High-Sensitivity CRP is Independently Associated with HDL-Mediated Cholesterol Efflux Capacity and HDL Remodeling in Patients with Coronary Artery Disease

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Research

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Abstract

**Background:** Cholesterol efflux capacity (CEC), a crucial atheroprotective function of high-density lipoprotein (HDL), has proven to be a reliable predictor of cardiovascular risk. Inflammation can damage CEC, but few studies have focused on the relationship between the systemic inflammation marker high-sensitivity C-reactive protein (hsCRP) and CEC in patients with coronary artery disease (CAD).

**Methods:** Thirty-six CAD patients and sixty-one non-CAD controls were enrolled in this observational, cross-sectional study. CEC was measured using a [³H] cholesterol loading Raw 264.7 cell model with apolipoprotein B-depleted plasma (a surrogate for HDL). Proton nuclear magnetic resonance (NMR) spectroscopy was used to assess HDL components and subclass distribution. hsCRP was measured with a latex particle, enhanced immunoturbidimetric assay.

**Results:** CEC was impaired in CAD patients compared to controls (11.9±2.3% vs. 13.0±2.2%, p=0.022). In the control group, CEC was positively correlated with enzymatically measured HDL cholesterol (HDL-C) levels (r=0.358, p=0.006) or NMR-determined HDL-C levels (r=0.416, p=0.001). However, in the CAD group, the significance of correlation disappeared (enzymatic method: r=0.216, p=0.206; NMR spectroscopy: r=0.065, p=0.708). Instead, we found that the level of hsCRP was negatively correlated with CEC (r=-0.351, p=0.036), and this relationship was not modified by CAD risk factors, HDL-C, and HDL subclasses. NMR showed that HDL particles shifted to larger ones in patients with high hsCRP levels, and this phenomenon was accompanied by decreased CEC.

**Conclusions:** In patients with CAD, the level of HDL-C cannot reflect HDL function, but hsCRP is independently associated with HDL dysfunction. The impaired correlation between HDL-C and CEC is possibly due to an inflammation-induced HDL subclass remodeling.

**Trial registration:** Chinese Clinical Trial Registry, ChiCTR1900020873. Registered on 21 January 2019 - Retrospectively

1. Introduction

Over the last few decades, epidemiological studies have confirmed a strong and inverse relationship between the level of high-density lipoprotein (HDL) cholesterol (HDL-C) and the risk of coronary artery disease (CAD) [1]. However, the role of HDL-C has been challenged by the failure of HDL-C raising trials using niacin or cholesteryl ester transfer protein (CETP) inhibitors [2, 3]. In addition, a genetically increased HDL-C does not necessarily translate to a decreased risk of myocardial infarction [4]. Even worse, higher HDL-C levels secondary to SCARB1 gene mutations lead to an increased risk of CAD [5]. A recent epidemiological study has also revealed that extremely high HDL-C levels are associated with increased CAD mortality [6]. These results highlight the potential limitations of using HDL-C levels, a
static mass-based parameter, to assess the risk of CAD, and call for investigations on more robust HDL functional markers for evaluating cardiovascular risks.

HDL particle exerts favorable effects against atherosclerosis, primarily by reverse cholesterol transport (RCT) [7]. A meta-analysis of 11 studies (n = 63,064 patients) has reported that the concentration of HDL particles was inversely related to cardiovascular events [8]. Cholesterol efflux capacity (CEC), a metric reflecting the ability of HDL particles as a cellular cholesterol acceptor, has been demonstrated to be inversely associated with subclinical atherosclerosis [9, 10], the incidence of cardiovascular events [11–13], and prognosis of CAD [14, 15]. The HDL particle numbers and CEC have been considered novel and reliable CAD markers [11] and may be better targets for intervention than HDL-C.

The measurement of CEC requires radiolabeled cholesterol and cultured cells, which is time-consuming and not applicable in clinical settings. It has been consistently observed that CEC was positively correlated with HDL-C levels in healthy populations [9, 12, 13, 16]. However, in patients with CAD, this relationship was inconsistent in different studies. Data from Khera, et al. [9] and Shao, et al. [17] showed that the correlation coefficients between HDL-C and CEC were 0.51 (p < 0.0001) and 0.31 (p < 0.05), respectively, while two other studies showed that the correlation was weak [18] or even inexistent [14].

