

LIPIDS AND BRAIN LIPIDES ET CERVEAU

Non enzymatic metabolites of polyunsaturated fatty acids: friend or foe

Valérie Bultel-Poncé, Thierry Durand*, Alexandre Guy, Camille Oger and Jean-Marie Galano

Institut des Biomolécules Max Mousseron IBMM, UMR 5247 CNRS – Université de Montpellier – ENSCM, Faculté de Pharmacie, 15. Av. Ch. Flahault, 34093 Montpellier Cedex 05, France

Received 14 September 2015 – Accepted 21 September 2015

Abstract – Under condition of oxidative stress, free radical mediated peroxidation of polyunsaturated fatty acids generates *in vivo*, cyclic metabolites like the isoprostanes, neuroprostanes, dihom-isoprostanes, isofuranes among a large number of key products which participate in many pathophysiological processes. These metabolites display a wide range of biological actions, and some of them are now the most reliable indicators of oxidative stress in humans. In this review, we will discuss several key points of our understanding of those cyclic polyunsaturated fatty acids derivatives, going from multi-step syntheses, analytical chemistry and biological activities.

Keywords: biomarkers / polyunsaturated fatty acid / radical peroxidation / isoprostanes / neuroprostanes / isofurans / total synthesis

Résumé – La peroxydation radicalaire des acides gras polyinsaturés, constituants majeurs des membranes cellulaires, conduit à de nombreux métabolites, comme les isoprostanes, les neuroprostanes, les dihom-isoprostanes, les isofuranes. Certaines isoprostanes sont à présent des marqueurs du stress oxydant cellulaire les plus significatifs utilisés en clinique. Ces lipides oxygénés possèdent également un large spectre d'activités biologiques. Cette revue fera le point sur notre dernière stratégie de synthèse organique multi-étapes pour obtenir ces molécules pures et sur des résultats récents issus de collaborations avec des collègues biologistes et cliniciens.

Mots clés : biomarqueurs / activités biologiques / peroxydation lipidique / acides gras polyinsaturés / isoprostanes / neuroprostanes / isofuranes

1 Introduction

In mitochondrial and microsomal membranes, molecular dioxygen can be transformed into several reactive oxygen species (ROS). Some of them can be seen as low reactive like the superoxide anion (O_2^-) which confers them their quality of regulators of several important biological functions. Under condition of oxidative stress (OS), the radical hydroxide (HO^\cdot), known to be preferentially formed through the Fenton and Haber-Weiss reactions (Fig. 1), is a more reactive species (along with peroxy HOO^\cdot) and has been implicated in the radical-mediated peroxidation of polyunsaturated fatty acids (PUFA) (Magder, 2006).

A living mammalian organism has many endogenous substances or enzymes able to maintain the ROS in a steady concentration or to trap the free radicals. In addition, the diet can supply exogenous anti-oxidant substances (like vitamins for example). However during particular circumstances if the overwhelming production of ROS cannot be countered

by antioxidant system, important biological molecules (lipids, proteins, nucleic acids...) will undergo radical mediated modifications.

In this review we will focus on lipids and especially on radical induced peroxidation of PUFAs by ROS (Jahn *et al.*, 2008). The hydroxide radical is able to abstract a hydrogen atom from a methylene group surrounded by the two-double bonds in PUFAs and thus to initiate the lipid peroxidation process. The progression of lipid peroxidation will promote several changes, loss of physiological function of cell membrane, inactivation of enzymes and further biochemical changes will have both desirable and undesirable effects. For example they are in part responsible of disorders like stroke, myocardial infarction, inflammatory disorder, cancers, diabetes, neurodegenerative diseases (Colavitti and Finkel, 2005; Il'yasova *et al.*, 2008; Janssen *et al.*, 2008; Steinberg *et al.*, 1989).

The most abundant PUFA in human organism is arachidonic acid (AA, C20:4 n-6) (Morrow *et al.*, 1990; Morrow and Roberts, 1994). Linked to phospholipids in the membranes, it is released in the free form by phospholipase A2 (PLA₂) and

* Correspondence: thierry.durand@umontpellier.fr

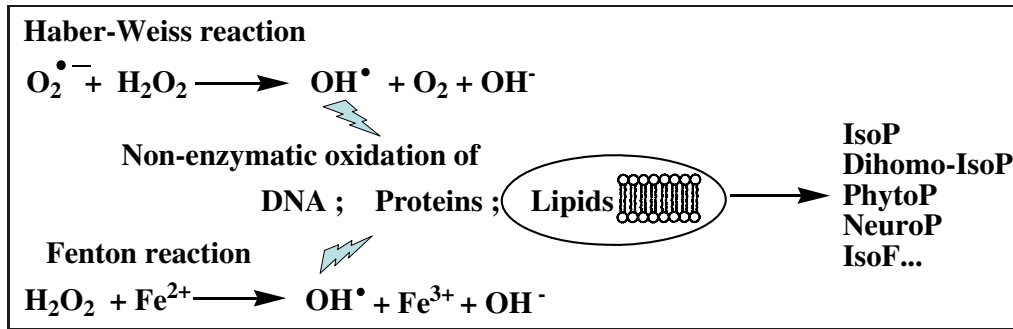


Fig. 1. Radical peroxidation of lipids

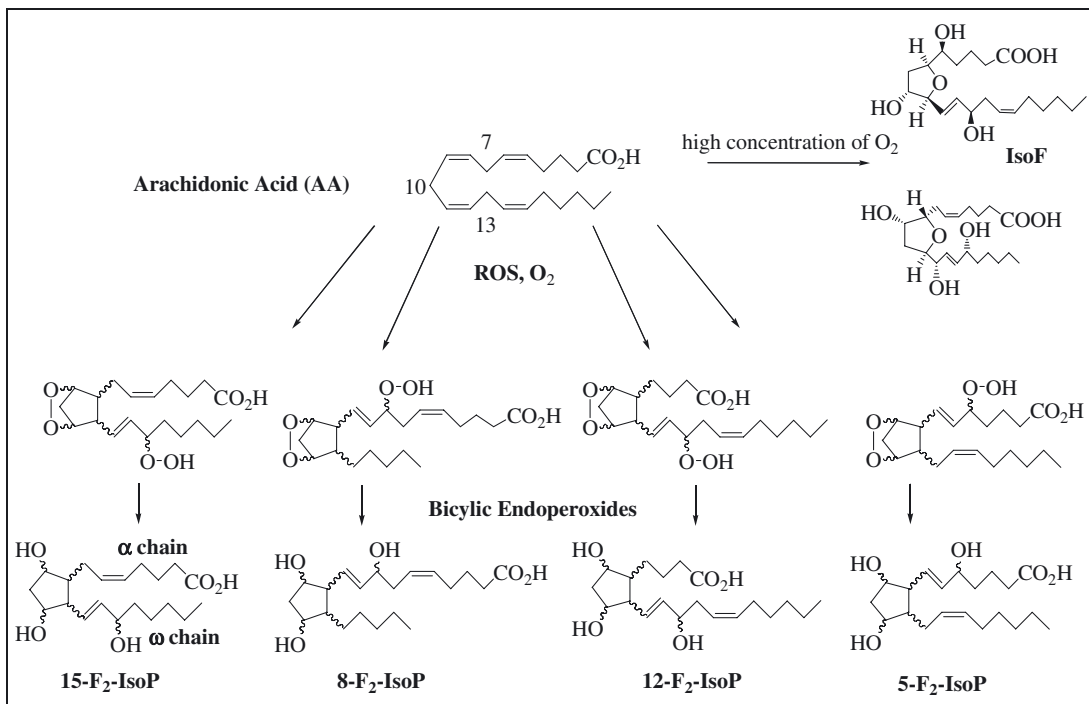


Fig. 2. F₂-IsoP products of AA peroxidation.

then can be used by cyclooxygenase enzymes to generate the biologically relevant prostaglandins (PGs) or the leukotrienes *via* lipoxygenase activity.

