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A B S T R A C T

Cyclic oxygenated metabolites are formed in vivo through non-enzymatic free radical reaction of n-6 and n-3 polyunsaturated fatty acids (PUFAs) such as arachidonic (ARA C20:4 n-6), adrenic (AdA 22:4 n-6), α-linolenic (ALA 18:3 n-3), eicosapentaenoic (EPA 20:5 n-3) and docosahexaenoic (DHA 22:6 n-3) acids. These cyclic com-pounds are known as isoprostanes, neuroprostanes, dihomo-isoprostanes and phytoprostanes. Evidence has emerged for their use as biomarkers of oxidative stress and, more recently, the n-3PUFA-derived compounds have been shown to mediate bioactivities as secondary messengers. Accordingly, this review will focus on the cy-clic oxygenated metabolites generated from AdA, ALA, EPA and DHA. This article is part of a Special Issue entitled "Oxygenated metabolism of PUFA: analysis and biological relevance".

Keywords:
Phytoprostanes
Dihomo-isoprostanes
Neuroprostanes
Isofurans
Biomarkers
Bioactive lipids

1. Introduction

Free radicals are implicated in a wide variety of human diseases [1] and the consequences are the oxidation of biomolecules, including DNA, proteins and lipids. Among lipids, polyunsaturated fatty acids (PUFAs) have reactive skipped dienes, or methylated interrupted double bonds, that can participate to form acyclic, and subsequently, stable cyclic oxygenated metabolites [2]. Since the discovery of F_2-isoprostanes (F_2-IsoPs) by Morrow et al. in 1990 derived from arachidonic acid (ARA, 20:4 n-6) peroxidation in vivo [3], an important field of research has been developed. Nowadays, elevation of F_2-IsoP levels in biological fluids (e.g. plasma and urines) is recognized as the reference biomarker for lipid peroxidation and oxidative stress [4]. Because of the high reactivity and short life span of free radicals, oxidative stress is evaluated by the measurement of damaged biological products, which can be considered as biomarkers of lipid peroxidation. Beyond their capacity of oxidative stress evaluation, isoprostanes also demonstrate to be biologically active [2,5,6].

Docosahexaenoic (DHA, 22:6 n-3) and eicosapentaenoic (EPA, 20:5 n-3) acids, the main n-3 PUFAs, form neuroprostanes (NeuroPs) [7,8] and EPA-derived isoprostanes [9], respectively, under free radical reactions. Also, the n-6 PUFA adrenic acid (AdA, 22:4) located in brain white matter and other tissues, such as the adrenal gland and kidney, is the precursor of dihomo-isoprostanes (dihomo-IsoPs) [10]. Finally α-linolenic acid (ALA, 18:3 n-3) from plants may be converted into phytoprostanes (PhytoPs) [11].

This review will focus on the isoprostanes, neuroprostanes, dihomo-isoprostanes and phytoprostanes generated from ALA, AdA, EPA and DHA with regard to (i) the synthesis of these new cyclic oxygenated metabolites of PUFA, (ii) the use of such lipid metabolites as biomarkers of oxidative stress in humans, and (iii) data relative to their biological activities in vitro and in vivo.

2. Biosynthesis of cyclic oxygenated metabolites of PUFA

In 1990, Roberts, Morrow and co-workers discovered novel prostaglandin (PG)-like isomers, which are named isoprostanes. In contrast to PG produced by cyclooxygenases, the mechanism of formation proceeds via a non-enzymatic free radical peroxidation of ARA esterified in phospholipids and not from free ARA [3]. The main structural characteristics compared to PGs are the *cis*-relationship of the side chains, the

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large number of potential isomers and the generation of racemic metabolites [12,13]. Once formed, the isoprostanes can then be released by phospholipases in the circulating fluids [14] (Scheme 1). Later, it was uncovered that PUFAs such as ALA, EPA, AdA and DHA having at least two-skipped diene units, i.e. three consecutive methylene interrupted units, can form isoprostane-like structures [2] (Scheme 2). In plants, ALA can generate PhytoPs [11] which were originally named dinor-IsoPs. DHA and EPA, the main n-3 PUFAs found in humans, release NeuroPs [7,8] and isoprostanes respectively, when subjected to free radical reactions [9]. Another important n-6 PUFA, AdA, highly present in brain white matter, is known to generate dihomo-IsoPs [10].

Similar to the biosynthesis of PGs, the intermediate G-IsoPs can lead to numerous types of IsoPs. Besides the F-type, E-, D-, A-, B-, and L-types (see ref. [2]) of IsoPs, PhytoPs and NeuroPs are also formed in vivo depending on the surrounding conditions of the membrane phospholipids (Scheme 3) [2].

In 2002, a completely novel structure of compounds was uncovered that follows the same free radical cascade pathway of IsoPs. The addition of molecular oxygen after initial cyclization leads to the generation of isofurans (IsoFs) from ARA [15]. It was shown that IsoF levels augment in elevated oxygen conditions (above 21%) while not so for IsoPs. Similar mechanisms are proposed to access such biosynthesis [2], including neurofurans (Scheme 4) [16,17] (NeuroFs) and dihomo-Isofurans [18] (dihomo-IsoFs) from DHA and AdA respectively.

