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Mass Spectrometric Analysis of Oxidized Eicosapentaenoic Acid Sodium Salt

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Abstract: Eicosapentaenoic acid (EPA) is an omega-3 polyunsaturated fatty acid (PUFA) with 20 carbon atoms and 5 carbon-carbon double bonds. Mammalian cells cannot synthesize long chain PUFAs such as EPA de novo, and, thus, the most effective way to enrich cells in EPA is by dietary intake of fish oils. EPA supplementation causes an increase in its concentration in plasma lipids and in cell membrane phospholipids. Many beneficial effects of EPA supplementation have been noted, including (1) the potential to sensitize cancerous tumors towards chemotherapy, (2) the promotion of cardiovascular health, and (3) the alleviation of some mental disorders, but results from clinical trials have sometimes been disparate. In this study, we report the use of mass spectrometry to investigate the autoxidation of EPA, thereby demonstrating the formation of a variety of oxidized products. The oxidative stress of the patient may affect the response to EPA and may, in part, explain divergent results from clinical trials.

Keywords: eicosapentaenoic acid, oxidation, mass spectrometry
Introduction

Eicosapentaenoic acid (EPA) is an omega-3 polyunsaturated fatty acid (PUFA) with 20 carbon atoms and 5 carbon-carbon double bonds and is designated as 20:5 (ω-3) (Fig. 1). Mammalian cells cannot synthesize long chain PUFAs de novo, although EPA can be synthesized from the essential fatty acid, α-linolenic acid (ALA), 18:3 (ω-3), but conversion is minimal. The most effective way to enrich cells in EPA is by dietary intake of fish oils.

There is an active interest in understanding the effects of dietary supplementation of EPA as evidenced by the number of trials exploring the use of this substance in improving human health. For instance, with respect to cancer, marine-derived lipids, such as EPA, have the potential to sensitize tumors toward chemotherapy and enhance the cytotoxicity of certain anti-cancer drugs while preserving (or possibly) enhancing the integrity of non-tumor tissue. PUFAs are known to increase the presence of reactive oxygen species in cancer cells and may contribute to improving neutrophil function thus enhancing the action of the innate immune system. Further studies, however, are necessary to understand the mechanism whereby PUFAs selectively sensitize tumor cells but not normal tissue toward chemotherapeutic drugs. Certain cancers also lead to muscle loss or wasting (known as cancer cachexia), with patients exhibiting reduced levels of plasma omega-3 lipids that continue to decline over the course of the disease. In this case, ongoing studies and trials indicate that fish oil supplements are beneficial.

There is also evidence from clinical trials and randomized controlled trials that omega-3 fatty acid supplementation decreases cardiovascular events and the risk of cardiac death. The exact mechanisms whereby omega-3 fatty acids contribute to decreasing cardiovascular events are not clear, although effects such as plaque stabilization, lipid profile alteration, triglyceride reduction, blood pressure reduction, inhibition of inflammation, or reduction in arrhythmia have been suggested. While the general trend might be that these oils are beneficial, other data indicate either insufficient evidence or even an increased risk of cardiac death among patients advised to take oily fish. The reasons for these discrepancies are not clear, although potential concerns have been raised with regard to the quality and rigor of some trials and the fact that in some studies, patients were already receiving cardioprotective agents, for example, statins, such that further supplementation with an omega-3 fatty acid may not have had an impact.

The effects of EPA supplementation have also been investigated in patients with schizophrenia and other mental health-related disorders. Certain lipids are integral to the brain and significant membrane abnormalities have been identified in the brains of schizophrenic patients. The enzyme phospholipase A (PLA2), which releases arachidonic acid, 20:4 (ω-6) from membrane phospholipids is thought to be overactive in schizophrenic brains. EPA supplementation may be beneficial since EPA may compete with arachidonic acid for incorporation into membrane phospholipids and thus inhibit the enzyme. While a pilot study indicated that EPA is beneficial in the treatment of schizophrenia, a more recent meta-analysis of EPA data does not indicate a favorable role in schizophrenia and related psychological disorders. However, there may be confounding effects in previous trials since a differential response to EPA may occur depending on the history of pharmacological intervention, and an overall conclusion may not be available as yet.