The unstable relationship between HDL-C and CEC may be subjected to dynamic changes in the components of HDL subclasses. Inflammation has been a well-established factor that affects HDL components and subclass distribution [19, 20], and patients with inflammatory connective tissue disease always present a decreased CEC [21], suggesting inflammatory markers may serve as surrogate parameters for HDL dysfunction.

Proton nuclear magnetic resonance (NMR) spectroscopy is an emerging technique that can provide a fine-grained snapshot of a person’s lipid metabolism. Using NMR-determined HDL subclasses can improve the mortality risk discrimination in the cardiac catheterization cohort [22] and predict the prognosis of patients with pulmonary arterial hypertension [23]. This study used NMR spectroscopy to provide more detailed information about the components of HDL and HDL subclasses. By simultaneously examining the level of high-sensitivity C-reactive protein (hsCRP), a sensitive marker of systemic inflammation, and HDL functional marker CEC, we intended to clarify the relationship between HDL-C and CEC and between hsCRP and CEC in patients with CAD.

2. Subjects And Methods

2.1 Study population

We recruited 36 CAD patients from June 2018 to December 2018 in the Department of Cardiovascular Medicine of the Second Xiangya Hospital of Central South University. Of these, 33 had acute coronary syndrome (ACS, including 4 ST-segment elevated myocardial infarction (MI), 14 non-ST-segment elevated MI, and 15 unstable angina), and 3 had stable angina. According to the guidelines of the European Society of Cardiology [24, 25], the diagnosis of MI was based on a combination criteria, namely the
increased level of serum high-sensitivity troponin T (hsTnT), with at least one value above the 99th percentile of the upper reference limit (0.0140 µg/L) and at least one of the following: (1) symptoms of ischemia; (2) new or presumably new significant ST-T wave changes or left bundle branch block on the 12-lead electrocardiogram (ECG); (3) development of pathological Q waves on the ECG; and (4) Imaging evidence of new or presumably new loss of viable myocardium or regional wall motion abnormality. MI with persistent (> 20 min) ST-segment elevation was diagnosed as ST-segment elevated MI; otherwise, it was diagnosed as non-ST-segment elevated MI. In patients with normal hsTnT levels that presented evidence of cardiac ischemia, the diagnosis was unstable angina. All patients with suspected CAD underwent coronary angiography, and the diagnosis was confirmed by a coronary angiography showing ≥ 50% stenosis in at least one main coronary artery. Sixty-one healthy controls (non-CAD) free from atherosclerotic disorders confirmed by coronary angiography or coronary computed tomography were recruited in the same period to our department. Patients during the gestational phase, under hormone therapy, taking corticosteroid or anti-inflammatory drugs were not included. Patients with human immunodeficiency virus (HIV) were not included either, because HIV infection has been reported to damage CEC [26]. Exclusion criteria included hemodynamic instability, a history of renal failure, hepatic function insufficiency, severe infections, autoimmunity disease, cancer, severe medical illnesses, heavy alcohol use (the average daily alcohol intake ≥ 40 g (for Female) or ≥ 80 g (for male)), or intensive exercise (comparable to a basketball competition) within one week before admission. Written informed consent was obtained from all the individuals included in this study. The research related to human use complied with all the relevant national regulations, institutional policies, and followed the tenets of the Helsinki Declaration, and has been approved by the Medical Ethics Committee of the Second Xiangya Hospital of Central South University. This trial was registered at the Chinese Clinical Trial Registry as ChiCTR1900020873.

2.2 Clinical and biochemical measurements

Demographic information, such as height, weight, blood pressure, and heart rate were measured and recorded. Fasting blood samples were collected the morning after admission. Routine blood, urine, and lipid profiles, including triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and HDL-C, were analyzed via the enzymatic method. hsCRP was measured with a latex particle, enhanced immunoturbidimetric assay. Cardiac troponin T was measured using a high-sensitivity assay. For the subsequent experiments, fresh plasma was aliquoted and stored at −80 °C.

2.3 ApoB-depleted plasma preparation

The plasma samples were thawed in a refrigerator at 4 °C before the experiment. According to the protocol of the previous experiment [27], 540 µL of heparin sodium solution (280 mg/mL, Aladdin, China) and 10 mL of a manganese chloride solution (1.06 mol/L, Aladdin, China) were mixed. Plasma was incubated for 30 min at 4 °C with a mixed solution (10:1 vol/vol) and then centrifuged at 1500 × g for 30 min. The supernatant was collected and if it was still turbid (especially samples with a high concentration of triglycerides), plasma was centrifuged again at 12000 × g for 10 min, and the lower liquid fraction was recovered for the next procedure. Compared to conventional ultracentrifugation, this
precipitation method is simpler and more efficient for HDL isolation [9]. A previous study revealed that heparin sodium/manganese chloride precipitation had a minor effect on HDL distribution [28].

