells *in vitro* and its release by dermal dendritic cells contributes to the facial flushing, which complicates administration of the hypolipidemic drug, niacin (5). PGD₂ mediates its effects via activation of D prostanoid receptors (DPs). DP1, a member of the prostanoid family of G protein-coupled receptors, mediates the vasorelaxant and bronchodilator effects (6, 7), whereas DP2, a G protein-coupled receptor of the formylmethionylleucylphenylalanine receptor subfamily regulates Th1 and Th2 switching in lymphocytes (8) and is also expressed on eosinophils and basophils (9).

Recent interest in PGD₂ has been prompted by the use of DP1 blockade as an adjunct to niacin therapy (10) and by the potential role of PGD₂ and its metabolites in the resolution of inflammation (11). However, DP1 is expressed on human platelets, and its activation *in vitro* results in a cyclic AMP-dependent inhibition of platelet function (12, 13). Nothing is known about the formation of PGD₂ or the consequences of its inhibition in hyperlipidemic patients. Aside from a potential role in cardiovascular disease, PGD₂ may be of importance in the resolution of inflammation. A metabolite of PGD₂, 15-deoxy- $\Delta^{12,14}$ -PGJ₂, has been postulated to activate peroxisome proliferator-activated receptor γ (14) and promote resolution of an inflammatory infiltrate (11). However, it remains to be determined by physicochemical methodology whether formation of 15-deoxy- $\Delta^{12,14}$ -PGJ₂ is indeed augmented during the resolution of human inflammation, and, although it can activate PPAR γ , the concentrations required are unlikely to be attained *in vivo* (15).

Attempts to assess the biosynthesis of PGD₂ have been constrained by a paucity of commercially available, sensitive, and specific methodology. Aside from asthma and mastocytosis

^{*} This work was supported in part by the Specialized Center in Clinical Research in Vascular Injury (Grant HL 83799), a Clinical and Translational Science Award (RR-023567), and Grant HL R01 073278 (to M. R.) from the National Institutes of Health and by an American Heart Association-Jon Holden DeHann Foundation scientist development grant (to M. W., Y. Y., and T. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[5] The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Figs. S1–S5.

¹ A McNeil 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.gcr.upenn.edu.

² The abbreviations used are: PGD₂, prostaglandin D₂; COX, cyclooxygenase; PGDS, PGD synthase; DP, D prostanoid receptor; PGF_{2 α} , prostaglandin F_{2 α} ; GC, gas chromatography; MS, mass spectrometry; MS/MS, tandem MS; tetranor PGDM, 11,15-dioxo-9 α -hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid; HPLC, high-performance liquid chromatography; WT, wild type; KD, knockdown; KO, knock-out; LPS, lipopolysaccharide; Cre, creatinine; PGEM, prostaglandin E₂ metabolite.

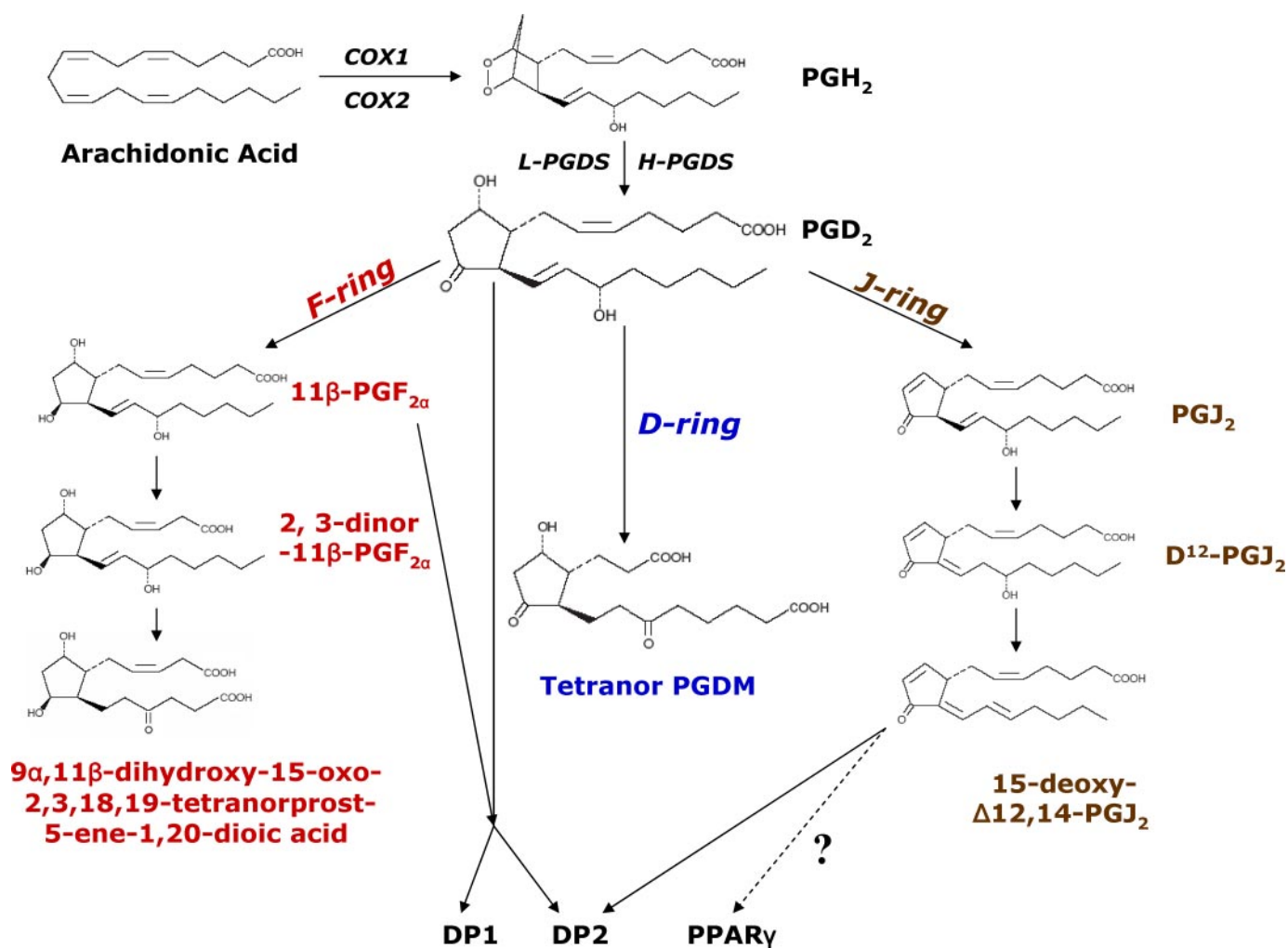


FIGURE 1. Biosynthetic pathway of PGD₂ and its derivatives.

(2–4), little information on biosynthesis of PGD₂ in humans has been acquired. Given the evanescence of primary PGs, biosynthesis is classically estimated by measurement of metabolites (16, 17). However, despite deletion of both DPs and PGDS enzymes, no metabolites of PGD₂ have been reported in mouse, preventing assessment of biosynthetic response to experimental manipulation in that species. Initial attempts at commercial assay development in humans have focused on 11β-PGF_{2α} (18) and 2,3-dinor-11β-PGF_{2α}. Both are formed as minor urinary metabolites in monkeys and in a human volunteer following infusion of radiolabeled PGD₂ (19). Indeed, paired analysis of 11β-PGF_{2α} by gas chromatography and mass spectrometry (GC/MS), and the commercially available immunoassay revealed poor concordance in the urine of patients with asthma (20, 21). Quantitative analysis of the major F-ring metabolite identified in urine after PGD₂ infusion in a volunteer, 9α,11β-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid (19) has been reported in human plasma and urine (22) and reflects nicely the marked augmentation of PGD₂ biosynthesis in systemic mastocytosis (4).

Here, we report the identification of a novel D-ring metabolite formed from infused PGD₂, 11,15-dioxo-9α-hydroxy-,

2,3,4,5-tetranorprostan-1,20-dioic acid (tetranor PGDM) as an abundant endogenous metabolite of PGD₂ in both human and mouse urine. The formation from PGD₂ of its F-, J-, and D-ring products is depicted in Fig. 1. Gene manipulations in mice and pharmacological studies in humans implicate COX-1 as the major source of PGD₂ as reflected by this metabolite in urine.

