Lipidomic approach provides new clues toward solving the mystery of accelerated atherosclerosis in diabetes

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Despite considerable progress in the management of major risk factors over recent decades, cardiovascular diseases (CVD) attributable to atherosclerosis are still a predominant cause of death worldwide. This is due, in large part, to the expansion of risk factors related to obesity such as insulin resistance and diabetes [1]. In fact, the risk of developing CVD in adults with diabetes is two to four times higher than in those without diabetes. Moreover, it is well established that insulin resistance and hyperglycemia promote atherosclerosis, leading to coronary artery disease. Yet the underlying mechanisms linking diabetes and atherosclerosis are still not completely understood. To overcome this lack of understanding, numerous studies have been conducted, using mouse models with targeted deletion in intimal cells, to evaluate the impact of impaired insulin signaling at the level of the arterial wall on atherosclerosis development and progression [2]. Taken together, this work highlights the critical consequences of altered insulin signaling, in both macrophages and endothelial cells, in atherogenesis and the formation of advanced plaques. In humans, comparative analyses of atherosclerotic plaques from diabetic and non-diabetic individuals have revealed some important differences in the quality and composition of plaques between these groups. Lesions from diabetic patients are characterized by a larger necrotic core, increased inflammation, neovascularization, intra-plaque hemorrhage, and calcification [3-7]. Higher macrophage and T-cell content, along with increased proportions of apoptotic macrophages and smooth muscle cells, may contribute to this larger necrotic area [4, 8]. When combined with the increased prevalence of thin-cap fibroatheroma and fibrocalcific atheroma, observed using Virtual Histology intravascular ultrasound in vessels of diabetic patients [5], these alterations of plaque morphology may explain the higher risk of acute coronary syndrome in diabetic patients. Indeed, coronary lesions of patients with diabetes mellitus are more vulnerable to rupture and subsequent thrombosis [3, 6].

Thorough analyses of human plaque composition are a powerful means to uncover molecular mechanisms involved in plaque disruption and thrombosis leading to acute coronary events in diabetic individuals. Previous analyses of diabetic atherosclerotic lesions revealed a higher area of lipid-rich atheroma than in specimens from patients without diabetes [3, 8]. Lipid composition is a critical determinant of plaque instability, as it is well established that plaques exhibiting increased lipid content, which is positively associated with macrophage accumulation, are more prone to plaque rupture [9]. Although free cholesterol (FC) and cholesterol esters (CE) account for the majority of lipids in human atherosclerotic lesions [10, 11], the recent development of ‘omic’ approaches, especially lipidomic analyses, now allow quantification of large sets of lipid species. Recent analyses of lipid species in human endarterectomy specimens by shotgun lipidomics resulted in the detection of 24 lipid species and the establishment of signature lipid profiles for vulnerable and stable plaques [10]. Interestingly polyunsaturated fatty acids (PUFA)-containing lipid species were detected in excess in human atherosclerotic lesions versus normal human specimens [10, 12], contributing to the lipid signature for vulnerable plaques [10].

In this issue of Atherosclerosis, Ménégaut et al. [13] performed a unique, targeted lipidomic analysis of human atherosclerotic lesions focused on PUFA-containing lipid species in both diabetic and non-diabetic patients, in order to identify specific lipid species which characterized diabetic