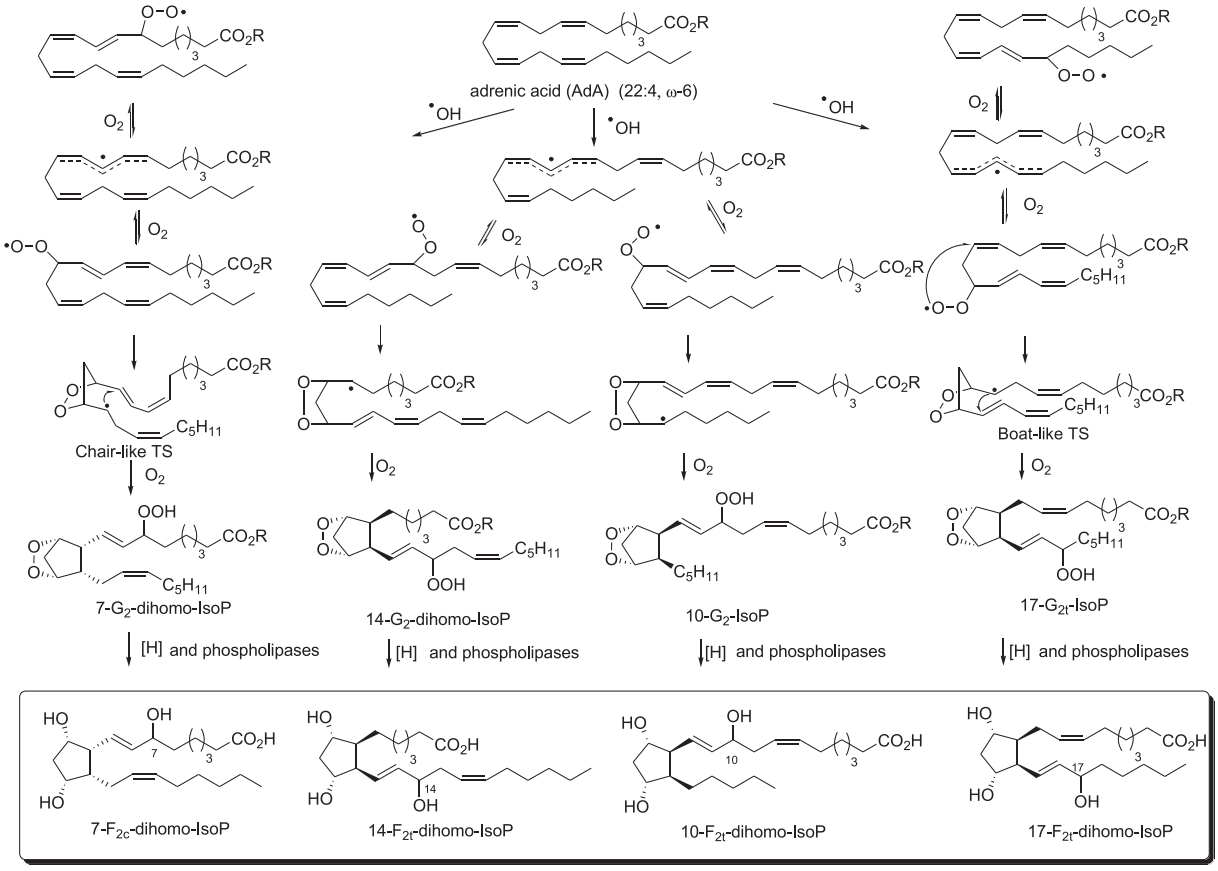
3. Chemical synthesis of cyclic oxygenated metabolites

The total synthesis of cyclic oxygenated metabolites is of greatest importance for the general understanding of their in vivo formation and biological functions, but also to explore their potential diagnostic applications. Different strategies to produce PhytoPs, IsoPs, dihomo-IsoPs and NeuroPs have been reported in the literature by organic chemists around the world (see few reviews [2,19,20]). All these strategies developed so far confirmed the biological importance of IsoPs, NeuroPs, and recently PhytoPs, and potential applications as biomarkers of oxidative stress in vivo. Lately, the successful synthesis of dihomo-isofuran compounds did uncover their potential as novel oxidative stress biomarkers [18].

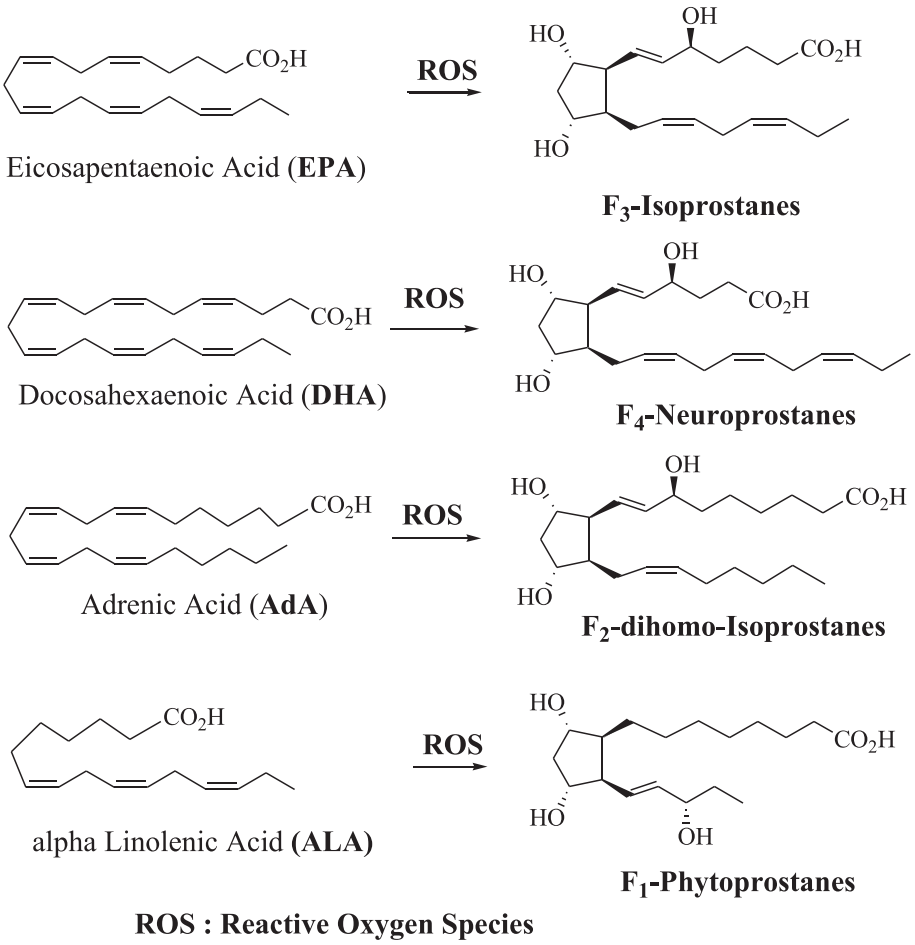
4. Cyclic oxygenated metabolites as biomarkers of lipid peroxidation

4.1. Eicosapentaenoic and docosahexaenoic acids

Among the identified n-3 fatty acids, EPA and DHA are the most notable ones for (patho)physiological functions. They are present in all human tissues at different levels, and DHA is foremost concentrated in the retina and brain. The structural occurrence of multiple double bonds allows the generation of oxygenated metabolites in the presence of free radicals. Oxidation of EPA can generate 6 series of F_3-IsoPs, of which 96 racemic derivatives can be potentially measured in vivo (see above Scheme 1 for details) [9]. From DHA, a total of 128 theoretical compounds can be formed at basal oxygen level in vivo and these are categorized into 8 regioisomer series (4, 7, 10, 11, 13, 14, 17 or 20) of NeuroPs, in which levels of 4- and 20-series are eminently high in vivo compared to the 6 other series [8,21]. The ratio between the type of oxygenated metabolites generated can also differ according to oxygen concentration, i.e. under hyperoxia NeuroFs are predominant over NeuroPs simply because of the differences in biosynthesis pathways (see above). At this stage, a total of 516 compounds of two families, each comprising 8 regioisomers, can be present in vivo [17].