EXPERIMENTAL PROCEDURES

Reagents

Authentic [²H₆]tetranor PGDM was kindly synthesized on request by Cayman Chemical Co. (Ann Arbor, MI). Authentic 2,3-dinor-11β-PGF_{2α}, [²H₄]11β-PGF_{2α}, and PGD₂ were purchased from the same source for use as standards. H₂¹⁸O was purchased from Cambridge Isotope Laboratories (Andover, MA). All mobile phases incorporated HPLC-grade solvents from Honeywell Burdick and Jackson. Reagent-grade acetic acid was purchased from Thermo Fisher Scientific. HPLC-grade ammonium hydroxide was purchased from Mallinckrodt Baker Chemicals. [¹⁸O₂]2,3-Dinor-11β-PGF_{2α} was prepared as previously described (23). Methoxyamine HCl was purchased from Sigma-Aldrich, Inc.

Urine Analysis

[²H₆]Tetranor PGDM, [¹⁸O₂]2,3-dinor-11β-PGF_{2α}, and [²H₄]11β-PGF_{2α} were added to 1 ml of human urine or 100 μl of mouse urine and were allowed to equilibrate for 15 min. One half of the urine volume of an aqueous solution of methoxyamine HCl (1 g/ml) was added and allowed to stand for 15 min at room temperature. The samples were purified by solid phase extraction using StrataX C18 cartridges (Phenomenex, Torrance, CA). The solid phase extraction 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 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 5% acetonitrile in water and filtered by centrifugation using 0.2-μm Nylon Microspin filters purchased from Alltech Associates (Deerfield, IL).

HPLC

A Shimadzu Prominence HPLC system (Shimadzu, Columbia, MD) consisting of two LC-20AD-vp pumps, a CBM-20A system controller, and an SIL-5000 autosampler was used for all chromatography. The HPLC column used was a 150 × 2 mm Luna C18(2) with 3-μm particles (Phenomenex, Torrance, CA). The mobile phase was generated from water (solvent A) and acetonitrile:methanol, 95:5 (solvent B), both containing 0.005% acetic acid adjusted to pH 5.7 with ammonium hydroxide. The flow rate was 0.2 ml/min. Separations were carried out with various linear solvent gradients.

Mass Spectrometry

A Thermo Finnigan TSQ Quantum Ultra tandem instrument (Thermo Fisher Scientific) equipped with a heated coaxial electrospray source and triple quadrupole analyzer was used in these studies. The electrospray ionization source was maintained at 240 °C and used nitrogen for both sheath and auxiliary gas at 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 collision-induced dissociation was maintained at 10 eV. The collision gas was argon at 1.5 mTorr. The analyzer was operated in the selected reaction monitoring mode for the analysis of urinary PGD₂ metabolites. For analysis of tetranor PGDM, the transitions monitored were *m/z* 385→336 for the endogenous material and *m/z* 391→342 for the deuterated internal standard. The collision energy was 15 eV. The transitions for [²H₄]11β-PGF_{2α} and endogenous 11β-PGF_{2α} were 357→197 and 353→193, respectively, with collision energy 24 eV. The transition for [¹⁸O₂]2,3-dinor-11β-PGF_{2α} was *m/z* 329→145 and *m/z* 325→145 for the endogenous material, collision energy 13 eV.

The product ion scan mode was used for spectral analysis of tetranor PGDM. Precursor ions (*m/z* 385 and 391 for endogenous tetranor PGDM and the [²H₆]tetranor PGDM internal standard, respectively) were collisionally activated at 15 eV

under 1.5-mTorr argon gas producing the collision-induced dissociation spectra.

Studies in Mice

All studies were performed following protocol review and approval by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Infusion Studies—Vehicle or PGD₂ (20, 150, and 500 μg) was infused intraperitoneal into 12-week-old male C57/BL6 mice (*n* = 5 per group). Urine was collected for 24 h in metabolic cages for analysis of PGD₂ metabolites.

PGDS Knock-outs—Urine was collected for 24 h from 3- to 4-month-old male wild-type (WT) mice, L-PGDS knock-out mice (kindly provided by Dr. Yoshihiro Urade, Osaka, Japan), and H-PGDS knock-out mice (kindly provided by Dr. Yoshihide Kanaoka, Boston, MA), all on a C57/BL6 background (*n* = 15 for WT and L-PGDS knock-outs and *n* = 16 for H-PGDS knock-outs).

COX Knockdown (KD) and Knock-out (KO) Mice—Urine was collected for 24 h from 2- to 3-month-old COX-1 KD mice (24), COX-1 KOs and their WT controls (*n* = 7–9 each), and COX-2 KOs (*n* = 15) and their WT controls (*n* = 7).

Clinical Studies

Five 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 of the University of Pennsylvania. All volunteers were apparently healthy on physical examination, were non-smokers, and refrained from all medications for 2 weeks before and then 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, 12 volunteers (6 male and 6 female) received a bolus injection of single dose 3 ng/kg bacterial lipopolysaccharide (LPS) under controlled conditions as we have previously described (25). Subjects were admitted to the Clinical and Translational Research Center the evening before the study, and an intravenous infusion of saline was commenced. The study involved a 60-h inpatient stay in the Clinical and Translational Research Center 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 PGD₂ metabolites were assessed in urines collected at the following time intervals before (–24 to –18, –18 to –12, –12 to –6, and –6 to 0 h) and after (0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 18, and 18 to 24 h) LPS administration. Data were plotted at the midpoint of each corresponding urine collection. Body temperature was recorded at the following time points before (–4 and –2 h) and after (0, 2, 4, 6, 8, 10, 12, 14, and 16 h) LPS administration.

In the second study, Niacin (600 mg) was administered to two healthy male volunteers. Urinary PGD₂ metabolites were assessed in spot urines collected at the following time points before (time 0) and after (1, 2, 3, 4, 5, and 6 h) niacin administration.

In a third study, 18 healthy volunteers (9 male and 9 female) received, in random order, a single dose of placebo or rofecoxib

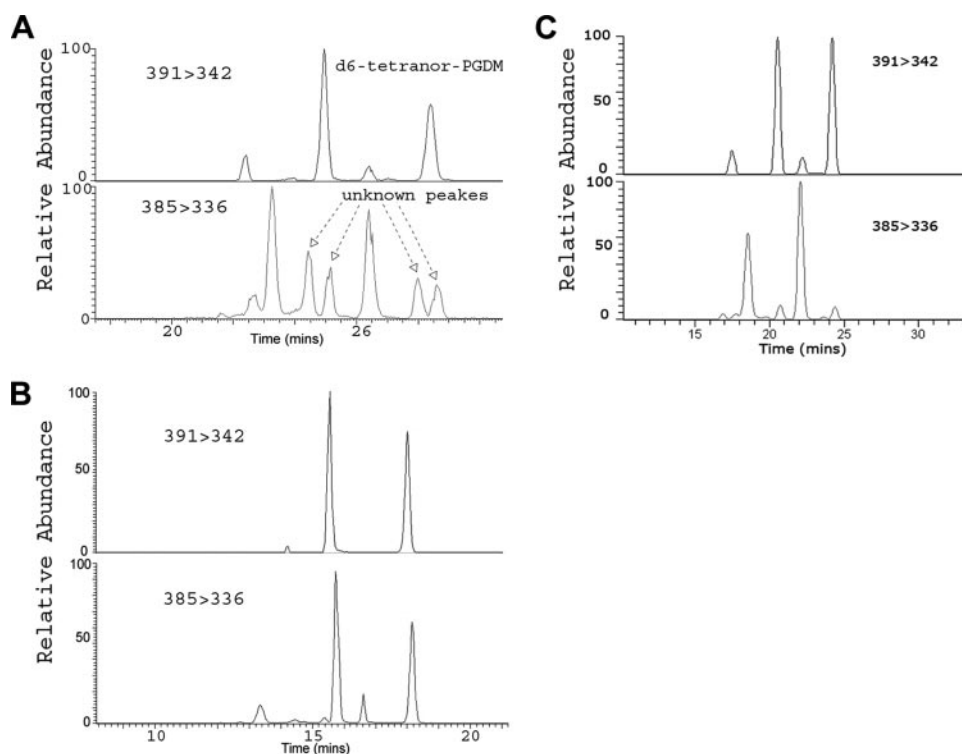


FIGURE 2. **HPLC-MS/MS of a major urinary PGD₂ metabolite.** Representative selected reaction monitoring chromatogram of [²H₆]tetranor PGDM (upper), and co-eluted peaks corresponding to the endogenous compound (lower) in mouse urine (A) and in mouse urine (B) following intraperitoneal administration of 500 μg of PGD₂, resulting in marked elevation of the endogenous compound and in human urine (C). Transitions characteristic of [²H₆]tetranor PGDM (*m/z* 391→342) and tetranor PGDM (*m/z* 385→336) are shown in the upper and lower panels, respectively.

