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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

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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 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

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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.

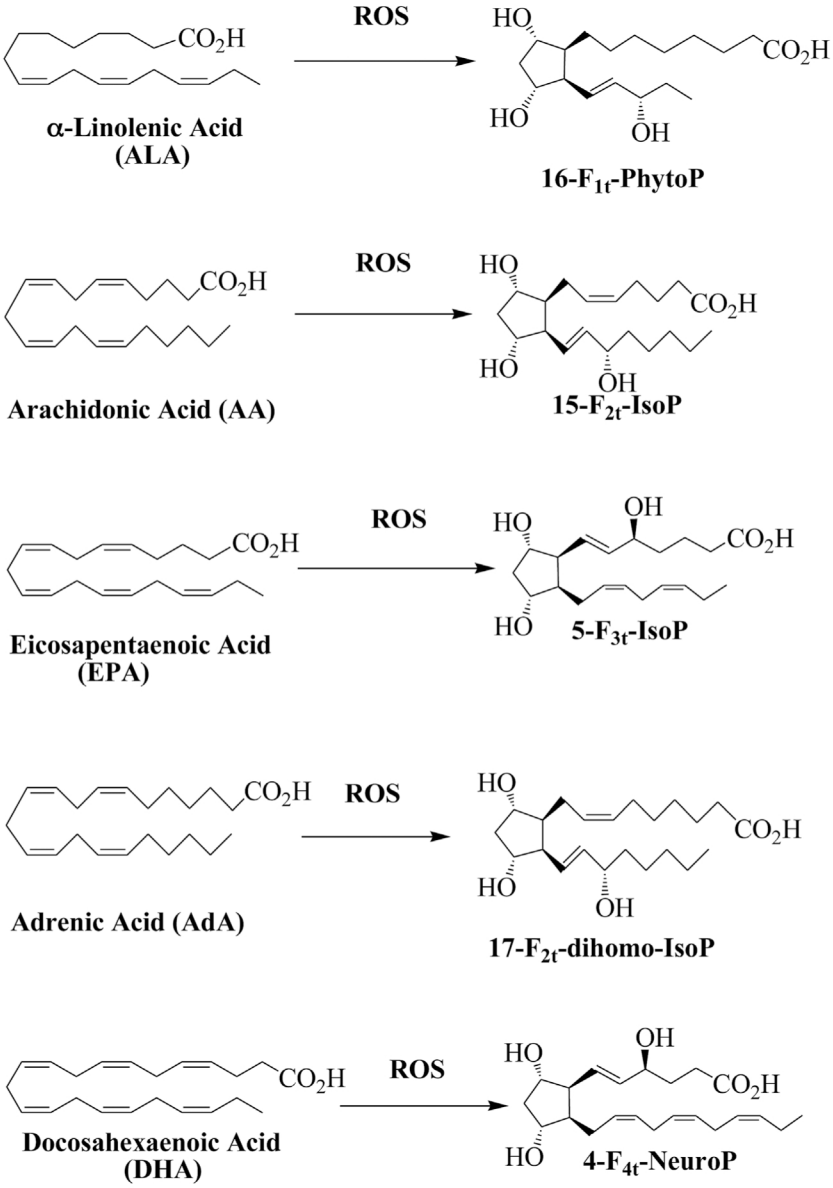

Figure 1. Chemical structures of phytoprostanes (PhytoP), isoprostanes (IsoPs), dihomo-Isoprostanes (dihomo-IsoPs) and neuroprostanes (NeuroP) generated from polyunsaturated fatty acids oxidation.

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 μM DHA prevented cellular arrhythmias. This anti-arrhythmic property was also observed in the presence of oxidants (1 μM H_2O_2) and not in antioxidants (1 μM 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_2O_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 (1μM) 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 (1μM). 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.

The authors have declared no conflict of interest.

References

[1] Dikalov, S., Cross talk between mitochondria and NADPH oxidases. *Free Radic. Biol. Med.* 2011, *51*, 1289–1301. DOI: 10.1016/j.freeradbiomed.2011.06.033

[2] Chance, B., Williams, G. R., Respiratory enzymes in oxidative phosphorylation.III. The steady state. *J. Biol. Chem.* 1955, *217*, 409–427.