Scheme 1. Example of F_2-dihomo-IsoPs synthesis from AdA. Initial hydrogen abstraction at one of the *bis*-allylic positions of AdA in membrane phospholipids generate a transient pentadienyl radical that is oxygenated at its terminal position to give pentadienyl peroxyl radicals. Irreversible O–C/C–C cyclizations (double 5-exo-trig cyclization) to available double bonds followed by the addition of oxygen and H-transfer yield G-type dihomo-IsoPs. Reduction of the hydroperoxide group then follows to produce the final products.



Scheme 2. Summary of the biosynthesis of F_1-PhytoPs, F_3-IsoPs, F_2-Dihomo-IsoPs, and F_4-NeuroPs from their respective PUFA precursors.

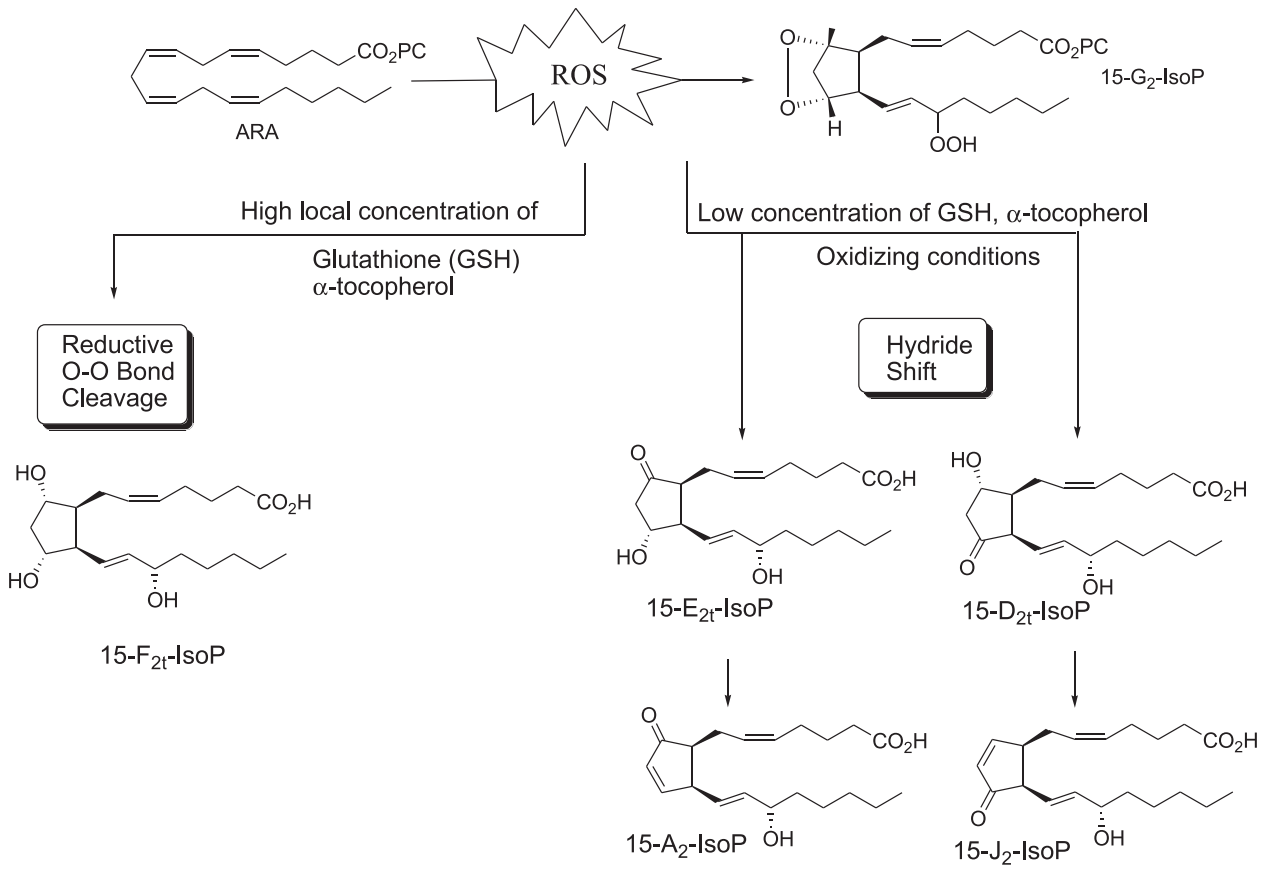
DHA is the main n-3 PUFA in vivo and is highly concentrated in the brain. It exists in the fetus through the maternal diet whereas it almost exclusively comes from food in humans. It is amenable to explore the role of NeuroPs and NeuroFs in the brain, however they were recently found to have multiple roles in other parts of the body, including the heart. Nevertheless, there is a smaller number of reports on NeuroPs and NeuroFs compared to IsoPs. To date, there is only a small number of synthetic standards that can be used for either gas chromatography–mass spectrometry (GC-NICI-MS or GC-NICI-MS/MS) or liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Table 1) [22].