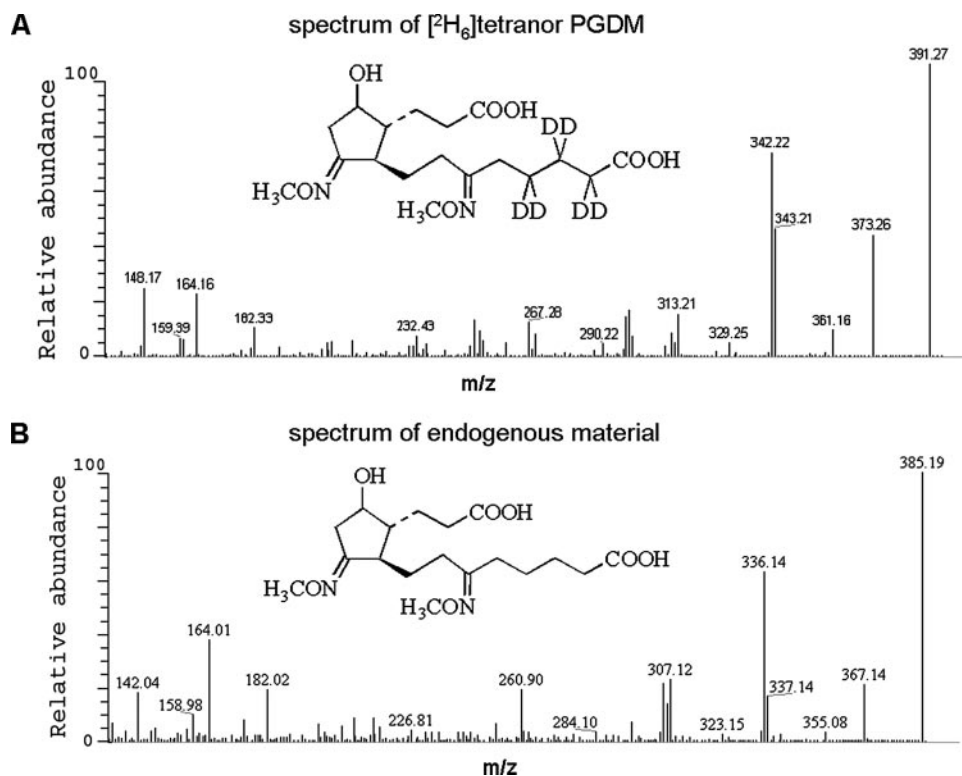


FIGURE 3. **Product ion analysis of tetranor PGDM.** A, product ion spectrum of [²H₆]tetranor PGDM (*m/z* 391) with *m/z* values of 373, 342, 313, 267, 182, 164, and 142. B, product ion spectrum of the endogenous tetranor PGDM (*m/z* 385) with *m/z* values of 367, 336, 307, 261, 182, 164, and 142. The differences in *m/z* values between these two groups were either 0 or 6 mass units reflecting fragments with or without deuterium.

(25 mg) under double-blind conditions, separated by washout periods of at least 2 weeks. Urinary PGD₂ metabolites were assessed at 0 and 4 h in spot urine samples that were collected 30 min after voiding.

In a fourth study, 8 healthy volunteers were orally administered 200 mg of celecoxib twice daily for 9 days. Measurements of urinary PGD₂ metabolites were performed predose and 4 h after drug administration on days 1 and 8.

In a fifth study, a single dose of aspirin (325 mg) was administered to 18 healthy volunteers (9 males and 9 females). Urinary PGD₂ metabolites were assessed in spot urine at time 0 and 4 h after dosing.

Data Analysis

Data are expressed after correction for urinary creatinine (Cre) concentrations and are reported as nanograms per milligram of 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.

RESULTS

Discovery of Tetranor PGDM—While measuring tetranor PGEM, the major urinary PGE metabolite in mouse urine (26), four other major peaks, apparently tetranor PGEM isomers, were observed in the mass chromatogram (Fig. 2A, lower panel). Because PGD₂ and PGE₂ are themselves structural isomers (27), it was surmised that some of these peaks might originate from PGD₂. When PGD₂ was infused into mice, two of these four peaks were increased dramatically (Fig. 2B, lower panel), consistent with the hypothesis that they corresponded to the analogous D-ring tetranor metabolites. Following addition of authentic [²H₆]tetranor PGDM to mouse urine, the deuterated compound coeluted with the endogenous material (Fig. 2, A and B). There are four chromatographic peaks for tetranor PGDM methoxyamine derivatives. However, the

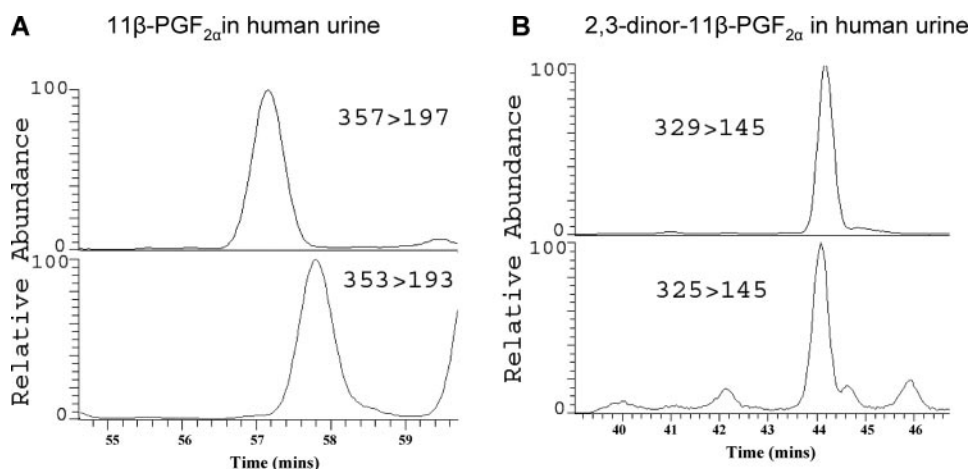


FIGURE 4. HPLC-MS/MS analysis of urinary 2,3-dinor-11 β -PGF_{2 α} and 11 β -PGF_{2 α} . A, representative selected reaction monitoring chromatogram of 11 β -PGF_{2 α} in human urine. Transitions characteristic of [²H₄]11 β -PGF_{2 α} (m/z 357 \rightarrow 197) and 11 β -PGF_{2 α} (m/z 353 \rightarrow 193) are shown in the upper and lower panels, respectively. Note that a peak corresponding to endogenous material co-eluting with the standard is not evident in the lower panel. B, representative selected reaction monitoring chromatogram of 2,3-dinor-11 β -PGF_{2 α} in human urine. Transitions characteristic of [¹⁸O₂]2,3-dinor-11 β -PGF_{2 α} (m/z 329 \rightarrow 145) and 2,3-dinor-11 β -PGF_{2 α} (m/z 325 \rightarrow 145) are shown in the upper and lower panels, respectively.

