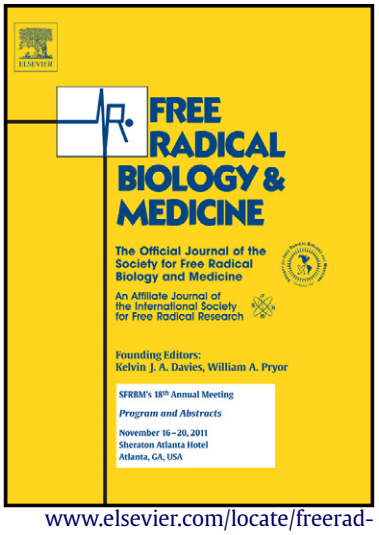

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PII: S0891-5849(15)00003-9
DOI: http://dx.doi.org/10.1016/j.freeradbiomed.2014.12.023
Reference: FRB12264

To appear in: *Free Radical Biology and Medicine*

Cite this article as: Alexander Y. Andreyev, Hui S. Tsui, Ginger L. Milne, Vadim V. Shmanai, Andrei V. Bekish, Maksim A. Fomich, Minhhan N. Pham, Yvonne Nong, Anne N. Murphy, Catherine F. Clarke, Mikhail S. Shchepinov, Isotope-Reinforced Polyunsaturated Fatty Acids Protect Mitochondria from Oxidative Stress, *Free Radical Biology and Medicine,* http://dx.doi.org/10.1016/j.freeradbiomed.2014.12.023

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Abstract Polyunsaturated fatty acid (PUFA) peroxidation is initiated by hydrogen atom abstraction at bis-allylic sites and sets in motion a chain reaction that generates multiple toxic products associated with numerous disorders. Replacement of bis-allylic hydrogens of PUFAs with deuterium atoms (D-PUFAs), termed site-specific isotope reinforcement, inhibits PUFA peroxidation and confers cell protection against oxidative stress. We demonstrate that structurally diverse deuterated PUFAs similarly protect against oxidative stress-induced injury in both yeast and mammalian (myoblast H9C2) cells. Cell protection occurs specifically at the lipid peroxidation step as formation of isoprostanes, immediate products of lipid peroxidation, is drastically suppressed by D-PUFAs. Mitochondrial bioenergetics function is a likely downstream target of oxidative stress and a subject of protection by D-PUFAs. Pre-treatment of cells with D-PUFAs is shown to prevent inhibition of maximal uncoupler-stimulated respiration as well as increased mitochondrial uncoupling, in response to oxidative stress induced by agents with diverse mechanisms of action, including t-butyl-hydroperoxide, ethacrynic acid, or ferrous iron. Analysis of structure activity relationship of PUFAs harbouring deuteriums at distinct sites suggests that there may be a mechanism supplementary to the kinetic isotope effect (KIE) of deuterium abstraction off the bis-allylic sites that accounts for the protection rendered by deuteration of PUFAs. Paradoxically, PUFAs with partially deuterated bis-allylic positions that retain vulnerable hydrogen atoms (e.g. monodeuterated 11-D_1-Lin) protect similarly to PUFAs with completely deuterated bis-allylic positions (e.g. 11,11-D_2-Lin). Moreover, inclusion of just a fraction of deuterated-PUFAs (20-50%) in the total pool of PUFAs preserves mitochondrial respiratory function and confers cell protection. The results indicate that the therapeutic potential of D-PUFAs may derive from the preservation of mitochondrial function.