2.4 Measurement of Cholesterol efflux capacity

The cholesterol efflux assay was performed according to established procedures [9, 13]. In brief, murine Raw 264.7 macrophages (ATCC, USA) were grown in the Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA), supplemented with 10% fetal bovine serum (Gibco, USA). Macrophages were plated on 48-well plates (300,000 cells/well). Subsequently, cells were loaded with 25 µg/mL acetylated LDL (Peking Union-Biology Co., Ltd, China) and 1 µCi/mL [3H] cholesterol (PerkinElmer, USA) for 24 hours. The macrophages were then washed with PBS (Gibco, USA). To upregulate the expression of ATP-binding cassette transporter A1 (ABCA1), cells were stimulated for 24 h with serum-free DMEM containing 0.3 mmol/L 8-Bromoadenosine 3',5'-cyclic monophosphate (Sigma, USA), then washed with PBS again and incubated with 2.8% (vol/vol) apoB-depleted plasma diluted in the medium for 6 hours. All steps were performed in the presence of 2 µg/mL of the acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibitor Sandoz 58–035 (Sigma, USA). The supernatant was collected and centrifuged to remove cellular debris. Cells on the plate were washed with PBS again and then incubated with a 0.1 mol/L NaOH solution for 30 min for cell lysis. The radioactivity within the supernatant and cells was determined by liquid scintillation counting (PerkinElmer, USA). CEC was calculated using the following equation: CEC (%) = 3H media / (3H media + 3H cells) × 100. All efflux experiments were performed in triplicate for each sample. Each plate contained blank control and positive control (50 µg/mL HDL, purchased from Peking Union-Biology Co. Ltd). A standard sample (pooled plasma from 20 individuals) was used to correct the inter-assay error.

2.5 Nuclear Magnetic Resonance Spectroscopy

The total plasma apolipoprotein A-I (apoA-I)-rich lipoprotein and 30 discrete HDL-related lipoproteins were measured by NMR spectroscopy at ProteinT Biotechnology Co., Ltd (Tianjin, China) by Bruker 600 MHz NMR spectrometer. In the context of the result, the HDL-C measured enzymatically was designated as ENZ-HDL-C, and the HDL-C measured by NMR was described as NMR-HDL-C. The lipoprotein-distribution-prediction method selected for the analysis was the commercial Bruker IVDr Lipoprotein Subclass Analysis (B.I.-LISA) method as previously described methods [23, 29]. HDL-related lipoproteins were classified into four subclasses, labeled numerically according to decreasing size and increasing density. The corresponding density (mg/L) of HDL1, HDL2, HDL3, and HDL4 was 1.063-1.100, 1.100-1.112, 1.112–1.125, and 1.125–1.210, respectively. The HDL1 subclass was the largest, while the HDL4 subclass was the smallest. Compared with the classic ultracentrifugation method obtained HDL, the sum of HDL1, HDL2, HDL3 is equivalent to the large HDL2 obtained by ultracentrifugation, and HDL4 is equivalent to the small HDL3 obtained by ultracentrifugation [30].

2.6 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 25.0. Plots were made using GraphPad Prism version 8.0. Normally distributed continuous data have been
expressed as mean ± standard deviation. Skewed distributed continuous data have been described as medians with interquartile ranges and were logarithmically transformed when necessary. Comparisons between categorical data were performed with the chi-square test, while continuous variables were assessed by t-test (for normal distribution) or nonparametric tests (for skewed distribution). The Pearson's correlation analysis was used to evaluate the associations between variables. Multiple linear regression analysis was performed to determine the variables with an independent significant association with CEC. In the correlation and regression analysis, a two-tailed p-value < 0.05 was considered statistically significant.