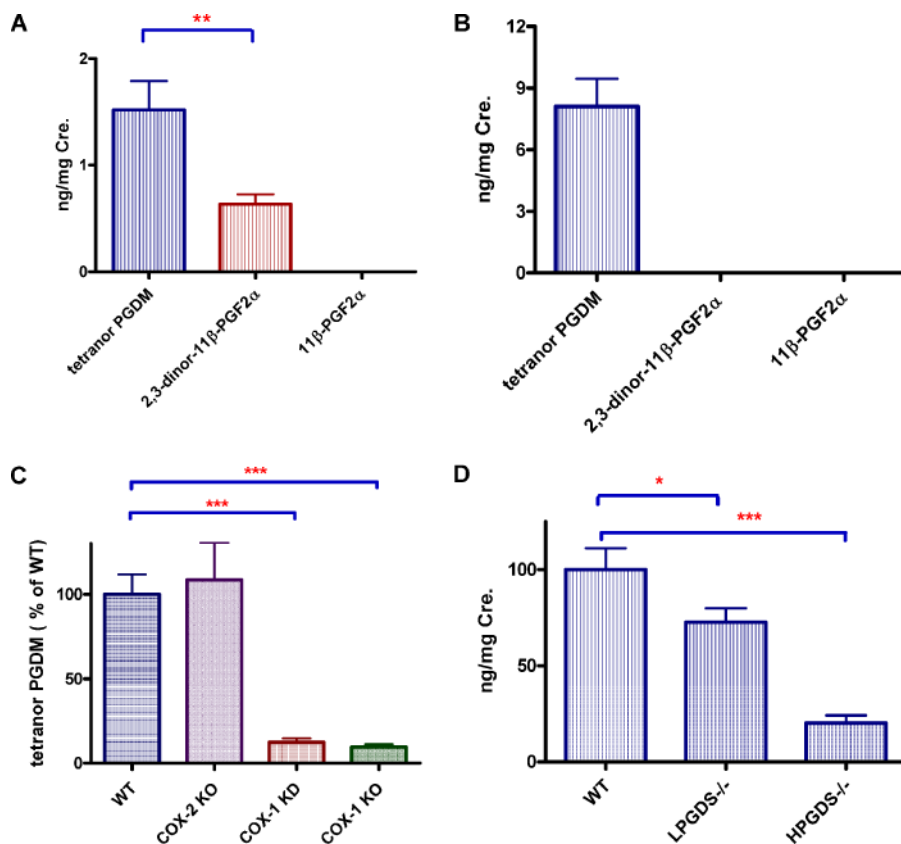


FIGURE 5. Biosynthesis of PGD₂ in humans and mice. A, urinary metabolites of PGD₂ were examined in human urine. Both tetranor PGDM and 2,3-dinor-11 β -PGF_{2 α} , but not 11 β -PGF_{2 α} , were detectable. B, urinary metabolites of PGD₂ were examined in mouse urine. Tetranor PGDM, but not 2,3-dinor-11 β -PGF_{2 α} or 11 β -PGF_{2 α} , was detectable. C, tetranor PGDM was depressed in urine from L-PGDS and H-PGDS knock-out mice compared with wild-type controls. D, tetranor PGDM was depressed in urine from COX-1 knockdown and COX-1 knock-out mice but not COX-2 knock-out mice compared with wild-type controls. Data shown are the mean \pm S.E. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

two smaller peaks were variably detectable. Mass spectral analysis of human urine also revealed the existence of tetranor PGDM, which differed from mouse only in its abundance relative to tetranor PGEM (Fig. 2C). Co-injection of extracts of

urine from mice infused with PGD₂ with extracts of human urine indicated that the endogenous peaks corresponding to tetranor PGDM in human urine corresponded to the peaks increased dose dependently by PGD₂ infusion in mice (supplemental Fig. S1).

Product ion analysis of the [²H₆]tetranor PGDM at m/z 391 gave rise to a series of major fragment ions with m/z values of 373, 342, 313, 267, 182, 164, and 142 (Fig. 3A), virtually identical to the product ion spectrum obtained from endogenous tetranor PGDM (m/z 385); m/z 367, 336, 307, 261, 182, 164, and 142 (Fig. 3B). The differences in m/z values between these two groups were either 0 or 6 mass units reflecting fragments with or without deuterium, again consistent with the original hypothesis. The transitions m/z 391 $>$ 336 and m/z 385 $>$ 336 are the same as those for tetranor PGEM, facilitating an integrated approach to lipidomic analysis (28). The assay was highly reproducible, and the D-ring metabolite was chemically stable at -20°C and -80°C (supplemental Fig. S2).

A study was performed to determine whether tetranor PGDM might activate the DP1 receptor on human platelets (12, 13). However, unlike PGD₂ itself, tetranor PGDM did not inhibit platelet aggregation (supplemental Fig. S3).

HPLC-MS/MS Analysis of Urinary 2,3-Dinor-11 β -PGF_{2 α} and 11 β -PGF_{2 α} —A representative selected reaction monitoring chromatogram of 11 β -PGF_{2 α} in human urine is shown in Fig. 4A. Transitions characteristic of [²H₄]11 β -PGF_{2 α} (m/z 357 \rightarrow 197) and 11 β -PGF_{2 α} (m/z 353 \rightarrow 193) are shown in the upper and lower panels, respectively. A detectable endogenous chromatographic peak that co-eluted with spike was absent from most human urine samples. Some peaks eluted close to, but not coincident with the internal standard, as in Fig. 4A. These peaks were further confirmed to be distinct from endogenous 11 β -PGF_{2 α} by addition of synthetic exogenous standards at the time of analysis. We surmise that these peaks represent F₂-isopros-

cident with the internal standard, as in Fig. 4A. These peaks were further confirmed to be distinct from endogenous 11 β -PGF_{2 α} by addition of synthetic exogenous standards at the time of analysis. We surmise that these peaks represent F₂-isopros-

Tetranor PGDM, a Major PGD₂ Metabolite

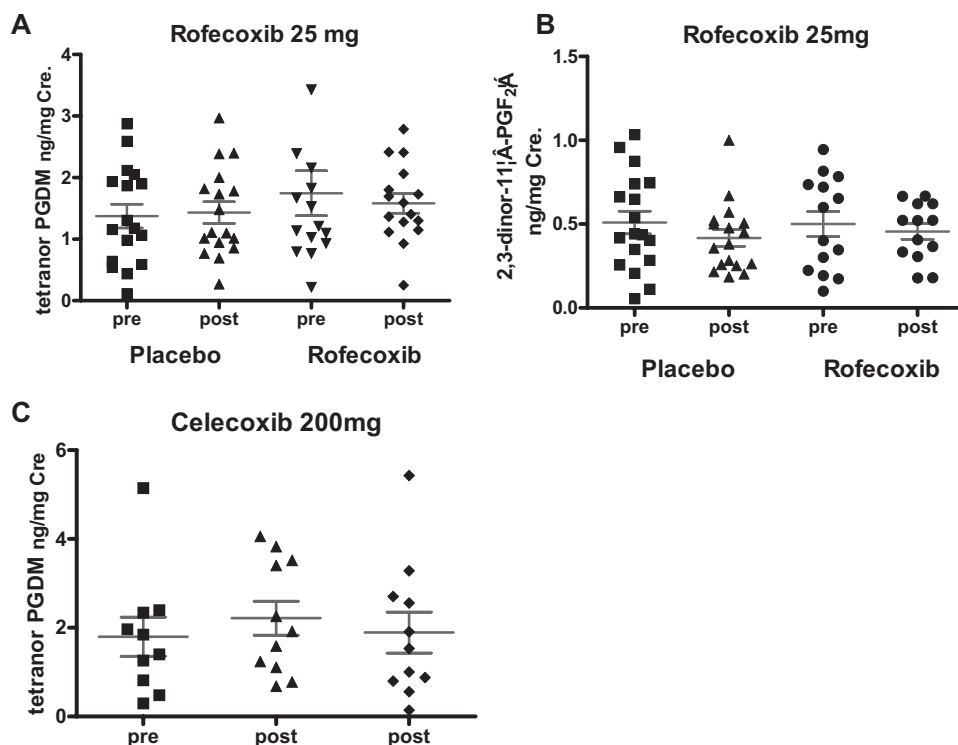


FIGURE 6. The effects of selective COX-2 inhibition by rofecoxib and celecoxib on excretion of major urinary PGD₂ metabolites. A, single dose rofecoxib failed to decrease significantly excretion of tetranor PGDM. B, single dose rofecoxib failed to decrease significantly excretion of 2,3-dinor-11β-PGF_{2α}. C, single dose and multiple doses of celecoxib failed to decrease significantly excretion of tetranor PGDM.

