Two sides of the same coin: NEO-PUFAs in Rett syndrome and post-infarction cardiac arrhythmias

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Marie Demion[1], Camille Oger[2], Claire Vigor[2], Jérôme Thireau[1], Jean-Yves Le Guennec[1], Thierry Durand[2], Jean-Marie Galano[2] and Jetty Chung-Yung Lee[3]

[1] Inserm U1046–UMR CNRS 9214 Physiologie et Médecine Expérimentale du cœur et des muscles–PHYMEDEX, Université de Montpellier, Montpellier, France
[2] Institut des Biomolécules Max Mousseron, (IBMM) UMR 5247, CNRS Université de Montpellier, ENSCM, Montpellier, France
[3] The University of Hong Kong, School of Biological Sciences, Hong Kong SAR

An imbalance between oxidants and antioxidants that favors the oxidants leads to a disruption of the redox signaling control and/or molecular damage. The action of the oxidants in a non-enzymatic process generates isoprostanoids from polyunsaturated fatty acids. In this review, we will focus on the effects of ω3-polyunsaturated fatty acids in two different pathogeneses related to chronic and acute oxidative stress, one in neurodevelopmental, also known as the Rett syndrome, and the second in myocardial infarction and cardiac arrhythmias, respectively. We hypothesize that ω3-polyunsaturated fatty acid supplementation displays antioxidant properties under a high oxidative stress situation, as in the Rett Syndrome, as well as protective properties of isoprostanoids from polyunsaturated fatty acids in pro-arrhythmic conditions.

Practical applications : In this review, we highlighted the role of omega 3- polyunsaturated fatty acids in 2 distinct pathologies where oxidative stress is elevated (in the Rett syndrome as an anti-oxidant molecule and in cardiac arrhythmias as messenger with biologic properties). The physiological relevance of these data open new unexplored pathways in integrative mechanism and thus potential new "non-drug" applications.

Keywords: Antioxidant / Bioactive / Biomarker / Cardiac Arrhythmias / PUFAs / Rett Syndrome / ROS

1 Introduction

Mitochondrion is the site for ATP synthesis by oxidative phosphorylation, as well as regulating apoptosis, redox status, and reactive oxygen species (ROS) production [1]. During the reverse electron transfer through the respiratory chain (from complex III to I) [2], free radical oxidants are generated [3]. However, ROS production is not limited to the mitochondria. Other organelles and cell types such as sarcoplasmic reticulum, T-tubules, or macrophages are potent sources of ROS due to the presence of NADPH oxidase. Alternative mechanisms to these main sources of ROS are arachidonic acid (AA) metabolism via the activation of various enzymes, mainly cyclooxygenase, lipoxygenase or cytochrome P450-dependent monooxygenase [4–7], nitric oxide (NO), and xanthine oxidase (XO) [8–11].

The intracellular redox balance is maintained [8, 12, 13] through antioxidant defenses such as enzymatic scavengers, namely superoxide dismutase (from SOD1 to SOD3), glutathione peroxidase (GPx group), and catalase, as well as non-enzymatic factors including flavonoids or vitamins C

Correspondence: Dr. Marie Demion, Inserm U1046–UMR CNRS 9214 Physiologie et Médecine Expérimentale du cœur et des muscles–PHY-MEDEX, Université de Montpellier, Montpellier, France
E-mail: marie.demion@inserm.fr
Fax: +334 674 152 42

Abbreviations: AdA, adrenic acid; **ALA,** α-linolenic acids; **DHA,** docoshexaenoic acid; **EPA,** eicosapentaenoic acid; **F_2-dihomo-IsoPs,** F_2-dihomo-isoprostanes; **F_3-IsoPs,** F_3-isoprostanes; **F_4-NeuroPs,** F_4-neuroprostanes; **F_1-PhytoPs,** F_1-phytoprostanes; **NEO-PUFAs,** non-enzymatic oxidation of polyunsaturated fatty acids; **OS,** oxidative stress; **PUFAs,** polyunsaturated fatty acids; **ROS,** reactive oxygen species; **RTT,** Rett syndrome; **RyR2,** Ryanodine Receptor Type 2; **SCD,** sudden cardiac death

