Specific Analysis in Plasma and Urine of 2,3-Dinor-5,6-dihydro-isoprostane F_{2\alpha}-III, a Metabolite of Isoprostane F_{2\alpha}-III and an Oxidation Product of \(\gamma\)-Linolenic Acid*

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F_{2\alpha}-isoprostanes (iPs) are free radical-catalyzed isomers of prostaglandin F_{2\alpha}. Circulating and urinary iPs have been used as indices of lipid peroxidation in vivo. Utilizing an \(^{18}\)O-labeled homologous internal standard, we developed a gas chromatography/mass spectrometry assay for the 2,3-dinor-5,6-dihydro (dihydro-dihydro) metabolite of iP F_{2\alpha}-III. Although urinary excretion of iP F_{2\alpha}-III reflects systemic lipid peroxidation, the metabolite is more abundant (median of 877 (range of 351–1831) versus 174 (range of 56–321) pg/mg of creatinin; \(p < 0.01\)) than the parent iP in urine and can be measured in plasma. Metabolite analysis may be preferable in plasma due to the abundance of arachidonic acid as a source of \textit{ex vivo} lipid peroxidation. Also, iP F_{2\alpha}-III may be formed in blood samples in a cyclooxygenase-dependent manner by platelets \textit{ex vivo}. By contrast, the metabolite is not formed by aggregated platelets (0.71 ± 0.08 versus 0.65 ± 0.09 pg/ml). Although the metabolite/parent ratio is altered in cirrhosis, urinary dinor-dihydro-iP F_{2\alpha}-III is elevated and increases further during reperfusion following orthotopic liver transplantation. In addition to its formation as an iPF_{2\alpha} metabolite, analysis of \(\gamma\)-linolenic acid autooxidation products and the compound present in freeze-thawed plasma suggests that \(\gamma\)-linolenic acid may also be an important source of dinor-dihydro-iP F_{2\alpha}-III.

Isoprostanes (iPs)\(^1\) are free radical-catalyzed products of arachidonic acid (1, 2). F_{2\alpha}-iPs, isomers of prostaglandin F_{2\alpha}, are the most studied species, but analogous isomers (iso-iso- and iso-tetra-iso- and iso-octa-iso- and iso-deca-iso) of other prostaglandins as well as of leukotrienes have been described (3, 4). Isoeicosanoids are formed initially in situ in the phospholipid domain of cell membranes by a free radical attack on polyunsaturated fatty acids. They are subsequently cleared by phopholipases, enter the general circulation, and are excreted in urine. Given their relative chemical stability, iPs represent attractive indices of oxidant stress \textit{in vivo}. Indeed, iPs have been measured in human atherosclerotic plaque and localized to monocytes/macrophages and vascular smooth muscle cells (5). Urinary excretion of discrete F_{2\alpha}-iPs is increased in humans and mice with hypercholesterolemia (6, 7). Suppression of urinary iP excretion with vitamin E in apoE-deficient mice retards atherogenesis, despite sustained hypercholesterolemia (7).

