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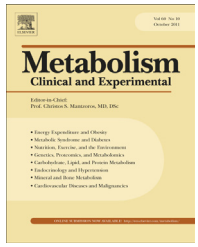

Impaired fluidity and oxidizability of HDL hydrophobic core and amphipathic surface in dyslipidemic men

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ABSTRACT

Objective. To examine and compare the composition, fluidity and oxidizability of HDL hydrophobic core and amphipathic surface of two groups of adult males (25 kg/m^2<BMI<30 kg/m^2), the former mixed dyslipidemic patients (MD) and the latter age- and BMI-matched healthy controls.

Methods and results. Pyrenyl-cholesteryl ester and pyrenyl-phosphatidylcholine, respectively incorporated in HDL core or surface were used for measuring both 2,2'-azobis-2-methyl-propanimidamide-dihydrochloride-induced peroxidation kinetics and fluidities of these regions. In comparison with the controls, MD HDL showed: a) higher free cholesterol to phospholipid ratio in surface and triacylglycerols to cholesteryl ester ratio in the core, b) higher malondialdehyde levels and lower alpha-tocopherol and beta-carotene to neutral lipid ratios, c) a more rigid surface and more fluid core, d) dramatically decreased lag-time and increased propagation rate of peroxidation kinetic in the core, but only an increased propagation rate on the surface.

Conclusion. These results suggest that better knowledge of the physical–chemical properties and oxidizability of HDL core and surface could contribute to better understanding of the mechanisms connecting HDL alteration to increased risk of CDV in MD.

1. Introduction

HDLs have been shown to exert a wide spectrum of anti-atherogenic activities, including the promotion of reverse cholesterol transport, anti-inflammatory actions in vasculature [1] and protection of LDL against oxidation [2]. Moreover, the capacity of HDL to accumulate [3,4] and neutralize [5] or transport oxidized lipids to the liver [6] suggests a role of HDL in the detoxification of these molecules in vivo. Recent evidence suggests that structural modification and composition alteration of HDL due to chronic inflammation and acute phase responses may result in loss of normal biological function of these lipoproteins [1,7]. Further, changes in lipid composition associated with some forms of dyslipidemia, such as hypercholesterolemia and hypertriacylglycerolemia, have been shown to influence the anti-atherogenic capacity of HDL [1].

In the western world, one of the most common forms of dyslipidemia is mixed dyslipidemia (MD) featuring elevated

Abbreviations: AAPH, 2,2′-azobis(2-methylpropionamidine) dihydrochloride; BMI, body mass index; CE, cholesteryl esters; CETP, cholesterol ester transfer protein; CRP, C-reactive protein, CVD, cardiovascular diseases; DTPA, diethylene triamine pentaacetic acid; HOMA-IR, homeostatic model of assessment for insulin resistance; LCAT, lecithin-cholesterol acyltransferase; LOOH, lipid peroxides; MDA, malondialdehyde; MD, mixed dyslipidemia; PyrPC, decanoyl γ-palmitoyl L-α phosphatidylcholine; PyrCE, cholesteryl (pyren-1-yl) hexanoate; PON, Paraoxonase; PUFA, polyunsaturated fatty acids; PL, phospholipids; TAG, triacylglycerols.

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serum concentrations of both cholesterol and triacylglycerols (TAG), and it is closely associated with an increased risk of CVD [8]. As the metabolism of HDL and VLDL is closely linked, an excessive increase of TAG in VLDL frequently results in enrichment in TAG and depletion of the cholesteryl esters (CE) of the HDL core [9]. In hypercholesterolemic patients, secondary hypertriacylglycerolemia can be promoted by several metabolic disorders, including obesity. Moreover, obesity has a more pronounced effect on other atherogenic lipid and lipoprotein alterations in hypercholesterolemic patients than in normo-cholesterolemic ones [10].

Oxidative modification of HDL has been suggested to be another pathway involved in the generation of less functional HDL [11]; however, while LDL oxidation has been widely investigated [12,13], the information on HDL oxidation and its role in atherogenesis is scarce. The oxidation of HDL lipids leads to the formation of a complex mixture of chemical compounds [14] such as conjugated dienes, arachidonic acid-derived isoprostanes, lipid peroxides , and aldehydes. Some of these aldehydes, such as malondialdehyde (MDA), can easily react with lipids and proteins giving rise to the formation of cross-links between lipid tails and amino-acid residues, which decrease the fluidity of the compartment in which these alterations have taken place. Such alterations may impair the activity of apoproteins and HDL-associated proteins and, therefore, the anti-atherogenic capacity of HDL. We have previously demonstrated that pyrene cholesteryl ester and pyrene phosphatidylcholine are selectively incorporated respectively into the hydrophobic core and surrounding amphipathic envelope of HDL, and are useful markers of the oxidizability of the region in which they are localized [15]. Furthermore, the diffusion-dependent formation of excited dimers (excimers) and the long excite state life-times make pyrene labelled lipids suitable for a very sensitive measure of changes in fluidity of the micro domains in which they are included [16,17] by measurement of the ratio between their monomer and excimer forms.