3. Results

3.1 Patient characteristics

The demographic and biochemical characteristics of the subjects have been shown in Table 1. The subjects in the CAD group were older, with a higher percentage of the male sex, diabetes, hypertension, statin use, and current smoking than subjects in the non-CAD group. Concentrations of TC, ENZ-HDL-C, and LDL-C were lower, but serum hsCRP was significantly higher in CAD patients than in the non-CAD controls (1.76 [0.88–4.05] vs. 0.91 [0.32–1.87], p = 0.004). Free glucose was also higher among CAD patients. The median hsTnT level in CAD patients was 0.0178 µg/L (0.0086–0.1667). Other parameters showed no statistically significant differences between the two groups. The interval from illness onset to admission for CAD patients has been presented in Supplementary Table 1. Twenty-nine (80.6%) of patients with CAD were admitted to the hospital more than 7 days after event onset, and 15 (41.7%) patients were admitted after more than 1 month of event onset, suggesting that most of our patients with CAD were beyond the acute stage (usually defined as within 7 days of event onset).
Table 1
Baseline Characteristics of the non-CAD (n = 61) group and the CAD (n = 36) group.

|                       | Non-CAD          | CAD              |
|-----------------------|------------------|------------------|
| **Age, y**            | 46.8 ± 13.3      | 60.8 ± 8.6**     |
| **Male sex, n (%)**   | 27 (44.3)        | 29 (80.6) **     |
| **BMI, kg/m²**        | 23.9 ± 3.8       | 24.8 ± 3.1       |
| **Diabetes, n (%)**   | 3 (4.9)          | 12 (33.3) **     |
| **Hypertension, n (%)**| 16 (26.2)      | 17 (47.2) *      |
| **Statin use, n (%)** | 4 (6.6)          | 27 (75.0) **     |
| **Current smoking, n (%)**| 11 (18.0)     | 15 (41.7) *      |
| **Drinking, n (%)**   | 9 (14.8)         | 7 (19.4)         |
| **TG, mg/dL**         | 117.80 (90.34-191.75) | 165.18 (105.62-229.40) |
| **TC, mg/dL**         | 170.01 ± 31.73   | 148.17 ± 36.89*  |
| **ENZ-HDL-C, mg/dL**  | 44.82 ± 10.37    | 36.04 ± 8.94**   |
| **LDL-C, mg/dL**      | 105.98 ± 26.78   | 89.74 ± 31.58*   |
| **hsCRP, mg/L**       | 0.91 (0.32–1.87) | 1.76 (0.88–4.05) ** |
| **hsTnT, µg/L**       | NA               | 0.0178 (0.0086–0.1667) |
| **Fasting glucose, mmol/L** | 4.50 (4.20-5.00) | 5.05 (4.4–6.18) ** |

CAD, coronary artery disease; BMI, body mass index; TG, triglycerides; TC, total cholesterol; ENZ-HDL-C, enzymatically measured high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity Troponin T; NA, not applicable. *p < 0.05, **p < 0.01.

3.2 Correlation between cholesterol efflux capacity and HDL-C levels

The CEC of the standard sample on each 48-well plate has been shown in Supplementary Fig. 1. The intra- and inter-assay coefficients of variation were 5.7% and 4.8%, respectively, which was comparable to previous studies [9, 31]. CEC in CAD group was significantly lower compared to the non-CAD group (11.9 ± 2.3% vs. 13.0 ± 2.2%, p = 0.022, Fig. 1). Correlation analysis showed that ENZ-HDL-C was positively correlated with CEC in the non-CAD group (r = 0.358, p = 0.006, Fig. 2A), while there was no significant correlation in the CAD group (r = 0.216, p = 0.206, Fig. 2B).
We also measured the level of HDL-C and other HDL-related lipoproteins using NMR spectroscopy (see Supplementary Table 2). Consistent with the results of enzymatic methods, NMR-HDL-C was positively correlated with CEC in non-CAD controls ($r = 0.416, p = 0.001$, Fig. 2C). However, in CAD patients, there was no correlation between NMR-HDL-C and CEC ($r = 0.065, p = 0.708$, Fig. 2D).