Despite the emerging evidence supporting the quantitation of iPs as an approach to the study of oxidant stress \textit{in vivo} (8, 9), little is known about their release from cell membranes or their metabolic disposition prior to excretion. Several analytical approaches to quantitation of F_{2\alpha}-iPs have been employed (2). Those based on GC/MS have usually measured a heterogeneous GC peak composed of an undetermined number of isomers. We have developed assays that measure a single isomer, which may be more reproducible. To date, we have developed methods for iP F_{2\alpha}-III (formerly 8-iso-prostaglandin F_{2\alpha}) (10), iP F_{2\alpha}-VI (formerly iP F_{2\alpha}-I) (11), and 8,12-iso-iP F_{2\alpha}-VI (12). We have reported coordinate elevation of distinct iPs in atherosclerotic plaque (5) in the brains of patients with Alzheimer’s disease (13) as well as in the urine of cigarette smokers (14), patients with hypercholesterolemia (6), and patients with chronic obstructive pulmonary disease (15). Immunoassays for iP F_{2\alpha}-III have been developed (16, 17), and some have been commercialized. However, the cross-reactivity of these antibodies with most other F_{\alpha}-iPs and their putative metabolites is unknown. Administration of labeled iP F_{2\alpha}-III to primates and one human suggested that close to 20% of administered radioactivity recovered in urine is 2,3-dinor-5,6-dihydroisoprostane F_{2\alpha}-III (dihydro-dihydro-iPF_{2\alpha}-III) (18). More recently, Chiabrando et al. (19) identified the presence of 2,3-dinor-iPF_{2\alpha}-III, in addition to the 2,3-dinor-5,6-dihydro metabolite, in human urine. Although this assay did not include use of homologous internal standards, the authors estimated the quantities of both metabolites in human urine as being roughly equivalent to the parent compound, iP F_{2\alpha}-III.

There is a substantial rationale for the development of sensitive and specific assays for iP metabolites. Thus, we have previously reported that iP F_{2\alpha}-III, unlike other iPs, may be formed by COX isozymes \textit{in vitro} or \textit{ex vivo} (10, 20). Although this pathway of formation contributes to an undetectable extent to urinary iP F_{2\alpha}-III excretion, even under conditions of COX activation (14), it may confound quantitation of iPs in blood samples (11, 16). Similarly, peroxidation of arachidonate substrate may represent an additional source of \textit{ex vivo} artifact in blood sampling, although this problem may be circumvented by analysis in urine, where there is considerably less substrate (6, 11). Measurement of a metabolite, if not formed in the cells of circulating blood, may bypass these sources of \textit{ex vivo} artifact.
Isoprostane Metabolite Analysis

(21) Morrow et al. (22) have recently reported a GC/MS assay for dinor-dihydro-iPF2-III using an 18O2-labeled homologous internal standard. We have independently developed a similar assay and report elevated levels in patients with hepatic cirrhosis. Urinary dinor-dihydro-iPF2-III is further increased during the reperfusion phase following hepatic transplantation. Unlike its parent compound, iPF2-III, this metabolite is not formed in plasma by activated platelets. However, dinor-dihydro-iPF2-III may also be formed as a primary iPF from the autoxidation of an alternative fatty acid substrate, y-linolenic acid (GLA). Although GLA may be a less prevalent substrate than arachidonic acid in human tissues, the similarity of the array of isomers observed following autoxidation of GLA and those observed in human urine and plasma implies that this may be a significant source of dinor-dihydro-iPF2-III. This confounding duality suggests that interpretation of dinor-dihydro-iPF2-III levels is less than straightforward. However, either as a primary iPF derived from GLA or as a metabolite of an arachidonic acid-derived iPF, urinary dinor-dihydro-iPF2-III, just like iPF2-III, reflects alterations in lipid peroxidation in vivo, even in patients with hepatic dysfunction.

EXPERIMENTAL PROCEDURES

Analysis in Urine—Synthetic dinor-dihydro-iPF2-III was generously provided by Cayman Chemical Co., Inc. (Ann Arbor, MI). An internal standard of [18O2]dinor-dihydro-iPF2-III was formed following the method of Pickett and Murphy (23). Unless otherwise specified, all organic solvents were of analytical grade (Burdick & Jackson, Muskegon, MI). Each 1-ml sample of urine was spiked with 1.5 ng of internal standard and acidified with formic acid to pH <3. The metabolite was extracted by solid-phase extraction (SPE) on a RapidTrace SPE workstation (Zymark Corp., Hopkinton, MA) using C18 EC cartridges (100 mg of sorbent; International Sorbent Technology, Mid Glamorgan, United Kingdom). Each sample was wet conditioned with 1 ml of ethyl acetate. After SPE, the sample was then dried under N2 and centrifuged at 10500 g for 10 min at 4 °C. The pH was then lowered to ~3.0 with 1 N HCl, and the solution was extracted with 3 volumes of EtOAc and dried under N2. The resulting solution was stored in 1 ml of CH2Cl2 at ~20 °C until analysis.