In 1990, Morrow *et al.* highlighted *in vivo* formation in human of the isoprostanes (IsoPs), isomeric compounds of the PGs, by a non enzymatic mechanism involving the free radical mediated peroxidation of AA. The IsoPs synthesized in the membranes of the cell by peroxidation of the still esterified AA. A large number of diastereoisomers can be generated and then can be released by a specific PLA₂ (Stafforini *et al.*, 2006).

The lipid peroxidation of AA begins with the abstraction of a hydrogen atom by HO[•], in one of the 3 bis-allylic positions at C-7, C-10 or C-13. The pentadienyl radical formed reacts with molecular dioxygen to form a peroxy radical which undergoes two consecutive 5-exo-trig cyclizations and the subsequent formed carbon centered allylic radical reacts one more time with dioxygen to generate a hydroperoxide intermediate after abstraction of another H-atom (Fig. 2). Finally, partial

and/or complete reductions yield the whole family of IsoPs with several type (A, D, E, F, J) and series (5, 8, 12, 15).

Isofurans (IsoFs) metabolites containing furan ring structures can also be formed in preference but not only, from AA, when oxygen tension is elevated (Fessel *et al.*, 2002). This was evidently shown in pigs, in which exposure of high oxygen tension augmented IsoFs production and reduced IsoPs production in the brain (Solberg *et al.*, 2012).

The increase of the IsoP concentration was demonstrated in several pathologies as for example in neurodegenerative and cardiovascular diseases, and IsoPs are now considered as the standard markers of the lipid peroxidation. Their quantification in urine and plasma allows a precise, non-invasive and representative measure of OS (Mas *et al.*, 2008; Michel *et al.*, 2008; Milne *et al.*, 2007; Vigor *et al.*, 2014).

The quantification processes were developed due to the availability of synthetic compounds provided by organic chemists who are able to produce large amount of pure compounds (Jahn *et al.*, 2008). Furthermore, molecules becoming

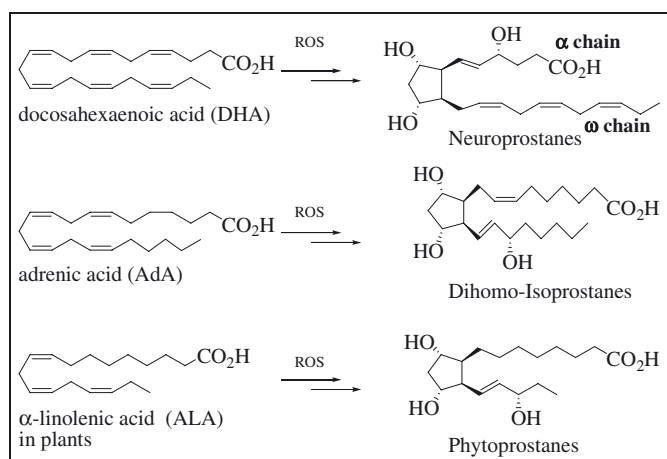


Fig. 3. Metabolites resulting of the radical peroxidation of PUFAs.

commercially available were studied and since then their biological roles were also uncovered (Galano *et al.*, 2015). For example, Morrow *et al.* (1990) showed that the 15-F_{2t}-IsoP injected in a rat kidney, in the peripheral vein or directly in the kidney, allowed the reduction of the blood pressure and the rate of filtration. Other IsoPs thus have biological activities which confer them a role of mediator in a context of OS (Brown and Marnett, 2011).

In mammals, this prolific pathway of peroxidation can occur with different PUFAs (Fig. 3), like docosahexaenoic acid (DHA, C22:6 n-3) enriched in the grey matter and retina, which yields the neuroprostanes (NeuroPs) (Nourooz-Zadeh *et al.*, 1998; Roberts, 1998) and neurofurans (NeuroFs) (Fessel *et al.*, 2002). Adrenic acid (AdA, C22:4 n-6) present in the myelin (white matter) and retina produces dihom-IsoPs, dihom-IsoFs (De La Torre *et al.*, 2015, 2014; Song *et al.*, 2008; VanRollins *et al.*, 2008). In plants, the phytoprostanes (PhytoPs) were described resulting from the radical peroxidation of α -linolenic acid (ALA, C18:3 n-3) (Imbusch and Mueller, 2000).

The lovely story of these molecules will be reported in this review. Herein you can find several outcomes in terms of syntheses, diagnosis and biological activities, gathering twenty five years of our group research, from our organic chemistry knowledge through smart multi-step synthesis strategies of IsoP derivatives, to our fruitful collaborations with biologists and clinicians around the world (Galano *et al.*, 2013, 2015).

2 Chemical synthesis

We have reported recently a simple and highly stereocontrolled strategy toward the total synthesis of IsoP derivatives based on a bicyclic α , β -epoxy ketone intermediate (Fig. 4) (Oger *et al.*, 2008).

The use of a bicyclo[3.3.0]octene scaffold permitted stereodirection of reagents allowing the facile 1,3-cis diol unit introduction *via* stereoselective epoxidation, diastereoselective ketone reduction, and regioselective epoxide opening otherwise not attainable with a simple cyclopentene framework.

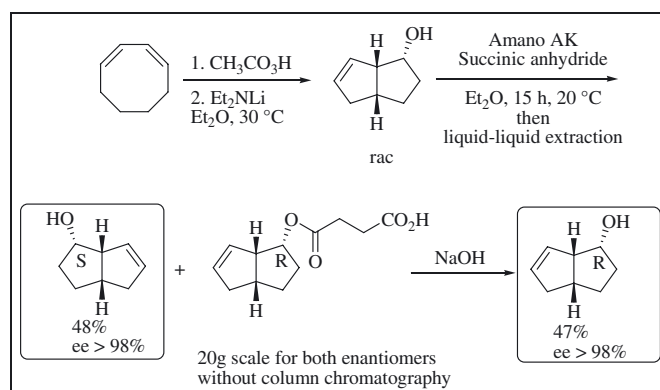


Fig. 4. Two key bicyclic alcohols.

The lateral chains were plugged by HWE and Wittig reactions, the allylic alcohol reduced and the final deprotections allowed us to validate this flexible strategy and to access IsoPs; dihom-IsoPs; NeuroPs (Figs. 5 and 6) (Brinkmann *et al.*, 2010; Oger *et al.*, 2010, 2012).

3 New biomarkers

3.1 Phytoprostanes

Supplementation with eicosapentaenoic acid (EPA; C20:5 n-3) and DHA has been reported to reduce lipid peroxidation products (F₂-IsoPs) formed from AA in healthy humans, as well as in patients with conditions associated with OS. While the shorter chain PUFA, ALA, is ubiquitous in plant cells and can serve as a precursor to EPA and DHA to human; its conversion to C20 and C22 PUFA seems to be inefficient. As described above, ALA can also undergo free radical oxidation, forming PhytoPs in all plants and leading to the accumulation of high concentrations of PhytoPs in plant pollens (Mariani *et al.*, 2007; Traidl-Hoffmann *et al.*, 2005). In a recent study with healthy male volunteers, Barden *et al.* (Barden *et al.*, 2009) examined the effect of ALA supplementation on F₁-PhytoPs and F₂-IsoPs concentrations in plasma and urine.