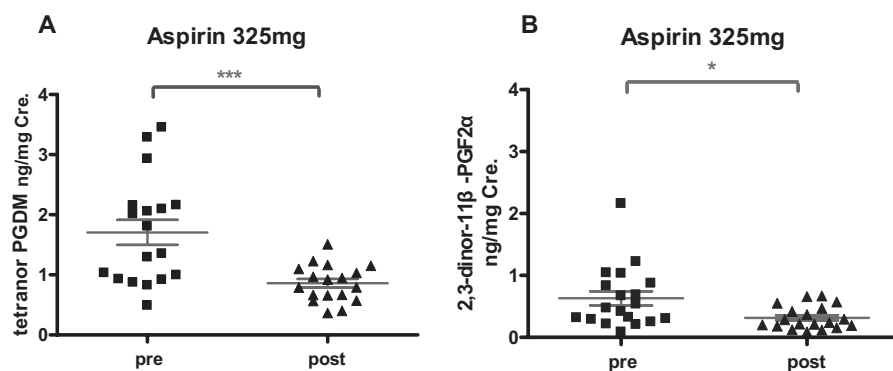


FIGURE 7. Nonselective COX inhibition by aspirin, 325 mg. Inhibition of systemic COX-1 and COX-2 with 325 mg of aspirin significantly decreased excretion of both tetranor PGDM (A) and 2,3-dinor-11β-PGF_{2α} (B).

tanones that would have the potential to compete with 11β-PGF_{2α} in an immunoassay and also may not have separated from endogenous 11β-PGF_{2α} under the elution conditions utilized in a GC/MS assay (29). Although 2,3-dinor-11β-PGF_{2α} was readily detectable in human urine, it required a long LC program (28) to achieve separation from interfering compounds. A representative selected reaction monitoring chromatogram of 2,3-dinor-11β-PGF_{2α} in human urine is shown in Fig. 4B. Urinary 2,3-dinor-11β-PGF_{2α} and 11β-PGF_{2α} were both below the limits of detection (~1 ng/mg Cre) in mouse urine (data not shown).

Comparative Levels of Endogenous Metabolites of PGD₂ Metabolites—In human urine, the comparative levels of tetranor PGDM and 2,3-dinor-11β-PGF_{2α} were 1.5 ± 0.3 ng/mg Cre and 0.6 ± 0.1 ng/mg Cre ($p < 0.01$), respectively, whereas

11β-PGF_{2α} was usually below the limits of detection (Fig. 5A). Tetranor PGDM was the only metabolite detectable in mouse urine at 8.1 ± 1.3 ng/mg Cre (Fig. 5B).

Enzymatic Contributions to the Biosynthesis of PGD₂—Both lipocalin (L)-like or hemopoietic (H) PGD synthase may form PGD₂ *in vitro*, but it is unclear which PGDS enzyme predominates under varied conditions *in vivo*. The enzymatic contributions to the biosynthesis of PGD₂ were examined by L-PGDS and H-PGDS mutant mouse models. Deletion of either PGDS significantly reduced biosynthesis of PGD₂. Tetranor PGDM was suppressed ~30% on average by deletion of L-PGDS ($p < 0.05$) and 80% on average by deletion of H-PGDS ($p < 0.0001$, Fig. 5C). Deletion of H-PGDS had a significantly greater ($p < 0.001$) impact on urinary tetranor PGDM than did deletion of L-PGDS in mice. Cross-regulation of PGDS enzymes differed in response to gene deletion. Expression of H-PGDS was up-regulated in the aorta of L-PGDS KO. By contrast, compensatory up-regulation of L-PGDS was not observed in H-PGDS KO (supplemental Fig. S4).

The comparative contribution to metabolite excretion of the COX enzymes in mice was addressed by comparing the impact of genetic manipulation of COX-1 and COX-2. Both KO and KD of COX-1 suppressed urinary tetranor PGDM by ~90%, whereas deletion of COX-2 failed to alter significantly metabolite excretion (Fig. 5D).

In humans, aspirin at a dose of 325 mg inhibits both COXs (30), whereas rofecoxib (25 mg) and celecoxib (200 mg) inhibit COX-2 selectively (31). Corresponding to the results in mice, inhibition of both COX-1 and COX-2 by aspirin, but not COX-2 alone by rofecoxib, depressed tetranor PGDM (Fig. 6A). Similar results were obtained with urinary 2,3-dinor-11β-PGF_{2α} (Fig. 6B). Both acute and chronic dosing with a second COX-2 inhibitor, celecoxib, also failed to depress tetranor PGDM (Fig. 6C).

Aspirin decreased urinary tetranor PGDM from 1.71 ± 0.21 ng/mg Cre to 0.86 ± 0.07 ng/mg Cre ($p < 0.001$, Fig. 7A) and 2,3-dinor-11β-PGF_{2α} decreased from 0.63 ± 0.11 ng/mg Cre to 0.32 ± 0.04 ng/mg Cre ($p < 0.01$, Fig. 7B). This dose of aspirin inhibits COX enzymes incompletely, as reflected by comparable suppression of prostaglandin E₂ metabolite (tetranor PGEM) and prostacyclin metabolite (PGIM). By contrast, urinary isoprostanes,

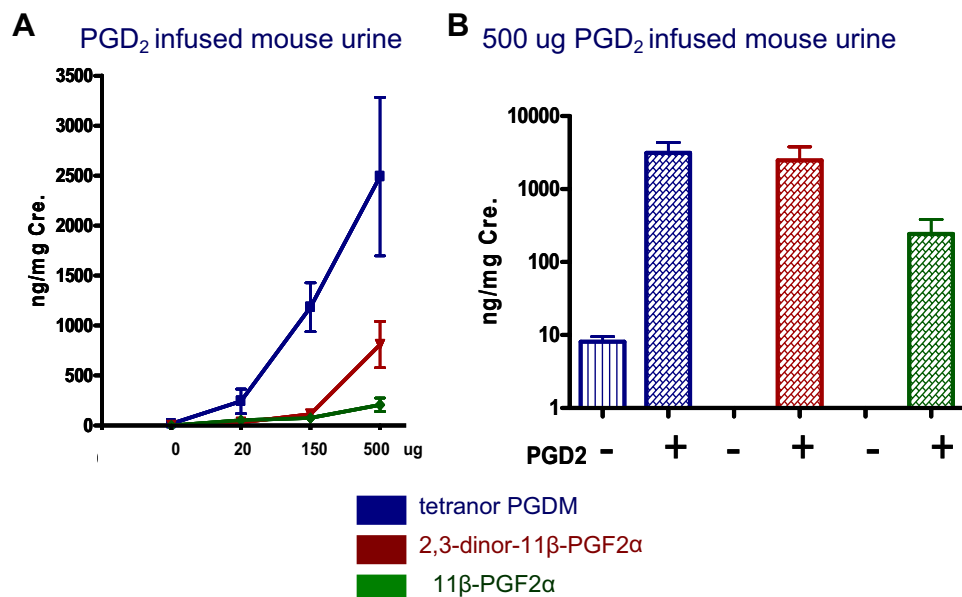


FIGURE 8. **Excretion of PGD₂ metabolites in PGD₂ infused mice.** A, urinary PGD₂ metabolites following intraperitoneal administration of PGD₂. B, comparison of urinary PGD₂ metabolites at 500 μ g of PGD₂ infusion. Infusion of PGD₂ dose dependently increased urinary tetranor PGDM > 2,3-dinor-11 β -PGF_{2 α} > 11 β -PGF_{2 α} .