and E. However, in the event of excess ROS, that is, oxidative stress (OS), a disruption of the redox signaling and control and/or molecular damage occurs [14], initiating certain biological processes including immune response of T-lymphocytes [15] or synaptic plasticity [16, 17]. Also, ROS production is found to increase in chronic diseases and aging, for example, in the development of hypertension [18] or neurodegenerative disorders such as Alzheimer or Parkinson's diseases [19–21], where both incidents involve non-enzymatic lipid peroxidation. It is known that OS induce the oxidation of lipids, proteins and/or DNA. For over two decades, it has been shown that non-enzymatic oxidation of polyunsaturated fatty acids (NEO-PUFAs) generates isoprostanoids and are noted as classic biomarkers of OS in biological systems [22–24]. The degree of unsaturation of PUFAs, oxygen concentration, or redox signaling modulates the rate of PUFAs peroxidation [25]. The chemical stability and the use of the proper analytical techniques allow scientists to quantify these molecules, which led to an in-depth understanding of the role of OS in human physiology and pathophysiology [22]. Recently, isoprostanoids were described not only as biomarkers but also appear to have biological functions. Indeed, F3-isoprostanes (F3-IsoPs), F4-neuroprostanes (F4-NeuroPs), F2- dihomo-isoprostanes (F2-dihomo IsoPs), and F1-phytoprostanes (F1-PhytoPs), which are respectively derived from eicosapentaenoic (EPA), docoshexaenoic (DHA), adrenic (AdA), and α-linolenic acids (ALA) (Fig. 1), were described to be potentially beneficial for health [26, 27].

In this review, we will focus on ROS in Rett syndrome (RTT) and sudden cardiac death (SCD), with a special emphasis on ω3-PUFA supplementation, leading to the decrease of disease severity through two different mechanisms.

2 Rett syndrome (RTT)

RTT (OMIM #312750) is a severe neurodevelopmental disease due to the X-linked mutation of the gene encoding methyl-CpG-binding protein 2 (MeCP2) [28]. Because of X chromosome inactivation, the individuals most affected are female heterozygotes, who are somatic mosaics for the normal and the mutant MeCP2 gene. Since they have only one X chromosome, males are rarely born with a MeCP2 mutation, but those affected display more severe disorders and early mortality [29]. The prevalence of RTT is estimated from 1:10,000 to 1:15,000 live female births [30]. Approximately 95% of the patients have confirmed MeCP2 mutation, in which eight to nine hotspot mutations account for more than 60% for all cases.

Following a normal development of 6–18 months the acquired cognitive, social, and motor skills of the RTT patients begin to deteriorate in four stages and develop

Marie Demion obtained her Ph.D. at the University of Poitiers in 2006 by characterizing a new ionic channel, the TRPM4 channel, in freshly isolated sinus node cells from mouse. She then moved at Paris for a Postdoctoral position, still working on the TRPM4 channel but in the immune system. In 2008, she got a permanent position at the University of Montpellier, as an Assistant Professor. She teaches mostly on cardiac physiology. She is now investigating the anti-arrhythmic properties of omega 3 polyunsaturated fatty acids at the cellular level.

Thierry Durand received his PhD at University of Montpellier I in 1990. After a postdoct at FIT in Melbourne, USA, he became CR-CNRS in 1991. He finished his Habilitation in 1996 and became DR in 2002. He is head of Department of Lipides at the Institute of Biomolécules Max Mousseron. His interests include the total synthesis of oxygenated cyclic and non cyclic metabolites of PUFAS, leukotrienes, iso-, phyto- and neuro-prostanes/furanes, as well as diH-PUFAs, branched Fatty Acids, lipophenols, lipopeptides and the understanding of the role of such bioactive lipids by developing collaborations with chemists, biochemists, biologists and clinicians.