The role of EPA may be controversial, but the indications are that EPA supplementation has, in some cases, shown promise in ameliorating or preventing several conditions. Therefore, it becomes important to understand the mechanisms of action. However, before biological mechanisms can be fully interpreted, it is essential to appreciate the chemical nature of EPA and the transformations that EPA can undergo. EPA shows reactivity toward oxygen, and is known to autoxidize readily. In this study, we investigated the autoxidation of EPA by mass spectrometry and report a number of oxidized products and their possible assignments. In this regard, we have reviewed some of the schemes involving the autoxidation of EPA.

Figure 1. Structure of eicosapentaenoic acid (EPA), 20:5 (ω-3).
Note: Positions 7, 10, 13, and 16 indicate bis-allylic (methyleneic) hydrogen locations.
Methods

Materials

The sodium salt of cis-5,8,11,14,17-eicosapentaenoic acid (EPA, ≥99% purity) was purchased from Sigma Aldrich (St. Louis, MO). The sodium salt of EPA was stored prior to use as received (under nitrogen) in the solid form at −80 °C. Henceforth, the sodium salt of EPA is referred to as “EPA.” Several portions of EPA (0.5 mg) in glass vials (approximately 2 cm in diameter and 6 cm in height) were allowed to oxidize in air at ambient temperature (22.2 °C–25.4 °C) over 2 to 4 days under conditions of ∼12 hours light and ∼12 hours dark. At the time of analysis, EPA was dissolved in molecular grade water (1 mL) from Cellgro (distilled; deionized; DNase, RNAse, and protease tested). While the sodium salt of EPA has limited solubility, we found that the amounts used (i.e. 0.5 mg/mL water) were fully soluble. Stock solutions were further diluted with molecular grade water before introduction to the mass spectrometer. When analyzing fresh EPA (i.e. when recording control mass spectra of the sodium salt of EPA), all manipulations were performed under nitrogen gas. In this regard, EPA was handled under nitrogen gas and dissolved in molecular grade water that had previously been purged with nitrogen.

Mass spectrometry

Native EPA and air-oxidized EPA samples were prepared as indicated above and dissolved in molecular grade water (0.17 mM). Samples were analyzed using an Applied Biosystems MDS SCIEX API 2000 instrument (AB SCIEX, Framingham, MA). The mass spectrometer was operated in negative ion mode with a mass range of m/z 250 to 600 amu, using a declustering potential (DP) of −60.0 V, a focusing potential (FP) of −400.0 V, and an entrance potential (EP) of −100.0 V. Nitrogen was used as both the sheath gas and the auxiliary gas. Data acquisition and analysis were performed using Analyst software, version 1.4. Samples were introduced via syringe injection at a flow rate of 10.00 μL/min. Spectra (100 cycles) were accumulated over a 5-minute period.

Results

Mass spectral analysis of autoxidized EPA

The sodium salt of EPA, a white powder, oxidizes readily in air as evidenced by a change in color to a yellow solid within a short period of time. When this process was monitored by mass spectrometry, several new species were observed within 0 to 4 days of exposure to air (Fig. 2). Mass spectral analysis of fresh native EPA gives rise to a main peak at 300.9 m/z (exact mass of acidic form of EPA = 302.2 amu), which corresponds to the deprotonated anion, that is, (exact mass-H)/z. The main peak at 300.9 m/z displays the correct isotopic pattern, giving two shoulders at 302.1 m/z (exact mass = 303.2 amu) and 303.1 m/z (exact mass = 304.2 amu) (Fig. 2, blue spectrum). While care was taken to exclude oxygen, some small peaks mainly centered at 317.0 m/z and 333.1 m/z were also observed, indicating that either the EPA contains some oxidized product, or else a small amount of oxidation occurred during the handling process. However, mass spectrometric analysis of several samples of fresh native EPA consistently revealed small amounts of oxidized product.