4.1.1. F_3-Isoprostanes as oxidative stress biomarkers

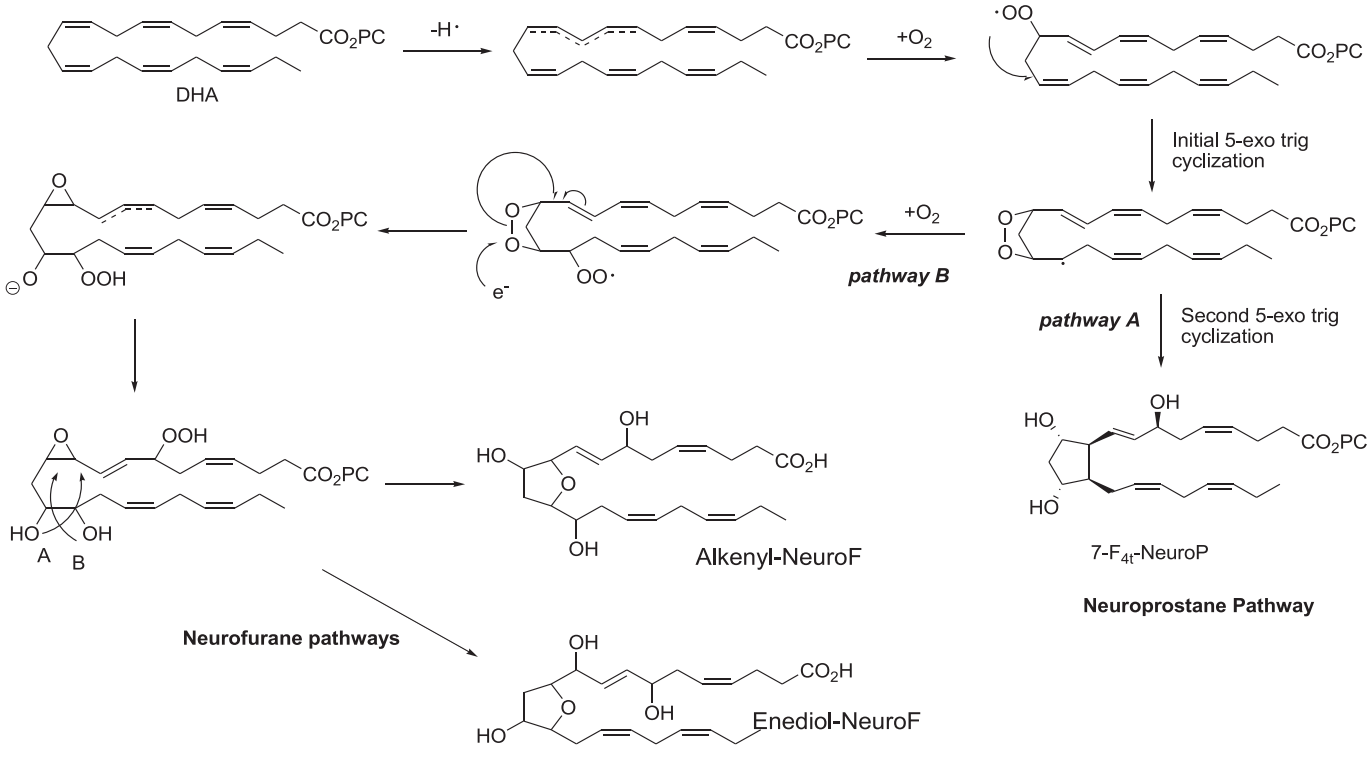
The level of F_3-isoprostanes (F_3-IsoPs) is a reliable index of specific lipid peroxidation of EPA and could be considered for an evaluation of oxidative stress in biological systems. In comparison to other biomarkers, they are chemically stable but there is still a lack of knowledge to appreciate them as such. Only recently that biological roles of F_3-IsoPs started to be investigated. In humans, stimulation of inflammation by injection of bacterial lipopolysaccharide (LPS) to healthy volunteers augmented urinary F_3-IsoPs within 4–6 h and an intravenous injection to mice showed no effect on systemic arterial pressure [23]. In dietary study, supplementation of fish oil to mice increased urinary F_3-IsoPs [23] and in $LDLR^{-/-}$ mice, it was found that western diet supplemented with EPA and/or DHA to reduce non-alcoholic steato-hepatitis (NASH) also increased urinary F_3-IsoPs as compared to controls [24]. Other investigations showed the acute exposure of fish to hydrogen peroxide increased F_3-IsoPs in muscles when compared to controls [25]. F_3-IsoPs were also assessed in invertebrates such as *Caenorhabditis elegans* in different environments, i.e. oxidative and aging conditions. In the report, whole organism including mitochondria and peroxisomes were measured, and F_3-IsoPs were shown to be elevated in conditions with mutated mitochondrial electron transport proteins, and in aged nematodes compared to young ones [26]. At cellular level, F_3-IsoPs were also measured in Jurkat cells to evaluate the physical effect of microbubbles during sonoporation, and it was shown to be elevated when compared to non-treated cells [27].

4.1.2. Neuroprostanes and neurofurans as oxidative stress biomarkers

The measurement of F_4-NeuroPs was initially performed in animals, such as normal rat and newborn pig brains, and the level was considerably higher in the cortex of the newborn pig brain [8]. Thereafter, F_4-NeuroPs were identified, in particular occipital and temporal lobes of brain tissues of Alzheimer's disease (AD) patients [28], and also in cerebrospinal fluid (CSF) of aneurismal subarachnoid hemorrhage patients. It was found to be nearly two-fold and ten-fold higher, respectively, compared to controls [8,29]. These findings opened a new area of research in neurodegenerative diseases as the involvement of reactive oxygen species (ROS) and oxidative stress is highly hypothesized in their developments. Thereafter, most of the observational studies (Table 1) focused on brain tissues of patients of periventricular white matter injury, mild cognitive impairment and in late stage of AD, transgenic mice, and in rare disease models such as Smith–Lemli–Opitz syndrome [17,30–33]. It was recently reported that, in *Mecp2* mutant mouse models of Rett syndrome (RTT), the brain concentration of F_4-NeuroPs is elevated in symptomatic and pre-symptomatic phases, in which the latter can lead to the onset of overt behavioral abnormalities. Moreover, *Mecp2*-null animals had inversed relationships between F_4-NeuroPs



Scheme 3. Generation of substituted isoprostanes through stabilization of G_2-IsoPs.



Scheme 4. Summary biosynthetic pathway of neurofuran DHA.

Table 1
Summary of observational studies reporting levels of neuroprostanes and neurofurans.