Dr. Jetty Chung-Yung Lee joined Yong Yoo Lin School of Medicine at National University of Singapore (NUS) as Research Fellow to research in antioxidant and oxidative stress in healthy community and clinical studies, focusing in neurodegenerative diseases. After 8 years at NUS, Dr. Lee was appointed as Assistant Professor by her Alma Mater, Hong Kong University to teach and research in Food and Nutritional Science at the School of Biological Sciences. Her current research focus is on polyunsaturated fatty acids and its oxidized mediators in health and diseases, and the environment and marine ecosystem.

autistic-like behavior with stereotypic hand movements. Autonomic and respiratory problems are frequent as well as gastrointestinal dysfunction or cardiac conduction disorders [31–39]. These phenotypes arise from the pleotropic effects of MeCP2, which is expressed very early
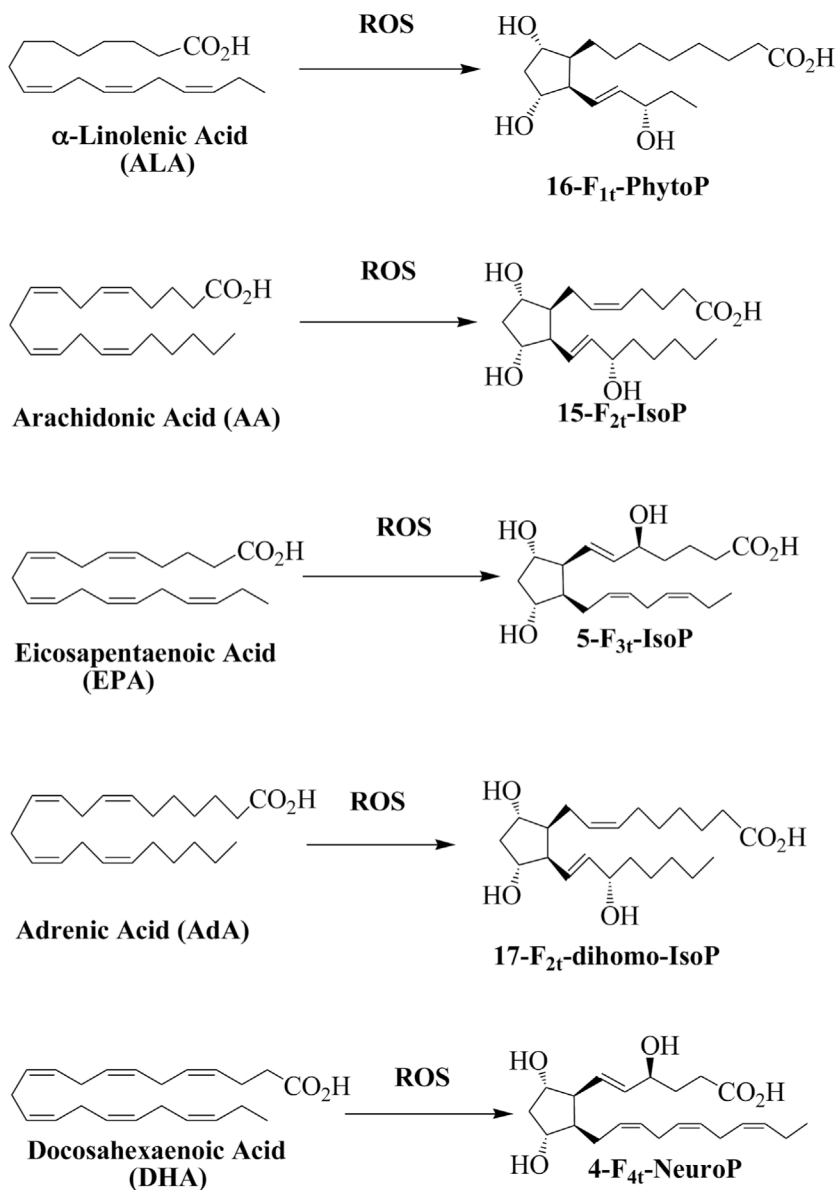
Figure 1. Chemical structures of phytoprostanes (PhytoP), isoprostanes (IsoPs), dihomo-Isoprostanes (dihomo-IsoPs) and neuroprostanes (NeuroP) generated from polyunsaturated fatty acids oxidation.

in neuronal progenitors and continues to be expressed into adulthood. The effects of *MeCP2* are mediated by diverse signaling, transcriptional, and epigenetic mechanisms.

