Nitrogen Dioxide Induces cis-trans-Isomerization of Arachidonic Acid within Cellular Phospholipids

DETECTION OF TRANS-ARACHIDONIC ACIDS IN VIVO*

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Oxygen free radicals oxidize arachidonic acid to a complex mixture of metabolites termed isoeicosanoids that share structural similarity to enzymatically derived eicosanoids. However, little is known about oxidations of arachidonic acid mediated by reactive radical nitrogen oxides. We have studied the reaction of arachidonic acid with NO2, a free radical generated by nitric oxide and nitrite oxidations. A major group of products appeared to be a mixture of arachidonic acid isomers having one trans-bond and three cis-double bonds. We have termed these new products trans-arachidonic acids. These isomers were chromatographically distinct from arachidonic acid and produced mass spectra that were nearly identical with mass spectra of arachidonic acid. The lack of ultraviolet absorbance above 205 nm and the similarity of mass spectra of dimethyloxazoline derivatives suggested that the trans-bond was not conjugated with any of the cis-bonds, and the C=C bonds were located at carbons 5, 8, 11, and 14. Further identification was based on comparison of chromatographic properties with synthetic standards and revealed that NO2 generated 14-trans-eicosatetraenoic acid and a mixture containing 11-trans-, 8-trans-, and 5-trans-eicosatetraenoic acids. Exposure of human platelets to submicromolar levels of NO2 resulted in a dose-dependent formation of 14-trans-eicosatetraenoic acid and other isomers within platelet glycerophospholipids. Using a sensitive isotopic dilution assay we detected trans-arachidonic acids in human plasma (50.3 ± 10 ng/ml) and urine (122 ± 50 ng/ml). We proposed a mechanism of arachidonic acid isomerization that involves a reversible attachment of NO2 to a double bond with formation of a nitroarachidonyl radical. Thus, free radical processes mediated by NO2 lead to generation of trans-arachidonic acid isomers, including biologically active 14-trans-eicosatetraenoic acid, within membrane phospholipids from which they can be released and excreted into urine.

Arachidonic acid is one of the most abundant polyunsaturated fatty acids found in the cellular membrane phospholipid bilayer. A characteristic structural feature of this fatty acid is a 20-carbon chain containing four cis-double bonds that form a molecule of 5Z,8Z,11Z,14Z-eicosatetraenoic acid. These double bonds are homoconjugated resulting in three bis-allylic methylene groups. Abstraction of a single hydrogen from one of these methylene groups via a homolytic cleavage of a C–H bond is a fundamental process of arachidonic acid metabolism by enzymatic as well as nonenzymatic reactions. Enzymatic processes lead to a family of biologically active lipids such as prostaglandins and leukotrienes, known collectively as the eicosanoids (1). Syndromes of oxidative stress elevate levels of free radicals that can directly target arachidonic acid bound to phospholipids. This generates a complex mixture of oxidized products, known as isoeicosanoids, that can be cleaved off by phospholipases, circulated, and excreted in urine. Isoprostaglandins (2, 3) and isoleukotrienes (4) are structurally similar to the native eicosanoids, and some of them display potent biological activity. Hydroxyl radical is a potent activator of polysaturated fatty acid peroxidation due to its high intrinsic oxidation potential. The oxidations of fatty acids are somehow limited by its short reactive half-life (~10−9 s) and occur at the diffusion controlled rates within a close distance to the site of OH radical formation.

Relatively less is known about transformations of polysaturated fatty acids induced by free radical nitrogen oxides. Nitric oxide reacts very slowly with olefins but quite fast with lipid peroxy and alkoxy radicals, which leads to unstable nitro and oxonitro derivatives of linoleic and linolenic acids, and by this mechanism nitric oxide is thought to terminate progression of lipid peroxidation (5).