The study protocol was as follows: thirty-six non smokers, 20–65 years of age, consumed 9 g/day of either flaxseed oil (62% ALA, 5.4 g/day) or olive oil (placebo) for 4 weeks in a parallel design. At baseline and after 4 weeks of supplementation, blood and a 24-h urine sample were collected for determination of concentrations of F₁-PhytoPs and F₂-IsoPs, and selected plasma fatty acids.

Compared with the group supplemented with olive oil, the flaxseed oil group showed significantly higher levels of ALA in plasma phospholipids ($p < 0.0001$), as well as significant elevations of F₁-PhytoPs in plasma ($p = 0.049$) and urine ($p = 0.06$). Flaxseed oil did not affect plasma or urinary F₂-IsoPs levels. The higher plasma F₁-PhytoP concentration in the flaxseed oil group most likely resulted from the increased plasma concentration of the ALA substrate and/or the greater F₁-PhytoPs content of the flaxseed oil (Fig. 7).

Recently the group of Gil-Izquierdo and colleagues investigated the relationship of OS and the content of PhytoPs in

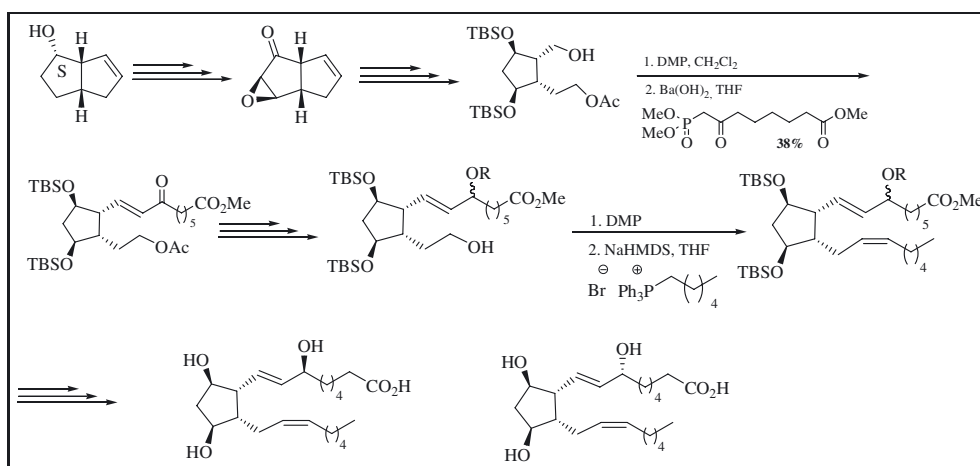


Fig. 5. Synthesis of *ent*-7- F_{21} -dihomo-IsoP and its epimer.

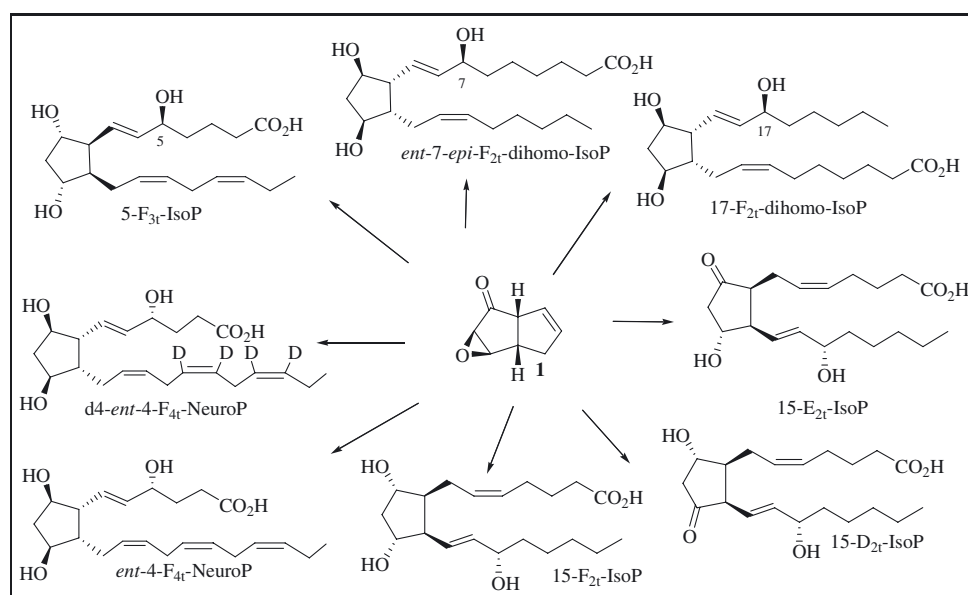


Fig. 6. A fully flexible approach.

plants. For example, Collado-Gonzales *et al.* have proposed a quick and accurate new analytical method by UHPLC-Qq-MS/MS which is able to identify free PhytoPs, in olive and refined sunflower oils (Collado-Gonzalez *et al.*, 2015). The recovery provided high extraction efficiencies ranging from 102.90% to 140.64% using Strata-XAW cartridge. The intra-day and inter-day variations for all target compounds ranged from 2.24% to 13.64% and 0.01% to 13.69%, respectively, and the accuracies for these parameters varied from 80.33% to 119.64% and from 80.34% to 119.90%, respectively. Results obtained reflect that refined sunflower presented more series of PhytoPs and a 20 and 8-fold higher quantity of PhytoPs than two types of olive oil: extra virgin olive oil and olive oil. The manufacture process could be the key for the different PhytoP production since most of the plant oils are subjected to a refining treatment. The results of this analysis performed in a single assay per sample are obtained within eight minutes, in addition, these advantages are linked to lower expenses in solvents.

From agronomical point of view in connection with food science and technology, future research is required to study how the production of PhytoPs is affected by different types of abiotic stress on olive oil and other plant oils. Nutritionally speaking, additional studies would be necessary to know the physiological effects of the PhytoPs in humans, since they show very similar structures than IsoPs and PGs, relevant bioactive compounds at physiological level.

Carrasco *et al.* studied almonds which have favourable contents of PUFA especially ALA (Carrasco-Del Amor *et al.*, 2015). This study represents the first approach to the quantitative and qualitative determination of the PhytoP profile in 11 almond cultivars under different agronomic conditions (conventional versus ecological, rain-fed versus irrigated). In the kernels have been identified 9- F_{11} -PhytoP, 9-*epi*-9- F_{11} -PhytoP, *ent*-16-*epi*-16- F_{11} -PhytoP, *ent*-16- F_{11} -PhytoP, 9- D_{11} -PhytoP, 9-*epi*-9- D_{11} -PhytoP, 16- B_1 -PhytoP and 9- L_1 -PhytoP. The total PhytoP content was in the range of 4.0 to 23.8 ng per 100 g. F_1 -PhytoPs predominated in all almond cultivars. L_1 -PhytoPs

