commentary

Human low density lipoprotein: the mystery of core lipid packing[1]

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LDL particles are very fascinating macromolecular assemblies of apolipoprotein B-100 and lipids and their structural features have attracted the attention of scientists for decades. There is a general consensus in the literature that LDL particles are organized into two major compartments, namely an apolar lipid core, comprised primarily of cholesteryl esters, triglycerides, and some free unesterified cholesterol and an outer amphipathic shell that surrounds the apolar core. This outer shell is composed of a phospholipid monolayer containing most of the free unesterified cholesterol and one single copy of apolipoprotein B-100 [for review, see Ref. (1)]. LDLs are highly heterogeneous in nature, varying in buoyant density, size, surface charge, and chemical composition (2). These intrinsic properties are intimately related to intravascular metabolism of LDL (3), atherogenicity (4), and the fate of the particle in the subendothelial space (5).

Of all circulating macromolecules in blood, LDLs are the only ones presently known to undergo a structural transition strikingly close to physiological temperature. Despite the identification of this reversible temperature-induced transition of apolar core lipids in LDL by Deckelbaum et al. (6) in the mid-1970s, the physiological role of this transition remains elusive. Most likely, the transition might play a role in the early progression of atherosclerosis through its effects on cellular pathways of LDL recognition. In view of this, many efforts have been made to clarify the molecular details and to establish structural models for this transition in terms of geometrical constraints and chemical lipid compositions.

There is broad scientific consensus that the core-located lipids are arranged in an ordered liquid-crystalline phase below the phase transition temperature. Above the transition temperature, however, the neutral lipids are organized in a fluid, oil-like, disordered state as demonstrated by early X-ray and neutron scattering studies (7, 8). The actual transition temperature of the core lipids varies between extremes of 15 and 35°C depending on the individual LDL particle. This value correlates well with the ratio of cholesteryl esters to triglycerides, being lower for higher triglyceride levels (6, 7).

Earlier structural models for LDL were principally based on an overall spherical particle shape with diameters in a size range from 18 to 25 nm. In these models, the internal core structure below the transition temperature was basically defined as a concentric spherical shell arrangement of cholesteryl esters. Variations within these models have been discussed on the basis of small angle X-ray scattering (SAXS) and neutron scattering experiments, and electron microscopic data (9, 10). Later on, cryo-electron microscopy (EM) studies suggested that snap-frozen LDL, with the core lipids already in the liquid-crystalline state, exhibit an oblate ellipsoid or discoidal overall particle shape (11, 12). Unexpectedly, in these cryo- images, the internal frozen lipid core was not necessarily centered radially. Instead, an ordered three-layer internal lamellar structure with a distance of about 3.6 nm between the layers was observed (12). This distance corresponded to the length of cholesteryl esters and was consistent with the characteristic periodicity observed in SAXS patterns for LDL below the transition temperature.

This new view of LDL divided the core into compartments separated by walls composed of ordered cholesteryl esters. With increasing triglyceride content, however, this ordered state condensation diminished and for high triglyceride content, the discoid features disappeared and predominantly spherical particles were observed (13). In contrast, diverse results were obtained for cholesteryl ester rich LDL particles, which were in the molten-lipid state above the phase transition before freezing (13, 14). These discrepancies most probably reflect the fact that the phase transition occurs extremely fast within milliseconds (15). Consequently, it seemed almost impossible to trap the lipids in the fluid-phase even by rapid plunge freezing.

However, as reported in this issue of the *Journal of Lipid Research*, Liu et al. (16) were successful in establishing an advanced fast freezing procedure to freeze LDL in a state above the phase transition. By doing so, they were able to capture an intermediate state in the transition from isotro-

[1]See referenced article, *J.Lipid Res.* 2011, 52: 256–262.

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DOI 10.1194/jlr.E013417

pic to liquid-crystalline. In the reconstructed 3D-images, the authors saw a phase coexistence, which is defined as simultaneous occurrence of layered and broken shell structures. For the first time, it was possible to visualize the nucleation process of cholesteryl esters within LDL. Indeed, this patch nucleation behavior permits the temporary formation of local molecular microenvironments as suggested previously in terms of trigylceride segregation (17). Beyond detailed information on the internal core lipid organization, the paper by Liu et al. (16) describes the direct impact of the core lipid phase transition on particle morphology and surface components. The findings are consistent with earlier studies, which provided evidence that the physical state of the core lipids induces changes in the local molecular dynamics of the surface monolayer (18, 19). Only recently, Ren et al. (20) also showed by cryoEM imaging, but starting from below the phase transition, that the surface structure of apolipoprotein B-100 follows that of the liquid crystalline core. In conclusion, these studies show that both the molecular organization and dynamics of LDL core lipids and the relationship to particle shape, size, and apolipoprotein B-100 conformation are important determinants of LDL function.

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