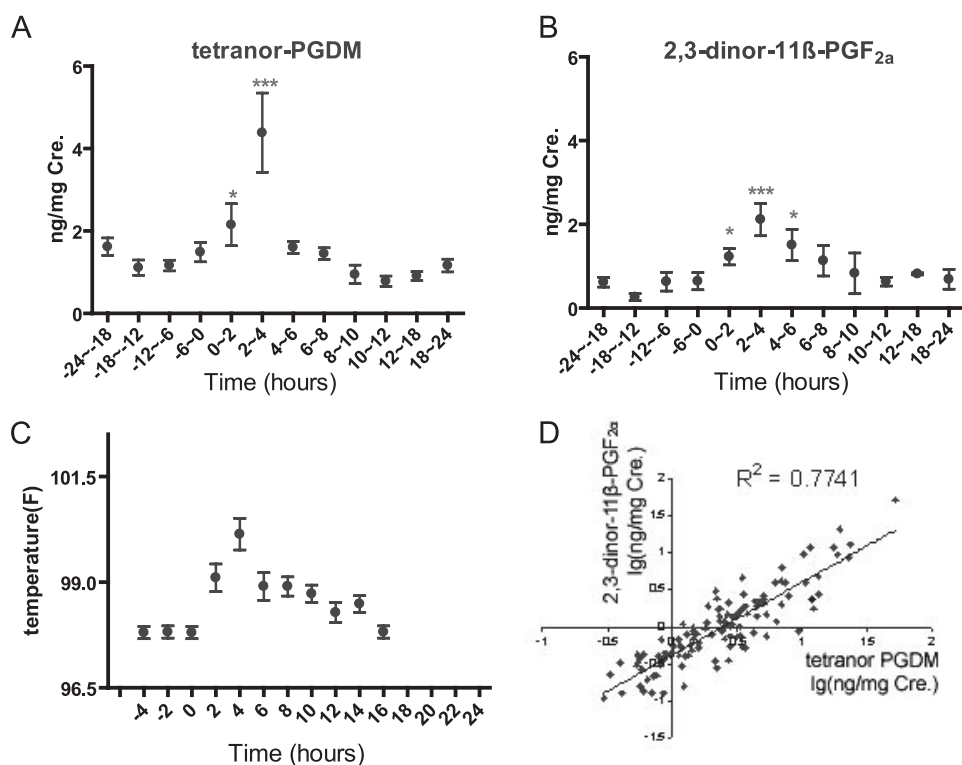


FIGURE 9. **LPS induced an increase in excretion of PGD₂ metabolites.** A, LPS induced a mean increase in tetranor PGDM from 1.49 ng/mg Cre at baseline to 2.15 ng/mg Cre at 2 h and 4.36 ng/mg Cre at 4 h after administration; B, LPS induced a mean increase in 2,3-dinor-11 β -PGF_{2 α} from 0.6 ng/mg Cre at baseline to 1.2 ng/mg Cre at 2 h and 2.1 ng/mg Cre at 4 h after administration. C, in healthy volunteers, temperature increased transiently during endotoxemia returning to baseline by 24 h after LPS. D, correlation between log transformed urinary tetranor PGDM and 2,3-dinor-11 β -PGF_{2 α} . Data shown are the mean \pm S.E. *, $p < 0.05$; ***, $p < 0.001$.

as reflected by the major F₂ isoprostane in urine, 8,12-*iso*-iPF_{2 α} -VI is unaltered by this regimen (supplemental Fig. S5).

Excretion of PGD₂ Metabolites in PGD₂-infused Mice—The comparative disposition of PGD₂ was examined by infusion of exogenous PGD₂. Urinary metabolites of PGD₂ increased dose

dependently in response to the infusion (Fig. 8A). The levels of tetranor PGDM, 2,3-dinor-11 β -PGF_{2 α} and 11 β -PGF_{2 α} attained after the maximal dose (500 μ g) of PGD₂ were 2498 \pm 792 ng/mg Cre, 809 \pm 346 ng/mg Cre, and 207 \pm 67 ng/mg Cre, respectively (Fig. 8B). Roughly 3–10% of infused PGD₂ was excreted as the tetranor PGDM metabolite in mouse urine. The fractional conversion to each metabolite appeared to be uninfluenced by dose.

Evoked Biosynthesis of PGD₂—LPS evokes a systemic inflammatory response in humans that is accompanied by regulated expression of both COX isozymes *ex vivo* and augmented biosynthesis of thromboxane A₂ and prostacyclin (25). LPS induced a mean increase in both tetranor PGDM (1.49 ng/mg Cre at baseline to 2.15 ng/mg Cre at 2 h and 4.36 ng/mg Cre at 4 h after administration, Fig. 9A) and correspondingly in 2,3-dinor-11 β -PGF_{2 α} from 0.6 ng/mg Cre to 1.2 ng/mg Cre and 2.1 ng/mg Cre, respectively (Fig. 9B). The peak pyrexial response to LPS (from an average 36.6 to 37.8 $^{\circ}$ C) occurred \sim 4 h after administration (Fig. 9C). The alterations in urinary tetranor PGDM and 2,3-dinor-11 β -PGF_{2 α} in response to LPS occur in a highly coordinated manner (Fig. 9D). Both metabolites fell after the inflammatory response and were not significantly different from basal levels during the resolution phase (8–10 h after LPS).

Oral administration of niacin, 600 mg, evoked an intense flush in both volunteers, primarily involving the face and upper part of the body. Flushing was most pronounced during the first hour after dosing and had dissipated after \sim 2–3 h. Both urinary tetranor PGDM and 2,3-dinor-11 β -PGF_{2 α} were elevated by niacin (Fig. 10, A and B), peaking 2–3 h and falling to basal levels 5–6 h after dosing. Again, reflecting the distinction from isoprostanes, urinary 8,12-*iso*-iPF_{2 α} -VI was unaltered by niacin administration (Fig. 10C).

DISCUSSION

PGD₂ is the predominant COX product of mast cells and contributes to the cutaneous flushing and hemodynamic dys-

Tetranor PGDM, a Major PGD₂ Metabolite

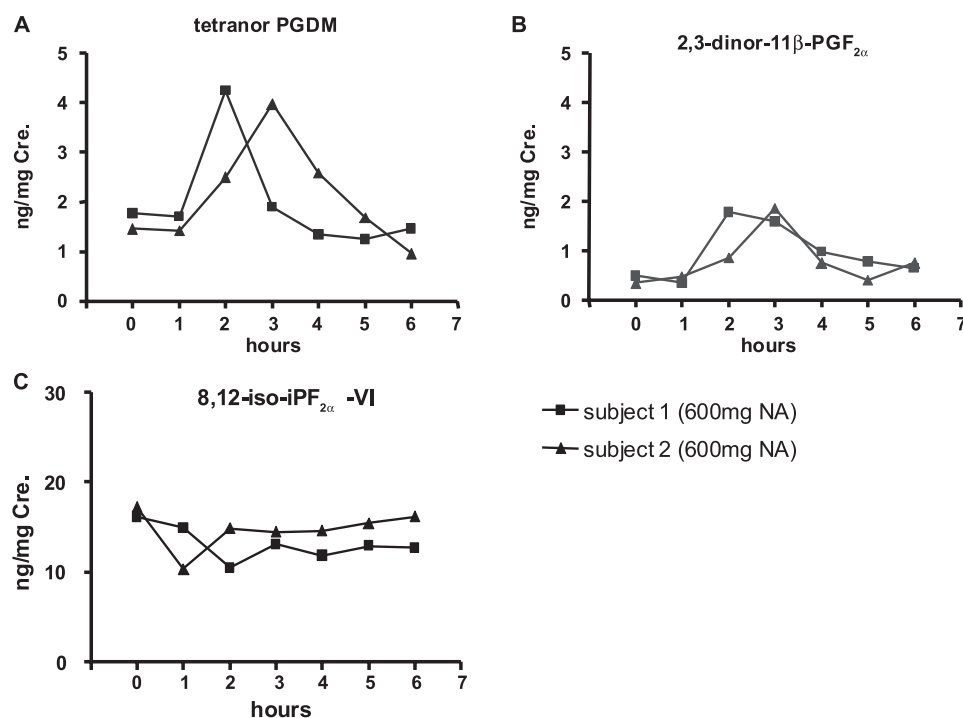


FIGURE 10. Niacin-evoked urinary PGD₂ metabolite excretion. Sequential measurement of tetranor PGDM (A), 2,3-dinor-11β-PGF_{2α} (B), and 8,12-iso-iPF_{2α}-VI (C) at various time points before (time 0) and after 600 mg of niacin administration to two healthy volunteers. Both urinary tetranor PGDM and 2,3-dinor-11β-PGF_{2α} were elevated by niacin, peaking 2–3 h and falling to basal levels 5–6 h after dosing. Urinary 8,12-iso-iPF_{2α}-VI is unaltered by niacin.

regulation that characterizes excessive mast cell activation (2, 4). Mast cells are evident in the potentially vulnerable shoulder region of human atherosclerotic plaques (32), and mast cell depletion retards atherogenesis in mice (33). Mast cells have recently been implicated in aortic aneurysm formation (34). Other cells of relevance to atherogenesis, among them macrophages, platelets, and leukocytes, may also generate PGD₂ (35, 36). Studies of L-PGDS also implicate PGD₂ in cardiovascular biology. Levels are induced in endothelial cells by laminar shear (37) and are elevated in the circulation after angioplasty (38) and in angina (39). However, lipocalins, such as L-PGDS, may subserve diverse biological functions (40); thus these observations only indirectly implicate PGD₂.