The *MeCP2* function is clearly to bind DNA. However, its precise role as an activator or repressor of transcription, a regulator of miRNA processing, or/and splicing or even as a regulator of chromosome looping or compaction is not known yet [29, 40–47]. As mentioned, the condition of RTT patients is subdivided into four stages from I to IV. Stage I represents a period in which girls have a developmental stagnation; stage II: a rapid regression; stage III: a stationary stage; and then stage IV when girls are in late motor deterioration. In comparison with other organs, the brain is the most vulnerable to ROS damage because of its high concentration of PUFAs, high metabolic rate, high cellular

iron level and "relatively" low antioxidant concentration [48]. In 1987, it was shown for the first time that there was a reduced antioxidant capacity, in particular ascorbic acid and glutathione [49], in the postmortem brain of one RTT patient. Reduced antioxidant defense was confirmed by low serum vitamin E levels in RTT patients, and subsequent oxidative damage of lipids and proteins [50, 51]. In 2004, oxidative damage of DNA due to epigenetic modification by free radicals was reported in RTT patients [52] and in 2008 F2-dihomo-IsoPs, NEO metabolites of AdA, were identified to be indicators of OS in human brain white matter [53]. Base on the apparent gap between the *MeCP2* mutation and the disease expression (as a function of time and phenotype severity), the search for an appropriate OS biomarker was needed to explain this gap. It was speculated that the white

matter was already damaged at the early stage of RTT, therefore De Felice et al. quantified plasma F2-dihomo-IsoPs from stage I to IV patients and compared them to healthy subjects. They demonstrated that plasma F2-dihomo-IsoP levels were three times higher in stage II to IV RTT patients and 100 times higher in stage I RTT patients, compared to the controls [54, 55].

Interestingly, RTT patients with preserved speech variant, which is the mildest form of the disease, showed a noticeable concentration of plasma F2-dihomo-IsoPs compared to healthy subjects, suggesting a relation between symptomatic severity and AdA peroxidation.

De Felice et al. [56] also evaluated OS markers in whole brains of Mecp2-null (pre-symptomatic, symptomatic, and rescued) and Mecp2-308 mutated (pre-symptomatic and symptomatic) mice, that is, animal models, which recapitulate the disease. They found that IsoPs (F2-IsoPs, F4-NeuroPs, F2-dihomo-IsoPs) increased before the onset of symptoms [56]. These results indicate an early insult of AdA during stage I of this disease. AdA is a critical component of myelin in brain white matter. The main function of myelin is to insulate axon, allowing Ranvier nodes to be formed and potential action to jump from one node to another. There is therefore a correlation between plasma F2-dihomo-IsoPs, AdA OS insult in the white matter and clinical onset of neurodegeneration.

To test the involvement of OS hypothesis in RTT patients, in a preliminary clinical study stage I RTT patients were supplemented with ω3-PUFAs (86 and 134 mg/kg b.w./day of DHA and EPA, respectively) for 6 months. Surprisingly, NEO metabolites from DHA and EPA were not increased in the plasma from RTT-supplemented patients but instead significantly decreased with NEO metabolites from AdA [57]. The fact that F2-dihomo-IsoPs decreased in the plasma of RTT-supplemented patients, without increasing F2-IsoPs or F4-NeuroPs, strongly suggests a significant lowering of OS. These results were also associated with the diminution of clinical severity scores, recovery of ambulation, purposeful hand movements, fewer hand stereotypies, and recovery of social engagement. Nonetheless, the mechanism of ω3-PUFAs supplementation leading to the decrease of OS still needs to be determined.

3 ω3-PUFAs: From antioxidant properties to biological effect of their oxidized products in cardiovascular pathology

The incident of cardiovascular diseases (CVD) is constantly increasing worldwide. According to the European Society of Cardiology (ESC), in 2015 more than 17 million deaths are due to CVD, of which 25% is accounted to sudden cardiac death (SCD) [58]. It is now well established that the propensity to sudden death comes from favorable substrate that are genetically related or acquired by electrical and/or mechanical modifications as well as the triggering of multiple external factors to provoke the fatal event. The major strategy to reduce SCD risk includes anti-arrhythmic drugs and defibrillator implantation. These symptomatic treatments are associated with stress (electrical shock) without inhibiting disease progression. Despite the fact that defibrillators are life-saving in SCD, they do not prevent disease. Therefore, it is necessary to develop new drugs, but to date such drugs have not been discovered.