Oxidation over several days revealed the formation of species with higher masses (Fig. 2A–C). For example, after 2 days of air oxidation, small clusters of ions centered at 317.0, 333.1, 348.9, and 365.0 m/z were observed to increase with a corresponding decrease of native EPA at 300.9 m/z (Fig. 2B, red spectrum). After 4 days of air oxidation, new species clustered at 380.7 m/z and 396.7 m/z appeared, accompanied by a decrease of parent EPA (300.9 m/z) together with a decrease in ions centered at 317.0 and 331.1 m/z (Fig. 2B, green spectrum). Notably, the average difference in mass between the peaks of greatest intensity in each cluster group is 15.97 ± 0.07 amu, corresponding to mass of an oxygen atom. Thus, the groups of ions centered at 317.0, 333.1, 348.9, 365.0, 380.7, and 396.7 m/z represent, respectively, the addition of 1, 2, 3, 4, 5, and 6 oxygen atoms to native EPA. The facts that (1) the relative intensities of these peaks changed with time and (2) higher masses were observed after longer exposures to air indicate that their presence is real and not an artifact of the mass spectrometric process. In some cases, where the peaks representing oxidized EPA ions are larger, it is possible to discern an isotopic pattern (which is also clearly apparent in non-oxidized EPA at 300.9 m/z).

While each cluster group can be ascribed to an ion having 1 to 6 additional oxygen atoms compared with the parent ion, it also appears that species within
Figure 2. Air oxidation of EPA leads to products of increased mass. Mass spectra of (A) native EPA (blue), and EPA oxidized in air for 2 and 4 days (red and green spectra, respectively). Native EPA gives a peak at 300.9 m/z (amu). (B) Magnified view of the same mass range as (a), with the mass of the dominant peak in each cluster group identified as the addition of 1, 2, 3, 4, 5, or 6 oxygen [O] atoms to EPA. (C) Magnified view of the 325–365 m/z range, highlighting the separation of peaks in each cluster of ions by 2.05 ± 0.04 (amu), representing the loss or gain of 2 hydrogen atoms. Peaks may also represent ions formed upon loss of water as, for instance, indicated for a species of mass 365.0 m/z losing H₂O to yield a species of mass 346.8 m/z.
each cluster group are separated by $2.05 \pm 0.04$ amu (Fig. 2C). This difference represents either an incremental gain or loss of 2 hydrogen atoms. While autoxidation pathways are covered in more detail below, possible mechanisms could include ring-opening of cyclic peroxides resulting in the formation of hydroxy compounds (i.e. gain of 2 hydrogen atoms) or, conversely, keto formation from an alcohol (i.e. loss of 2 hydrogen atoms). Loss of water from compounds could also result (i.e. loss of 18 amu).

The species that result from autoxidation of EPA appeared and disappeared at different rates, as shown by plots of peak intensity versus time (Fig. 3). The peak intensity of the parent EPA ion decreased over 4 days (Fig. 3A). Several species in the cluster groups with 1 or 2 oxygen atom additions

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Variation of peak intensity with time. Peak intensities are monitored over time (0–4 days) to indicate the rate at which ions appear or disappear. (A) Parent EPA ion at 300.9 m/z and the cluster group of ions with (B) 1 oxygen [O] atom addition, (C) 2 oxygen atoms, (D) 3 oxygen atoms, (E) 4 oxygen atoms, (F) 5 oxygen atoms, and (G) 6 oxygen atoms.
(designated as EPA + 1[O] and EPA + 2[O] in Fig. 3B and C, respectively) also disappeared over 4 days. Conversely, some species (indicated by peaks at 312.9, 326.8, 328.8, and 330.8 m/z) were formed during this time, perhaps from precursors that are being reduced in intensity. The clusters of peaks representing the addition of 3, 4, 5, and 6 oxygen atoms all increased with time (Fig. 3D–G). Notably, the higher-mass species (e.g. EPA + 5[O] and EPA + 6[O]) only started to appear after 2 days, indicating that the higher-mass oxidized species are formed from the lower-mass oxidized species and that oxygen addition is a sequential process.