NO2 is a toxic free radical found in biological systems as a product of spontaneous oxidation of NO and enzymatic oxidations of nitrite (6). NO2 is also an air pollutant and has been implicated to cause pulmonary edema and fibrosis, bronchitis, asthma, and possibly cancer (7). NO2 is a potent oxidant that causes lipid peroxidation (8, 9); however, the reaction of NO2 with arachidonic acid has not been characterized. Oxidation of nitric oxide to NO2 is significantly accelerated within the hydrophobic phase of cellular phospholipid bilayer (10). This intramembrane reaction is facilitated by the much higher solubility of nitric oxide in hydrophobic layer of phospholipids than in the aqueous phase. Thus, it is possible that a significant amount of NO2 may be formed under aerobic conditions within the cellular phospholipid bilayer. These observations raise the possibility of novel nonenzymatic, free radical pathways involved in arachidonate transformations by NO2. In this study, we established the chemical structures of major products formed from this reaction, and we found that the predominant process mediated by NO2 leads to a new group of lipids, which we have termed trans-arachidonic acids.
trans-Arachidonic Acids

Materials—Arachidonic acid was from Sigma. [1-14C]Arachidonic acid (specific activity, 57 Ci/mmol) was from NEN Life Science Products. [5,6,8,9,11,12,14,15-d6]Arachidonic acid (isotopic purity, >98%) was from BioMol (Plymouth Meeting, MI). Nitrogen dioxide was from Matheson (E. Rutherford, NJ). All solvents were of highest chromatographic grade.

Preparation of NO2 with Arachidonic Acid—In a typical experiment, arachidonic acid (100 µg) was dissolved in 1 ml of hexane, and sodium arachidonate (100 µg) was dissolved in 1 ml of phosphate buffer (0.5 mM, pH 7.4). d6-Arachidonic acid (10 µg) was mixed with [1-14C]arachidonic acid (10,000 cpm) and was used to prepare the internal standards for quantitative analyses. Nitrogen dioxide was prepared shortly before reaction with arachidonic acid as described (11). Briefly, about 1 ml of liquid was collected from the original NO2 tank. NO2 gas was delivered into arachidonate solutions either via bubbling using helium as a carrier gas (~0.1 ml/min) or was sampled using a 50-µl gas-tight syringe. The final concentrations of NO2 were 43–430 nM. The reaction was carried for additional 3–5 min, and the lipids were isolated by extraction with organic solvents. The extracts were dissolved in small volume of methanol and analyzed by HPLC.1 In some experiments the lipid extracts were treated with sodium borohydride to reduce hydroperoxides.

HPLC Analyses—HPLC analyses were performed on a HP1050 system (Hewlett-Packard) using C18 column (250 × 4.6 mm, Beckman Instruments). Samples were eluted with a gradient of acetonitrile in water (62.5% increased to 100% in 60 min), and the effluent was monitored by an on-line UV diode array detector. Fractions were collected by a Gilson FC 203B fraction collector. In the experiments where [1-14C]arachidonic acid was used as a substrate for NO2, the effluent was also monitored by the on-line radioactivity monitor to detect radioactive labeled products.

Preparation of Derivatives—Pentafluorobenzyl (PFB) and methyl esters were prepared as described (13). Dimethylxiloxane (DMOX) derivatives were prepared as described (14) by treatment of fatty acids with 2-amino-2-methylpropanol (Aldrich) in a microvial at 150 °C for 1 h. After cooling, samples were dried under a stream of nitrogen, extracted with ethyl acetate, and finally purified by HPLC. The DMOX derivatives of compound I and arachidonic acid eluted at 28 and 23.5 min, respectively. N,O-Bis(trimethylsilyl) trifluoroacetamide was used to convert hydroxyl groups into trimethylsilyl (TMS) derivatives. Samples were finally dissolved in n-decane, and 1-µl aliquots were analyzed by GC/MS. The samples were hydrogenated by bubbling hydrogen gas through a solution of sodium hypophosphite. The samples were finally dissolved in methanol (1.55 ml) and centrifuged. The methanolic solution was evaporated to near dryness, dissolved in 1 ml of water, and extracted with ethyl acetate. The lipid extracts were purified by HPLC and analyzed by GC/MS as described above. The fractions containing trans-arachidonic acids were collected and dried. The residue was derivatized with PFB bromide and analyzed by GC/MS. Ions at m/z 303 and 311, corresponding to endogenous trans-arachidonic acids and deuterium-labeled internal standard, were monitored.

