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Deuterium-reinforced polyunsaturated fatty acids protect against atherosclerosis by lowering lipid peroxidation and hypercholesterolemia

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ABSTRACT

Background and aims: Oxidative modification of lipoproteins is a crucial step in atherosclerosis development. Isotopic-reinforced polyunsaturated fatty acids (D-PUFAs) are more resistant to reactive oxygen species-initiated chain reaction of lipid peroxidation than regular hydrogenated (H-)PUFAs. We aimed at investigating the effect of D-PUFA treatment on lipid peroxidation, hypercholesterolemia and atherosclerosis development.

Methods: Transgenic *APOE*3-Leiden.CETP* mice, a well-established model for human-like lipoprotein metabolism, were pre-treated with D-PUFAs or control H-PUFAs-containing diet (1.2%, w/w) for 4 weeks. Thereafter, mice were fed a Western-type diet (containing 0.15% cholesterol, w/w) for another 12 weeks, while continuing the D-/H-PUFA treatment.

Results: D-PUFA treatment markedly decreased hepatic and plasma F_2-isoprostanes (approx. −80%) and prostaglandin $F_2\alpha$ (approx. −40%) as compared to H-PUFA treatment. Moreover, D-PUFAs reduced body weight gain during the study (−54%) by decreasing body fat mass gain (−87%) without altering lean mass. D-PUFAs consistently reduced plasma total cholesterol levels (approx. −25%), as reflected in reduced plasma non-HDL-cholesterol (−28%). Additional analyses of hepatic cholesterol metabolism indicated that D-PUFAs reduced the hepatic cholesterol content (−21%). Sterol markers of intestinal cholesterol absorption and cholesterol breakdown were decreased. Markers of cholesterol synthesis were increased. Finally, D-PUFAs reduced atherosclerotic lesion area formation throughout the aortic root of the heart (−26%).

Conclusions: D-PUFAs reduce body weight gain, improve cholesterol handling and reduce atherosclerosis development by reducing lipid peroxidation and plasma cholesterol levels. D-PUFAs, therefore, represent a promising new strategy to broadly reduce rates of lipid peroxidation, and combat hypercholesterolemia and cardiovascular diseases.

1. Introduction

Atherosclerotic vascular disease, comprising heart attacks, stroke, aortic aneurysms, and peripheral vascular disease, is the

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most frequent cause of death in the Western world [1,2]. The impact of the atherosclerosis pandemic is predicted to increase worldwide over the next few decades, despite recent progress in lipid-lowering therapy [2,3].

Increased retention of low-density lipoprotein (LDL) in the vessel wall and subsequent oxidative modification is a crucial step in the pathogenesis of atherosclerosis [4]. Polyunsaturated fatty acids (PUFAs) can get oxidized through either enzymatic or non-enzymatic pathways. Non-enzymatic damage can be initiated by both 2-electron- and 1-electron-oxidants, through an addition to a double bond, or, more typically (mostly for 1-electron-oxidants) by H-atom abstraction off a *bis*-allylic methylene group. Once the radical is formed, the ensuing chain reaction of lipid peroxidation (LPO) multiplies the destruction. A smorgasbord of the down-stream products of LPO include toxic carbonyls, which further exacerbate the atherosclerosis-related damage, through the modification of lipids [5] and apoB within LDL [6] (Fig. 1). Aldehyde-modified LDL-apoB is scavenged by macrophages in an uncontrolled manner, leading to foam cell formation and initiation of the atherosclerotic lesion [6]. Reactive carbonyls as well as oxysterols, cholesterol oxidation products, contribute to atherosclerosis mainly through their ability to induce inflammation, oxidative stress and apoptosis [7,8]. Isoprostanes are another LPO-derivative from PUFA oxidation (Fig. 1), although some (*e.g.* 8-iso-prostaglandin-F_2) can also be produced enzymatically. These bioactive molecules can promote atherosclerosis development among others via inducing inflammation and enhancing endothelial/immune cell interaction [9].

Yet, oral antioxidants have not provided the obvious solution to this LPO problem. The most likely reason for this is that it has proven impractical to supplement an organism with sufficient antioxidants to block ongoing LPO chain reactions [10,11], specifically when endogenous protective antioxidant mechanisms are disrupted due to other underlying clinical conditions. In addition, some antioxidants may induce adverse effects as for instance, in the presence of an excess of LDL, vitamin E (α-tocopherol) may act as a pro-oxidant [12,13].