Model of study	Sample	Metabolites measured	Method of measurement	Reference
Human				
Smokers and age-matched controls; Ischemic-stroke patients and age-matched controls	Plasma	F_4-NeuroPs	GC-NICI-MS	[38,39]
Pre-eclampsia patients and normal pregnant women	Plasma of maternal and umbilical cord blood	F_4-NeuroPs	GC-NICI-MS	[40]
High cardiovascular risk subjects	Plasma	F_4-NeuroPs	GC-NICI-MS	Seet et al. (2013)
Adolescent Rett Syndrome patients and age-and gender-matched healthy controls	Plasma	F_4-NeuroPs	GC-NICI-MS/MS	[37]
Influenza A patients and age-matched controls	Plasma	F_4-NeuroPs	GC-NICI-MS	[41]
PD patients and age-matched healthy controls	Plasma	F_4-NeuroPs	GC-NICI-MS	[36]
Healthy subjects	Urine	7-F_{4c}-NeuroP	GC-NICI-MS LC-MS/MS	[88]
Non-aSAH control patients and aSAH patients	CSF	F_4-NeuroPs	GC-NICI-MS	[29]
Patients with severe aSAH and TBI, and age-and gender-matched healthy controls	CSF	4(*RS*)-F_{4t}-NeuroP	GC-NICI-MS	[89]
Normal and patients with aSAH	CSF	F_4-NeuroPs	GC-NICI-MS	[90]
AD and healthy controls	Brain: Occipital, temporal and parietal lobes	F_4-NeuroPs	GC-NICI-MS	[28]
AD patients and age- and gender-matched healthy controls	Post-mortem brain samples: superior and middle temporal gyri, hippocampus, inferior parietal lobule and cerebellar cortex	Total (D_2/E_2-IsoPs + D_4/E_4-NPs) F_4-NeuroPs	GC-NICI-MS	[30]
Early PWMI and age-matched controls	Post-mortem brain samples	F_4-NeuroPs	GC-NICI-MS	[31]
Healthy control, MCI, and late AD patients	Post-mortem brain samples of: frontal and occipital lobes, inferior parietal lobule, and hippocampus	F_4-NeuroPs	GC-NICI-MS	[91]
Animals				
AD and age-matched control; rats; newborn pigs	CSF, whole rat brain, brain cortex of newborn pig	F_4-NeuroPs	GC-NICI-MS	[8]
Fischer 344 rats	Young and old brains	F_4-NeuroPs	GC-NICI-MS	Youssef et al. (2003)
Tg 2576 mice: Transgenic mice model of AD	Brain cortex	F_4-NeuroPs NeuroFs	GC-NICI-MS LC-MS/MS	[17]
Newborn pigs	Brain prefrontal cortex	F_4-NeuroPs NeuroFs	GC-NICI-MS	[35]
Dhcr7-KO Mice Dhcr7-KO (Dhcr7$^{tm1Gst/J}$): genetic mouse model of Smith–Lemli–Opitz syndrome	Brain and liver	F_4-NeuroPs	GC-NICI-MS	[33]
Preterm pigs	Brain prefrontal and medial prefrontal cortex	F_4-NeuroPs NeuroFs	LC-MS/MS	[18]
Mecp2 mutant mouse models of Rett Syndrome including *Mecp2*-null (pre-symptomatic, symptomatic, and rescued) and *Mecp2*-308 mutated (pre-symptomatic and symptomatic) mice and wild type littermates	Plasma and brain	F_4-NeuroPs	GC-NICI-MS/MS	[34]

NeuroPs: neuroprostanes; NeuroP: neuroprostane; NeuroFs: neurofurans; GC-NICI-MS: gas chromatography–mass spectrometry, negative chemical ionization; LC-MS/MS: liquid chromatography tandem mass spectrometry; CSF: cerebrospinal fluid; AD: Alzheimer's disease; PWMI: periventricular white matter injury; MCI: mild cognitive impairment; PD: Parkinson's disease; aSAH: aneurysmal subarachnoid hemorrhage; TBI: traumatic brain injury.

levels and brain weight and body weights [34]. RTT is a known neurodevelopment disorder by loss of function due to mutation in the gene encoding the methyl-CpG binding protein 2 (MECP2), and considered as a genetic model of infantile autism in female.

In a recent study, two specific regioisomers of F_4-NeuroPs, 4(*RS*)-4-F_{4t}-NeuroP and 10-F_{4t}-NeuroP, were quantified in brain prefrontal and medial prefrontal cortex of preterm pigs and it was found that 4(*RS*)-4-F_{4t}-NeuroP was predominant, indicating that not all types of NeuroPs may be actively released under oxidative stress conditions. Not many data (Table 1) are yet available on NeuroFs. A quantitative method was recently developed [16], and NeuroFs were measured in the brain cortex and cerebellum of AD transgenic mice and reported to be predominant in the cortex [17]. An increase in oxygen tension to >21% in newborn pigs has been shown to substantially increase levels of NeuroFs in the brain prefrontal cortex [35].

Evaluation in brain tissues in humans and rodents revealed F_4-NeuroPs to be a valuable biomarker of neuronal disorders. Similar findings were found in plasma of Parkinson's disease patients. Seet et al. [36] have shown that F_4-NeuroP plasma levels of Parkinson's disease patients were elevated when compared to controls and were independent of the severity of the disease. In addition, plasma of adolescent RTT patients had increased levels of free F_4-NeuroPs in comparison with healthy controls, and the levels correlated with the severity of the illness [37]. Aside from neurodegenerative diseases, onset of ischemic-stroke also showed to be associated with augmented plasma concentrations of free and esterified F_4-NeuroPs, and the levels remained high after the onset, especially for the free form [38]. Levels of F_4-NeuroPs were also investigated in cigarette smokers who had augmented plasma concentrations in comparison to controls. The concentration was further elevated in the smokers immediately after cigarette smoking session [39]. Furthermore, plasma of maternal and cord blood of pre-eclampsia women had higher F_4-NeuroPs levels compared to normal pregnant women [40]. Nevertheless, not all observational studies showed elevated in vivo F_4-NeuroPs levels; it was suppressed in plasma of patients with influenza A, and remained lower than in controls at 3 months post-infection [41]. In cells, the effect of sonoporation in Jurkat cells showed no difference in F_4-NeuroPs concentrations when compared to non-treated cells [27].

