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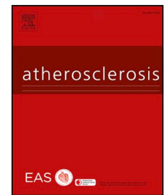
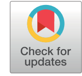


Increased electronegativity of high-density lipoprotein in uremia patients impairs its functional properties and is associated with the risk of coronary artery disease



Chiz-Tzung Chang[a,b], Ming-Yi Shen[c,d], Ju-Yi Hsieh[a], Chia-Ming Chang[a], Hsin-Yi Liao[e], Fang-Yu Chen[a], Chung Y. Hsu[b], Chao-Yuh Yang[f], Chao-Jung Chen[d,e,g,*]

[a] *Cardiovascular Research Laboratory and Division of Nephrology, China Medical University Hospital, No 2 Yu-Der Rd, Taichung, 40447, Taiwan*
[b] *College of Medicine, China Medical University, No 91, Hsueh-Shih Rd, Taichung, 40402, Taiwan*
[c] *Graduate Institute of Clinical Medical Science, China Medical University, No 91, Hsueh-Shih Rd, Taichung, 40402, Taiwan*
[d] *Medical Research, China Medical University Hospital, 2 Yu-Der Rd, Taichung, 40447, Taiwan*
[e] *Proteomic Core Laboratory, China Medical University Hospital, 2 Yu-Der Rd, Taichung, 40447, Taiwan*
[f] *Department of Medicine, Baylor College of Medicine, 1 Bayor Plaza, Houston, TX, TX77030, USA*
[g] *Graduate Institute of Integrated Medicine, China Medical University, No 91, Hsueh-Shih Rd, Taichung, 40402, Taiwan*

HIGHLIGHTS

- Antioxidant lipoproteins were less abundant in uremia H5 than in control H5.
- Carbamylation of ApoA1/ApoCIII contributes to increased electronegativity of HDL.
- Antioxidant, antiapoptotic, and cholesterol efflux of HDL were reduced in uremia H5.
- H5% could be a prediction marker for the development of CAD in uremia patients.

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ABSTRACT

Background and aims: Uremia patients have impaired high-density lipoprotein (HDL) function and a high risk of coronary artery disease (CAD). Increased lipoprotein electronegativity can compromise lipoprotein function, but the effect of increased HDL electronegativity on HDL function and its association with CAD in uremia patient are not clear.

We aimed to assess HDL electronegativity and various properties of HDL in uremia patients and investigate whether electronegative HDL is a risk factor for CAD in these individuals.

Methods: HDL from 60 uremia patients and 43 healthy controls was separated into 5 subfractions (H1—H5) with increasing electronegativity by using anion-exchange chromatography. Lipoprotein content was analyzed by gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight-mass spectrometry. HDL anti-oxidant, anti-apoptosis and cholesterol efflux activities were examined by fluorescence-based assays.

Results: The percentage of H5 HDL (H5%) was significantly higher in uremia patients than in controls ($p < 0.001$). The concentration of apolipoprotein (Apo) AI was lower and apolipoprotein modifications were more prevalent in uremia HDL subfractions than in control HDL subfractions. Carbamylation of ApoAI and ApoCIII was increased in more electronegative HDL subfractions from uremia patients. Anti-oxidant activity, anti-apoptotic activity, and cholesterol efflux capability were reduced in HDL subfractions from uremia patients when compared with control HDL subfractions. Multiple logistic regression analysis showed that H5% was associated with CAD risk in uremia patients.

Conclusions: In HDL of uremia patients, increased electronegativity is accompanied by compositional changes and impaired function. Our findings indicate that increased H5% is associated with increased CAD risk in uremia patients.

* Corresponding author. No 91, Hsueh-Shih Rd, Taichung, 40402, Taiwan.
E-mail address: cjchen@mail.cmu.edu.tw (C.-J. Chen).

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1. Introduction

Previous studies have shown that lipoproteins with increased electronegativity have altered composition and function, but the mechanisms underlying these changes in high-density lipoprotein (HDL) have not been completely identified [1–3]. HDL contains major types of apolipoprotein (Apo), including mainly ApoAI and ApoAII, and, in smaller amounts, ApoE and ApoC [4]. In uremia patients, dyslipidemia results in abnormal lipoprotein metabolism and composition, which further contribute to atherosclerosis and increased risk of atherosclerotic cardiovascular disease [5–8]. HDL is known to be anti-atherogenic because of its anti-oxidant properties, and anti-apoptotic activity in endothelial cells. HDL also plays an important role in reverse cholesterol transport (RCT), the process by which cholesterol is removed from macrophages and peripheral tissue and transported to the liver for excretion [9,10]. Recently, studies have shown that biochemical alterations in HDL may destroy HDL function in patients with uremia [11]. Changes in protein cargo, inflammatory protein levels, or lipoprotein modifications may compromise cholesterol efflux capacity (CEC) and hamper the ability of HDL to prevent endothelial cell apoptosis, oxidation, and atherosclerosis [12,13]. Notably, CEC was inversely associated with the incidence of cardiovascular events in a population-based cohort [14].