In the univariate analysis, as shown in Table 2, CEC was positively correlated with the levels of total plasma apolipoprotein A-I ($r = 0.369, p = 0.004$), HDL-phospholipids ($r = 0.338, p = 0.009$), HDL-free cholesterol ($r = 0.282, p = 0.032$), HDL-apoA-I ($r = 0.400, p = 0.002$), and HDL-apoA-II ($r = 0.340, p = 0.009$) in the non-CAD group. In the CAD group, however, these parameters showed no correlation with CEC.
Table 2
Pearson's correlation analysis between cholesterol efflux capacity and NMR-determined total plasma apolipoprotein A-I-rich lipoprotein and HDL-related lipoproteins in the non-CAD (n = 61) group and the CAD (n = 36) group.

|                        | Non-CAD |         | CAD   |         |
|------------------------|---------|---------|-------|---------|
|                        | r       | p       | r     | p       |
| Total plasma apoA-I    | 0.369   | 0.004   | 0.086 | 0.618   |
| Total-HDL-triglycerides| 0.014   | 0.918   | -0.190| 0.267   |
| Total-HDL-phospholipids| 0.338   | 0.009   | -0.028| 0.871   |
| Total-HDL-free cholesterol| 0.282 | 0.032   | 0.228 | 0.182   |
| Total-HDL-apoA-I       | 0.400   | 0.002   | 0.070 | 0.687   |
| Total-HDL-apoA-II      | 0.340   | 0.009   | -0.071| 0.681   |
| HDL1-cholesterol       | 0.285   | 0.030   | -0.159| 0.354   |
| HDL1-triglycerides     | 0.116   | 0.384   | -0.230| 0.178   |
| HDL1-phospholipids     | 0.231   | 0.081   | -0.194| 0.256   |
| HDL1-free cholesterol  | 0.246   | 0.063   | 0.089 | 0.604   |
| HDL1-apoA-I            | 0.223   | 0.092   | -0.179| 0.297   |
| HDL1-apoA-II           | 0.165   | 0.217   | -0.258| 0.128   |
| HDL2-cholesterol       | 0.289   | 0.028   | -0.116| 0.499   |
| HDL2-triglycerides     | 0.035   | 0.795   | -0.220| 0.197   |
| HDL2-phospholipids     | 0.208   | 0.116   | -0.209| 0.222   |
| HDL2-free cholesterol  | 0.181   | 0.173   | 0.044 | 0.798   |
| HDL2-apoA-I            | 0.221   | 0.096   | -0.198| 0.247   |
| HDL2-apoA-II           | 0.137   | 0.305   | -0.233| 0.171   |
| HDL3-cholesterol       | 0.319   | 0.015   | 0.015 | 0.933   |
| HDL3-triglycerides     | 0.021   | 0.878   | -0.205| 0.231   |
| HDL3-phospholipids     | 0.236   | 0.074   | -0.091| 0.600   |
| HDL3-free cholesterol  | 0.210   | 0.113   | 0.112 | 0.516   |

CEC, cholesterol efflux capacity; NMR, nuclear magnetic resonance; HDL, high-density lipoprotein; CAD, coronary artery disease; ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II. Boldface type emphasizes significant changes.
|                 | Non-CAD | CAD    |
|----------------|---------|--------|
| HDL3-apoA-I    | 0.246   | 0.063  |
| HDL3- apoA-II  | 0.155   | 0.246  |
| HDL4-cholesterol| 0.224   | 0.091  |
| HDL4-triglycerides | 0.077 | 0.566  |
| HDL4-phospholipids | 0.206 | 0.120  |
| HDL4- free cholesterol | 0.194 | 0.145  |
| HDL4-apoA-I    | 0.238   | 0.072  |
| HDL4-apoA-II   | 0.182   | 0.171  |

CEC, cholesterol efflux capacity; NMR, nuclear magnetic resonance; HDL, high-density lipoprotein; CAD, coronary artery disease; ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II. Boldface type emphasizes significant changes.

3.3 In the CAD group, cholesterol efflux capacity was negatively correlated with the hsCRP level

To investigate which factor correlated with HDL-mediated CEC in CAD patients, univariate analysis was performed. There was no correlation between CEC and age, body mass index, severity of coronary stenosis (expressed as the Gensini score), hsTnT, or the serum levels of TG, TC, LDL-C, fasting glucose (see Supplementary Table 3). However, we found that CEC was negatively correlated with the hsCRP level ($r=-0.351$, $p = 0.036$, Fig. 2E). Then, we divided 36 CAD patients into hsCRP-low (n = 18) and hsCRP-high (n = 18) groups by using the median hsCRP level value (1.75 mg/L) as the criterion (the values of hsCRP in all patients have been shown in Supplementary Fig. 2). The baseline characteristics, the concentrations of total HDL lipids and apolipoproteins were comparable in two groups, except for the significantly higher level of hsTnT in the hsCRP-high group (see Supplementary Table 4). Nonetheless, CEC was significantly lower in the hsCRP-high group than in the hsCRP-low group (11.3 ± 2.2% vs. 12.6 ± 2.1%, $p = 0.038$, Fig. 2F).