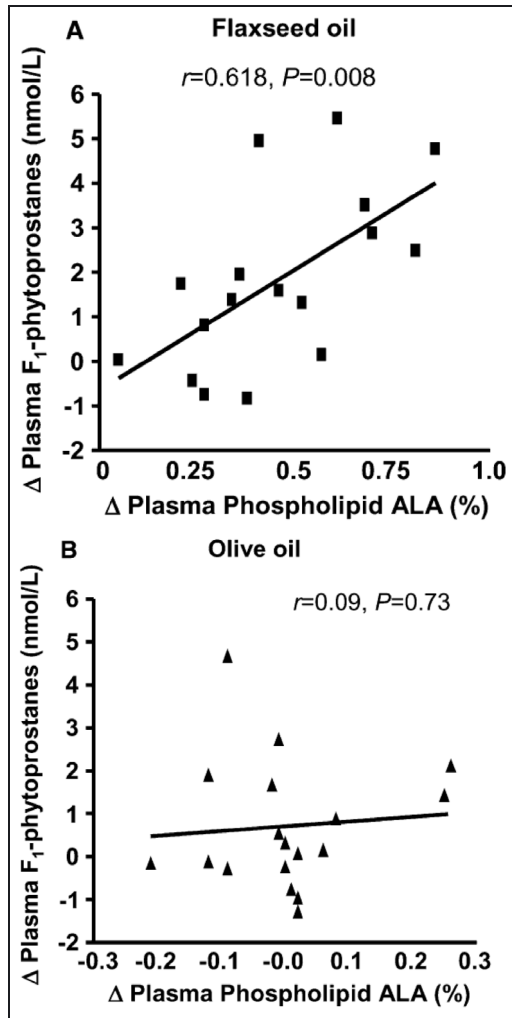


Fig. 7. Correlation between changes in plasma F₁-PhytoP and changes in plasma PL-ALA after supplementation for 4 weeks with 9 g/day of either olive and flaxseed oil (Barden *et al.*, 2009).

were minor components while D₁-PhytoPs were only detected in cultivars 'Colorada' and 'Avellanera'. The PhytoP profile varied greatly depending on the genotype, but was also affected by factors such as the agricultural system (conventional or ecological) and irrigation. The ecological system promoted the synthesis of D₁-PhytoPs. Almonds from rain-fed trees had lower individual and total PhytoP concentrations than those under irrigation, even though non-irrigation led to the detection of the 16-F₁-PhytoP. Consequently, irrigation and ecological techniques applied to almonds could be considered as actions to enhance their PhytoP content and hence their potential beneficial effects on human health.

PhytoPs have been studied in several plant species, but information regarding the natural occurrence of this large family of biologically active oxidized lipids in macroalgae is still scarce. Barbosa *et al.* (2015) studied the free PhytoP composition of 24 macroalgae species belonging to Chlorophyta, Phaeophyta, and Rhodophyta using an UHPLC-QqQ-MS/MS method (Barbosa *et al.*, 2015). The PhytoP profiles varied greatly among all samples, F₁-PhytoP and L₁-PhytoP being

the predominant and minor classes, respectively. No correlation between the amounts of ALA in alga material and PhytoP content was found. Therefore, it was hypothesized that the observed variability could be species-specific or result from interspecific interactions. This study provides new insight about the occurrence of PhytoPs in macroalgae and opens doors for future exploitation of these marine photosynthetic organisms as sources of potentially bioactive oxylipins, encouraging their incorporation in food products, nutraceutical and pharmaceutical preparations for human health.

3.2 Neuroprostanol and isofurans

OS may contribute to the pathogenesis of pre-eclampsia, a life-threatening disorder of pregnancy that adversely affects the mother and the baby (Barden *et al.*, 2004). In 1996 Barden *et al.* showed that plasma F₂-IsoP level raises in proteinuric pre-eclampsia (Barden *et al.*, 1996). In a recent study IsoFs, F₄-NeuroPs and F₂-IsoPs were quantified in maternal plasma and cord blood of women with pre-eclampsia and normal pregnancies (Barden *et al.*, 2012). Women with pre-eclampsia had significantly elevated maternal IsoFs and F₄-NeuroPs, but no F₂-IsoPs. Cord blood F₄-NeuroPs were elevated among neonates of women with pre-eclampsia. Interestingly, cord blood IsoFs were approximately 5-fold higher than those found in maternal plasma. This could reflect the oxidative challenge presented at birth, when there is transition from a relatively low intra-uterine oxygen environment to a significantly higher extra uterine oxygen environment. Maternal F₄-NeuroPs were not significantly correlated with cord blood F₄-NeuroPs in pre-eclamptic and in normal pregnancies, suggesting the origin of cord F₄-NeuroPs may be independent of maternal plasma. In normal pregnancy birth weight was negatively related to maternal F₂-IsoPs, IsoFs and F₄-NeuroPs.

The brain is vulnerable to oxidative insult because of high oxygen requirements for its metabolism and high PUFA composition, in particular DHA. Thus F₄-NeuroPs are considered as specific markers of brain OS. Aneurysmal subarachnoid hemorrhage (aSAH) and traumatic brain injury (TBI) are associated with devastating central nervous system (CNS) injury. In two case-controlled studies we have shown a significant increase in cerebrospinal fluid (CSF) IsoFs in aSAH and TBI patients compared with their respective age- and gender-matched controls. aSAH patients also had significantly increased levels of CSF F₄-NeuroPs and F₂-IsoPs. Patients with TBI had significantly increased CSF F₄-NeuroPs but F₂-IsoPs were similar to control (Corcoran *et al.*, 2011). These data confirm that CNS injury, in case of aSAH or TBI, results in increased OS and as DHA is the brain major PUFA, F₄-NeuroP levels in CSF could be a much more specific indicator of neurological dysfunction than F₂-IsoP. Hsieh *et al.* have shown that increased F₄-NeuroPs in CSF of patients with aSAH correlated with poor neurological outcome (Hsieh *et al.*, 2009). They suggested that F₄-NeuroPs might be more useful than F₂-IsoPs in CSF to predict outcome and interpret the role of hemorrhage in aSAH. Although Farias *et al.* showed increased F₂-IsoPs during rat brain ischemia, the E₂/D₂-IsoPs were increased to a greater extent, suggesting the latter may better markers of OS in brain ischemia (Farias *et al.*, 2008).

The anti-atherogenic effects of n-3 (omega) fatty acids, EPA and DHA are well recognized but the impact of dietary intake on bioactive lipid mediator profiles remains unclear. Such a profiling effort may offer novel targets for future studies into the mechanism of action of omega 3 fatty acids. Gladine *et al.* studied the impact of DHA supplementation on the profiles of PUFA oxygenated metabolites and their contribution to atherosclerosis prevention (Gladine *et al.*, 2014). A special emphasis was given to the non-enzymatic metabolites knowing the high susceptibility of DHA to free radical-mediated peroxidation and the increased OS associated with plaque formation. Atherosclerosis prone mice (LDLR^{2/2}) received increasing doses of DHA (0, 0.1, 1 or 2% of energy) during 20 weeks leading to a dose-dependent reduction of atherosclerosis ($R^2 = 0.97$, $p = 0.02$), triglyceridemia ($R^2 = 0.97$, $p = 0.01$) and cholesterolemia ($R^2 = 0.96$, $p < 0.01$). Targeted lipidomic analyses revealed that both the profiles of EPA and DHA and their corresponding oxygenated metabolites were substantially modulated in plasma and liver. Notably, the hepatic level of F₄-NeuroPs was strongly correlated with the hepatic DHA level. Moreover, unbiased statistical analysis including correlation analyses, hierarchical cluster and projection to latent structure discriminate analysis, revealed that the hepatic level of F₄-NeuroPs was the variable most negatively correlated with the plaque extent ($p < 0.001$) and along with plasma EPA-derived diols, was an important mathematical positive predictor of atherosclerosis prevention. Thus, oxygenated n-3 PUFAs, and F₄-NeuroPs in particular, are potential biomarkers of DHA-associated atherosclerosis prevention. While these may contribute to the anti-atherogenic effects of DHA, further *in vitro* investigations are needed to confirm such a contention and to decipher the molecular mechanisms of action.