Previous attempts to study biosynthesis of PGD₂ have relied particularly on commercially available assays of 11β-PGF_{2α}. This compound can be formed from PGD₂ *in vitro* by bovine PGF synthase (18) and was the earliest metabolite detectable by Liston and Roberts after administration of radiolabeled PGD₂ in a human (19) and itself retains biological activity, causing bronchoconstriction when inhaled by humans (41). The hemodynamic instability in patients with systemic mastocytosis is thought to reflect metabolism of PGD₂, and other vasodilator D-ring metabolites, to vasoconstrictor F-ring metabolites, such as 11β-PGF_{2α}. However, although endogenous concentrations of 11β-PGF_{2α} have been reported in human urine, there has been poor concordance between estimates based on immunoassay and GC/MS. Here, we failed to detect urinary 11β-PGF_{2α} using a more specific approach, HPLC-MS/MS. This raises the possibility that cross-contaminating substances, particularly F₂ isoprostanes (29), may have confounded, in some

instances, analysis of 11β-PGF_{2α} by GC/MS. However, we did identify endogenous 2,3-dinor-11β-PGF_{2α} in human urine. Liston and Roberts detected predominantly F-ring metabolites in the 39% of administered radioactivity recovered after administration of radiolabeled PGD₂ to a volunteer. The most abundant of these metabolites was 9α,11β-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid (22). Surprisingly, they failed to detect tetranor PGDM; indeed the detected D-ring metabolites accounted for <4% of administered radioactivity. It is unknown whether this reflected a feature of this particular individual's metabolism or technical factors. In the present studies tetranor PGDM was detected in the urines of all mice and humans studied under basal conditions. Indeed the levels in urine exceeded not only 2,3-dinor-11β-PGF_{2α}, but also the reported levels of 9α,11β-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid in healthy humans (22). Furthermore, tetranor PGDM is thus far uniquely detectable in mouse urine, affording the possibility of integrating estimates of biosynthesis with experimental manipulations in mice in which elements of the PGD₂ synthesis/response system have been genetically manipulated. Future studies may reveal biological activity for this metabolite, but unlike PGD₂ itself, it failed to inhibit platelet aggregation, an effect transduced via DP1 (12, 13).

Formation of urinary tetranor PGDM is altered by modulation of PGD₂ biosynthesis in both mice and humans. Deletion of either H- or L-PGDS enzymes suppresses incompletely its excretion in mice. Deletion of H-PGDS had a significantly greater impact on urinary tetranor PGDM than did deletion of L-PGDS. This may reflect in part distinctions in cross-regulation of the synthases: whereas LPGDS was not up-regulated in HPGDS KOs, compensatory up-regulation of HPGDS was observed in aortic tissue in LPGDS KOs. Genetic manipulation in mice and pharmacological studies in humans both indicate that COX-1 is the dominant source of PGD₂, as reflected by this metabolite, under physiological conditions. Consistent with these observations, very low doses of aspirin that favor inhibition of COX-1 may induce asthma in susceptible subjects (42, 43). This phenomenon is thought to reflect both suppression of PGD₂, together with endoperoxide redirection to augment leukotriene biosynthesis (20). Experiments *in vitro* indicate that both COX enzymes have the capacity to contribute to PGD₂ production. For example, PGD₂ appears to derive in roughly equal amounts from COX-1 and COX-2 in liver macrophages under basal and LPS-stimulated conditions (44), whereas in mast cells, by contrast, PGD₂ is initially derived from secretory

phospholipase A₂ and COX-1 followed by sustained formation by cytoplasmic phospholipase A₂ and COX-2 (45). The availability of a urinary tetranor PGDM as a biomarker of PGD₂ biosynthesis will permit definition of the relative contribution of the two enzymes to its formation in response to experimental or disease-related perturbation in humans and mice.

There is considerable interest in the importance of PGD₂ and its metabolites both in the mediation and resolution of inflammation (11, 46). A tractable model of an acute inflammatory response in humans is to administer LPS, which evokes transient flu-like symptoms with pyrexia and a hemodynamic response. We have previously shown that both COX-1 and COX-2 are expressed *ex vivo* coincident with this symptomatic response and that biosynthesis of both thromboxane and prostacyclin, as reflected by their major urinary metabolites, is markedly augmented coincident with increased expression of the enzymes (25). Deletion of H-PGDS undermines resolution of the delayed type hypersensitivity evoked by injection of methylated bovine serum albumin in mice (47). Here, we show that tetranor PGDM increased markedly during the inflammatory response to LPS in humans and that this response is highly correlated with the less abundant 2,3-dinor-11 β -PGF_{2 α} . However, rather than a further increase, both metabolites fell during the resolution phase.

Morrow and colleagues have previously demonstrated that niacin evokes facial flushing by prompting release of PGD₂ by dermal dendritic cells (5). Given that niacin is the only currently available hypolipidemic drug that elevates high density lipoprotein (48), it is hoped that co-administration of a DP1 antagonist might reduce this complication and enhance compliance. However, DP1 activation elevates cAMP and inhibits aggregation of human platelets (49), raising concern that it might function as an endogenous modulator of platelet activation *in vivo*, much like PGI₂ (50). Urinary tetranor PGDM reflects the increase in biosynthesis evoked by niacin and should facilitate elucidation of the role of PGD₂ in cardiovascular disease.

In summary, here we report a novel, abundant, D-ring urinary PGD₂ metabolite 11,15-dioxo-9 α -hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid, tetranor PGDM, which is detectable in mouse and human urine. Analysis of this compound reflects modulated biosynthesis of PGD₂ in both species and will complement the use of genetic and pharmacological probes in the further elucidating the biology of PGD₂ *in vivo*.

Acknowledgments—We appreciate technical help from Wenxuan Li, Helen Zou, Matthew Stetz, Azri Mohd, Yubing Yao, and Eileen Callaghan.