Comparison of the total intensity of peaks in the 290 to 450 m/z region shows that ~30% of the total intensity is lost after 2 days and is further reduced after 4 days (~50% that of the original spectrum). While a comparison of total intensity may not provide a consistent method of comparing spectra, the indication is that there is an overall loss of intensity. Although the loss of intensity is not fully understood, it is possible that some of the species formed are not detected by the mass spectrometric process or else occur at masses outside the region shown. Although oxidized EPA appears soluble giving rise to clear, yellow solutions, it is also possible that the solubility of some of the oxidized species is limited.

**Discussion**

In this study, we sought to identify the types of products formed when the sodium salt of EPA is autoxidized in air at ambient temperature. To the best of our knowledge, this represents the first study in which the autoxidation process was allowed to occur in the solid phase in the presence of air and light but in the absence of any solvent or exogenously-added peroxyde/free-radical initiating species. Previous mass spectrometric studies of in vitro oxidized EPA products have involved the addition of free-radical initiating compounds to EPA dissolved in organic solvents. Gas chromatography coupled with mass spectrometry (GC-MS) provides a sensitive method for characterizing products of PUFA oxidation, but often requires the derivatization of products prior to analysis. To prevent additional modification that may occur during the GC-MS process, an alternative method may be used that utilizes liquid chromatography to separate compounds before being introduced to the ion source and characterized by tandem mass spectrometry (LC-MS/MS). These methods have previously been successfully applied to profiling oxidized lipids, for instance, in plasma and urine samples. In the current study, we applied electrospray ionization mass spectrometry to the direct observation of oxidized EPA species.

Our results indicated that species containing up to 6 oxygen atoms result from air oxidation (Fig. 2). In addition to these species, we also observed ions that were separated by 2.05 ± 0.04 amu from the main peak of each cluster group (and, in some cases, by 18 amu between peaks of different cluster groups). In order to make potential assignments, it is necessary to consider schemes of oxygen addition and autoxidation, as described below.

**Basic products of EPA autoxidation**

Unsaturated fatty acids are susceptible to oxidation due to the fact that the formation of a free radical on a carbon atom located between 2 carbon-carbon double bonds (otherwise known as a bis-allylic position) is remarkably stable. Thus, it is relatively easy to remove a hydrogen atom from EPA to produce a radical that can interact with an oxygen molecule. EPA has four bis-allylic moieties, centered at C-7, C-10, C-13, and C-16 (Fig. 1).

Scheme 1 illustrates a mechanism of EPA oxygenation, which is based on the mechanism of arachidonic acid oxygenation. EPA is similar in structure to arachidonic acid in that it has a 20-carbon backbone but, unlike arachidonic acid, EPA has 5 carbon-carbon double bonds (versus 4 in arachidonic acid) and is an omega-3 fatty acid (rather than arachidonic acid which is an omega-6 fatty acid). Due to their similarities in structure, similar mechanisms may apply to oxygenation of both EPA and arachidonic acid. Lipid oxidation can be initiated by certain transition metals, such as iron, but physiologically, lipid oxidation may occur enzymatically in a selective manner. For instance, oxygenation of arachidonic acid by the cyclooxygenase enzymes (COX-1 and COX-2) initiates the cascade leading to prostanoid (PG) formation. Oxygenation by COX occurs when arachidonic acid lies in the cyclooxygenase channel with the C-13 position adjacent to tyrosine-385 in the polypeptide backbone. The catalytic process involves tyrosine cation radical production,
Physiologically, EPA oxidation is expected to occur enzymatically but, under situations of oxidative stress, nonenzymatic routes may also occur in living systems. EPA is prone to peroxidation and can be oxidized nonenzymatically, often resulting in prostaglandin-like substances, called isoprostanes.

When only lipid radicals and oxygen are involved, the process is known as “autoxidation,” but similar mechanisms may be involved in both enzymecatalyzed lipid oxidation and autoxidation. Thus, Scheme 1 illustrates a mechanism of EPA oxygenation with initial hydrogen abstraction occurring at the C-13 position. Rearrangement leads to radical formation at the C-11 position with subsequent radical trapping of oxygen, followed by the formation of a 5-membered endoperoxide ring, cyclization, further rearrangement and a second oxygenation at C-15. Reduction results in the production of F₃ isoprostane (also known as PGF₃α).