PUFAs are essential nutrients as they are not synthesized in mammalian tissues and have to be supplied through the diet. Following ingestion, PUFAs are quickly incorporated into lipid structures throughout the body. Deuterium is a stable hydrogen isotope that has natural abundance (150 ppm in ocean water) and is accordingly recognized by living systems as a normal, natural subtype of hydrogen. Deuterium incorporated into PUFAs at bis-allylic positions (D-PUFAs) gives rise to a well-known "kinetic isotope effect" [14], as a result of which, reactions involving cleavage of a C-H bond are slowed down in the C-D bond. As the abstraction step by reactive oxygen species (ROS) is repeated throughout the chain of LPO events, the protective effect of D-PUFAs is multiplied, thus resulting in a larger total beneficial effect when compared to exposure with normal (H-)PUFAs. As a result, we have been able to show that D-PUFAs, that are specifically deuterated at the bis-allylic positions, are resistant to LPO [15–18], and can mitigate several pathologies, including important aspects of cellular damage in Friedreich's ataxia [19] and Parkinson's disease [20,21].

As oxidative stress is a crucial step in atherosclerosis development and there is likely interplay between oxidative stress and lipid metabolism, we aimed to investigate the effect of D-PUFA treatment on LPO, hypercholesterolemia and atherosclerosis development in *APOE*3-Leiden.CETP* mice. This hyperlipidemic model is a well-established model for human-like lipoprotein metabolism. Unlike hyperlipidemic apoE- and LDLR-deficient mice, they have an intact, albeit attenuated, apoE-LDLR clearance pathway for cholesterol-enriched lipoprotein remnants [22,23]. As

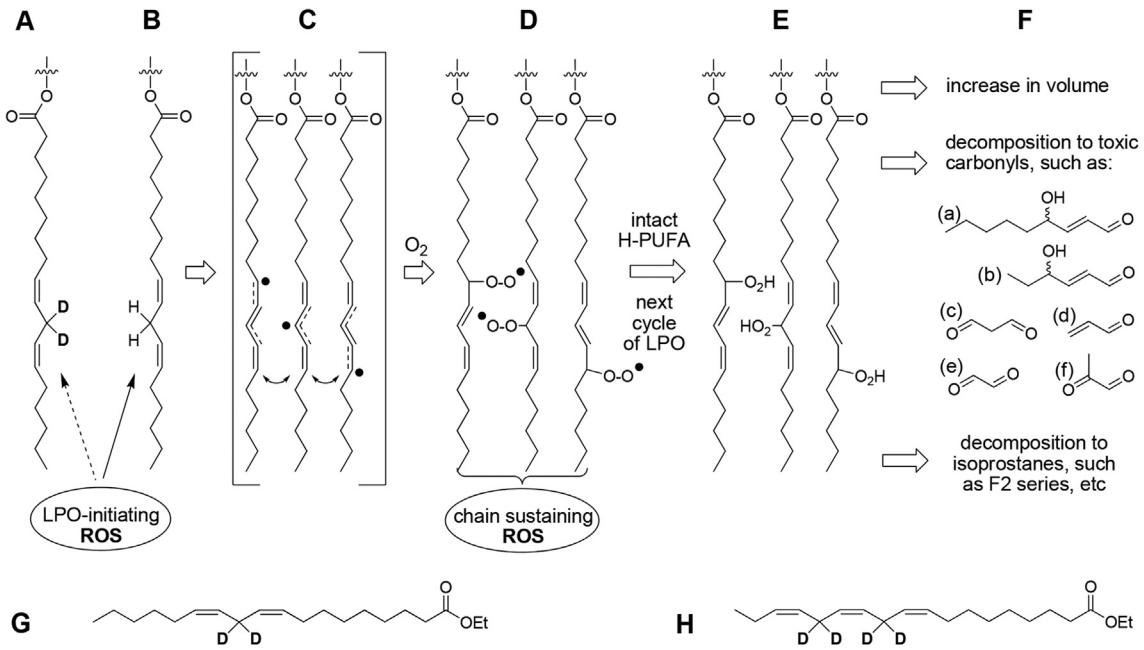

Fig. 1. Protective effect of D-PUFAs on LPO.
(A) D-PUFAs inhibit the rate-limiting step of ROS-driven abstraction off a bis-allylic site. (B) ROS-driven hydrogen abstraction off a bis-allylic site generates resonance-stabilized free radicals (C), which quickly react with abundant molecular oxygen to form lipid peroxyls (D). These newly formed ROS species (L-OO.) abstract hydrogen off a neighbouring PUFA molecule (turning themselves into lipid peroxides LOOH (E)), thus sustaining the chain by reacting with another molecule of (B). LOOH (E), which have greater volume compared to non-oxidized lipids, further decompose through multiple pathways (particularly upon a prior enzymatic elongation/extension, not shown here, into higher PUFAs such as arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), into a smorgasbord of species (F), such as isoprostanes (*e.g.* F_2-isoprostanes), reactive carbonyls, for instance 4-hydroxynonenal (4-HNE; a), 4-hydroxyhexenal (4-HHE; b), malondialdehyde (MDA; c), acrylic aldehyde (acrolein, AA; d), oxalic aldehyde (OA; e), methylglyoxal (MGA; f). The chain is eventually terminated by a chain-terminating antioxidant or homologous recombination (not shown). The ethyl esters of D2-linoleic acid (G) and D4-linolenic acid (H) used in this study.

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