RESULTS

Arachidonic acid reacted readily with NO2 in a dose-dependent manner generating two major compounds (I and II) and a complex mixture of less abundant products (Fig. 1). Compounds I and II eluted after the peak of arachidonic acid, suggesting that they were relatively less polar than arachidonic acid. Purified material in peaks I and II did not show [1-14C]arachidonic acid (specific activity, 57 Ci/mmol) as a substrate for NO2 in phosphate buffer. The compound I and II eluted after the peak of arachidonic acid, suggesting that they were relatively less polar than arachidonic acid. Purified material in peaks I and II did not show [1-14C]arachidonic acid (specific activity, 57 Ci/mmol) as a substrate for NO2 in phosphate buffer. The compound I and II eluted after the peak of arachidonic acid, suggesting that they were relatively less polar than arachidonic acid. Purified material in peaks I and II did not show [1-14C]arachidonic acid (specific activity, 57 Ci/mmol) as a substrate for NO2 in phosphate buffer.

The regression analysis produced a formula for a correlation line (r2 = 0.999) that allowed conversion of retention times of analyzed compounds into their C values.

Preparation of trans-Arachidonic Acids—Epoxycosatrienic acids (EET) were prepared from arachidonic acid and m-chloroperoxycyclohexene as described (16). 14,15-EET and, separately, a mixture of 5,6-, 8,9-, and 11,12-EET (200–400 µg) were dissolved in dry tetrahydrofuran (100 µl) and mixed with a solution of triphenylphosphine in tetrahydrofuran (final concentration 0.1 mM) (17). The reaction was carried out in a glass tube, under nitrogen, in a block heated to 100 °C for 40 min. The products were extracted with ethyl acetate and purified by HPLC. Z,9,Z,11,14-EET cosatricentric acid (14E-AAA) was synthesized as described (18). Briefly, this synthesis involved a Wittig reaction between (Z)-t-butylidihydroxyilloylhept-3-enal and the ylide of (3Z,6E)-dodeca-3,6-dienyl-triphenylphosphonium bromide. The C19 tetraenoic ether was isolated and transformed in three steps to a methyl ester of 14E-AAA in 81% overall yield (18). The 14E-AAA methyl ester was hydrolyzed with lithium hydroxide in aqueous methanolic solution (10:1). The stereoisomeric purity of 14E-AAA (PFB derivative) was >99% as established by GC/MS.

Preparation of trans-Arachidonic Acids in Human Platelets—A concentrate of fresh human platelets was obtained from Hudson Valley Blood Bank (Elmsford, NY), and platelet suspensions in phosphate buffer were prepared as described (13). Platelets were exposed to NO2 and were subjected to microfugal centrifugation (10,000 cells/ml) of phosphate buffer (0.5 mM, pH 7.4) were mixed with NO2 solution in helium (1–20 µl; final concentration, 0.88–0.7 µM) delivered with a gas-tight syringe, and the cells were stirred for an additional 3–5 min at room temperature. Total platelet lipids were extracted with chloroform/methanol using a Bligh and Dyer protocol without acidification. The lipid extracts were dried under nitrogen and hydrolyzed in 1 N NaOH for 1.5 h, at 100 °C for 2 h. Fatty acids were then extracted with ethyl acetate. Prior to hydrolysis, 5 ng of d6-trans-arachidonic acid was added as internal standard. Lipids were purified by HPLC, and the fractions containing trans-arachidonic acids were collected and dried. The residue was derivatized with PFB bromide and analyzed by GC/MS. Ions at m/z 303 and 311, corresponding to endogenous trans-arachidonic acids and deuterium-labeled internal standard, were monitored.