3.3 Dihomo-isoprostanes

Rett syndrome (RTT) is a pervasive abnormality of development affecting almost exclusively females, which is included among the autism spectrum disorders. RTT is caused in up to 95% of cases by mutations in the X-linked methyl-CpG binding protein 2 (MeCP2) genes (De Felice *et al.*, 2012). Although over 200 different MeCP2 mutations have been reported to cause RTT, nine most frequent ones (hotspot mutations) are known to comprise more than three quarters of all the reported pathogenic mutations. The disease shows a wide phenotypical heterogeneity, with at least 4 distinct major clinical presentations, i.e., typical, preserved speech, early seizure variant, and congenital variant. Clinical evidence indicates that F₂-IsoPs and F₄-NeuroPs are involved in the intimate pathogenic mechanisms of RTT. Plasma levels of free F₂-IsoPs are significantly higher in the early stages of RTT, as compared with the late natural progression of typical RTT. Until recently it was thought that the predominant central nervous system damage in RTT occurred in gray matter. However, the relative abundance in myelin of the precursor AdA and the increased level of F₂-dihomo-IsoPs, strongly confirm an early and severe damage to the brain white matter as suggested by previous brain MRI evidence. Thus F₂-dihomo-IsoPs can be considered early markers of lipid peroxidation in RTT (De Felice *et al.*, 2011). F₄-NeuroPs also appear to be important biomarkers in

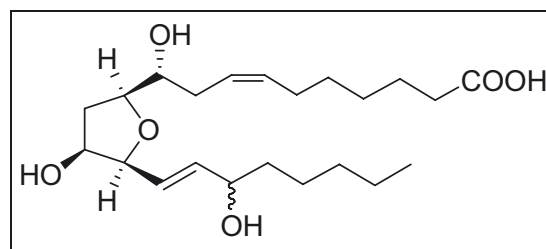


Fig. 8. 10-epi-17(RS)SC- Δ^{15} -11-dihomo-IsoF.

RTT. Plasma F₄-NeuroP levels correlate with disease severity in RTT and are significantly related to neurological symptoms severity, mutation type and clinical presentation. Therefore, F₄-NeuroPs may play a major role along the biochemical pathway from MeCp2 gene mutation to clinical evidence, proving that a DHA oxidation process occurs.

3.4 Dihomo-isofurans/neurofurans

Neurofurans (NeuroFs) and dihomom-isofurans (dihomo-IsoFs) are produced *in vivo* by non-enzymatic free radical pathways from DHA and AdA, respectively. As these metabolites are produced in minute amounts, their analyses in biological samples remain challenging. We performed syntheses of NeuroFs and dihomom-IsoFs (Fig. 8), thanks to an enantiomerically enriched intermediate, which allowed, for the first time, access to both families: the alkenyl and enediol (De La Torre *et al.*, 2015, 2014).

Owing to this formation, quantitation of specific NeuroF and dihomom-IsoF in biological samples was attainable and we reported the presence of 4(RS)-ST- Δ^5 -8-NeuroF and 7(RS)-ST- Δ^8 -11-dihomom-IsoF in rat brain and heart tissues. It is also the first report to show concentration of known NeuroP and dihomom-IsoP in the heart tissue. The concentration difference of 4(RS)-ST- Δ^5 -8-NeuroF between the heart and the brain indicates that it is a robust indicator for macro- and micro-vascular function, considering disparate *in situ* oxygen tension of the tissues. These DHA and AdA metabolites are presently in testing for various pathological models as OS biomarkers and bioactive compounds.

4 New bioactive lipids

Isoprostanes and analogs are not only biomarkers of lipid peroxidation but also mediators of oxidant injury.

4.1 Isoprostanes

IsoPs are vasoconstrictors in many species and various vascular beds, modulate platelet activity and monocyte adhesion, and induce proliferation of endothelial and smooth muscle cells.

IsoPs mediate their biological effects by activation and/or inhibition of several prostanoid receptors, among them the thromboxane receptor (TP), prostaglandin F₂ receptor (FP),

prostaglandin E₂ subtype 3 receptor (EP3), prostaglandin D₂ subtype 2 receptor (DP2) and by activation of the peroxisome proliferators activated receptor gamma (PPAR γ) (Milne *et al.*, 2011).

The vasomotor action of 15-F_{2t}-IsoP has been investigated in isolated human saphenous and umbilical veins, in bronchial, radial and internal mammary arteries, and in pulmonary vasculature as well as placental and maternal vessels. In contrast to 15-F_{2t}-IsoP, 5-F₂-IsoP-series do not contribute to the vasoconstriction mediated by IsoP. Besides vasoconstriction and platelet activation, IsoPs also enhance the vascular reperfusion damage after myocardial infarction; pioneering cardiac smooth muscle apoptosis and scar formation. In this scenario, formation of collateral and new vasculature outgrowth is essential for cardiac function recovery. The complex interplay of pro-angiogenic growth factors, IsoPs and the role of the TP receptors have been investigated thoroughly in different primary human endothelial cells. Low concentrations of 15-F_{2t}-IsoP promoted endothelial cell migration. In contrast, higher concentrations of several E-, A- and F-series IsoPs inhibited the VEGF-induced migration and tube formation of endothelial cells. These effects were abolished either by TP receptors blockade or alternatively by short hairpin RNA-mediated knock down of the TP receptors.

Taken together, these findings highlight the role of 15-F_{2t}-IsoP but also of other IsoPs in vascular homeostasis and there by provide a new rationale for TP receptors blockade (Benndorf *et al.*, 2008).

The retina is enriched with long chains PUFAs and is constantly exposed to light, rendering it highly vulnerable to OS. Because OS plays a key role in the pathogenesis of ocular neuropathies such as glaucoma and triggers spontaneous generation of long chain PUFA metabolites in retina, it is significant to delineate effect of these novel compounds on retinal pharmacology. So far, the pharmacological role for the 15-F₂-IsoP on neurotransmission in mammalian ocular tissues is well documented. However, the effect of the 5-F₂-IsoP-series on ocular tissues has not been described. In a recent study, we elucidated the pharmacological actions, *in vitro* of the 5-F₂-IsoP epimer pair, 5-*epi*-5-F_{2t}-IsoP (C5-OH in *R*-position) and 5-F_{2t}-IsoP (C5-OH in *S*-position) on excitatory glutamate release (using [3H]D-aspartate as a marker) in bovine retina (Hou *et al.*, 2004; Jamil *et al.*, 2012; Zhao *et al.*, 2009). Whereas 5-*epi*-5-F_{2t}-IsoP elicited a concentration-dependent inhibitory action, the 5-(*S*)-OH-epimer, 5-F_{2t}-IsoP displayed a more potent, biphasic inhibitory action on the neurotransmitter release, suggesting that spatial side chain orientation at the C5-position is required for the biphasic response. Consistent with the later observation, a biphasic profile of activity been reported for 15-F_{2t}-IsoP on the regulation of sympathetic and excitatory neurotransmission in the mammalian anterior uvea and retina, respectively. Contrary to 5-F_{2t}-IsoP, the 15-F_{2t}-IsoP lacks the hydroxyl side chain at C5 position. It is therefore apparent that additional factors contribute to the biphasic pattern of IsoP-response on neurotransmitter release. Because the effect of their 15-F₂-IsoP-counterparts is largely dependent on activation of prostanoid receptors, Jamil *et al.* (2012) examined the role of prostanoid receptors in the inhibitory action of the 5-*epi*-5-F_{2t}-IsoP. The inhibitory action of this 5-F₂-IsoP was