Autoxidation of GLA—y-Linolenic acid (10 mg) and benzoyl peroxide (12.5 mg) were dissolved in 1 ml of CH2Cl2 and allowed to stand at room temperature for 72 h. Triphenylphosphine (5 mg) was added, and after standing for 30 min, the solution was dried under a stream of nitrogen. The reaction mixture was stored in 1 ml of acetonitrile at ~20 °C until analysis.

Design of Clinical Studies—Two clinical studies were performed. The first assessed the capacity of human platelets to generate the metabolite. The second addressed its formation in patients with liver disease. In the platelet study, 20 ml of blood was drawn from the antecubital veins of five volunteers (three males) aged 27–56 years (mean of 35.2 years). Blood was centrifuged at 2000 × g for 15 min at room temperature, and 2.4 ml of the platelet-rich plasma (PRP) was removed. The remainder of the sample was centrifuged at 1000 × g at 4 °C for 10 min. A 2.4-ml aliquot of this platelet-poor plasma supernatant was removed and stored on ice until used. Fifteen microliters of collagen (Chromolog Corp., Havertown, PA) was added to each of the PRP samples, which were stirred at 37 °C for 5 min to induce platelet aggregation and then placed in 5% acetic acid in methanol. Samples were transferred to a clean test tube, and protein mass was denatured and precipitated by the addition of 2 ml of ice-cold 10% isopropyl alcohol in acetonitrile.

The samples were then supplemented with 91.75 pg/ml [18O2]dinor-dihydro-iPF2-III and 400 pg of [18O2] iPF2-III, acidified, and centrifuged. The supernatant was transferred to a glass test tube for automated solid-phase extraction. C18 cartridges were conditioned with 1 ml of ethanol followed by 0.3 ml of pH 3 buffer. The sample was loaded and rinsed with 1 ml of pH 3 buffer and 1 ml of 25% ethanol in water with 0.1% acetic acid. The column was dried with 10 ml of air, and the sample was collected in 1 ml of ethyl acetate. This was purified by straight-phase SPE using Isolute NH2 cartridges (International Sorbent Technology). The sample was loaded, rinsed with 50% acetonitrile in ethanol, and collected in 5% acetic acid in methanol. This was dried, and the pentafluorobenzyl derivative was formed. TLC purification was performed as described for urine samples. iPF2-III was separated from the metabolite by scraping at the level of the visualization standard after the second TLC step, and a tert-butyldimethylsilyl ether derivative was formed as described previously (10). The ions monitored by GC/MS were at m/z 547.4 and 543.4 for the dinor-dihydro compound and at m/z 699.5 and 695.5 for iPF2-III.

Healthy volunteers (n = 32) used as controls in the liver study were aged 20–57 years (15 males). Patients with liver disease, including some scheduled to undergo orthotopic liver transplantation, were recruited from the Hepatology Clinic at the University of Pennsylvania Medical Center. Their clinical details are provided in Table I. All patients who participated in the study consented to do so, or if unable to give consent, this was obtained on their behalf from family members.

Base-line urine samples were obtained prior to orthotopic liver transplantation. Intraoperative samples were obtained prior to the start of the anhepatic phase of surgery, prior to reperfusion, 15–30 and 90–120 min after reperfusion of the newly grafted liver. Samples were immediately placed on ice and then stored at ~80 °C until analysis.