[3] Panov, A., Dikalov, S., Shalbuyeva, N., Hemendinger, R., et al., Species- and tissue-specific relationships between mitochondrial permeability transition and generation of ROS in brain and liver mitochondria of rats and mice. *Am. J. Physiol. Cell Physiol.* 2007, *292*, C708–C718. DOI: 10.1152/ajpcell.00202.2006

[4] Zuo, L., Christofi, F. L., Wright, V. P., Bao, S., et al., Lipoxygenase-dependent superoxide release in skeletal muscle. *J. Appl. Physiol. Bethesda. Md. 1985* 2004, *97*, 661–668. DOI: 10.1152/japplphysiol.00096.2004

[5] Woo, C. H., Eom, Y. W., Yoo, M. H., You, H. J., et al., Tumor necrosis factor-alpha generates reactive oxygen species via a cytosolic phospholipase A2-linked cascade. *J. Biol. Chem.* 2000, *275*, 32357–32362. DOI: 10.1074/jbc.M005638200

[6] Woo, C. H., Lee, Z. W., Kim, B. C., Ha, K. S., et al., Involvement of cytosolic phospholipase A2, and the subsequent release of arachidonic acid, in signalling by rac for the generation of intracellular reactive oxygen species in rat-2 fibroblasts. *Biochem. J. 348 Pt* 2000, *3*, 525–530.

[7] Okabe, E., Kato, Y., Kohno, H., Hess, M. L., et al., Inhibition by free radical scavengers and by cyclooxygenase inhibitors of the effect of acidosis on calcium transport by masseter muscle sarcoplasmic reticulum. *Biochem. Pharmacol.* 1985, *34*, 961–968.

[8] Zuo, L., Zhou, T., Pannell, B. K., Ziegler, A. C., et al., Biological and physiological role of reactive oxygen species-the good, the bad and the ugly. *Acta Physiol. Oxf. Engl.* 2015, *214*, 329–348. DOI: 10.1111/apha.12515

[9] Cocco, T., Di Paola, M., Papa, S., Lorusso, M., Arachidonic acid interaction with the mitochondrial electron transport chain promotes reactive oxygen species generation. *Free Radic. Biol. Med.* 1999, *27*, 51–59.

[10] Pou, S., Keaton, L., Surichamorn, W., Rosen, G. M., Mechanism of superoxide generation by neuronal nitric-oxide synthase. *J. Biol. Chem.* 1999, *274*, 9573–9580.

[11] Wong, M. S.-K., Vanhoutte, P. M., COX-mediated endothelium-dependent contractions: From the past to recent discoveries. *Acta Pharmacol. Sin.* 2010, *31*, 1095–-1102. DOI: 10.1038/aps.2010.127

[12] Calabrese, V., Cornelius, C., Mancuso, C., et al., Cellular stress response: A novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity. *Neurochem. Res.* 2008, *33*, 2444–-2471. DOI: 10.1007/s11064-008-9775-9

[13] Ott, M., Gogvadze, V., Orrenius, S., Zhivotovsky, B., Mitochondria, oxidative stress and cell death. *Apoptosis Int. J. Program Cell Death* 2007, *12*, 913–922. DOI: 10.1007/s10495-007-0756-2.

[14] Sies, H., Oxidative stress: A concept in redox biology and medicine. *Redox Biol.* 2015, *4*, 180–183. DOI: 10.1016/j.redox.2015.01.002

[15] Belikov, A. V., Schraven, B., Simeoni, L., T cells and reactive oxygen species. *J. Biomed. Sci.* 2015, *22*, 85. DOI: 10.1186/s12929-015-0194-3

[16] Ma, T., Hoeffer, C. A., Wong, H., Massaad, C. A., et al., Amyloid β-induced impairments in hippocampal synaptic plasticity are rescued by decreasing mitochondrial superoxide. *J. Neurosci. Off. J. Soc. Neurosci.* 2011, *31*, 5589–5595. DOI: 10.1523/JNEUROSCI. 6566–10.2011