We performed the following study, in which we investigated the composition, oxidizability and fluidity of the hydrophobic core and surrounding amphipathic envelope of HDL in two adult male groups with the aim of providing more detailed information on the features of HDL in MD patients. The first group consisted of overweight and moderately obese MD patients and the second of age- and body mass index (BMI)-matched healthy controls.

2. Materials and methods

2.1. Materials

Analytical grade chemicals and solvents and doubly-distilled water were used. β-(Pyren-1-yl) decanoyl γ-palmitoyl L-α phosphatidylcholine (PyrPC), cholesteryl (pyren-1-yl) hexanoate (PyrCE), diethylene triamine pentaacetic acid (DTPA), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), paraoxon (diethyl-p-nitrophenyl phosphate) phenyl acetate and fatty acid methyl ester and liposoluble vitamin standards were from Sigma Aldrich (Milan, Italy) and organic solvents from Merck (Merck Italy, Milan, Italy).

2.2. Subjects

The study included 25 overweight or moderate obese (25 kg/m^2<BMI<30 kg/m^2) men with mixed dyslipidemia defined as serum total cholesterol≥5.7 mmol/mL and TAG between 1.7 mmol/L and 5.75 mmol/L. For comparison a control population composed of 25 age- and BMI-matched men with serum cholesterol<5.17 mmol/L and TAG<1.49 mmol/L was also enrolled. Exclusion criteria were HDL cholesterol<1.03 mmol/L, endocrine, metabolic and kidney disease, or medical problems other than obesity. All subjects were non-smokers and had not modified the weight, taken antioxidants, or drugs known to affect lipoprotein metabolism during the two months preceding the enrollment and were consuming less than 25 g/day of alcohol. Homeostatic model of assessment for insulin resistance [HOMA-IR, (fasting blood glucose × insulin) / 22.5, normal range≤2.6] [18] and serum high-sensitivity C-reactive protein (CRP, normal range<0.5 mg/dL) levels were chosen as marker of insulin resistance and systemic inflammation, respectively, and subjects with HOMA and CRP values out of the normal range were excluded. Furthermore, subjects with a current or recent illness were excluded from the study, because modifications of lipoprotein composition and oxidative stress were described in human patients during the acute phase response [19]. The study was approved by the ethics committee of the Istituti Clinici di Perfezionamento di Milano and was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000. Subjects gave their written consent to the study.

Blood pressure and physical data were determined during a complete clinical examination. Blood samples were obtained after overnight fast by venipuncture and were collected in EDTA-containing Vacutainer tubes. Clinical chemistry parameters were determined under strictly standardized conditions with dedicated commercial kits. Plasma was prepared by centrifugation at 3000 rpm for 20 min at 4 °C and thereafter used for the determination of paraoxonase activity and the preparation of lipoproteins. The activity of paraoxonase-1 (PON-1), an enzyme that circulates mostly tightly associated with HDL [20], was assayed using two synthetic substrates: paraoxon (diethyl-p-nitrophenyl phosphate) and phenyl acetate. Paraoxonase activity was calculated using the molar extinction coefficient of p-nitrophenol (18.290 M^{-1} cm^{-1}); whereas arylesterase activity was calculated using the molar extinction coefficient of phenyl acetate (1310 M^{-1} cm^{-1}) [21].

2.3. Lipoprotein isolation and characterization

Lipoproteins were isolated from plasma as previously described [22] by adapting procedure 16 in ref. [23] to "Optima Max" tabletop ultracentrifuge (Beckman Coulter). To remove albumin completely, the HDL fraction (density, 1.063–1.210 g/ml) was subjected to a second centrifugation [procedure 15 in ref. [23]]. After separation, HDLs were dialyzed and their levels of proteins, cholesterol (total and free), phospholipid, triacylglycerols, α-tocopherol and β-carotene were determined as previously described [15]. After the extraction of amphipathic and non-polar lipids [12], the fatty acid profile of these lipids was determined as previously described [24].

Malondialdehyde (MDA) concentration was measured fluorometrically as described by Conti et al. [25].