RESULTS

Sensitivity and Specificity of the Assay—Dinor-dihydro-iPF2-III was easily identifiable in urine and clearly separated from other compounds of the same mass (Fig. 1). The intra-assay variability was 3.35%, and the interassay variability was 4.47% on a single sample assayed six times on 4 separate days.
The assay was linear over a range of 0–8000 pg of added authentic material (Fig. 2). Twenty-four-hour urine samples collected from 32 healthy volunteers gave a mean urinary dinor-dihydro-iPF$_{2\alpha}$-III level of 540.7 ± 61 (range of 132–1434) pg/mg of creatinine. Values in males (541 ± 90.5 pg/mg of creatinine, aged 22–57 years) were not significantly different from those in females (540.4 ± 85.5 pg/mg of creatinine, aged 20–56 years). There was no significant relationship ($r^2$ = 0.008) between urinary dinor-dihydro-iPF$_{2\alpha}$-III and age in the total group. Paired analysis of both compounds was performed in a subset of 12 healthy individuals. Urinary levels of the dinor-dihydro metabolite were significantly higher (median of 876.9 (range of 351–1831) pg/mg of creatinine) than those of iPF$_{2\alpha}$-III (median of 174 (range of 56–321) pg/mg of creatinine; $p < 0.01$) in these individuals.

iPF$_{2\alpha}$-III and Its Dinor-dihydro Metabolite in Urine—Both urinary iPF$_{2\alpha}$-III and dinor-dihydro-iPF$_{2\alpha}$-III were analyzed in a subset of 12 healthy volunteers and 11 patients with chronic liver disease. Urinary iPF$_{2\alpha}$-III was elevated in liver disease, as we have previously reported (26, 27). The urinary dinor-dihydro metabolite was also elevated (3750 ± 876.4 versus 876.9 ± 148 pg/mg of creatinine) in patients with chronic liver disease compared with controls. The ratio of the dinor-dihydro compound to parent compound, iPF$_{2\alpha}$-III, was 5.3 ± 8.5 in healthy controls ($n = 12$) and 3.3 ± 0.56 in age- and gender-matched patients ($n = 22$) with chronic liver disease. The difference between these ratios attained statistical significance ($p < 0.005$).

The dinor-dihydro compound is not formed by activated platelets. Levels of the parent isoprostane rose from 2.4 ± 0.62 pg/ml in platelet-poor plasma to 29.2 ± 7.5 pg/ml in aggregated PRP ($p < 0.0001$). By contrast, levels of the metabolite were unchanged (0.71 ± 0.08 versus 0.65 ± 0.09 pg/ml; $p =$ not significant) (Fig. 3).
Elevated Metabolite Excretion during Hepatic Reperfusion—Ten patients due to undergo orthoptic liver transplantation were studied. Eight were male. Preoperative urinary metabolite levels in these patients with chronic liver disease were also significantly \( p < 0.01 \) elevated (1914.5 ± 416.6 pg/mg of creatinine). Serial intraoperative samples were available from seven of these patients. Urinary dinor-dihydro-iPF\(_{2\alpha}^{-}\)-III increased further \( p < 0.01 \) during the reperfusion phase after hepatic ischemia, peaking at 3213.7 ± 686.4 pg/mg of creatinine 90–120 min post-reperfusion. Excretion of the metabolite decreased over the next several days to the range observed in healthy volunteers (Fig. 4).

Formation as a Primary Isoprostane from GLA—Autoxidation of GLA \textit{in vitro} gave rise to a series of peaks, the largest of which coeluted with the internal standard for dinor-dihydro-iPF\(_{2\alpha}^{-}\)-III (Fig. 5). The pattern of peaks observed in plasma closely resembles that of autooxidized GLA. Furthermore, the large increase in all peaks after plasma is subjected to freeze-thaw treatment is likely analogous to the observation by Morrow et al. (28) of similar increases in the concentration of arachidonic acid-derived F\(_2\)-iPs after such treatment. The fact that a similar pattern of peaks was observed in normal urine (Fig. 5) further implies that a significant portion of urinary dinor-dihydro-iPF\(_{2\alpha}^{-}\)-III is also derived from autooxidized GLA. A schematic route for the formation of GLA-derived iPs is depicted in Fig. 6.