[17] Brieger, K., Schiavone, S., Miller, F. J., Krause, K.-H., Reactive oxygen species: From health to disease. *Swiss Med. Wkly.* 2012, *142*, w13659. DOI: 10.4414/smw.2012.13659

[18] Nazarewicz, R. R., Dikalova, A. E., Bikineyeva, A., Dikalov, S. I., Nox2 as a potential target of mitochondrial superoxide and its role in endothelial oxidative stress. *Am. J. Physiol. Heart Circ. Physiol.* 2013, *305*, H1131–H1140. DOI: 10.1152/ajpheart.00063.2013

[19] Musiek, E. S., Cha, J. K., Yin, H., Zackert, W. E., et al., Quantification of F-ring isoprostane-like compounds (F4-neuroprostanes) derived from docosahexaenoic acid in vivo

in humans by a stable isotope dilution mass spectrometric assay. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2004, *799*, 95–102.

[20] Nourooz-Zadeh, J., Liu, E. H., Yhlen, B., Anggård, E. E., et al., F4-isoprostanes as specific marker of docosahexaenoic acid peroxidation in Alzheimer's disease. *J. Neurochem.* 1999, *72*, 734–740.

[21] Halliwell, B., Oxidative stress and neurodegeneration: Where are we now?. *J. Neurochem.* 2006, *97*, 1634–1658. DOI: 10.1111/j.1471-4159.2006.03907.x

[22] Galano, J.-M., Mas, E., Barden, A., Mori, T. A., et al., Isoprostanes and neuroprostanes: Total synthesis, biological activity and biomarkers of oxidative stress in humans. *Prostaglandins Other Lipid Mediat.* 2013, *107*, 95–102. DOI: 10.1016/j.prostaglandins.2013.04.003

[23] Kadiiska, M. B., Peddada, S., Herbert, R. A., Basu, S., et al., Biomarkers of oxidative stress study VI. Endogenous plasma antioxidants fail as useful biomarkers of endotoxin-induced oxidative stress. *Free Radic. Biol. Med.* 2015, *81*, 100–106. DOI:10.1016/j.freeradbiomed.2015.01.006

[24] Milne, G. L., Yin, H., Hardy, K. D., Davies, S. S., et al., Isoprostane generation and function. *Chem. Rev.* 2011, *111*, 5973–5996. DOI: 10.1021/cr200160h

[25] Wang, W., Yang, H., Johnson, D., Gensler, C., et al., Chemistry and biology of ω-3 PUFA peroxidation-derived compounds. *Prostaglandins Other Lipid Mediat.* 2016, DOI: 10.1016/j.prostaglandins.2016.12.004

[26] Durand, T., Bultel-Poncé, V., Guy, A., El Fangour, S., et al., Isoprostanes and phytoprostanes: Bioactive lipids. *Biochimie.* 2011, *93*, 52–60. DOI: 10.1016/j.biochi.2010.05.014

[27] Galano, J.-M., Lee, J. C.-Y., Gladine, C., Comte, B., et al., Non-enzymatic cyclic oxygenated metabolites of adrenic, docosahexaenoic, eicosapentaenoic and α-linolenic acids; bioactivities and potential use as biomarkers. *Biochim. Biophys. Acta* 2015, *1851*, 446–455. DOI: 10.1016/j.bbalip.2014.11.004

[28] Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., et al., Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* 1999, *23*, 185–188. DOI: 10.1038/13810

[29] Chahrour, M., Zoghbi, H. Y., The story of Rett syndrome: from clinic to neurobiology. *Neuron* 2007, *56*, 422–437. DOI: 10.1016/j.neuron.2007.10.001

[30] Laurvick, C. L., de Klerk, N., Bower, C., Christodoulou, J., et al., Rett syndrome in Australia: A review of the epidemiology. *J. Pediatr.* 2006, *148*, 347–352. DOI: 10.1016/j.jpeds.2005.10.037