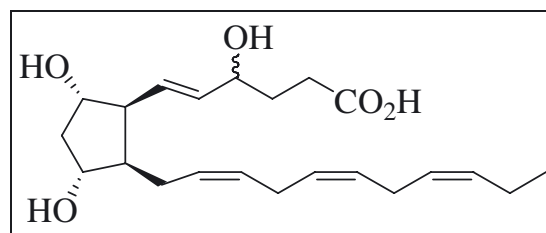


Fig. 9. 4(*RS*)-4-F_{4t}-NeuroP.

reversed by the prostanoid EP1- (SC-51322; SC-19220) and EP4-(AH 23848) receptor antagonists but not the EP1–3/DP-(AH 6809) and DP/TP receptor antagonist (BAY-u3405). Due to the prominent role that glutamate plays in the physiology of the retina as the major excitatory neurotransmitter and in neuronal excitotoxicity, the ability of 5-F₂-IsoP to attenuate excitatory neurotransmitter release could have significant pathophysiological implications in mammalian retina. It is conceivable that these endogenously derived AA-metabolites could modulate progression of ocular neuropathies and provide a new target for diagnostic and/or therapeutic strategies in the management of ocular neuropathies. Taken together, these data support a modulatory role for 5-F₂-IsoP epimer pair, 5-*epi*-5-F_{2t}-IsoP and 5-F_{2t}-IsoP on excitatory neurotransmitter release in bovine retina, *in vitro*. Whereas the allylic hydroxyl group at position C5 contributes to the apparent biphasic pattern of response exhibited by 5-F_{2t}-IsoP, the prostanoid EP1 and EP4 account for its inhibitory effect on excitatory neurotransmitter release (Opere *et al.*, 2005).

4.2 Neuroprostanes

There is considerable evidence that an enriched n-3 PUFA diet confers cardioprotective effects due primarily to the two main PUFAs, EPA and DHA (GISSI-Prevenzione Investigators, 1999; Judé *et al.*, 2003). This large prospective study showed that the most marked effect of DHA and EPA supplementation is a reduction of sudden cardiac death in the months following a cardiac infarction. This benefit has been explained, in part, by a reduction in arrhythmias and systolic cardiac failure. The anti-arrhythmic effects of n-3 PUFA have been confirmed in animal models of cardiac infarction by ligation of the left coronary artery. These and other studies in single cardiac cells have shown that EPA and DHA can modulate the activity of ion channels, the transmembrane proteins responsible for the electrical activity of the heart. However, it has been suggested that oxygenated metabolites of EPA and DHA may also play a role in these actions. In this regard it has been shown that some of the effect of DHA on rat cardiac ion channels was due to an oxidative metabolite of DHA (Judé *et al.*, 2003).

Le Guennec *et al.* tested different F₂-IsoP, F₃-IsoP and F₄-NeuroP on arrhythmias induced by isoprenaline and stimulation frequency of isolated ventricular mice cardiac cells. Among them, some F₄-NeuroPs have anti-arrhythmic properties (AAP) (IC₅₀ \approx 100 nM) (Le Guennec, 5 December 2012). The main isomer, 4(*RS*)-4-F_{4t}-NeuroP (Fig. 9), showed potent

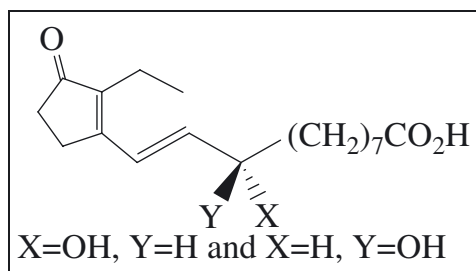


Fig. 10. 9-L₁-PhytoP.

dose-dependent AAP *in cellulo* and also *in vivo* in PMI mice. At the cellular level, the mechanism of action is unlikely to be due to a β -blocker effect, but the AAP can instead be explained by a rycal-like effect; in particular, stabilization of the RyR2 complex with FKBP12.6 (Andersson and Marks, 2010; Roy, 2015).

4.3 Phytostanes

PhytoPs formed in higher plants from ALA, are highly active lipids in plant kingdom and in humans. Experimental evidence strongly suggests that in plants PhytoPs act as endogenous mediators able to protect cells from damages under various conditions (Conconi *et al.*, 1996, Thoma *et al.*, 2003), especially those related to OS (Loeffler *et al.*, 2005). Since humans are potentially exposed to PhytoPs, which can be absorbed after oral ingestion of vegetable food or by inhalation of pollen (Gilles *et al.*, 2009), it is important to identify their activities in animal cells (Gutermuth *et al.*, 2007).

Minghetti *et al.* (2014) investigated the possible effect of 9-L₁-PhytoP (Fig. 10) on cells of the central nervous system (CNS), which are particularly susceptible to OS. Two experimental models were used: the SH-S5Y5 cell line, from a human neuroblastoma as a neuronal model and primary cultures of oligodendrocyte progenitors (OLs) from neonatal rat brain.

9-L₁-PhytoP can exert protective effects on cells in CNS, in particular, they protect neuronal cells not yet differentiated, such as those found in the adult brain from damages related to OS. They also promote the differentiation of OLs, through mechanisms which involve in part the activation of the nuclear receptor and ligand dependent transcription factor PPAR γ .

5 Conclusions

Our understanding of the role of PUFA peroxidation in the pathogenesis of various human diseases is at an early stage. We know that free radical-induced autoxidation of PUFAs occurs in numerous pathological conditions from cardiovascular disorders to cancers and neurodegenerative diseases. Early work with animal tissues indicated that ROS, or radicals formed after exposure to toxicants, are the initial triggers for oxidative injury to membranes. Subsequently, it was shown that IsoP generation accompanied lipid peroxidation injury in mammalian tissues. The exact PUFA derivatives generated will

depend on the fatty acid composition of the tissue, an important consideration for the use of IsoPs or NeuroPs as an organ-specific biomarker. Plant cells subjected to OS also produce cyclopentenone prostanoids, termed PhytoPs, by a non-enzymatic mechanism. Through our knowledge of organic chemistry, we can contribute to clinical and basic research by developing novel synthetic approaches and providing samples for biological and analytical work. A number of new approaches for chiral synthesis of IsoPs, PhytoPs NeuroPs are now available. Some of these products may be used as markers for the diagnosis and management of patients and will need to be measured accurately and precisely. The contribution of each of these unique PUFA derivatives to tissue and organ damage has to be clearly ascertained within a complex network of signalling molecules and mediators.

Acknowledgements. We thank our co-workers who are cited in the references. We thank CNRS, the French Ministry of Education and Research, for their continuous support of our research in this field and a part of this work was supported by the University Montpellier.