DISCUSSION

We report the presence of dinor-dihydro-iPF\(_{2\alpha}^{-}\)-III in human urine and plasma using a highly reproducible, specific, and sensitive GC/MS-based assay. Previously, Roberts et al. (18) reported that intravenous administration of labeled iPF\(_{2\alpha}^{-}\)-III to non-human primates and to a human volunteer resulted in \( \sim 20\% \) of the radioactivity in urine being accounted for by this compound. Thus, it is likely that endogenous iPF\(_{2\alpha}^{-}\)-III is a source of endogenous urinary dinor-dihydro-iPF\(_{2\alpha}^{-}\)-III in the present study. Chiabrando et al. (19) have identified the presence of both dinor-dihydro-iPF\(_{2\alpha}^{-}\)-III and 2,3-dinor-iPF\(_{2\alpha}^{-}\)-III in human urine. They demonstrated that excretion of the metabolites was elevated in smokers and was not depressed in individuals given the COX inhibitor naproxen. Their approach to quantitation is accompanied by several caveats, however. Due to the lack of homologous stable isotope-labeled internal standards, they could not control for the efficiency of the immunoaffinity extraction. Also, the possibility exists for differential efficiency of MS fragmentation between the \(^{[2H_4]}\text{iPF}_{2\alpha}^{-}\)-III in-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Effect of platelet aggregation on dinor-dihydro-iPF\(_{2\alpha}^{-}\)-III and iPF\(_{2\alpha}^{-}\)-III. PRP and platelet-poor plasma were prepared as described under "Experimental Procedures." PRP was aggregated with collagen, and all samples were then extracted, purified, derivatized, and analyzed by GC/MS as described under "Experimental Procedures."}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{Urinary dinor-dihydro-iPF\(_{2\alpha}^{-}\)-III excretion before and after liver transplantation. Levels were elevated pre-transplant, increased further with reperfusion of the grafted liver, and returned to the level of healthy volunteers (dotted lines) within 4 days of transplant. \textit{intra-op}, intraoperative.}
\end{figure}
Isoprostane Metabolite Analysis

2503

![GC/MS analysis after purification and derivatization.](image)

**FIG. 5.** GC/MS analysis after purification and derivatization. All traces represent selected ion monitoring at m/z 543. A, autoxidized GLA; B, normal human urine; C, freeze-thawed human plasma; D, normal human plasma. Samples were either supplemented before workup (B and D) or co-injected (A and C) with synthetic [18O2]dinor-dihydro-iPF2-III. For clarity, the selected ion monitoring trace for the standard (m/z 547), which coeluted with the peaks marked with asterisks, is not shown.

ternal standard and the two metabolites.

Urinary dinor-dihydro-iPF2-III, just like iPF2-III (26, 27, 29, 30), is increased in patients with hepatic cirrhosis, as are other markers of oxidant stress (26, 30). In the present study, there was an additional transient increment in metabolite excretion during the reperfusion phase following orthoptic liver transplantation. We have previously described such an increment in urinary iPF2-III coincident with coronary reperfusion after ischemia with thrombolytic drugs, cardiopulmonary bypass (31), or angioplasty (32). Interestingly, in the present study, urinary excretion of the dinor-dihydro compound fell to levels within the normal range after reperfusion, suggesting correction of the preoperative syndrome of oxidant stress. We are presently exploring the possibility that later increases in metabolite excretion might presage episodes of transplant rejection.