[31] Ciccoli, L., De Felice, C., Leoncini, S., Signorini, C., et al., Red blood cells in Rett syndrome: Oxidative stress, morphological changes and altered membrane organization. *Biol. Chem.* 2015, *396*, 1233–1240. DOI: 10.1515/hsz-2015-0117

[32] Cronk, J. C., Derecki, N. C., Litvak, V., Kipnis, J., Unexpected cellular players in Rett syndrome pathology. *Neurobiol. Dis.* 2016, *92*, 64–71. DOI: 10.1016/j.nbd.2015.05.005

[33] De Felice, C., Maffei, S., Signorini, C., Leoncini, S., et al., Subclinical myocardial dysfunction in Rett syndrome. *Eur. Heart J. Cardiovasc Imaging* 2012, *13*, 339–345. DOI: 10.1093/ejechocard/jer256

[34] De Felice, C., Signorini, C., Leoncini, S., Pecorelli, A., et al., The role of oxidative stress in Rett syndrome: An overview. *Ann. N. Y. Acad. Sci.* 2012, *1259*, 121–135. DOI: 10.1111/j.1749-6632. 2012.06611.x

[35] De Felice, C., Rossi, M., Leoncini, S., Chisci, G., et al., Inflammatory lung disease in Rett syndrome. *Mediators Inflamm.* 2014 *2014*, 560120. DOI: 10.1155/2014/560120

[36] Gonnelli, S., Caffarelli, C., Hayek, J., Montagnani, A., et al., Bone ultrasonography at phalanxes in patients with Rett syndrome: A 3-year longitudinal study. *Bone* 2008, *42*, 737–742. DOI: 10.1016/j.bone.2007.12.003

[37] Julu, P. O., Kerr, A. M., Apartopoulos, F., Al-Rawas, S., et al., Characterisation of breathing and associated central autonomic dysfunction in the Rett disorder. *Arch. Dis. Child.* 2001, *85*, 29–37.

[38] Lebwohl, B., Ludvigsson, J. F., Green, P. H. R., Celiac disease and non-celiac gluten sensitivity. *BMJ* 2015, *351*, h4347.

[39] Signorini, C., De Felice, C., Leoncini, S., Durand, T., et al., Altered erythrocyte membrane fatty acid profile in typical Rett syndrome: Effects of omega-3 polyunsaturated fatty acid supplementation. *Prostaglandins Leukot. Essent. Fatty Acids* 2014, *91*, 183–193. DOI: 10.1016/j.plefa.2014.08.002

[40] Chen, L., Chen, K., Lavery, L. A., Baker, S. A., et al., MeCP2 binds to non-CG methylated DNA as neurons mature, influencing transcription and the timing of onset for Rett syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 2015, *112*, 5509–5514. DOI: 10.1073/pnas.1505909112

[41] Cheng, T.-L., Wang, Z., Liao, Q., Zhu, Y., et al., MeCP2 suppresses nuclear microRNA processing and dendritic growth by regulating the DGCR8/Drosha complex. *Dev. Cell* 2014, *28*, 547–560. DOI: 10.1016/j.devcel.2014.01.032

[42] Georgel, P. T., Horowitz-Scherer, R. A., Adkins, N., Woodcock, C. L., et al., Chromatin compaction by human MeCP2. Assembly of novel secondary chromatin structures in the absence of DNA methylation. *J. Biol. Chem.* 2003, *278*, 32181–32188. DOI: 10.1074/jbc.M305308200

[43] Horike, S., Cai, S., Miyano, M., Cheng, J. F., et al., Loss of silent-chromatin looping and impaired imprinting of DLX5 in Rett syndrome. *Nat. Genet.* 2005, *37*, 31–40. DOI: 10.1038/ng1491

[44] Li, Y., Wang, H., Muffat, J., Cheng, A. W., et al., Global transcriptional and translational repression in human-embryonic-stem-cell-derived Rett syndrome neurons. *Cell Stem Cell* 2013, *13*, 446–458. DOI: 10.1016/j.stem.2013.09.001