An important difference between enzyme-catalyzed lipid oxygenation and autoxidation is that enzyme-directed oxygenations are stereospecific. Autoxidation of arachidonic acid is well elucidated and can occur at different bis-allylic positions. In this regard, autoxidation of EPA can, in principle, be initiated at any of the C-7, C-10, C-13, or C-16 sites. Novel oxidation products of EPA have previously been identified using mass spectrometric techniques including Ag⁺ coordination ionspray and atmospheric pressure chemical ionization mass spectrometry. Importantly, these oxidation products were observed both in vitro and in vivo. Scheme 2 illustrates the collection of hydroperoxyeicosapentaenoic acid (HpEPE) and hydroxyeicosapentaenoic (HEPE) isomers that could form following initiation at the 4 different bis-allylic positions. According to Scheme 2, the peaks observed at 317.0 and 333.1 m/z (Fig. 2) represent the addition of 1 and 2 oxygen atoms to EPA, respectively, and can potentially be ascribed to HEPE and HpEPE isomers of EPA (exact masses = 318.2 amu and 334.2 amu, respectively).

Monocyclic and serial cyclic peroxide formation
Monocyclic and serial cyclic peroxide products of EPA have previously been investigated. Scheme 3A represents the type of monocyclic (1,2-dioxolane) and serial cyclic peroxides that can, in principle, form which is responsible for stereoselective abstraction of the 13-pro-S hydrogen from the C-13 position and thus initiating oxygenation. EPA can readily replace arachidonic acid in the lipid bilayer and act as a substrate for the COX enzymes.
when initial hydrogen abstraction occurs at C-13, followed by rearrangement to yield a carbon radical at C-11 to allow initial oxygen addition, that is, (1) to (3) in Scheme 1. In this particular example, EPA can potentially gain 4 oxygen atoms (monocyclic peroxide), or else peroxidation can go further yielding a structure with 6 oxygen atoms (serial cyclic peroxide).

The position of initial hydrogen abstraction and subsequent location of the carbon radical (with respect to its proximity to carbon-carbon double bonds)
determine the extent of peroxidation and dioxolane formation. For instance, structures are possible with only 2 oxygen atom additions (i.e., when oxygen first adds to a carbon radical on either C-5 or C-18) or up to 8 oxygen atom additions (i.e., when oxygen first adds to C-9 or C-14 radicals). For example, hydrogen abstraction at C-7 leading to a radical on C-9 allows addition of 4, 6, or 8 oxygen atoms, as shown in Scheme 3B. When hydrogen abstraction at C-7 instead leads to radical formation on C-5, only 2 oxygen atom additions are possible with the result that 5-HpEPE is formed.

In summary, depending on the position of carbon radical formation, it is possible to form HpEPEs (2 oxygen atoms; exact mass = 334.21 amu), monocyclic peroxides (4 oxygen atoms; exact mass = 366.20 amu) and serial cyclic peroxides containing 2 dioxolane rings (6 oxygen atoms, exact mass = 398.19 amu).
Eicosanoids that are produced with arachidonic acid formed, with products containing 3 double bonds. When COX metabolizes EPA, the PG-3 series is formed, with products containing 3 double bonds. However, when COX metabolizes EPA, the PG-3 series is formed, with products containing 3 double bonds. EPA can readily replace arachidonic acid in the lipid bilayer and act as a substrate for the COX enzymes. When arachidonic acid is the COX substrate, the prostaglandin (PG-2) series of products is formed, where the number 2 corresponds to the number of carbon-carbon double bonds in the product. However, when COX metabolizes EPA, the PG-3 series is formed, with products containing 3 double bonds. Eicosanoids that are produced with arachidonic acid as the initiating substrate are often associated with proatherogenic and proinflammatory properties. In contrast, eicosanoids derived from EPA are associated with less biological activity and therefore have less of an impact on the process of inflammation or proliferation. For instance, oxidized EPA significantly inhibited human neutrophil and monocyte adhesion to endothelial cells by preventing expression of the endothelial adhesion receptor via a mechanism involving activation of the peroxisome proliferator-activated receptor α (PPARα) and subsequent inhibition of NF-κB. 