4.1.3. Neuroprostanes and neurofurans as biomarkers in metabolic studies

Intervention studies on NeuroPs were mainly conducted on analyses of their effects on specific areas of rodent brain and rare diseases (Table 2). Studies were highly focused on the effect of anti-inflammatory and antioxidant agents in brain upon injury by chemical adulteration, and viral or bacterial infections. Both types of agents showed to be effective in reducing F_4-NeuroPs levels after injury, in particular in the cerebrum [42–45]. Levels of F_4-NeuroPs in ischemic-induced rodent brain (cerebral cortex, hippocampus, gray matter) were also determined after α-lipoic acid treatment but no significant effect was found [46]. However, treatment with melatonin after brain

Table 2
Summary of metabolic studies reporting levels of neuroprostanes and neurofurans.

Model of study and type of samples	Treatment	Metabolite measured	Outcome of the study	Reference
Human				
Type 2 diabetic patients and age matched controls: plasma	Zinc supplementation	F_4-NeuroPs	Supplementation did not change free or total (free + esterified) F_4-NeuroPs levels.	[92]
Adolescent Rett Syndrome patients: plasma	ω-3 PUFA (fish oil)	F_4-NeuroPs	Supplementation of ω-3 PUFA for 6 and 12 months decreased free F_4-NeuroPs.	[37]
Young children of Rett Syndrome patients: plasma	ω-3 PUFA (fish oil)	F_4-NeuroPs	Supplementation of ω-3 PUFA for 6 months decreased free F_4-NeuroPs	[60]
Adolescent Rett Syndrome patients: plasma	ω-3 PUFA (fish oil)	F_4-NeuroPs	Supplementation of ω-3 PUFA for 6 and 12 months decreased free F_4-NeuroPs and was inversely correlated to left ventricular systolic velocity of blood flow.	[80]
Rat				
Sprague–Dawley: cerebrum, plasma and urine	Subcutaneous injection of KA	F_4-NeuroPs	Only, cerebrum F_4-NeuroPs elevated 2 h post KA treatment. Plasma and urine were not affected by KA.	[42]
Sprague–Dawley: brain	Ethanol withdrawal	F_4-NeuroPs	F_4-NeuroPs were elevated in the cerebral cortex and brainstems of rats with ethanol withdraw versus control.	[93]
Sprague–Dawley: brain	Carbonfuran with/without memantine HCl and atropine sulfate	F_4-NeuroPs	Carbonfuran increased F_4-NeuroPs alone and then inhibited by memantine HCl and atropine sulfate compared to non-treated rat.	[94]
Ischemia-induced Sprague–Dawley: brain ipsilateral striatum, ipsilateral cortex, contralateral striatum, and contralateral cortex	Treatment with or without tamoxifen	F_4-NeuroPs	Tamoxifen reduced ipsilateral cortex, contralateral striatum, striatum and contralateral cortex F_4-NeuroPs compared to non-treated rats.	[48]
Wistar: brain	Manganese chloride	F_4-NeuroPs	F_4-NeuroPs levels of rats treated manganese chloride increased compared to control group.	[95]
Hypoxia-ischemia induced Sprague–Dawley: brain, prefrontal cortex	Melatonin	F_4-NeuroPs NeuroFs	F_4-NeuroPs decreased on both side of prefrontal cortex and NeuroFs decreased on right side of prefrontal cortex in rats treated with melatonin after 24 h from the hypoxia-ischemia injury.	[47]
Mice				
C57BL/6: cerebrum	Pre-treated with indomethacin and ibuprofen for 2 weeks and then ICV injection with KA or LPS	F_4-NeuroPs	F_4-NeuroPs elevated after KA and LPS exposure, the latter had higher increase. Treatment of indomethacin or iburopfen reduced F_4-NeuroPs.	[42]
C57BL/6: cerebrum	1. ICV LPS with or without N-tert-butyl-α-phenylnitrone and NSAIDs 2. ICV LPS with or without α-tocopherol or γ-tocopherol 3. ICV kainic acid with or without α-tocopherol, N-tert-butyl-a-phenylnitrone, or ibuprofen	F_4-NeuroPs	1. F_4-NeuroPs elevated after LPS exposure and then inhibited by N-tert-butyl-α-phenylnitrone and NSAIDs. 2. Elevated F_4-NeuroPs by LPS reduced by α-tocopherol or γ-tocopherol. Kainic acid increased F_4-NeuroPs and was reduced by α-tocopherol, N-tert-butyl-a-phenylnitrone, or ibuprofen.	[43–45]
ATM −/− and ATM +/+: forebrain and cerebellum	Supplementation of EUK-189 is a low-molecular weight salen-manganese for 84 days.	F_4-NeuroPs NeuroFs	Both F_4-NeuroPs and NeuroFs decreased after supplementation compared to placebo but was statistically significant for NeuroFs only.	[49]
BALB/c: brain	Intracerebral inoculation of either herpes simplex virus type 1 or normal saline into the left cerebral hemisphere	F_4-NeuroPs	F_4-NeuroPs elevated after acute herpes simplex virus type 1 infection.	[96]
TTg2576: left forebrain cerebral cortex, hippocampus, and deep gray matter	α-Lipoic acid supplementation.	F_4-NeuroPs	No effect of α-Lipoic acid on F_4-NeuroPs levels compared to non-treated mice.	[46]
C57B6J: brain cortex and cerebellum	Induced vitamin E deficiency	F_4-NeuroPs	Lower α-tocopherol levels were associated with lower F_4-NeuroPs.	[97]
L-gulono-γ-lactone oxidase (C57BL/6J): brain, medial cortex and cerebellum	Low and high dose of ascorbic acid supplementation	F_4-NeuroPs	Low L-gulono-γ-lactone oxidase mice with low ascorbic acid showed elevated F_4-NeuroPs in cerebellum compared to high L-gulono-γ-lactone oxidase mice with high ascorbic acid and wild type.	[98]
$APPS_{we}$/PSEN1ΔE9 bigenic: cortical tissues	Low vitamins C and E, and high vitamins C and E supplementation	F_4-NeuroPs	Treatment of low vitamins C and E but not high vitamins C and E reduced F_4-NeuroPs compared to control.	[99]
Tg 2576, transgenic mice model of AD: liver samples	Carbon tetrachloride	F_4-NeuroPs NeuroFs	Carbon tetrachloride injection increased F_4-NeuroPs after 1 h and after 2.5 h for NeuroFs compared to controls.	[17]
Atherosclerosis (*$LDLR^{-/-}$*): liver samples	DHA	F_4-NeuroPs	Supplementation of DHA increased F_4-NeuroPs in dose-dependent manner. This increase had inverse relationship with risk of atherosclerosis.	[53]
Fish				
Marine (medaka): body muscle	Hydrogen peroxide	F_4-NeuroPs	F_4-NeuroPs elevated after 2 h and 6 h exposure in male and female medaka respectively.	[25]