plaques. Analyses of carotid atheroma plaque samples from 31 diabetic and 48 non-diabetic patients undergoing endarterectomy allowed the authors to quantify 57 lipid species in atherosclerotic lesions from both diabetic and non-diabetic individuals. Although overall lipid composition in plaques was similar in patients regardless of diabetes status, Ménégaut et al. detected more FC in diabetic atherosclerotic lesion when normalized for the total amount of phosphatidylcholines (PC) or CE, corroborating previous observations from Chen et al. [11]. Reasons for increased plaque FC in diabetes may include impaired cholesterol esterification or hydrolysis, as proposed by the authors, and/or defective macrophage free cholesterol efflux caused by advanced glycation end products [14]. Such accumulation of FC in diabetic atherosclerotic lesions likely contributes to increased necrotic core size and instability by promoting macrophage apoptosis [15]. However, morphologic examination of lesions in the present study [13] revealed no significant difference in necrotic core areas between plaques from diabetic and non-diabetic patients. Although major changes in fatty acid (FA) distribution in glycerophospholipids and CE could have been expected in plaques from diabetic patients versus control subjects, Ménégaut et al. [13] did not detect significant FA alterations in PC, phosphatidylethanolamines (PE), or CE in their cohort. Nevertheless, a thorough analysis of lysophosphatidylcholines (LPC), especially of the sn-1 and sn-2 isomers, provided very interesting results. Overall lesion LPC species and contents quantified in the present study were similar to those previously reported by Thukkani et al. with 16:0 LPC > 18:0 LPC > 18:2 LPC and 20:4 LPC; their content being elevated in atherosclerotic aortae compared to normal aortae [12]. Although the majority of LPC species remained unchanged, the amount of a single 20:4 LPC (sn-2 isomer) specie, sn-2 arachidonoyl-lysophosphatidylcholine (2-AA-LPC), was found to be robustly increased in diabetic plaques. It is interesting to note that *plasma* 20:4 LPC was previously reported to be positively associated with stable coronary artery disease (CAD) [16]. Whether accumulation or generation of this specie within the plaque, versus its exclusion to plasma, is associated with lesion progression in diabetes is an intriguing possibility.

The striking increase in 2-AA-LPC in plaques from diabetic subjects observed by Ménégaut et al. [13] very likely resulted from local hydrolysis of diacyl-PC. A search for enzymes with phospholipase A1 activity which could be responsible for production of 2-AA-LPC in diabetic plaques led the authors to propose endothelial lipase (EL) and calcium-independent $PLA_2\gamma$ ($iPLA_2\gamma$, PNPLA8) as potential candidates. Indeed, convincing immunohistochemistry experiments indicated that these two enzymes were expressed in plaques from diabetic subjects, and more precisely in plaque macrophages, with EL expression being significantly increased in diabetic lesions. EL has been reported to be highly expressed in macrophages in advanced human atherosclerotic lesions [17] and inhibition of its expression in human macrophages can attenuate proinflammatory cytokine expression and secretion [18]. However, the role of EL in atherosclerosis remains controversial, since *Lipg* deficiency in *Apoe*$^{-/-}$ mice fed a western diet has been shown to either increase susceptibility to atherosclerosis [19] or to have no effect on atherosclerosis development [20]. In humans, some *LIPG* variants have been associated with coronary artery disease [21] or microvascular complications in type 2 diabetic patients [22]. Moreover, serum EL concentration is increased in type 2 diabetic patients, and is associated with subclinical inflammation [23]. Nonetheless, it must be kept in mind that EL catalyzes the hydrolysis of

both PC and TG [24], albeit with a higher specificity for PC, and exhibits both PLA1 and PLA2 activities [25]. It is therefore unlikely to be the only enzyme responsible for 2-AA-LPC production in atherosclerotic plaques. In contrast, iPLA$_2\gamma$ (*PNPLA8*) catalyzes the highly selective production of 2-AA-LPC [26]. Transgenic iPLA$_2\gamma$ mice are characterized by a marked increase of 2-AA-LPC production in mitochondria [26], while iPLA$_2\gamma$-deficient mice fed a western diet exhibit impaired mitochondrial oxidation of fatty acids and are resistant to obesity and insulin resistance [27], highlighting the major role of iPLA$_2\gamma$ in maintaining optimal bioenergetic mitochondrial function. Although a growing body of evidence suggests that iPLA$_2\gamma$ could contribute to the pathophysiology of diabetes [27] and thrombosis [29], the potential role of iPLA$_2\gamma$ in atherogenesis and acute coronary events is largely unknown and warrants further investigation. Given the evidence provided by Ménégaut et al.[13], that iPLA$_2\gamma$ is expressed in human atherosclerotic lesions, and more specifically in plaque macrophages, there is strong rationale for generation of mouse models deficient in macrophage *Pnpla8* to help elucidate the role of this lipase in atherosclerosis during diabetes.