Arachidonic acid reacted readily with NO2 in a dose-dependent manner generating two major compounds (I and II) and a complex mixture of less abundant products. Compound I and II eluted after the peak of arachidonic acid, suggesting that they were relatively less polar than arachidonic acid. Purified material in peaks I and II did not show UV light absorbance above 205 nm. Table I shows that compounds I and II eluted after the peak of arachidonic acid, suggesting that they were relatively less polar than arachidonic acid. Purified material in peaks I and II did not show UV light absorbance above 205 nm. Table I shows that compounds I and II eluted after the peak of arachidonic acid, suggesting that they were relatively less polar than arachidonic acid. Purified material in peaks I and II did not show UV light absorbance above 205 nm.

The abbreviations used are: HPLC, high pressure liquid chromatography; PTSA, para-toluenesulfonic acid; PFBA, pentafluorobenzyl; DMOX, 4,4-dimethylxiloxane; TMS, trimethylsilyl; GC/MS, gas chromatography/mass spectrometry; EET, epoxycosatrienic acids(s); AA, arachidonic acid.

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double bonds in I and II produced a compound indistinguishable of arachidonic acid. The spectra contained ions characteristic of arachidonic acid and a mixture of trans-arachidonic acids, respectively. The location of C=C bonds was established by analysis of mass differences in a series of characteristic ions. Sequential cleavage of each of the carbon-carbon bonds directed by the ionized dimethyloxazoline moiety led to a series of ions differing by 14 units (CH₂). An advance by 0.3–0.5 units relative to compounds I and II was identified as arachidonic acid and a mixture of trans-arachidonic acids, respectively. 

![Fig. 1. Representative chromatogram showing detection of products from the NO₂/arachidonic acid reaction by HPLC.](image)

**Fig. 1.** Representative chromatogram showing detection of products from the NO₂/arachidonic acid reaction by HPLC. In this experiment, 100 μg of arachidonic acid was dissolved in hexane and bubbled with NO₂ (470 μM) for 3 min. Lipids were analyzed on a C18 column (250 × 4.6 mm) and separated with a gradient of acetonitrile in water (62.5–100% in 50 min). The inset shows the tandem electrospray mass spectrometry of product I following collision-induced decomposition of the molecular anion at m/z 303. Material in peaks labeled AA, I, and II produced similar spectrum and was identified as arachidonic acid and a mixture of trans-arachidonic acids, respectively. 

| Solvent         | Compound I (%) | Compound II (%) | Oxidized products (%) |
|-----------------|----------------|-----------------|-----------------------|
| Hexane          | 57.6           | 7.2             | 35.1                  |
| Phosphate buffer| 18.3           | 3.2             | 11.1                  |

shown are the percentages of total peak area of products absorbing at 205 nm from chromatograms obtained by HPLC analyses as shown in Fig. 1 (n = 2–5). Compounds I and II were identified as a mixture of arachidonic acid isomers having one or more trans-double bonds, whereas oxidized products contained prostaglandin F₂, hydroxyeicosatetraenoic acids, epoxyeicosatrienoic acids, nitrohydroxyeicosatrienoic acids, and nitroeicosatetraenoic acids. 

**TABLE I**

Relative abundance of products generated from the reaction of nitrogen dioxide with arachidonic acid on the formation of compounds I and II. A similar profile of metabolites was obtained from the treatment of arachidonyl phosphatidylcholine with NO₂ in phosphate buffer followed by a mild alkaline hydrolysis (not shown).