Scheme 5 indicates that following bicyclic endoperoxide (PGG₃) formation, reduction occurs forming PGH₂, which can then be converted to several different types of eicosanoids, depending on the available enzymes. In the presence of PGE synthase (PGES) or PGDS, the products PGE, and PGD, can form. Interestingly, it has previously been noted that both PGE₂ and PGD₂ (the analogous products of arachidonic acid metabolism) can form independent of COX. In general, although the products that result from enzymatic catalysis and autoxidation may be similar, there may be important distinctions in cis-trans orientations of side chains with respect to the prostane ring, with non-enzymatic routes favoring racemic mixtures. However, for COX-independent formation of PGE₂ and PGD₂, the products were found to be identical to COX-derived PGE₂ and PGD₂. Thus, it may be possible for PGE₃ and PGD₃ to result from autoxidation. PGA₂ and PGJ₁ (not shown) result from dehydration of PGE₃ and PGD₃, respectively. Interestingly, it has previously been found that incubation of EPA with free-radical generating compounds in ethanol leads to the identification by mass spectrometry of PGA₂ and PGJ₁-like products (termed A₃/J₃ isoprostanes). Thus, products similar to PGA₂ and PGJ₁ may also result during autoxidation.

Further products requiring enzymatic catalysis include PGI₁ (catalyzed by prostacyclin synthase), which is likely unstable and converts to 6-keto-PGF₃α (as PGI₁ converts readily to 6-keto-PGF₁α). Importantly, the arachidonate-derived PGI₁ is a potent vasodilator and inhibitor of platelet aggregation. Platelet-derived thromboxane synthase would convert PGH₂ to thromboxane B₂, the stable hydrolysis product of TxA₂. Notably, the arachidonate-derived

**Hydrogen abstraction at multiple bis-allylic carbon positions**

Hydrogen abstraction that occurs at multiple bis-allylic carbon positions in EPA can result in structures that contain multiple hydroxy and/or peroxyl adducts. This is illustrated in Scheme 4 for 5-HpEPE that results from initial hydrogen abstraction at C-7. Subsequent removal of a hydrogen atom from C-10 results in diHpEPE, which has another bis-allylic position at C-16 that can undergo further hydrogen abstraction. The resulting structures include triHpEPE and triHEPE. The triHEPE compounds are also known as resolvins and have been found to have anti-inflammatory properties. Notably, in human endothelial cells, aspirin therapy causes COX-2 to switch its catalytic activity from forming prostanoids to producing specific HEPEs and novel trihydroxy-containing resolvins. In particular, 5,12,18-triHEPE, also known as RvE1, has been associated with having potent anti-inflammatory and analgesic properties.

**Products of enzymatic oxygenation versus autoxidation**

EPA can readily replace arachidonic acid in the lipid bilayer and act as a substrate for the COX enzymes. When arachidonic acid is the COX substrate, the prostaglandin (PG-2) series of products is formed, where the number 2 corresponds to the number of carbon-carbon double bonds in the product. However, when COX metabolizes EPA, the PG-3 series is formed, with products containing 3 double bonds. Eicosanoids that are produced with arachidonic acid
**TxA2** has properties completely opposed to **PGI2**, in that it is a potent inducer of platelet aggregation and vasoconstrictor. A diet rich in EPA has been found to lead to both **PGI2** and **TxA2** production and a concomitant reduction in thrombogenic **TxA2**.40

**Possible assignments of EPA autoxidation products**

Possible assignments of EPA autoxidation products observed by mass spectrometry are listed in Table 1. Compounds, outlined in Schemes 1–5, that result from
non-enzymatic oxidation of EPA include HEPEs, HpEPEs, monocyclic peroxides, bicyclic endoperoxides, serial cyclic peroxides, and potentially a series of compounds with several hydroxyl or peroxyl moieties are included as possible products. Table 1 shows that several isomers are possible for a single peak (and not all possible isomers are considered). Other species were also noted that are incrementally $2.05 \pm 0.04$ amu lower or higher in mass than the main ions in each cluster group representing a gain of $1–6$ oxygen atoms (Fig. 2). This mass is consistent with 2 hydrogen atoms. Possible mechanisms that involve a gain of
Table 1. Examples of possible assignments of mass spectrometric peaks.

