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Aging affects high-density lipoprotein composition and function ☆

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ABSTRACT

Most coronary deaths occur in patients older than 65 years. Age associated alterations in the composition and function of high-density lipoproteins (HDL) may contribute to cardiovascular mortality. The effect of advanced age on the composition and function of HDL is not well understood.

HDL was isolated from healthy young and elderly subjects. HDL composition, cellular cholesterol efflux/uptake, anti-oxidant properties and paraoxonase activity were assessed. We observed a 3-fold increase of the acute phase protein serum amyloid A, an increased content of complement C3 and proteins involved in endopeptidase/protease inhibition in HDL of elderly subjects, whereas levels of apolipoprotein E were significantly decreased. HDL from elderly subjects contained less cholesterol but increased sphingomyelin. Most importantly, HDL from elderly subjects showed defective antioxidant properties, lower paraoxonase 1 activity and was more rapidly taken up by macrophages, whereas cholesterol efflux capability was not altered.

These findings suggest that aging alters HDL composition, resulting in functional impairment that may contribute to the onset/progression of cardiovascular disease.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide. CVD rises dramatically with age and is of major concern in the increasing elderly population. Epidemiological studies have shown that high-density lipoprotein (HDL) cholesterol levels are inversely associated with risk for CVD [1]. The protective effect of HDL has been classically attributed to its ability to promote reverse cholesterol transport, a series of processes by which HDL is able to transport cholesterol from the periphery back to the liver for excretion [2]. Of particular interest, the ability of HDL to promote cholesterol efflux was found to be a better predictor for CVD than HDL-cholesterol [3]. In addition to its role in reverse cholesterol transport, HDL was found to inhibit low-density lipoprotein oxidation, to inhibit the secretion of pro-inflammatory mediators from macrophages, to reduce adhesion molecule expression on endothelial cells, to stimulate nitric oxide formation and to promote vasodilatation [4–8].

However, latest failures of HDL-cholesterol raising drugs and a recent study that showed no causal association between risk for myocardial infarction and genetically raised plasma HDL-cholesterol have called into question whether HDL-cholesterol is a suitable surrogate marker for HDL-related risk assessment [9,10].

Recent proteomic studies provided convincing evidence that inflammation alters the protein composition of HDL thereby generating dysfunctional or even pro-atherogenic forms of HDL [11] by enriching pro-inflammatory proteins such as serum amyloid A (SAA), apoC-III or complement component 3 [12–20]. In addition, inflammation leads to marked alterations in the lipid moiety of HDL, highlighted by a significant reduction in phospholipids [21–23].

These important studies linked compositional alterations of HDL with functional impairment of HDL, suggesting that even in the absence of low HDL-cholesterol levels, dysfunctional HDL may be causally involved in the development and progression of cardiovascular disease. Therefore, it is becoming increasingly apparent that direct measures of HDL function are needed rather than relying on surrogate markers such as the concentration of HDL-cholesterol.

Data on the effect of aging on HDL composition and function are limited. Associations of inflammation with age-related pathologies are documented; however, there is little information available how healthy aging impacts HDL composition and function. Initial studies reported that HDL from elderly subjects has a reduced potency to promote cholesterol efflux and to inhibit LDL oxidation [24,25]. In the present study, we assessed the impact of healthy aging on HDL composition and function.

2. Methods

2.1. Characteristics of study subjects and blood collection

All subjects were considered healthy and clinical characteristics are given in Table 1. Exclusion criteria included any history of cardiovascular

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disease, pregnancy, obesity, dyslipidemia, renal disease and diabetes. No subjects showed clinical signs of inflammation. Study subjects were free of lipid-lowering medication and anti-inflammatory drugs.

Blood was sampled from healthy subjects after obtaining written informed consent, according to a protocol approved by the Institutional Review Board of the Medical University of Graz (Nr.: 21-523 ex 09/10). Blood was collected in serum tubes (Greiner, Kremsmünster, Austria).

2.2. Isolation of HDL

Serum density was adjusted with potassium bromide (Sigma-Aldrich Corporation, Vienna, Austria) to 1.24 g/mL and a two-step density gradient was generated in centrifuge tubes (16 × 76 mm, Beckman Instruments) by layering the density-adjusted plasma (1.24 g/mL) underneath a NaCl-density solution (1.006 g/mL) as described [26,27]. Tubes were sealed and centrifuged at 90.000 rpm for 4 h in a 90 Ti fixed angle rotor (Beckman Instruments, Krefeld, Germany). After centrifugation, the HDL-containing band was collected, desalted via PD10 columns (GE Healthcare, Vienna, Austria) and immediately used for experiments or stored at −70 °C.

2.3. Determination of plasma and HDL lipid composition

Levels of total cholesterol, non-esterified cholesterol, triglycerides, choline-containing phospholipids (DiaSys Diagnostic Systems GmbH, Holzheim, Germany) and sphingomyelin (Cayman Europe, Tallinn, Estonia) were measured enzymatically with commercially available kits. Sphingomyelin values were subtracted from total choline-containing phospholipids to quantify phosphatidylcholine. LDL cholesterol was calculated according to the Friedewald equation using HDL cholesterol values measured in the supernatant of the phosphotungstic acid/$MgCl_2$ precipitation.