The authors further found that plaque 2-AA-LPC was positively correlated with glycated hemoglobin levels and was associated with the presence of diabetes in multivariable logistic regression models [13], providing important clues as to the molecular mechanisms of atherosclerosis progression in diabetes. While 2-AA-LPC content alone may not discriminate unstable versus stable plaques in diabetic patients, 2-AA-LPC is a key branch point metabolite in cellular eicosanoid metabolism [27]. Indeed, 2-AA-LPC can be deacylated by lysophospholipases leading to the generation of glycerolphosphatidylcholine and the release of free arachidonic acid (AA). Subsequently, AA can be oxidized by lipoxygenases (LOXs), cyclooxygenases (COXs), and cytochrome P450 epoxygenases to form eicosanoids such as hydroxyeicosatetraenoic acids, prostaglandins (PG), and epoxyeicosatrienoic acids, respectively. In addition, 2-AA-LPC can be converted to 2-arachidonoyl-lysophospatidic acid by lysophospholipase D, or to endocannabinoid 2- arachidonoyl-glycerol by lysophospholipase C which may serve as substrates for COX2, 12-LOX and 15-LOX to generate glycerol esters, PG, 12- and 15-hydroperoxymetabolites, respectively. Oxidized lipids generated through LOX and COX pathways of AA metabolism are potent lipid mediators exhibiting growth, vasoactive, chemotactic, oxidative, and proinflammatory properties that can contribute to atherosclerosis development, especially in a diabetic context [28]. Moreover, increased COX-2 expression in macrophages from diabetic atherosclerotic lesions has been associated with increased expression of receptor for AGE (RAGE) which could further contribute to plaque destabilization [8], as RAGE deficiency can attenuate the development of atherosclerosis in diabetes [29]. Whether the increased supply of 2-AA-LPC in the diabetic lesion promotes LOX- and COX-mediated generation of proinflammatory lipids, upregulation of RAGE, and subsequent plaque progression remain to be investigated.

To conclude, the findings reported by Ménégaut et al. [13] in this issue of Atherosclerosis offer new clues toward understanding the mystery of accelerated atherosclerosis in diabetes, by introducing AA metabolism, and specifically 2-AA-LPC, as potential key players in this process. Although further investigations are required to elucidate the spectrum of 2-AA-LPC action in atherogenesis and its

potential role in subsequent plaque progression (Figure), the present study provides new rationale for the therapeutic use of apolipoprotein A-I mimetics [30] and omega-3 PUFAs [31] in diabetes, as these molecules may limit the deleterious effects of AA-derived oxidized lipids.

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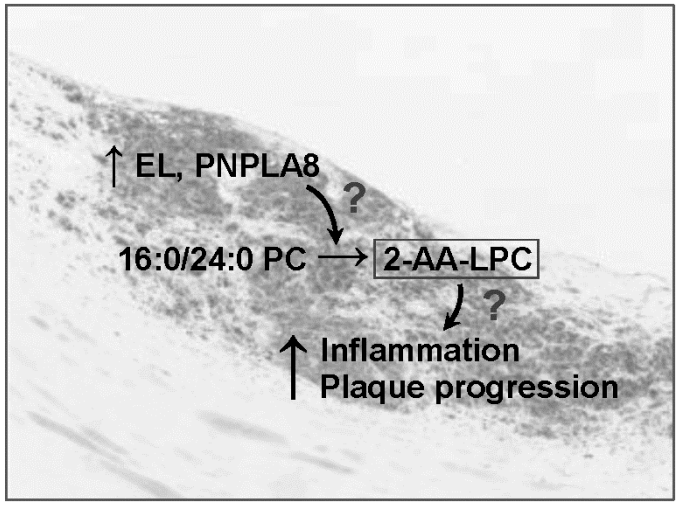

Figure. Working model for the increased accumulation and potential pathological role of 2-AA-LPC in human diabetic atherosclerotic plaques. Increased expression and/or activity of EL and/or PNPLA8 in plaque macrophages leads to increased local generation of 2-AA-LPC from diacyl-PC. 2-AA-LPC accumulation contributes to further inflammation and subsequent plaque progression. 2-AA-LPC, sn-2 arachidonoyl-lysophosphatidylcholine; EL, endothelial lipase; PNPLA8, calcium-independent $PLA_2\gamma$; PC, phosphatidylcholines.