REFERENCES

- Urade, Y., and Hayaishi, O. (2000) *Vitamins Hormones* **58**, 89–120
- O'Sullivan, S., Dahlen, B., Dahlen, S. E., and Kumlin, M. (1996) *J. Allergy Clin. Immunol.* **98**, 421–432
- Bochenek, G., Nagraba, K., Nizankowska, E., and Szczeklik, A. (2003) *J. Allergy Clin. Immunol.* **111**, 743–749
- Roberts, L. J., 2nd, Sweetman, B. J., Lewis, R. A., Austen, K. F., and Oates, J. A. (1980) *N. Engl. J. Med.* **303**, 1400–1404
- Morrow, J. D., Parsons, W. G., 3rd, and Roberts, L. J., 2nd (1989) *Prostaglandins* **38**, 263–274
- Williams, T. J., and Peck, M. J. (1977) *Nature* **270**, 530–532
- Matsuoka, T., Hirata, M., Tanaka, H., Takahashi, Y., Murata, T., Kabashima, K., Sugimoto, Y., Kobayashi, T., Ushikubi, F., Aze, Y., Eguchi, N., Urade, Y., Yoshida, N., Kimura, K., Mizoguchi, A., Honda, Y., Nagai, H., and Narumiya, S. (2000) *Science* **287**, 2013–2017
- Nagata, K., Tanaka, K., Ogawa, K., Kemmotsu, K., Imai, T., Yoshie, O., Abe, H., Tada, K., Nakamura, M., Sugamura, K., and takano, S. (1999) *J. Immunol.* **162**, 1278–1286
- Nagata, K., Hirai, H., Tanaka, K., Ogawa, K., Aso, T., Sugamura, K., Nakamura, M., and Takano, S. (1999) *FEBS Lett.* **459**, 195–199
- Cheng, K., Wu, T. J., Wu, K. K., Sturino, C., Metters, K., Gottesdiener, K., Wright, S. D., Wang, Z., O'Neill, G., Lai, E., and Waters, M. G. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103**, 6682–6687
- Gilroy, D. W., Colville-Nash, P. R., Willis, D., Chivers, J., Paul-Clark, M. J., and Willoughby, D. A. (1999) *Nat. Med.* **5**, 698–701
- Oelz, O., Oelz, R., Knapp, H. R., Sweetman, B. J., and Oates, J. A. (1977) *Prostaglandins* **13**, 225–234
- Bushfield, M., McNicol, A., and MacIntyre, D. E. (1985) *Biochem. J.* **232**, 267–271
- Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) *Cell* **83**, 803–812
- Bell-Parikh, L. C., Ide, T., Lawson, J. A., McNamara, P., Reilly, M., and FitzGerald, G. A. (2003) *J. Clin. Invest.* **112**, 945–955
- McAdam, B. F., Catella-Lawson, F., Mardini, I. A., Kapoor, S., Lawson, J. A., and FitzGerald, G. A. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 272–277
- Catella, F., Healy, D., Lawson, J. A., and FitzGerald, G. A. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 5861–5865
- Watanabe, K., Iguchi, Y., Iguchi, S., Arai, Y., Hayaishi, O., and Roberts, L. J., 2nd (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 1583–1587
- Liston, T. E., and Roberts, L. J. (1985) *J. Biol. Chem.* **260**, 13172–13180
- Misso, N. L., Aggarwal, S., Phelps, S., Beard, R., and Thompson, P. J. (2004) *Clin. Exp. Allergy* **34**, 624–631
- Bochenek, G., Nizankowska, E., Gielicz, A., Swierczynska, M., and Szczeklik, A. (2004) *Thorax* **59**, 459–464
- Morrow, J. D., Guzzo, C., Lazarus, G., Oates, J. A., and Roberts, L. J., 2nd (1995) *J. Invest. Dermatol.* **104**, 937–940
- Pickett, W. C., and Murphy, R. C. (1981) *Anal. Biochem.* **111**, 115–121
- Yu, Y., Cheng, Y., Fan, J., Chen, X. S., Klein-Szanto, A., Fitzgerald, G. A., and Funk, C. D. (2005) *J. Clin. Invest.* **115**, 986–995
- McAdam, B. F., Mardini, I. A., Habib, A., Burke, A., Lawson, J. A., Kapoor, S., and FitzGerald, G. A. (2000) *J. Clin. Invest.* **105**, 1473–1482
- Cheng, Y., Wang, M., Yu, Y., Lawson, J., Funk, C. D., and FitzGerald, G. A. (2006) *J. Clin. Invest.* **116**, 1391–1399
- Hamberg, M., and Fredholm, B. B. (1976) *Biochim. Biophys. Acta* **431**, 189–193
- Song, W. L., Lawson, J. A., Wang, M., Zou, H., and FitzGerald, G. A. (2007) *Methods Enzymol.* **433**, 51–72
- O'Sullivan, S., Mueller, M. J., Dahlén, S., and Kumlin, M. (1999) *Prostaglandins Other Lipid Mediators* **57**, 149–165
- FitzGerald, G. A., Oates, J. A., Hawiger, J., Maas, R. L., Roberts, L. J., 2nd, Lawson, J. A., and Brash, A. R. (1983) *J. Clin. Invest.* **71**, 676–688
- Fries, S., Grosser, T., Price, T. S., Lawson, J. A., Kapoor, S., DeMarco, S., Pletcher, M. T., Wiltshire, T., and FitzGerald, G. A. (2006) *Gastroenterology* **130**, 55–64
- BanderLaan, P. A., and Reardon, C. A. (2005) *J. Lipid Res.* **46**, 829–838
- Sun, J., Sukhova, G. K., Wolters, P. J., Yang, M., Kitamoto, S., Libby, P., MacFarlane, L. A., Clair, J. M., and Shi, G. P. (2007) *Nat. Med.* **13**, 719–724
- Sun, J., Sukhova, G. K., Yang, M., Wolters, P. J., MacFarlane, L. A., Libby, P., Sun, C., Zhang, Y., Liu, J., Ennis, T. L., Knispel, R., Xiong, W., Thompson, R. W., Baxter, B. T., and Shi, G. P. (2007) *J. Clin. Invest.* **117**, 3359–3368
- MacDermot, J., Kelsey, C. R., Waddell, K. A., Richmond, R., Knight, R. K., Cole, P. J., Dollery, C. T., Landon, D. N., and Blair, I. A. (1984) *Prostaglandins* **27**, 163–179
- Parsons II, W. G., and Roberts II, L. J. (1988) *J. Immunol.* **141**, 2413–2419
- Taba, Y., Sagaguri, T., Miyagi, M., Abumiya, T., Miwa, Y., Ikeda, T., and Mitsumata, M. (2000) *Circ. Res.* **86**, 967–973
- Inoue, T., Takayanagi, K., Morooka, S., Uehara, Y., Oda, H.,

- Seiki, K., Nakajima, H., and Urade, Y. (2001) *Thromb. Haemost.* **85**, 165–170
39. Eguchi, Y., Eguchi, N., Oda, H., Seiki, K., Kijima, Y., Matsu-ura, Y., Urade, Y., and Hayaishi, O. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 14689–14694
40. Flower, D. R. (1996) *Biochem. J.* **318**, 1–14
41. Beasley, C. R., Robinson, C., Featherstone, R. L., Varley, J. G., Hardy, C. C., and Church, M. K. (1987) *J. Clin. Invest.* **79**, 978–983
42. Barr, R. G., Kurth, T., Stampfer, M. J., Buring, J. E., Hennekens, C. H., and Gaziano, J. M. (2007) *Am. J. Respir. Crit. Care Med.* **175**, 120–125
43. Catella-Lawson, F., Reilly, M. P., Kapoor, S. C., Cucchiara, A. J., DeMarco, S., Tournier, B., Vyas, S. N., and FitzGerald, G. A. (2001) *N. Engl. J. Med.* **345**, 1809–1817
44. Dieter, P., Scheibe, R., Jakobsson, P. J., Watanabe, K., Kolada, A., and Kamionka, S. (2000) *Biochem. Biophys. Res. Commun.* **276**, 488–492
45. Reddy, S. T., and Hershman, H. R. (1997) *J. Biol. Chem.* **272**, 3231–3237
46. Gilroy, D. W., Colville-Nash, P. R., McMaster, S., Sawatzky, D. A., Willoughby, D. A., and Lawrence, T. (2003) *FASEB J.* **17**, 2269–2271
47. Trivedi, S. G., Newson, J., Rajakariar, R., Jacques, T. S., Hannon, R., Kanaoka, Y., Eguchi, N., Colville-Nash, P., and Gilroy, D. W. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103**, 5179–5184
48. Carlson, L. A. (2005) *J. Intern. Med.* **258**, 94–114
49. Giles, H., Leff, P., Bolofo, M. L., Kelly, M. G., and Robertson, A. D. (1989) *Br. J. Pharmacol.* **96**, 291–300
50. FitzGerald, G. A., Smith, B., Pederson, A. K., and Brash, A. R. (1984) *N. Engl. J. Med.* **310**, 1065–1068

Tetranor PGDM, an Abundant Urinary Metabolite Reflects Biosynthesis of Prostaglandin D₂ in Mice and Humans

Wen-Liang Song, Miao Wang, Emanuela Ricciotti, Susanne Fries, Ying Yu, Tilo Grosser, Muredach Reilly, John A. Lawson and Garret A. FitzGerald

J. Biol. Chem. 2008, 283:1179-1188.

doi: 10.1074/jbc.M706839200 originally published online November 8, 2007

Access the most updated version of this article at doi: [10.1074/jbc.M706839200](https://doi.org/10.1074/jbc.M706839200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

Supplemental material:

<http://www.jbc.org/content/suppl/2007/11/09/M706839200.DC1>

This article cites 50 references, 16 of which can be accessed free at <http://www.jbc.org/content/283/2/1179.full.html#ref-list-1>