NeuroPs: neuroprostanes; NeuroFs: neurosfurans; LPS: lipopolysaccharide; KA: Kainic acid; ICV: Intracerebroventricular; AD: Alzheimer's disease; NSAID: non-steroidal anti-inflammatory drug; DHA: docosahexaenoic acid; PUFA: polyunsaturated fatty acid; ATM: ataxia–telangiectasia mice.

ischemic injury reduced concentrations of F_4-NeuroPs in both sides of prefrontal cortex, and particularly NeuroFs in the right side [47]. Likewise, tamoxifen also lowered F_4-NeuroPs in brain striatum and cortex of ischemic-induced rodent brain [48]. Supplementation of *Ataxia-telangiectasia*$^{-/-}$ mice with salen-manganese (catalytic scavenger of hydrogen peroxide and cytoprotective agent) was able to reduce both levels of F_4-NeuroPs and NeuroFs in the forebrain and cerebellum, with a stronger effect on NeuroFs. Ataxia-telangiectasia also known as Louis–Bar Syndrome, is a recessive disease that can lead to cerebellar degeneration and genomic instability as well as suppression of immune functions [49]. Such findings are important in alleviating oxidative stress role in neurological diseases related to brain prefrontal cortex, as it has been associated with bipolar disorders and schizophrenia, and to cognitive dysfunction [50,51].

In the transgenic mice model of AD (*Tg 2576*), the injection of carbon tetrachloride (CCl_4), a strong inducer of lipid peroxidation, raised levels of F_4-NeuroPs and NeuroFs in the liver with a peak at 1 h and 2.5 h [17]. This is comparable to data reported by Morrow et al. [52] on F_2-IsoPs, showing a raise in liver, 2 h after an oral administration of CCl_4. Regardless of the type of rodent used in the model, it appears that the generation of F_4-NeuroPs and NeuroFs is rapid, like for F_2-IsoPs. Gladine et al. showed that liver samples of LDLR$^{-/-}$ mice supplemented with different doses of DHA had increased F_4-NeuroP levels in a dose-dependent manner. The increase correlated with reduced plaque formation and atherosclerotic lesions, strongly suggesting that F_4-NeuroPs might play an active role in atherosclerosis prevention [53].

Aside from the brain and liver, F_4-NeuroPs levels were measured in plasma of type 2 diabetic patients, before and after zinc supplementation and no significant change was observed [54]. The effect of hydrogen peroxide on these compounds was recently evaluated in marine fish, showing a rate of increase in F_4-NeuroPs levels faster in males than in females [25].

4.2. Adrenic acid

AdA is the third most important PUFA in the brain, after DHA and ARA, and particularly in the myelin lipids. It is also abundant in the adrenal gland and kidney. AdA is not found in everyday diet but is formed from ARA by chain elongation. Similar to ARA, AdA is metabolized by COX into dihomo-PGs derivatives [55–57]. The non-enzymatic peroxidation of AdA leads to the release of dihomo-IsoP derivatives and was first reported by VanRollins et al. in 2008 [10]. The F_2-dihomo-IsoPs are present as 4 regio-isomers (7, 10, 14 or 17) in which 7- and 17-series are the most present in vivo. Recently, dihomo-IsoFs which can add up to 256 metabolites were discovered and quantified in pig brain samples [58].

4.2.1. F_2-dihomo-IsoPs and F_2-dihomo-IsoFs as oxidative stress biomarkers

In 2008, the discovery of F_2-dihomo-IsoPs showed in particular that the formation of F_2-dihomo-IsoPs and F_4-NeuroPs was of phospholipid origin and time-dependent (gray matter, white matter and myelin) [10]. The ratio of AdA/DHA in the different samples was consistent with F_2-dihomo-IsoPs to NeuroPs at homeostatic conditions, but after in vitro oxidation, the myelin lipids of the white matter consistently showed highest increases compared to the gray matter (154% *vs* 50% at 24 h). Furthermore, in urine of adult volunteers, F_2-dihomo-IsoPs were not detected, whereas in brain tissues of Alzheimer's disease patients' frontal lobe white matter, a 2-fold higher level compared to healthy volunteers was reported.

In 2014, a novel isofuran derivative of AdA, termed dihomo-IsoF was synthesized; the compound 17(*RS*)-SC-Δ^{15}-11-dihomo-IsoF [58]; represents one of the 8 series of dihomo-IsoF (each series with its 32 potential isomers). Quantitation in brain of preterm pigs showed that, out of the isofuran derivatives of ARA, DHA and AdA, dihomo-IsoF was the highest and had level comparable to 4-F_{4t}-NeuroPs which can potentially be valuable for assessing neuronal disorders. Knowing that AdA level was more than 10 times lower than DHA, and that both PUFAs mainly represent gray and white matter, respectively, This suggests that both dihomo-IsoFs and 4-F_{4t}-NeuroPs should be used as complementary biomarkers when evaluating neuronal damage and disease.

F_2-dihomo-IsoPs were also measured in RTT patients and found to be an early disease biomarker. It was shown that these metabolites were two-orders of magnitude elevated in plasma of first stage RTT patients compared to controls [59]. These data indicate for the first time, that quantification of F_2-dihomo-IsoPs in plasma can represent an early biomarker of the disease. Further research on RTT showed that 6 months of n-3 fish oil supplementation can dramatically reduce the clinical severity of the disease together with a significant decrease in all the examined oxidative stress biomarkers and mainly of F_2-dihomo-IsoPs (by 82%) [60].