Because lipoproteins are complex, it is difficult to comprehensively analyze HDL protein modifications and functional changes. However, understanding these changes in HDL may help guide the direction of treatment development for cardiovascular disease in uremia patients. Recently, we developed the subfractionation of HDL from healthy adults into 5 subfractions (H1−H5) of increasing electronegativity using anion-exchange chromatography [4]. This method provided good resolution for the separation of lipoproteins to facilitate the analysis of protein modifications and functions. Using our previously established techniques, we examined biochemical and functional changes in HDL subfractions from uremia patients. We found that increased electronegativity in uremic HDL subfractions reduced its anti-oxidant, anti-apoptotic, and CEC properties. Furthermore, an increase of the most electronegative HDL subfraction, H5 (H5%), was associated with coronary artery disease (CAD) risk in uremia patients.

2. Patients and methods

2.1. Blood sample collection

Blood was collected from all study participants after fasting, and before hemodialysis therapy in uremia patients. CAD was defined as > 50% stenosis in at least one epicardial artery on coronary angiography [15]. The control subjects were all hospital workers and their CAD risks were excluded by their medical history and electrocardiogram. We did not record statin use history in the study because our national insurance reimbursement for statin use was restricted to periods when individual patients had plasma cholesterol above 200 mg/dl, which made comparisons between groups highly difficult. Our methods adhered to the Declaration of Helsinki. The experimental protocols were approved by the China Medical University & Hospital Research Ethics Committee, Taiwan (reference number: CMUH-102-REC3-142). Blood sampling was performed after getting written informed consent from patients.

2.2. HDL purification

Plasma was separated from blood cell using centrifugation immediately after blood sampling. The freshly collected plasma was added with cocktail protease inhibitor (Roche Applied Sciences, Indianapolis, IN), anti-microbial agent of sodium azide (0.06% wt/vol), and anti-oxidation agent of EDTA (0.06% wt/vol). Plasma (8 mL) was added with 2 mL of PBS and centrifuged (64,000 rpm, 20 min, 4 °C) to remove the upper chylomicron fraction. The lower layer of liquid was added with potassium bromide (KBr) and mixed well to adjust the plasma density to 1.063 g/mL. After centrifugation (64,000 rpm, 4 h, 4 °C), the upper layer of LDL was removed. Then, the sample solution was added with KBr to increase its density to 1.210 g/mL. After centrifugation (64,000 rpm, 5 h, 4 °C), the upper layer of HDL was collected and dialyzed against the buffer solution of 20 mM Tris-HCl/0.5 mM EDTA (pH = 8.0) at 4 °C with three buffer changes in 24 h.

2.3. Subfractionation of HDL

HDL was loaded onto a UnoQ12 anion-exchange column with a fast-protein liquid chromatography (FPLC) device (GE Healthcare, Pittsburg, PA). HDL was eluted sequentially according to electronegativity using a multistep gradient buffer B (1 M NaCl in buffer A). The peaks were grouped according to the following elution and gradient profile: isocratic at 0% B for 10 min, increase gradient from 0% to 15% B for 10 min; and then from 15% to 20% B for 30 min, isocratic at 20% B for 10 min; increase gradient from 20% to 40% B for 25 min; and then from 40% to 100% B for 10 min, isocratic at 100% B for 10 min, and then returned to 0% B in 5 min. Eluents were monitored at 280 nm and then pooled into five subfractions named H1, H2, H3, H4, and H5. H1 was eluted at 18–28 min, H2 at 28–32 min, H3 at 32–48 min, H4 at 48–60 min, and H5 at 60–80 min. H1 was the least electronegative HDL subfraction and H5 was the most electronegative HDL subfraction [1,4].

2.4. Agarose gel electrophoresis

To confirm electric charge differences among HDL subfractions of uremia patients and controls, 8 μg of each HDL subfraction was loaded onto a 0.7% agarose gel (90 mM Tris, 80 mM borate, and 2 mM EDTA, pH = 8.2) and separated using electrophoresis at 100 V for 2 h, as previously described [1]. Gels were stained with Coomassie blue.

2.5. SDS-PAGE

Proteins (2 μg) of each HDL subfraction were solubilized in 10% SDS and separated on 4%–20% SDS gels at 30 mA for 1 h at room temperature. Commercial human apolipoproteins were used as standards (Academy Bio-Medical Co., Houston, TX). Gels were stained with Coomassie blue.

2.6. MALDI-TOF-MS (matrix-assisted laser desorption/ionization-time of flight-mass spectrometry) analysis

Dialyzed H1−H5 samples (10 μl) were added with 500 μl of ethanol-acetoacetate solvent (1:1) with vortex mixing for 1 min. After centrifugation (12000 × g, 5min, 4 °C) to remove the lipid layer, the protein pellet from H1−H5 was obtained and dissolved with 0.1% formic acid. Protein samples (1 μl) was applied on a polydimethylsiloxane-coated MALDI plate [16] and mixed with 1 μl of saturated sinapinic acid matrix (30% ACN/0.1 %TFA). After co-crystallization of protein sample and matrix, the protein sample was analyzed using a MALDI-TOF-MS instrument (Ultraflex III TOF/TOF, Bruker Daltonics, Germany). MALDI-TOF was operated in linear positive ion mode with 25-kV of accelerating voltage and was calibrated in the mass range of 5000–40000 m/z by using calibration standard 1 and 2 kits (Bruker Daltonics).

2.7. Analysis of H1−H5 proteome by data-independent acquisition (DIA) approach

To identify proteome of H1−H5, nanoLC-MS/MS was performed with a nanoflow ultra performance liquid chromatography (nanoUPLC) system (UltiMate 3000 RSLCnano system, Dionex, Amsterdam, Netherlands) coupled with a hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer (maXis impact, Bruker). Sample was injected into a

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