3.4 In CAD patients, HDL particles underwent extensive remodeling with a high level of hsCRP

To explore why CEC reduced despite the level of total HDL lipids and major apolipoproteins remained unchanged between the hsCRP-high and the hsCRP-low group, we compared HDL subclass distribution in the two groups. In the hsCRP-high group, the concentrations of lipids and major apolipoproteins in the largest HDL subclass (HDL1) were significantly higher, while those in the smallest HDL subclass (HDL4) were significantly lower than those in the hsCRP-low group (Fig. 3). Further analysis showed that the levels of lipids and major apolipoproteins in HDL1 were positively correlated with the level of hsCRP, while in HDL4 there was a negative correlation (see Supplementary Table 5).
3.5 Multiple linear regression analysis of cholesterol efflux capacity in patients with CAD

To clarify whether the relationship between hsCRP and CEC was independent, multiple linear regression analysis was performed. Cardiovascular risk factors including age, sex, LDL-C, diabetes, current smoking, body mass index, TG (log-transformed), and hsTnT (log-transformed) were included as covariates. Adjustments were made for ENZ-HDL-C, NMR-HDL-C, HDL1-cholesterol, and HDL4-cholesterol. Results showed that hsCRP had an independent relationship with HDL-mediated CEC, regardless of conventional CAD risk factors, hsTnT, HDL-C, and HDL subclasses (Table 3).

| Linear-Regression Covariates | Beta Coefficient per 1-SD increase in lg(hsCRP) (95% CI) | p   |
|-----------------------------|--------------------------------------------------------|-----|
| Cardiovascular risk factors | -0.019 (-0.035 to -0.004)                               | 0.016|
| Cardiovascular risk factors, ENZ-HDL-C | -0.018 (-0.035 to -0.002)                               | 0.027|
| Cardiovascular risk factors, NMR-HDL-C | -0.019 (-0.035 to -0.004)                               | 0.019|
| Cardiovascular risk factors, HDL1-cholesterol | -0.020 (-0.038 to -0.002)                               | 0.032|
| Cardiovascular risk factors, HDL4-cholesterol | -0.019 (-0.035 to -0.003)                               | 0.023|

Cardiovascular risk factors included: age, male sex, LDL-C, diabetes, current smoking, body mass index, triglycerides (log-transformed), hsTnT (log-transformed). hsCRP, high-sensitivity C-reactive protein; CAD, coronary artery disease; ENZ-HDL-C, enzymatically measured high-density lipoprotein cholesterol; NMR-HDL-C, NMR-determined high-density lipoprotein cholesterol. Boldface type emphasizes significant changes.

4. Discussion

HDL has been recognized as a traditional protective factor against atherosclerosis. Early epidemiological studies have consistently shown that HDL-C is inversely correlated with cardiovascular risk [1]. However, subsequent attempts for drug therapies that aim to raise HDL-C levels with niacin [3] or CETP inhibitor [2] have both been in vain. HDL-mediated RCT is the key protective function of HDL. CEC, a metric defined by ex vivo experiments which reflects the first and rate-limiting step of RCT, has been demonstrated to be more valuable than HDL-C in predicting CAD risks [9–12, 14, 15]. However, CEC is susceptible to impairment in many disease states.
Our results showed that compared to non-CAD controls, CEC was lower in CAD patients and was not significantly related to HDL-C. The reduced CEC in CAD patients has already been well described in a high-quality study [9] but the correlation between HDL-C and CEC was inconsistent in previous reports. For example, the correlation coefficient between HDL-C and CEC was 0.51 (p < 0.0001) in the study by Khera et al. [9] (combining 442 CAD patients and 351 controls) but it was −0.09 in the study by Zhang et al. (313 CAD patients) [14]. The discordance could result from the different study populations, as most of the patients in Zhang et al.’s study and our study had ACS, while Khera et al.’s study excluded ACS patients. In addition to CAD, the CEC of HDL is also impaired in other diseases. Our previous observation found that patients with end-stage renal disease exhibited significantly decreased CEC compared to controls [10]. HIV infection has been reported to induce structural and functional changes in HDL particles [26]. A similar impairment of CEC was also found in patients with type 1 [32] or type 2 diabetes [12] and autoimmune diseases [21].