References

- Andersson DC, Marks AR. 2010. Fixing ryanodine receptor Ca²⁺ leak – a novel therapeutic strategy for contractile failure in heart and skeletal muscle. *Drug Discov. Today: Dis. Mech.* 7: e151–e157.
- Barbosa M, Collado-Gonzalez J, Andrade PB *et al.* 2015. Nonenzymatic alpha-Linolenic Acid Derivatives from the Sea: Macroalgae as Novel Sources of Phytostanes. *J. Agric. Food Chem.* 63: 6466–6474.
- Barden A, Beilin LJ, Ritchie J, Croft KD, Walters BN, Michael CA. 1996. Plasma and urinary 8-iso-prostane as an indicator of lipid peroxidation in pre-eclampsia and normal pregnancy. *Clin. Sci. (Lond)* 91: 711–718.
- Barden AE, Mori TA, Dunstan JA, *et al.* 2004. Fish oil supplementation in pregnancy lowers F₂-isoprostanes in neonates at high risk of atopy. *Free Radic. Res.* 38: 233–239.
- Barden A, Croft K, Durand T, Guy A, Mueller M-J, Mori TJ. 2009. F₁-phytostanes following ALA supplementation in men: A randomised controlled trial. *J. Nutr.* 10: 1890–1895.
- Barden AE, Corcoran TB, Mas E, *et al.* 2012. Is There a Role for Isofurans and Neuroprostanoids in Pre-Eclampsia and Normal Pregnancy? *Antioxidants Redox Signaling* 16: 165–169.
- Benndorf RA, Schwedhelm E, Gnann A, Taheri R, Kom G, Didie M, *et al.* 2008. Isoprostanes Inhibit Vascular Endothelial Growth Factor-Induced Endothelial Cell Migration, Tube Formation, and Cardiac Vessel Sprouting In Vitro, As Well As Angiogenesis *In Vivo* via Activation of the Thromboxane A₂ Receptor: A Potential Link Between Oxidative Stress and Impaired Angiogenesis. *Circ. Res.* 103: 1037–1046.
- Brinkmann Y, Oger C, Guy A, Durand T, Galano JM. 2010. Total synthesis of 15-D₂- and 15-epi-15E₂-Isoprostanes. *J. Org. Chem.* 75: 2411–2414.
- Brown HA, Marnett LJ. 2011. Lipid biochemistry, metabolism, and signaling. *Chem. Rev.* 111: 5817–6512.
- Carrasco-Del Amor AM, Collado-Gonzalez J, Aguayo E, Guy A, Galano JM, Durand T, Gil-Izquierdo A. 2015. Phytostanes in almonds: identification, quantification, and impact of cultivar and type of cultivation. *RSC Adv.* 5: 51233–51241.

- Colavitti R, Finkel T. 2005. Reactive Oxygen Species as Mediators of Cellular Senescence. *IUBMB Life* 57: 277–281.
- Collado-Gonzalez J, Medina S, Durand T, *et al.* 2015. New UHPLC/QqQ-MS/MS method for quantitative and qualitative determination of free phytoprostanes in foodstuffs of commercial olive and sunflower oils. *Food Chem.* 178: 212–220.
- Conconi A, Miquel M, Browse JA, Ryan CA. 1996. Intracellular levels of free linolenic and linoleic acid increase in tomato leaves in response to wounding. *Plant Physiol.* 111: 797–803.
- Corcoran TB, Mas E, Barden AE, *et al.* 2011. Are Isofurans and Neuroprostanes Increased After Subarachnoid Hemorrhage and Traumatic Brain Injury? *Antioxidants Redox Signal.* 15: 2663–2667.
- De Felice C, Signorini C, Durand T, *et al.* 2011. F2-dihomo-isoprostanes as potential early biomarkers of lipid oxidative damage in Rett syndrome. *J. Lipid Res.* 52: 2287–2297
- De Felice C, Signorini C, Leoncini S, *et al.* 2012. The Role of Oxidative Stress in Rett Syndrome: an overview. *Ann. NY Acad. Sci.* 1259: 121–135.
- De La Torre A, Lee YY, Oger C, *et al.* 2014. Synthesis, discovery, and quantitation of dihydro-isofurans: Biomarkers for in vivo adrenic acid peroxidation. *Angew. Chem. Int. Ed. Engl.* 53: 6249–6252.
- De La Torre A, Lee YY, Mazzoni A, *et al.* 2015. Total syntheses and in vivo quantitation of novel neurofuran and dihydro-isofuran derived from docosahexaenoic acid and adrenic acid. *Chemistry A Eur. J.* 21: 2442–2446.
- Farias SE, Basselin M, Chang L, Heidenreich KA, Rapoport SI, Murphy RC. 2008. Formation of eicosanoids, E2/D2 isoprostanes, and docosanoids following decapitation-induced ischemia, measured in high-energy-microwaved rat brain. *J. Lipid Res.* 49: 1990–2000.
- Fessel JP, Porter NA, Moore KP, Sheller JR, Roberts LJ. 2002. Discovery of lipid peroxidation products formed in vivo with a substituted tetrahydrofuran ring (isofurans) that are favored by increased oxygen tension. *Proc. Natl. Acad. Sci. U.S.A.* 99: 16713–16718.
- Galano J-M, Mas E, Barden A, *et al.* 2013. Isoprostanes and neuroprostanates: Total synthesis, biological activity and biomarkers of oxidative stress in humans. *Prostaglandins Other Lipid Mediat.* 107: 95–102.
- Galano J-M, Lee JC-Y, Gladine C, *et al.* 2015. Non-enzymatic cyclic oxygenated metabolites of adrenic, docosahexaenoic, eicosapentaenoic and α -linolenic acids; bioactivities and potential use as biomarkers. *Biochim. Biophys. Acta* 1851: 446–455.
- Gilles S, Mariani V, Bryce M, *et al.* 2009. Pollen-Derived E1-Phytoprostanates Signal via PPAR- γ and NF- κ B-Dependent Mechanisms. *J. Immunol.* 182: 6653–6658.
- GISSI-Prevenzione Investigators. 1999. *Lancet* 354: 447–455.
- Gladine C, Newman JW, Durand T, *et al.* 2014. Lipid Profiling following Intake of the Omega 3 Fatty Acid DHA Identifies the Peroxidized Metabolites F4-Neuroprostanates as the Best Predictors of Atherosclerosis Prevention. *PLoS One* 9: e89393.
- Gutermuth J, Bewersdorff M, Traidl-Hoffmann C, *et al.* 2007. Immunomodulatory effects of aqueous birch pollen extracts and phytoprostanates on primary immune responses *in vivo*. *J. Allergy Clin. Immunol.* 120: 293–299.
- Hou X, Roberts LJ, Gobeil F, *et al.* 2004. Isomer-specific contractile effects of a series of synthetic F2-isoprostanes on retinal and cerebral microvasculature. *Free Radic. Biol. Med.* 36: 163–172
- Hsieh YP, Lin CL, Shiue AL, *et al.* 2009. Correlation of F4-neuroprostanates levels in cerebrospinal fluid with outcome of aneurysmal subarachnoid hemorrhage in humans. *Free Radic. Biol. Med.* 47: 814–824.
- Il'yasova D, Ivanova A, Morrow JD, Cesari M, Pahor M. 2008. Correlation between two markers of inflammation, serum C-reactive protein and interleukin 6, and indices of oxidative stress in patients with high risk of cardiovascular disease. *Biomarkers* 13: 41–51.
- Imbusch R, Mueller MJ. 2000. Formation of isoprostane F(2)-like compounds (phytoprostanates F(1)) from alpha-linolenic acid in plants. *Free Radic. Biol. Med.* 28: 720–726.
- Jahn U, Galano JM, Durand T. 2008. Beyond prostaglandins—chemistry and biology of cyclic oxygenated metabolites formed by free-radical pathways from polyunsaturated fatty acids. *Angew. Chem. Int. Ed. Engl.* 47: 5894–5955.
- Jamil J, Wright A, Harrison N, *et al.* 2012. Regulation of [(3)H]d-aspartate release by the 5-F(2t)-isoprostane and its 5-epimer in isolated bovine retina. *Neurochem. Res.* 37: 574–582.
- Janssen LJ. 2008. Isoprostanes and lung vascular pathology. *Am. J. Respir. Cell Mol. Biol.* 39: 383–389.
- Judé S, Bedut S, Roger S, *et al.* 2003. Peroxidation of docosahexaenoic acid is responsible for its effects on I TO and I SS in rat ventricular myocytes. *Br. J. Pharmacol.* 139: 816–822
- Le Guennec J-Y, Galano JM, Oger C, *et al.* 5 December 2012. Methods and pharmaceutical composition for the treatment and prevention of cardiac arrhythmias. European patent EP12306519.3.
- Loeffler C, Berger S, Guy A, *et al.* 2005. B1-phytoprostanates trigger plant defense and detoxification responses. *Plant Physiol.* 137: 328–340.
- Magder S. 2006. Reactive oxygen species: toxic molecules or spark of life? *Critical Care* 10: 208–215.
- Mariani V, Gilles S, Jakob T, *et al.* 2007. Immunomodulatory mediators from pollen enhance the migratory capacity of dendritic cells and license them for Th2 attraction. *J. Immunol.* 178: 7623–7631.
- Mas E, Michel F, Guy A, *et al.* 2008. Quantification of urinary F2-Isoprostane with 4(RS)-F4t-Neuroprostane as an internal standard using gas chromatography-mass spectrometry. *J. Chromatogr. B* 10: 5087–5090.
- Michel F, Bonnefont-Rousselot D, Mas E, Drai J, Thérond P. 2008. Biomarkers of lipid peroxidation: analytical aspects. *Ann. Biol. Clin.* 66: 605–620.
- Milne GL, Yin H, Brooks JD, Sanchez S, Roberts II LJ, Morrow JD. 2007. Quantification of F2-isoprostanes in biological fluids and tissues as a measure of oxidant stress. *Methods Enzymol.* 433: 113–126.
- Milne GL, Yin H, Hardy KD, Davies SS, Roberts LJ. 2011. Isoprostane Generation and Function. *Chem. Rev.* 111: 5973–5996.
- Minghetti L, Salvi R, Lavinia Salvatori M, Ajmone-Cat MA, *et al.* 2014. Nonenzymatic oxygenated metabolites of \pm -linolenic acid B1- and L1-phytoprostanates protect immature neurons from oxidant injury and promote differentiation of oligodendrocyte progenitors through PPAR- δ activation. *Free Radic. Biol. Med.* 73: 41–50.
- Morrow JD, Roberts II LJ. 1994. Mass spectrometry of prostanoids: F2-isoprostanes produced by non-cyclooxygenase free radical-catalyzed mechanism. *Methods Enzymol.* 233: 163–174.
- Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts II LJ. 1990. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc. Natl. Acad. Sci. USA* 87: 9383–9387.
- Nourooz-Zadeh J, Liu EH, Anggard E, Halliwell B. 1998. F4-isoprostanes: a novel class of prostanoids formed during peroxidation of docosahexaenoic acid (DHA). *Biochem. Biophys. Res. Commun.* 242: 338–344