[45] Maunakea, A. K., Chepelev, I., Cui, K., Zhao, K., Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. *Cell Res.* 2013, *23*, 1256–1269. DOI: 10.1038/cr.2013.110

[46] Yazdani, M., Deogracias, R., Guy, J., Poot, R. A., et al., Disease modeling using embryonic stem cells: MeCP2 regulates nuclear size and RNA synthesis in neurons. *Stem Cells Dayt. Ohio.* 2012, *30*, 2128–2139. DOI: 10.1002/stem.1180

[47] Chahrour, M., Jung, S. Y., Shaw, C., Zhou, X., et al., MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science* 2008, *320*, 1224–1229. DOI: 10.1126/science.1153252

[48] Gerlach, M., Ben-Shachar, D., Riederer, P., Youdim, M. B., Altered brain metabolism of iron as a cause of neurodegenerative diseases? *J. Neurochem.* 1994, *63*, 793–807.

[49] Brücke, T., Sofic, E., Killian, W., Rett, A., et al., Reduced concentrations and increased metabolism of biogenic amines in a single case of Rett-syndrome: A postmortem brain study. *J. Neural Transm.* 1987, *68*, 315–324.

[50] De Felice, C., Ciccoli, L., Leoncini, S., Signorini, A., et al., Systemic oxidative stress in classic Rett syndrome. *Free Radic. Biol. Med.* 2009, *47*, 440–448. DOI: 10.1016/j.freeradbiomed.2009.05.016

[51] Sierra, C., Vilaseca, M. A, Brandi, N., Artuch, R., et al., Oxidative stress in Rett syndrome. *Brain Dev.* 2001, *23*, S236–S239.

[52] Valinluck, V., Tsai, H.-H., Rogstad, D. K., Burdzy, A., et al., Oxidative damage to methyl-CpG sequences inhibits the binding of the methyl-CpG binding domain (MBD) of methyl-CpG binding protein 2 (MeCP2). *Nucleic Acids Res.* 2004, *32*, 4100–4108. DOI: 10.1093/nar/gkh739

[53] VanRollins, M., Woltjer, R. L., Yin, H., Morrow, J. D., et al., F2-dihomo-isoprostanes arise from free radical attack on adrenic acid. *J. Lipid Res.* 2008, *49*, 995–1005. DOI: 10.1194/jlr.M700503-JLR200

[54] Durand, T., De Felice, C., Signorini, C., Oger, C., et al., F(2)-Dihomo-isoprostanes and brain white matter damage in stage 1 Rett syndrome. *Biochimie* 2013, *95*, 86–90. DOI: 10.1016/j.biochi.2012.09.017

[55] De Felice, C., Signorini, C., Durand, T., Oger, C., et al., F2-dihomo-isoprostanes as potential early biomarkers of lipid oxidative damage in Rett syndrome. *J. Lipid Res.* 2011, *52*, 2287–2297. DOI: 10.1194/jlr.P017798

[56] De Felice, C., Della Ragione, F., Signorini, C., Leoncini, S., et al., Oxidative brain damage in Mecp2-mutant murine models of Rett syndrome. *Neurobiol. Dis.* 2014, *68*, 66–77. DOI: 10.1016/j.nbd.2014.04.006

[57] De Felice, C., Signorini, C., Durand, T., Ciccoli, L., et al., Partial rescue of Rett syndrome by ω-3 polyunsaturated fatty acids (PUFAs) oil. *Genes Nutr.* 2012, *7*, 447–458. DOI: 10.1007/s12263-012-0285-7

[58] Authors/Task Force Members, Priori, S. G, Blomström-Lundqvist, C., et al., 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC)Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). *Eur. Heart J.* 2015, *36*, 2793–2867. DOI: 10.1093/eurheartj/ehv316

[59] Katz, A. M., Messineo, F. C., Lipid-membrane interactions and the pathogenesis of ischemic damage in the myocardium. *Circ. Res.* 1981, *48*, 1–16.