Although measurement of parent iP's in urine reflects systemic and not just renal generation of iP's (26, 27, 31, 32), analysis of a metabolite rather than the parent iP may be advantageous in certain circumstances. We have previously demonstrated that iPF2-III, apparently alone among the F2 isoprostanes, can be formed in a COX-dependent manner by platelets in

vitro and in serum ex vivo (10, 16). Both COX-1 in platelets (10) and COX-2 in monocytes (20) have the capacity to form iPF2-III. Although this enzymatic pathway does not appear to contribute detectably to urinary concentrations of the iP (11), even in syndromes of COX activation (14), this may not be the case for plasma-based assays. Thus, COX-1 activation in platelets will occur ex vivo after blood sampling and may confound estimates of iPF2-III actually circulating in vivo. For example, thromboxane A2, the major COX metabolite formed by platelets (33), is rapidly hydrolyzed to thromboxane B2, which is biologically inactive, but chemically relatively stable and readily measured in biological fluids. However, continuing platelet activation ex vivo, even when samples are harvested into a mixture including a COX inhibitor, results in an uncontrolled source of artifact ex vivo (21). In the case of thromboxane, this is readily addressed by measurement of a metabolite (11-dehydro-thromboxane B2) that is not formed by cells of the circulating blood (21), but rather in tissues such as liver, lung, and kidney. We now report that the dinor-dihydro metabolite of iPF2-III, unlike the parent isoprostane, is not formed by aggregated human platelets.

In the case of the iP's, a second form of potential artifact is lipid peroxidation ex vivo. This may also be more of a problem for plasma-based assays of parent iP's, where the content of the arachidonic acid substrate is much greater than in urine. Again, this problem may be bypassed by measurement of a metabolite that is not formed in blood. Formation of parent iP's ex vivo in urine has not been detected in carefully collected samples (11).

In addition to these theoretical advantages of a metabolite assay in plasma, such compounds are sometimes more abundant than the parent compound in urine. In contrast to the findings of Chiabrando et al. (19), we report that this is also the case for dinor-dihydro-iPF2-III. The greater abundance of the metabolite renders it more tractable for various forms of analysis. In this case, the GC/MS method we describe for the metabolite is more facile than that for the parent compound, iPF2-III (10). Use of the trimethylsilyl derivative allows for a reaction time of 10 min as opposed to 12 h for the tert-butyldimethylsilyl, a GC retention time of 8 versus 13 min, and a smaller sample volume of urine (1 versus 5 ml). Although lacking the elegant simplicity possible with immunoaffinity extraction, this method takes advantage of reasonably priced, commercially available, single-use SPE cartridges and TLC plates, avoiding the necessity of raising, purifying, characterizing, and coupling antibodies (19).

The availability of assays for iP metabolites will permit initial studies of iP disposition. Although we do not know the tissues in which this or other metabolites are formed, the liver seems one likely source by analogy with dinor metabolites of prostaglandins and thromboxane. Indeed, the dinor-dihydro metabolite is formed from iPF2-III by rat hepatocytes in vitro (19). Consistent with the likelihood of a hepatic contribution to metabolite biosynthesis in humans, the ratio of metabolite to parent in the urine of patients with cirrhosis is less than that observed in healthy individuals. However, even in liver disease, urinary levels of both the metabolite and the parent iP are elevated compared with controls, suggesting that a change in iP generation predominates over one in iP disposition. Paired analysis of parent plus metabolite will permit further elucidation of alterations in generation versus disposition of iP's in human disease. Biological effects have been attributed to iPF's, including iPF2-III (2). The extent to which metabolic disposition contributes to the inactivation of such effects of parent iP's in vivo is presently unknown. Recently, Basu (34) described an additional compound, 2,3,4,5-tetranor-15-keto-13,14-dihydro-
iPF$_{2\alpha-III}$, as a major metabolite in the rat.