- Oger C, Brinkmann Y, Bouazzaoui S, Durand T, Galano J-M. 2008. Stereocontrolled Access to Isoprostanes via a Bicyclo[3.3.0]octene Framework. *Org. Lett.* 10: 5087–5090.
- Oger C, Bultel-Poncé V, Guy A, *et al.* 2010. The Handy Use of Brown's P2-Ni Catalyst for a Skipped Diyne Deuteration: Application to the Synthesis of a [D(4)]-Labeled F(4t)-Neuroprostane. *Chemistry A Eur. J.* 16: 13976–13980.
- Oger C, Bultel-Poncé V, Guy A, Durand T, Galano J-M. 2012. Total Syntheses of Isoprostanes Derived from Adrenic Acid and EPA. *Eur. J. Org. Chem.* 2012: 2621–2634.
- Opere CA, Zheng W, Huang J, Adewale A, Kruglet M, Ohia SE. 2005. Dual effects of isoprostanes on the release of [3H]D-aspartate from isolated bovine retinae: Role of arachidonic acid metabolites. *Neurochem. Res.* 30: 129–137.
- Roberts II LJ, Montine TJ, Markesbery WR, *et al.* 1998. Formation of isoprostane-like compounds (neuroprostanes) in vivo from docosahexaenoic acid. *J. Biol. Chem.* 273: 13605–13612.
- Roy J, Oger C, Thireau J, *et al.* 2015. Nonenzymatic lipid mediators, neuroprostanes, exert the antiarrhythmic properties of docosahexaenoic acid. *Free Radic. Biol. Med.* 86: 269–278.
- Solberg R, Longini M, Proietti F, Vezzosi P, Saugstad OD, Buonocore G. 2012. Resuscitation with supplementary oxygen induces oxidative injury in the cerebral cortex. *Free Radic. Biol. Med.* 53: 1061–1067.
- Song W-L, Lawson JA, Reilly D, *et al.* 2008. Neurofurans, Novel Indices of Oxidant Stress Derived from Docosahexaenoic Acid. *J. Biol. Chem.* 283: 6–16.
- Stafforini DM, Sheller JR, Blackwell TS, *et al.* 2006. Release of Free F2-isoprostanes from Esterified Phospholipids Is Catalyzed by Intracellular and Plasma Platelet-activating Factor Acetylhydrolases. *J. Biol. Chem.* 281: 4616–4623.
- Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Wilztum JL. 1989. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* 320: 915–924.
- Thoma I, Loeffler C, Sinha AK, *et al.* 2003. Cyclopentenone isoprostanes induced by reactive oxygen species trigger defense gene activation and phytoalexin accumulation in plants. *Plant J.* 34: 363–375.
- Traidl-Hoffmann C, Mariani V, Hochrein H, *et al.* 2005. Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. *J. Exp. Med.* 201: 627–636.
- VanRollins M, Woltjer RL, Yin H, Morrow JD, Montine TJ. 2008. F2-Dihomo-isoprostanes arise from free radical attack on adrenic acid. *J. Lipid Res.* 49: 995–1005.
- Vigor C, Bertrand-Michel J, Pinot E, *et al.* 2014. Non-enzymatic lipid oxidation products in biological systems: Assessment of the metabolites from polyunsaturated fatty acids. *J. Chromatog. B* 964: 65–78.
- Zhao M, Destache C, Ohia S, Opere C. 2009. Role of prostanoid production and receptors in the regulation of retinal endogenous amino acid neurotransmitters by 8-isoprostaglandin *ex vivo*. *Neurochem. Res.* 34: 2170–2180.

Cite this article as: Valérie Bultel-Poncé, Thierry Durand, Alexandre Guy, Camille Oger, Jean-Marie Galano. Non enzymatic metabolites of polyunsaturated fatty acids: friend or foe. OCL 2016, 23(1) D118.