Electrospray mass spectrometry of compounds I and II produced strong anions at m/z 303. Collisional activation of ion m/z 303 revealed major fragment ions at m/z 285 (loss of H₂O), 259 (loss of CO₂), and 205 (loss of C₉H₁₅) (Fig. 1). GC/MS analyses of compounds I and II (PFB derivatives) produced prominent ions at m/z 303 and revealed a characteristic pattern of peaks having retention time 0.1–0.45 min longer than the PFB ester of arachidonic acid (Fig. 2). This retention time difference corresponded to an increase of C value by 0.3–0.5 relative to arachidonic acid (21, 3). The mass spectrometric data suggested that compounds I and II contained several isomers having the molecular mass of 304 units and were likely to be isomers of arachidonic acid having altered double bond location and/or configuration. Catalytic reduction of I and II with hydrogen gas and rhodium revealed a single chromatographic peak showing a mass spectrum with an ion at m/z 311. Thus, reduction of double bonds in I and II produced a compound indistinguishable from saturated arachidonic acid, eicosanoic acid.

The location of C=C bonds in compound I was established by GC/MS analysis of DMOX derivatives. These derivatives have been useful in establishing the double bond position in fatty acids (14), including arachidonic acid (19). The DMOX derivative of compound I eluted at the relative retention time (C value) of 21.66, e.g., 0.33 units more than the DMOX derivative of arachidonic acid. The spectra contained ions characteristic for DMOX derivatives at m/z 113 (base peak) and 126, and the molecular ion appeared at m/z 357. The location of the C=C bonds was established by analysis of mass differences in a series of characteristic ions. Sequential cleavage of each of the carbon-carbon bonds directed by the ionized dimethyloxazoline moiety led to a series of ions differing by 14 units (CH₂). An advance by 0.3–0.5 units relative to compounds I and II was identified as arachidonic acid and a mixture of trans-arachidonic acids, respectively. The location of C=C bonds was established by analysis of mass differences in a series of characteristic ions. Sequential cleavage of each of the carbon-carbon bonds directed by the ionized dimethyloxazoline moiety led to a series of ions differing by 14 units (CH₂). An advance by 0.3–0.5 units relative to compounds I and II was identified as arachidonic acid and a mixture of trans-arachidonic acids, respectively. 

**Fig. 2.** Comparison of chromatograms obtained by GC/MS analyses of PFB esters of AA and products I and II via a selected ion monitoring of anion m/z 303.
identified a major product of arachidonic acid/NO₂ reaction as a mixture of four mono-trans-arachidonic acids (Scheme I).

Mass spectra of material in fractions eluting at 8–10 min (Fig. 1) revealed a compound having molecular mass of 367 units that is likely to have nitro and hydroxyl groups attached to the arachidonyl chain. Mass spectrum of a major component (PFB and TMS derivative) revealed ions at m/z 438 (M–PFB, relative abundance 100%), 391 (M–PFB-HNO₂, 2%), 348 (M–PFB-TMSOH, 38%), and 301 (m/z 348-HNO₂, 1%). Electron ionization mass spectrum of this derivative revealed characteristic ions at m/z 619 (M⁺, 1.4%), 572 (M⁺-HNO₂, 1%), 529 (M⁺-TMSOH, 10%), 232 (M⁺-NO₂CHCH(OTMS)C₅H₁₁, 9%), 173 (TMSOCH(CH₃)₂CH₂, 29%), and 181 (C₆F₅CH₂, 100%) (not shown). This spectrum was consistent with the structure of 14-nitro-15-hydroxy-eicosatrienoic acid. Minor products of the NO₂/arachidonic acid reaction were identified as having structures consistent with isomers of hydroxyeicosatetraenoic acids, EET, prostaglandin F, and nitroeicosatetraenoic acids and were not analyzed further.