A common feature of the above diseases is that inflammation is a major part of their pathophysiological mechanism. The inflammatory process can influence HDL components, shifting HDL proteome to inflammatory profile [33], and some of these uninvited guests can be detrimental to CEC. For example, the enrichment of serum amyloid A (SAA) or apolipoprotein C-III (ApoC-III) in HDL can impair HDL-mediated CEC [19, 31]. In addition to the change of HDL components, inflammation can also lead to modification of apoA-I, the major protein of HDL. Our early experimental study has found that myeloperoxidase (MPO)-mediated tryptophan oxidation of apoA-I was associated with decreased cholesterol efflux [34]. Modified apoA-I was also abundant in the plasma recovered from patients with CAD [17]. Although we did not measure the level of serum MPO or modified apoA-I in this study, correlation analysis revealed that the level of apoA-I (determined by NMR) was not correlated with CEC in CAD patients, suggesting that apoA-I modification could be a reason for HDL dysfunction in this study.

Our study showed that hsCRP was inversely correlated with CEC in CAD patients. The correlation remained significant after adjusting other conventional risk factors, HDL-C levels, and HDL subclasses. This independent role of hsCRP to CEC has not been reported in patients with CAD, although univariate negative correlation has been noted before [17]. Inflammation in patients with CAD mainly derives from the activation of inflammatory pathways by various atherogenic risk factors. IL-1-IL-6-CRP axis is one of the most critical inflammatory pathways, and its activation can explain our findings. Cells in the atheroma (e.g., endothelial cells, macrophages, smooth muscle cells, etc.) produce IL-1β when exposed to stimuli (e.g., oxidized lipoproteins, disturbed blood flow, etc.). IL-1β strongly augments the production of IL-6 by various cell types. IL-6 induces liver to produce acute-phase proteins, such as CRP and SAA [35, 36]. We speculated that the activation of the IL-1-IL-6-CRP axis increased the level of hsCRP, and at the same time, led to the SAA-mediated CEC impairment. Inflammation was the possible reason behind the negative correlation between hsCRP and CEC.

hsCRP is a valuable marker of CAD. In people without a history of vascular disease, the risk ratios for CAD per 1-SD higher of log_{e}CRP concentration were 1.37 (1.27–1.48) after adjustment for conventional risk factors [37]. In patients with CAD, it has been shown that patients with higher hsCRP baseline level
(usually $\geq 2 \text{ mg/L}$) have significantly higher risk of major adverse cardiovascular events (MACE) compared to those with lower hsCRP baseline level (usually $< 2 \text{ mg/L}$) [38, 39]. hsCRP is not only a risk marker but can also predict the clinical benefit of antiatherogenic agents. Results from the JUPITER trial showed that participants without dyslipidemia but who had hsCRP levels higher than 2 mg/L can benefit more from rosuvastatin treatment [40]. In the CANTOS trial, participants with canakinumab, a monoclonal antibody targeting IL-1$\beta$, who achieved an on-treatment hsCRP level $< 2 \text{ mg/L}$ had a 25% reduction in the risk of MACE, while no significant benefit was observed in participants whose on-treatment hsCRP concentration was 2 mg/L or above [41]. Recent results from the COLCOT trial also showed that the non-specific anti-inflammation agent colchicine reduced stroke and coronary revascularization in patients with myocardial infarction, accompanied by a large reduction (> 65%) of the hsCRP level [42]. On the other hand, treatment with another anti-inflammation medicine, methotrexate, did not reduce the risk of MACE, as there was no decrease in the hsCRP levels following treatment [43]. Results from these high-quality studies and the current study consistently revealed the potential role of IL-1-IL-6-CRP axis in CAD and underscored the importance of hsCRP testing in patients with CAD.

In our study, the HDL subclass distribution was also determined by NMR spectroscopy. Our results showed that CAD patients with above median hsCRP levels exhibited a different HDL subclass distribution compared to patients with hsCRP levels below the median, namely the increased large HDL particles (HDL1) and the decreased small HDL particles (HDL4). Partial correlation analysis revealed that even after controlling for hsTnT levels, age, LDL-C levels, and diabetes, hsCRP levels remained positively correlated with HDL1-cholesterol levels, suggesting that the systematic inflammatory status of CAD was the underlying reason for HDL subfraction-distribution abnormality. HDL is a heterogeneous population of particles with sizes ranging from 7 nm-14 nm in diameter [7], and HDL particles are known to shift toward larger ones as a result of inflammation [20, 46]. The mechanism by which inflammation affects HDL remodeling is still not fully understood. In our study, the underlying reason, as mentioned before, could be the increased SAA levels [47] or the altered activity of plasma enzymes related to HDL metabolism, such as CETP [48].