Among SCD causes, aging (>65 years old) brings about chronic remodeling of the cardiac muscle from hypertension, valvular disease, and/or repeated coronary ischemia. During repeated ischemia events, irreversible loss of cardiomyocytes is observed, which is also associated with the modification of local metabolism [59]. Excess amount of ROS production during ischemia is a potential trigger of arrhythmia genesis, as well as an activator of cellular remodeling [60–64]. These modifications can lead to arrhythmias that might be lethal. It has been shown that free fatty acids and prostanoid accumulation in the ischemic zone is correlated to the severity of the ischemia but also with the development of arrhythmias [65].

However, the mechanism linking fatty acid and prostanoid accumulation to arrhythmia remains unclear; some prostanoid receptors show pro-arrhythmic or anti-arrhythmic properties [66, 67]. The discrepancy of the effects could depend on which type of fatty acid the prostanoids originate from (AA or EPA) [68, 69].

Studies show ω3-PUFAs clearly prevent arrhythmias and SCD related with myocardial infarction and atrial fibrillation in in vivo and ex vivo models, and these effects involve enzymatic and non-enzymatic metabolites from ω3-PUFA oxidation [70–72]. In fact, Le Guennec et al. were the first to hypothesize that these effects are mediated by NEOPUFAs [73, 74]. They demonstrated that the incubation of isolated murine cardiomyocytes in the presence of $10 \mu M$ DHA prevented cellular arrhythmias. This anti-arrhythmic property was also observed in the presence of oxidants ($1 \mu M$ H_2O_2) and not in antioxidants ($1 \mu M$ Vitamin E). Indeed, the anti-arrhythmic effect was much stronger in the presence of H_2O_2 when compared to DHA alone. They then demonstrated that the oxidized product responsible for the effect was a DHA NEO metabolite, namely 4(RS)-4-F4T-Neuroprostane (4-F4T-NeuroP). In collaboration with Durand et al., who synthesized numerous NEO-PUFAs, they found 4-F4T-NeuroP to reduce cellular arrhythmias and the effective concentration (EC50) was close to 100 nM. The underlying mechanism for the observation is contributed by the ability of 4-F4T-NeuroP to maintain calcium homeostasis in the cells. The diastolic calcium concentration was comparable to the concentration observed in control conditions whereas it was increased in arrhythmic cells. The augmentation in the arrhythmic cells was caused by the calcium leak from the sarcoplasmic reticulum through type 2 Ryanodine receptor

(RyR2), a major intracellular calcium channel involved in the excitation-contraction coupling. This leak was prevented in the presence of 4-F4T-NeuroP, DHA or DHA + H$_2$O$_2$. The normalization was aligned with post-transduction modification of RyR2 protein where carbonylation and S-nitrosylation were the two alterations observed in the pathological state of the cardiomyocytes. The modification induced a leaky channel function, which is responsible for the intracellular calcium elevation and thus cellular arrhythmias [73].

4-F4T-NeuroP is also active in vivo. In mice, 4-F4T-NeuroP (1 μM) reduced arrhythmias evoked by a β-adrenergic agonist (2.5 mg/kg) after myocardial infarction. The reduction was equivalent to those observed using a reference anti-arrhythmic drug, the β-antagonist carvedilol (1 μM). Altogether, the study indicated a novel therapeutic opportunity for the use of natural products, NEO-PUFAs, by explicitly showing the cellular mechanism of 4-F4T-NeuroP mediating prostanoid receptor activation and in mice addressing its adverse effects.

4 Conclusions

The study suggests that ω6-PUFAs replacement by ω3-PUFAs in the diet increase DHA and EPA incorporation, by substituting AA and modifying metabolic processes. From this review, we propose the hypothesis that OS can be modulated by ω3-PUFAs, as in the Rett syndrome, or can generate metabolites of ω3-PUFAs and exert cardioprotection in myocardial infarction. The physiological relevance of these data open new unexplored pathways in integrative mechanisms. Moreover, OS is not only a consequence of an unbalanced redox system but also a signal with downstream adapted (or not) responses.

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