2.4. Biochemical quantification of HDL-associated proteins

ApoA-I, apoA-II, apoC-II, apoC-III and apoE (Greiner, Flacht, Germany) were determined by immunoturbidimetry. All lipoprotein analyses were performed on an Olympus AU640 analyzer (Olympus Diagnostika, Hamburg, Germany). Serum amyloid A (SAA) (Life Technologies, Vienna, Austria) and clusterin (BioVendor R&D, Candler, NC) were determined using enzyme-linked immunosorbent assays.

2.5. LC–MS/MS analysis

Proteomic profiling of HDL was performed as previously described [28]. HDL was digested with trypsin and the resulting peptides were separated by nano-HPLC. The sample was ionized in the nanospray source equipped with nanospray tips and analyzed in a LTQ-FT mass spectrometer (Thermo Scientific, Waltham, US). The standard deviation of spectral counts was below 10% between duplicates. Spectral counts were recorded and used for data analysis by searching the human SwissProt public database downloaded on May 4th 2011 with Spectrum Mill Rev. A.03.03.084 SR4 (Agilent Technologies, Vienna, Austria). Detailed search criteria were: trypsin; max. missed cleavage sites: 2; carbamidomethylation at cysteine as fixed modification; variable modification: oxidized methionine; precursor mass tolerance +/− 0.05 Da; product mass tolerance +/− 0.7 Da. Protein hits were subjected to automatic validation by Spectrum mill: for precursor charge of 2: score threshold 6.0, percent scored peak intensity (%SPI) threshold 60.0, Fwd-Rev score threshold 2.0 and rank 1-2 score threshold 2.0; for precursor charge of 1: score threshold 6.0, %SPI threshold 70.0, Fwd-Rev score threshold 2.0 and rank 1-2 score threshold 2.0; for precursor charge of 3: score threshold 8.0, %SPI threshold 70.0, Fwd-Rev score threshold 2.0 and rank 1–2 score threshold 2.0.

Table 1
Clinical characteristics of study subjects.

	Young	Elderly
n	26	20
Age (yr)	26.6 (25.4–28.7)	67.2 (65.4–69.2)*
Male/female	13/13	9/11
CRP (mg/dL)	0.1 (0.0–0.2)	0.9 (0.5–2.9)
Total cholesterol (mg/dL)	174 (159–195)	225 (200–239)*
Triglycerides (mg/dL)	68 (54–102)	114 (89–130)
HDL-cholesterol (mg/dL)	55 (47–69)	56 (44–68)
LDL-cholesterol (mg/dL)	97 (85–113)	134 (122–160)*
SAA (mg/dL)	0.8 (0.4–1.5)	2.0 (0.8–7.9)**

Results are given as medians with interquartile range in brackets. Significances were accepted at the level of *P < 0.05.

2.6. Advanced oxidation protein products (AOPP) assay

AOPPs have been determined as described previously with modifications [29,30]. Briefly, serum was depleted of apoB-containing lipoproteins with polyethylenglycol (PEG). 400 μL PEG-solution (20% PEG in 200 mmol/L glycine, pH = 7.4) was added per mL serum and incubated for 20 min at RT. Precipitate was pelleted (10,000 rpm, 30 min, 4 °C) and the supernatant used for AOPP detection. Subsequently, 10 μL apoB-depleted serum was mixed with 40 μL 0.2 mol/L citrate buffer and incubated for 2 min on a shaker. Afterwards, absorbance was measured at 340 nm. AOPP were calibrated with chloramine-T (linear within the range of 0 to 100 μmol/L) and were expressed as μmol/L of chloramine-T equivalents.

2.7. Arylesterase activity assay

Ca^{2+}-dependent arylesterase activity was determined with a photometric assay using phenylacetate as the substrate. HDL (0.5 μg protein) was added to 200 μL buffer containing 100 mmol/L Tris, 2 mmol/L $CaCl_2$ (pH 8.0) and phenylacetate (1 mmol/L). The rate of hydrolysis of phenylacetate was monitored by the increase of absorbance at 270 nm and readings were taken every 30 s at room temperature to generate a kinetic plot. The slope from the kinetic chart was used to determine ΔAb_{270nm}/min. Enzymatic activity was calculated with the Beer—Lambert Law from the molar extinction coefficient of 1310 $mol^{1}*L^{-1}*cm^{-1}$ for phenylacetate.

2.8. Lp-PLA2 activity assay

Lp-PLA2 was measured using commercially available photometric assay (Cayman Europe, Talinn, Estonia).

2.9. LCAT, PLTP and CETP activity assay

LCAT was measured with a commercially available kit from Merck (Darmstadt, Germany). PLTP and CETP were measured with assay kits from Abnova (Eubio, Vienna, Austria).

2.10. Determination of the anti-oxidative capacity of HDL

The anti-oxidative activity of HDL was determined as previously described [31]. Briefly, dihydrorhodamine (DHR) was suspended in DMSO to a 50 mmol/L stock, which was diluted in HEPES (20 mmol/L HEPES, 150 mmol/L NaCl, pH 7.4) to a 50 μmol/L working reagent. 7.5 μg HDL protein was placed in a 384-well, 15 μL of DHR working reagent was added and the volume completed to 100 μL with HEPES buffer. The increase in fluorescence due to the oxidation of DHR was measured every 2 min for 1 h at 538 nm. The increase in fluorescence per minute was determined for samples containing only DHR and for samples containing DHR and individual HDL probes from study subjects.