Development of a sensitive quantitative assay enabled us to investigate the occurrence of trans-arachidonic acids in cells exposed to NO₂ and in vivo. Analyses of human platelets exposed to NO₂ (0.08–0.7 μM) revealed that trans-arachidonic acids were formed within platelets in a dose-dependent manner (Fig. 5). The arachidonic acid isomers from platelets coeluted with deuterium-labeled and synthetic trans-arachidonic acids standards. NO₂ induced formation of 14E-AA, and, relatively, 2.5-fold more of other mono-trans-arachidonic acids, possibly a mixture of 11E-, 8E-, and 5E-AA, products obtained from the reaction of AA and NO₂, and products obtained from the reaction of AA and PTSA.

### Table II

Comparison of mass spectra of arachidonic acid and compound I

| Compound I m/z (intensity) | Arachidonic acid m/z (intensity) |
|---------------------------|---------------------------------|
| 140 (1.1)                 | 140 (1.1)                       |
| 166 (11.8)                | 166 (10.3)                      |
| 180 (4.7)                 | 180 (3.7)                       |
| 206 (6.7)                 | 206 (6.0)                       |
| 220 (4.6)                 | 220 (4.9)                       |
| 246 (8.2)                 | 246 (6.6)                       |
| 260 (1.4)                 | 260 (1.6)                       |
| 286 (2.5)                 | 286 (2.3)                       |
| 357 (5.8)*                | 357 (3.9)*                      |

* Molecular ion.

### Scheme 1. Structures of arachidonic acid isomers.
plasma levels of trans-arachidonic acids were $50.3 \pm 10$ ng/ml ($n = 4$), whereas human urine levels were $122 \pm 50$ pg/ml ($n = 3$) (Fig. 6).

**DISCUSSION**

Oxidations of arachidonic acid by reactive oxygen radicals generate a complex family of oxidized lipids known as isoeicosanoids. Initially formed arachidonate hydroperoxy radicals or hydroperoxides have been detected as intermediate products in formation of isoprostanes and isoleukotrienes. We describe here a new unique family of free radical-generated lipids derived from NO$_2$-mediated isomerization of the arachidonic acid double bonds that does not appear to involve hydroperoxides. Formation of trans-arachidonic acids within cellular membranes is a new aspect of NO$_2$ biochemistry and may have profound influence on cellular membrane properties. Several isomers having distinct chromatographic mobility were observed and appeared to have one trans-bond and three cis-bonds. Thus, four such isomers of arachidonic acid can potentially be generated (Scheme I). Therefore, we have termed the arachidonic acid isomers having a single trans-double bond ($E$ configuration) as trans-arachidonic acids. Analytical data provided evidence that NO$_2$ changed arachidonate double bond geometry without rearrangement. Analyses by GC/MS detected one sharp peak that had the retention time identical with the synthetic 14E-AA and another, broadened peak that appeared to contain several components and coeluted with a mixture of trans-arachidonic acids prepared from epoxyeicosatrienoic acids and triphenylphosphine. NO$_2$ also caused formation of smaller amounts of material (compound II) that probably contained arachidonate isomers having more than one trans-bond. Although the relative proportion of the arachidonate trans-isomers remains to be established, the 14E-AA was separated from arachidonic acid and other trans-isomers on a gas capillary column. This isomer cochromatographed with two synthetic standards, and comparison of the retention times allowed identification of 14E-AA as a product from the reaction of NO$_2$ with arachidonic acid. We observed that the profile of trans-arachidonic acids produced by NO$_2$ was nearly identical to that from the reaction of arachidonic acid with PTSA, a reagent known to induce cis-trans-isomerization of olefins that does not rearrange double bonds (21).