4.3. α-Linolenic acid

ALA is an essential fatty acid and precursor in humans of the n-3 PUFAs such as EPA and DHA. It is found in algae and higher plants, and can be highly enriched in certain oils such as linseed oil (up to 65% total fatty acid (FA). Despite being an essential FA, it is a minor component of animal tissues and mainly used for energy, elongation and desaturation to a more complex PUFA, and oxygenated by lipoxygenases. ALA can be oxidized non-enzymatically to produce oxygenated cyclic compounds known as phytoprostanes (PhytoPs). They were first described in 1998 by Mueller and Parchmann in plants [11]. Only two series can be found, the 9th and 16th series, and so far the families found in plants are the F-, D-, E-, A, deoxy-J, B- and L-PhytoPs [61–64]. PhytoPs are known to be biomarkers of oxidative stress in plants and biologically active but their impact in humans is still unknown. The only study of PhytoPs in human was conducted by Barden et al. [65] who found that supplementation of flaxseed oil (62% of ALA, 5.4 g/d) increased plasma levels of F_1-PhytoPs as well as plasma ALA, but to a rather low extent. It is clear that PhytoPs remain to date under-investigated, as a potential biomarker of oxidative stress and it may be limited to plants and vegetable oils.

5. Bioactive lipids and potential signaling molecules

The biological activities of non-enzymatic-derived cyclic oxygenated metabolites are well recognized but the focus has been mainly on IsoPs (from ARA) [66]. Of the F_2-IsoPs, 15-F_{2t}-IsoP is the most studied where it is able to induce vasoconstriction and platelet activation. In addition, F_2-IsoPs induces inflammation by expression of adhesion molecules (e.g. ICAM-1) leading to enhanced adhesion of monocytes, mitogenesis of smooth muscle cells and proliferation of fibroblasts. Collectively, they are all related to the pathogenesis of atherosclerosis and associated with cardiovascular diseases (see [2] for details regarding the mechanisms of action).

In contrast, the biological activities of n-3 derived IsoPs and NeuroP remain largely unexplored probably because of the difficulties linked to their chemical synthesis and their analysis in vivo. However, several lines of evidence in the literature showed that some peroxidized metabolites of n-3 PUFAs are bioactive molecules and substantially beneficial. The group of Sethi et al. was the first to demonstrate that peroxidation of EPA and DHA contributes to anti-inflammatory activities [67]. The authors showed that ex vivo oxidation of EPA and DHA (obtained with $CuSO_4$ and ascorbic acid) reduced the adhesion of U937 monocytes to endothelial cells and decreased the expression of adhesion molecules whereas EPA and DHA had no effect. The nature of peroxidized metabolites from EPA and DHA was not identified at this time but the authors demonstrated that the peroxidation of EPA and DHA was a mandatory prerequisite to make them bioactive on endothelial cells. The reduction of adhesion molecules expression was later linked to the inhibition of NFκB binding activity and associated with PPARγ dependent mechanism [68]. Another study from the same group showed that endothelial

cells exposed to oxidized EPA reduced the production of inflammatory cytokines (IL-8, MCP-1) [69].

The bioactivity of oxidized EPA and DHA was also investigated in hepatocytes and found to be associated with ApoB100 degradation [70] and iNOS expression [71]. Moreover, the impact of EPA and DHA on the ionic potential of cardiac function is well-defined [72] and it has been shown that this effect, at least in rat cardiac ventricular myocytes, is due to peroxidation of DHA [73]. In support, one of the oxidized DHA product, 4(*RS*)-4-F_{4t}-NeuroP has been recently elucidated to have cardiac anti-arrhythmic properties and potentially be a therapeutic agent [74].

Morrow and coworkers, pioneers in the in vivo identification of IsoPs and NeuroPs, were the first to speculate that EPA and DHA-related isoprostanes could be bioactive molecules involved in atherosclerosis prevention [75]. This hypothesis was more thoroughly investigated using an integrated approach associating a dose–response intervention study with DHA in $LDLR^{-/-}$ mice, targeted lipidomic analysis, and unbiased statistical analysis including correlation analyses, hierarchical cluster and projection to latent structure discriminate analysis [53]. The main finding of this study was the identification of F_4-NeuroPs that negatively correlated with plaque size and the best predictive variable of atherosclerosis prevention. Altogether, the results reinforced the hypothesis that F_4-NeuroPs could contribute to the anti-atherogenic effects of DHA. In parallel, several in vitro experiments showed that EPA and DHA-related isoprostanes (namely 15-A_{3t}-IsoP, J_3-IsoP, A_4/J_4-NeuroP and 4-F_{4t}-NeuroP) possessed potent anti-inflammatory properties linked notably to the inhibition of the NFκB and/or Nrf-2 pathways [76–79]. However, contrasting outcome was found in supplementation of RTT patients with n-3 PUFA from fish oil in which plasma free F_4-NeuroPs concentrations were lower when compared to controls. Also, the level of free F_4-NeuroPs released was inversely associated with cardiac left ventricular systolic function in the RTT patients after fish oil supplementation [37,60,80].

The biological activities of PhytoPs have been mainly demonstrated in plants. They can trigger the first adaptive responses to oxidative stress by inducing notably the expression of genes involved in detoxification and secondary metabolism [81,82]. It is evidently shown in humans, where consumption of plant food sources rich in ALA or inhalation of pollen had relatively noticeable levels of PhytoP in vivo [83,84]. However, reports on bioactivity of PhytoPs in the mammalian systems are scarce and to date, findings mainly rely on in vitro experiments, despite their occurrence has been demonstrated in vivo in humans [85]. Nevertheless, different types of PhytoPs (mainly the cyclopentenone ring structure, A-, deoxy-J-, and their precursors E- and D- expect the B-type structures) have been shown to exert potent inflammatory regulation properties illustrated by the inhibition of nitric oxide production by LPS-stimulated mice macrophages (RAW 264.7) via NFkB inhibition [83], and by the decreased dendritic cell IL-12 production and stimulation of TH2 polarization of naïve T cells [86]. Another study showed putative anti-aggregation properties of ent-16-F_1-PhytoP [65]. Recently, Minghetti et al. investigated the neuroprotective effects of B_1-PhytoPs, by protecting undifferentiated neuronal cells (SH-SY5Y cells) against oxidative stress induced by hydrogen peroxide, and promoting myelination [87].

6. Conclusions

In this review, we have discussed findings showing that non-enzymatically-derived cyclic oxygenated metabolites of n-3 and n-6 PUFA, i.e. isoprostanes, neuroprostanes, dihomo-isoprostanes and phytoprostanes can be useful oxidative stress biomarkers, especially in neuronal disorders and diseases, but can also exhibit bioactivities contributing to signaling and regulatory events in vivo. There is scope to explore how the relative prevalence of these oxygenated cyclic products of PUFA can elucidate the beneficial or detrimental effects of their precursor fatty acids in different (patho)physiological conditions.

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