Little is known of the effects of substrate modification on iP generation. Peroxidation products of both eicosapentaenoic acid and docosahexaenoic acid have been described (35). Interestingly, aside from its formation as a metabolite of arachidonic acid-derived iP (iPF$_{2\alpha-III}$), the dinor-dihydro compound may theoretically also be formed as a primary iP from GLA, which itself may be formed as a degradation product of arachidonic acid. Interestingly, iPF$_{2\alpha-III}$ is degraded to dinor-dihydro-iPF$_{2\alpha-III}$ by the same peroxisomal mechanism that converts arachidonic acid to GLA (Fig. 6). Dinor isoprostane regioisomers formed from a-linolenic acid in plants have previously been described (36). We present evidence consistent with the identity of dinor-dihydro-ip$_{2\alpha-III}$ in human urine and plasma to a primary autoxidation product of GLA. We cannot discriminate between the two potential sources of the dinor-dihydro compound in the present study. However, the pattern of peaks observed in urine and plasma bears a striking similarity to the pattern obtained from the autoxidation of GLA, suggesting that this may be a significant source of the compound. Indeed, a more appealing nomenclature for the compound would be 2,3-dinor-ip$_{2\alpha-III}$ (37), which is unencumbered by the implication of a metabolic derivation from iPF$_{2\alpha-III}$.

In summary, we describe the detection of dinor-dihydro-iPF$_{2\alpha-III}$ (2,3-dinor-ip$_{2\alpha-III}$) in human plasma and urine using a homologous standard in a GC/MS assay. Urinary excretion of the compound was elevated in patients with cirrhosis and was increased further during the reperfusion phase of hepatic transplantation. Irrespective of whether it is formed as a metabolite of arachidonic acid-derived iP or directly from GLA, it is not formed by activated platelets and is more abundant and more readily measured than iPF$_{2\alpha-III}$ in urine; and urinary levels reflect lipid peroxidation in vivo.