Several mechanisms may be involved in the formation of a trans-bond in arachidonic acid. The similarity with PTSA product profile suggested that generation of the trans-arachidonic acids by NO$_2$ may occur via a free radical mechanism. It is possible that NO$_2$ initially attaches to a double bond and forms a nitroarachidonyl radical. The rearrangement of this radical followed by elimination of NO$_2$ is likely to form a trans-bond (Fig. 7, arrow a). One piece of evidence supporting formation of the nitroarachidonyl radical comes from detection of 14-nitro-15-hydroxyeicosatrienoic acid among the products of the NO$_2$/arachidonic acid reaction (Fig. 7). This compound may originate from trapping of oxygen to the nitroarachidonyl radical or from attachment of the second molecule of NO$_2$. Hydrolysis of such a nitro nitrite intermediate would produce a nitro alcohol (Fig. 7, arrow b). This mechanism appears to be more likely because we noticed that nitrohydroxyarachidonic acids can be isolated without reduction of samples by sodium borohydride. The NO$_2$-mediated isomerization of arachidonic acid appeared to be an efficient process and exceeded formation of hydroperoxyeicosatetraenoic acids, which accounted for only $5\%$ of total products. In aerobic solutions the isomerization must compete with scavenging by oxygen. For the trans-isomers to be formed at observed yields, the rate of rotation of the ni-
troarachidonyl radical and elimination of NO₂ would have to be greater than attachment of oxygen to this radical. In addition, these reactions must be faster than disproportionation of NO₂. According to the work by Prütz et al. (22), NO₂ generates arachidonate radicals with a rate (−10⁶ m⁻¹ s⁻¹) that is greater than the disproportionation to nitrite and nitrate. Thus, NO₂-induced cis-trans-isomerization of arachidonic acid is kinetically favorable in aerobic aqueous solutions.

Studies describing the effects of NO₂ on living systems have focused almost exclusively on the toxicity of inhaled NO₂ (7). Many of these studies have established that the toxic effects of NO₂ could be correlated with increased lipid peroxidation. Our findings suggest that formation of trans-arachidonic acids within cellular phospholipids may represent an additional aspect of NO₂-induced toxicity. Increased levels of trans-arachidonic acids could contribute to changes of the membrane asymmetry and fluidity that have been noted to occur following exposure to NO₂ (23, 24). Fatty acids with trans-bonds are known to have much different physical properties, e.g., a higher melting point than analogous cis-isomers. Our data suggest that isomerization of arachidonic acid is likely to occur in biological systems following exposure to NO₂, which at low concentrations exists almost exclusively as a monomer (22). In addition to changes of the membrane biochemistry, the free trans-arachidonic acids are likely to modulate the activity of cyclooxygenases and lipoxygenases. A recent study has described inhibition of platelet aggregation by 14E- AA that coincides with inhibition of thromboxane synthesis and generation of unique metabolites (25).

Although the origin of trans-arachidonic acids in human urine remains to be determined, by analogy to the cyclooxygenase derived prostaglandins as well as isoprostaglandins, these compounds may derive at least in part from local formation in the kidney. trans-Arachidonic acids have not been detected in human plasma previously, and their rather high concentration warrants further study. It has been known that trans-linoleic acids that are found in processed foods, hydrogenated fats, and dairy products could be desaturated and elongated to certain trans-arachidonic acids in the rat liver (19). A diet enriched in fatty acids containing trans-isomers has been suspected as a risk factor in coronary artery disease and other disorders (26); however, the effects of trans-fatty acids on health outcome are not fully understood (27, 28). In particular, the possibility that trans-arachidonic acids are formed within cells via a free radical mechanism involving NO₂ has not been explored. Increased amounts of trans-isomers of arachidonic acid and possibly of other polyunsaturated fatty acids may originate from inhaled as well as endogenously formed NO₂. Because trans-fatty acids are not produced by the hydroxyl radical, the detection and quantification of trans-arachidonic acids in vivo may be used as a specific index to assess the degree of cellular injury mediated by NO₂. The present study provides a basis for such an investigation. Studies into the mechanisms of cellular activation and trans-arachidonic acids may clarify their importance in vivo in syndromes such as inflammation, thrombosis, and ischemia-reperfusion injury in which damage to cellular membrane phospholipids coincides with oxidant stress.

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