[60] Fukuda, K., Davies, S. S., Nakajima, T., Ong, B. H., et al., Oxidative mediated lipid peroxidation recapitulates proarrhythmic effects on cardiac sodium channels. *Circ. Res.* 2005, *97*, 1262–1269. DOI: 10.1161/01.RES.0000195844.31466.e9

[61] Morita, N., Sovari, A. A., Xie, Y., Fishbein, M. C., et al., Increased susceptibility of aged hearts to ventricular fibrillation during oxidative stress. *Am. J. Physiol. Heart Circ. Physiol.* 2009, *297*, H1594–H1605. DOI: 10.1152/ajpheart.00579.2009

[62] Sato, D., Xie, L.-H., Sovari, A. A., Tran, D. X., et al., Synchronization of chaotic early afterdepolarizations in the genesis of cardiac arrhythmias. *Proc. Natl. Acad. Sci. U. S. A.* 2009, *106*, 2983–2988. DOI: 10.1073/pnas.0809148106

[63] Sovari, A. A., Cellular and molecular mechanisms of arrhythmia by oxidative stress. *Cardiol. Res. Pract.* 2016, *2016*, 9656078. DOI: 10.1155/2016/9656078

[64] Sia, Y. T., Parker, T. G., Liu, P., Tsoporis, J. N., et al., Improved post-myocardial infarction survival with probucol in rats: Effects on left ventricular function, morphology, cardiac oxidative stress and cytokine expression. *J. Am. Coll. Cardiol.* 2002, *39*, 148–156.

[65] Chien, K. R., Han, A., Sen, A., Buja, L. M., et al., Accumulation of unesterified arachidonic acid in ischemic canine myocardium. Relationship to a phosphatidylcholine deacylation-reacylation cycle and the depletion of membrane phospholipids. *Circ. Res* 1984, *54*, 313–322.

[66] Birincioglu, M., Olmez, E., Aksoy, T., Acet, A., The role of prostaglandin synthesis stimulation in the protective effect of captopril on ischaemia-reperfusion arrhythmias in rats in vivo. *Pharmacol. Res.* 1997, *36*, 299–304. DOI: 10.1006/phrs.1997.0232

[67] Takayama, K., Yuhki, K., Ono, K., Fujino, T., et al., Thromboxane A2 and prostaglandin F2alpha mediate inflammatory tachycardia. *Nat. Med.* 2005, *11*, 562–566. DOI: 10.1038/nm1231

[68] Li, Y., Kang, J. X., Leaf, A., Differential effects of various eicosanoids on the production or prevention of arrhythmias in cultured neonatal rat cardiac myocytes. *Prostaglandins* 1997, *54*, 511–530.

[69] Mechiche, H., Grassin-Delyle, S., Robinet, A., Nazeyr-ollas, P., et al., Prostanoid receptors involved in regulation of the beating rate of neonatal rat cardiomyocytes. *PLoS ONE 7*:e45273. 2012, DOI: 10.1371/journal.pone.0045273

[70] Billman, G. E., Kang, J. X., Leaf, A., Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs. *Circulation* 1999, *99*, 2452–2457.

[71] Leaf A., Albert C. M., Josephson M., Steinhaus, D., et al, Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake *Circulation* 2005, *112*, 2762–2768 DOI: 10.1161/CIRCULATIONAHA.105.549527

[72] Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: Results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico *Lancet Lond. Engl.* 1999, *354*, 447–455.

[73] Roy, J., Oger, C., Thireau, J., Roussel, J., et al., Nonenzymatic lipid mediators, neuroprostanes, exert the antiarrhythmic properties of docosahexaenoic acid. *Free Radic. Biol. Med.* 2015, *86*, 269–278. DOI: 10.1016/j.freeradbiomed.2015.04.014

[74] Roy, J., Le Guennec, J.-Y., Galano, J.-M., Thireau, J., et al., Non-enzymatic cyclic oxygenated metabolites of omega-3 polyunsaturated fatty acid: Bioactive drugs. *Biochimie*. 2015, *120*, 56–61. DOI: 10.1016/j.biochi.2015.06.010