REFERENCES

1. Morrow, J. D., Hill, K. E., Burk, R. F., Nammour, T. M., Bahr, K. F., and Roberts, L. J., II (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9833–9837.
2. Lawson, J. A., Rokach, J., and FitzGerald, G. A. (1999) J. Biol. Chem. 274, 2441–2444.
3. Morrow, J. D., Award, J. A., Wu, A., Zackert, W. E., Daniel, V. C., and Roberts, L. J., II (1996) J. Biol. Chem. 271, 23185–23190.
4. Harrison, K. A., and Murphy, R. C. (1996) J. Biol. Chem. 271, 17273–17278.
5. Pratico, D., Iuliano, L., Mauriello, A., Spagnoli, L., Lawson, J. A., Rokach, J., Mlacloft, J., Violi, F., and FitzGerald, G. A. (1997) J. Clin. Invest. 100, 2028–2034.
6. Reilly, M., Pratico, D., Delanty, N., D’Immo, G., Tremoli, E., Rader, D., Kapoor, S., Rokach, J., Lawson, J., and FitzGerald, G. A. (1998) Circulation 98, 2822–2828.
7. Pratico, D., Tangirala, R. K., Rader, D. J., Rokach, J., and FitzGerald, G. A. (1998) Nat. Med. 4, 1193–1198.
8. Witztum, J. L., and Berliner, J. A. (1995) Curr. Opin. Lipidol. 9, 441–448.
9. Rokach, J., Khanapure, S. P., Hwang, S. W., Adiyaman, M., Lawson, J. A., and FitzGerald, G. A. (1997) Prostaglandins 54, 823–851.
10. Pratico, D., Lawson, J. A., and FitzGerald, G. A. (1995) J. Biol. Chem. 270, 9800–9808.
11. Pratico, D., Barry, O. P., Lawson, J., Adiyaman, M., Hwang, S. W., Khanapure, H., Rokach, J., and FitzGerald, G. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 95, 3449–3454.
12. Lawson, J. A., Li, H., Rokach, J., Adiyaman, M., Hwang, S. W., Khanapure, S. P., and FitzGerald, G. A. (1998) J. Biol. Chem. 273, 29295–29301.
13. Pratico, D., Trojanski, J., Lee, V., Rokach, J., and FitzGerald, G. A. (1997) FASEB J. 12, 1777–1783.
14. Reilly, M., Delanty, N., Lawson, J. A., and FitzGerald, G. A. (1998) Circulation 94, 19–25.
15. Pratico, D., Basili, S., Vieri, M., Cordova, C., Violi, F., and FitzGerald, G. A. (1998) Am. J. Respir. Crit. Care Med. 158, 1709–1714.
16. Wang, Z., Ciabattoni, G., Creminon, C., Lawson, J. A., FitzGerald, G. A., Patrono, C., and Macleod, J. (1995) J. Pharmacol. Exp. Ther. 275, 94–100.
17. Davi, G., Ciabattoni, G., Consoli, A., Mezzetti, A., Faleo, A., Santarone, S., Pennesi, E., Vitacolonna, E., Bucciarrelli, T., Costantini, F., Capani, F., and Patrono, C. (1999) Circulation 99, 224–229.
18. Roberts, L. J., Moore, K. P., Zackert, W. E., Oates, J. A., and Morrow, J. D. (1996) J. Biol. Chem. 271, 20617–20620.
19. Chiabrando, C., Valagussa, A., Rivolta, C., Durandt, T., Guy, A., Zucato, E., Villa, P., Rossi, J.-C., and Faneli, R. (1999) J. Biol. Chem. 274, 1313–1319.
20. Pratico, D., and FitzGerald, G. A. (1996) J. Biol. Chem. 271, 8919–8924.
21. Catella, F., Healy, D., Lawson, J. A., and FitzGerald, G. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 93, 5861–5865.
22. Morrow, J. D., Zackert, W. E., Yang, J. P., Kubits, E. H., Callewaert, D., Dworski, R., Kanai, K., Taber, D., Moore, K., Oates, J. A., and Roberts, L. J. (1999) Anal. Biochem. 289, 326–331.
23. Pickett, W. C., and Murphy, R. C. (1981) Anal. Biochem. 111, 115–121.
24. Llobat-estelles, M., Sevillano-Cabeja, A., and Campine-Falo, P. (1989) Analyst 114, 597–602.
25. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
26. Murphy, E. A., Barry, O. P., Burke, A., Lucey, M. E., Lawson, J. A., Rokach, J., and FitzGerald, G. A. (1999) J. Clin. Invest. 104, 805–813.
27. Pratico, D., Juliano, L., Basili, S., Ferro, D., Camastra, C., Cordova, C., Riggs, O., FitzGerald, G. A., and Violi, F. (1998) J. Invest. Med. 46, 51–57.
28. Morrow, J. D., Harris, T. M., and Roberts, L. J., II (1990) Anal. Biochem. 184, 1–10.
29. Aleykin, S. I., Leo, M. A., Aleykin, M. K., and Lieber, C. S. (1998) Alcohol Clin. Exp. Res. 22, 192–196.
30. Leo, M. A., Aleykin, S. I., Siegel, J. H., Kasmin, F. E., Aleykin, M. K., and Lieber, C. S. (1997) Am. J. Gastroenterol. 92, 2069–2072.
31. Delanty, N., Reilly, M., Lawson, J. A., McCarthy, J., Wood, F., FitzGerald, D. J., and FitzGerald, G. A. (1997) Circulation 95, 2492–2499.
32. Reilly, M., Delanty, N., Roy, L., O’Callaghan, P., Crean, P., and FitzGerald, G. A. (1997) Circulation 96, 3314–3320.
33. Hamberg, M., Svensson, J., and Samuelsson, B. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 3824–3828.
34. Basu, S. (1998) FEBS Lett. 428, 32–36.
35. Nourooz-Zadeh, J., Liu, E. H., Yhlen, B., Anggard, E. E., and Halliwell, B. (1999) J. Neurochem. 72, 734–740.
36. Parchmann, S., and Mueller, M. J. (1998) J. Biol. Chem. 273, 32650–32655.
37. Rokach, J., Khanapure, S. P., Hwang, S. W., Adiyaman, M., Lawson, J. A., and FitzGerald, G. A. (1997) Prostaglandins 54, 853–873.