In conclusion, we found that HDL-C levels could not reflect the functional status of HDL in patients with CAD, and the impaired correlation between HDL-C levels and CEC was possibly due to inflammation-induced HDL-subclass remodeling. The inflammatory marker, hsCRP, had an independent and negative relationship with CEC in CAD patients. As a relatively small group of patients was taken into account, further studies with larger cohorts including stable CAD patients, ACS patients, and mechanistic studies will need to be undertaken to further verify our conclusions and to elucidate the underlying mechanism for CEC impairment and HDL remodeling.

**Abbreviations**

HDL
High-density lipoprotein; HDL-C: High-density lipoprotein cholesterol; CETP: Cholesteryl ester transfer protein; RCT: Reverse cholesterol transport; CEC: Cholesterol efflux capacity; CAD: Coronary artery disease;
hsCRP: High-sensitivity C-reactive protein; hsTnT: High-sensitivity Troponin T; MI: Myocardial infarction; HIV: Human immunodeficiency virus; ECG: Electrocardiogram; NMR: Nuclear magnetic resonance; ACS: Acute coronary syndrome; TG: Triglycerides; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; DMEM: Dulbecco's Modified Eagle's Medium; ABCA1: ATP binding cassette transporter A1; ACAT: Acyl-coenzyme A: cholesterol acyltransferase; ENZ-HDL-C: High-density lipoprotein cholesterol measured enzymatically; NMR-HDL-C: High-density lipoprotein cholesterol measured by NMR spectroscopy; SPSS: Statistical Package for Social Sciences; HDL1: The largest HDL subclass defined by NMR spectroscopy; HDL4: The smallest HDL subclass defined by NMR spectroscopy; MPO: Myeloperoxidase; ApoA-I: Apolipoprotein A-I; ApoA-II: Apolipoprotein A-II; ApoB: Apolipoprotein B; ApoC-III: Apolipoprotein C-III; SAA: Serum amyloid A; MACE: Major adverse cardiovascular events

Declarations

Ethics approval and consent to participate: Written informed consent was obtained from all the individuals included in this study. The research related to human use complied with all the relevant national regulations, institutional policies, and followed the tenets of the Helsinki Declaration, and has been approved by the Medical Ethics Committee of the Second Xiangya Hospital of Central South University.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: None.

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Authors’ Contributions: Among the authors, DP, BY, XT conceived the hypotheses and analyses. LM, XT, JC, TZ, XG, JK collected samples and data. XT and SW conducted the experiments. XT performed statistical analysis and drafted the paper. DP, BY, TZ refined interpretation and the final manuscript. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Figures
Figure 1

Comparison of CEC between non-CAD controls (n=61) and CAD patients (n=36). CEC, cholesterol efflux capacity; CAD, coronary artery disease; * p <0.05.
Figure 2

Correlation between CEC and ENZ-HDL-C in (A) non-CAD controls and (B) CAD patients. Correlation between CEC and NMR-HDL-C in (C) non-CAD controls and (D) CAD patients. (E) Correlation between hsCRP levels (log-transformed) and CEC in CAD patients (n=36); (F) Comparison of CEC between hsCRP-low CAD patients (hsCRP <1.75 mg/L, n=18) and hsCRP-high CAD patients (hsCRP ≥1.75 mg/L, n=18).

CEC, cholesterol efflux capacity; CAD, coronary artery disease; ENZ-HDL-C, enzymatically measured high-
density lipoprotein cholesterol; NMR-HDL-C, NMR measured high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; CEC, cholesterol efflux capacity; CAD, coronary artery disease; * p <0.05.

Figure 3

Comparison of 24 HDL-subclass related lipoproteins between hsCRP-low CAD patients (hsCRP <1.75 mg/L, n=18) and hsCRP-high CAD patients (hsCRP ≥1.75 mg/L, n=18). HDL subclasses were labeled numerically according to decreasing size and increasing density. HDL, high-density lipoprotein; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II. Data have been expressed as mean ± SD; * p <0.05, ** p <0.01.

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