<table>
<thead>
<tr>
<th>Observed mass (anion) m/z (amu)</th>
<th>Formula</th>
<th>Possible assignments of the neutral form</th>
<th>Exact mass (amu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300.9 C_{20}H_{30}O_{2}</td>
<td>EPA (1)</td>
<td>1 oxygen addition</td>
<td>302.22</td>
</tr>
<tr>
<td>317.0 C_{20}H_{30}O_{3}</td>
<td>HEPE (Scheme 2) (9), (11), (13), (15), (17), (19), (21), (23)</td>
<td>318.22</td>
<td></td>
</tr>
<tr>
<td>330.8 C_{20}H_{28}O_{4}</td>
<td>PGA, (PGJ, (Scheme 5) (43)</td>
<td>2 oxygen additions</td>
<td>332.20</td>
</tr>
<tr>
<td>333.1 C_{20}H_{30}O_{4}</td>
<td>HpEPE (Scheme 2) (8), (10), (12), (14), (16), (18), (20), (22)</td>
<td>334.21</td>
<td></td>
</tr>
<tr>
<td>348.9 C_{20}H_{30}O_{5}</td>
<td>Hydroxy-HpEPE (Scheme 4) (31)</td>
<td>3 oxygen additions</td>
<td>350.21</td>
</tr>
<tr>
<td>351.0 C_{20}H_{28}O_{5} F_{3}</td>
<td>triHEPE (Scheme 4) (37)</td>
<td>4 oxygen additions</td>
<td>352.22</td>
</tr>
<tr>
<td>365.0 C_{20}H_{30}O_{6}</td>
<td>Bicyclic endoperoxide, PGH_{3} (Scheme 1) (6)</td>
<td>5 oxygen additions</td>
<td>366.20</td>
</tr>
<tr>
<td>380.7 C_{20}H_{30}O_{7}</td>
<td>Hydroxy-dihpEPE (Scheme 4) (36)</td>
<td>6 oxygen additions</td>
<td>382.20</td>
</tr>
<tr>
<td>396.7 C_{20}H_{30}O_{8}</td>
<td>Serial cyclic peroxide (Scheme 3) (25), (27)</td>
<td></td>
<td>398.19</td>
</tr>
</tbody>
</table>

2 hydrogen atoms include ring-opening of cyclic peroxides resulting in hydroxyl compounds or, conversely, keto formation from an alcohol involves a loss of 2 hydrogen atoms. Dehydration could also contribute, as this would result in a loss of 18 amu.

Conclusion

Lipid peroxidation can be a function of physiological as well as pathophysiological processes. Dissecting which processes are the result of normal function versus those that are the result of disease and aging might be difficult. Given that enzymatic processes might also be accompanied by lipid peroxidation by non-enzymatic routes, it may be possible that, given their oxidative stress level, patients may differ in their response to EPA and thus give rise to divergent results in clinical trials. Given the propensity of EPA to react with oxygen in the absence of exogenously added free-radical initiators, it is important that patient populations receive the same quality of EPA to ensure that results from clinical trials are comparable. However, it may be possible that differential responses to EPA may still occur given the oxidative stress level of the patient.

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Author Contributions

Conceived and designed the experiments: RKU. Analyzed the data: RKU, KDJ. Wrote the first draft of the manuscript: RKU. Contributed to the writing of the manuscript: RKU, KDJ. Agree with manuscript results and conclusions: RKU, KDJ. Jointly developed the structure and arguments for the paper: RKU, KDJ. Made critical revisions and approved final version: RKU, KDJ. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.
Disclosures and Ethics
As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests. Provenance: the authors were invited to submit this paper.

References
1. Anderson BM, Ma DW. Are all n-3 polysaturated fatty acids created equal? Lipids Health Dis. 2009;8:33.

