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Association of Cholesterol Uptake Capacity, a Novel Indicator for HDL Functionality, and Coronary Plaque Properties: An Optical Coherence Tomography-based Observational Study

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Running head: Cholesterol uptake capacity and coronary plaques

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Abbreviations

ABCA1: ATP-binding cassette transporter A1

apoA1: apolipoprotein A1

apoB: apolipoprotein B

BODIPY: boron dipyrromethene difluoride

CAD: coronary artery disease

cAMP: cyclic adenosine monophosphate

CEC: cholesterol efflux capacity

CUC: cholesterol uptake capacity

CVD: cardiovascular disease

HbA1c: hemoglobin A1c

HDL: high-density lipoprotein

hs-CRP: high sensitivity C-reactive protein

LDL: low-density lipoprotein

OCT: optical coherence tomography

PCI: percutaneous coronary intervention

TG: triglycerides

Abstract

Background: Cholesterol efflux from atherosclerotic lesion is a key function of high-density lipoprotein (HDL). Recently, we established a simple, high-throughput, cell-free assay to evaluate the capacity of HDL to accept additional cholesterol, which is herein referred to as "cholesterol uptake capacity (CUC)".

Objective: To clarify the cross-sectional relationship between CUC and coronary plaque properties.

Methods: We enrolled 135 patients to measure CUC and assess the morphological features of angiographic stenosis by optical coherence tomography (OCT). We estimated the extent of the lipid-rich plaque by multiplying the mean lipid arc by lipid length (lipid index). The extent of the OCT-detected macrophage accumulation in the target plaque was semi-quantitatively estimated using a grading system.

Results: Lipid-rich plaque lesions were identified in 125 patients (92.6%). CUC was inversely associated with the lipid index ($R = -0.348$, $P < 0.0001$). In addition, CUC was also inversely associated with macrophage score (R =

-0.327, $P < 0.0001$). Conversely, neither circulating levels of HDL cholesterol nor apoA1 showed a similar relationship.

Conclusions: We demonstrated that CUC was inversely related to lipid-rich plaque burden and the extent of macrophage accumulation, suggesting that CUC could be useful for cardiovascular risk stratification.

Key words: high-density lipoprotein (HDL); cholesterol uptake capacity (CUC); cholesterol efflux capacity (CEC); coronary plaque; optical coherence tomography (OCT); lipid index; macrophage score

1. Introduction

Accumulated epidemiological evidence has demonstrated an inverse relationship between high-density lipoprotein cholesterol (HDL-C) concentration and the risk of cardiovascular disease (CVD) [1-3]. However, there is controversy regarding the causal relationship between HDL-C and cardiovascular disease. Cholesterol ester transfer protein (CETP) inhibitors have been developed to raise HDL-C levels. Until recently, randomized controlled trials of CETP inhibitors have failed to reduce the risk of CVD[4-6]. Finally, the REVEAL trial has demonstrated that the CETP inhibitor anacetrapib reduced cardiovascular events in patients undergoing statin treatment[7]. However, cardiovascular benefits of anacetrapib might be attributed to lowering low-density lipoprotein cholesterol (LDL-C), rather than raising HDL-C by CETP inhibition [8]. Recently, the Framingham Offspring Study has demonstrated that isolated low HDL-C levels do not predict risk when low-density lipoprotein (LDL-C) and triglyceride (TG) levels are completely normal[9]. Moreover, recent reports have demonstrated that extremely high HDL-C levels were associated with an increase rather than a decrease in mortality[10, 11]. Conversely, recent cohort studies have demonstrated that that CEC of HDL, a dynamic rate of the initial step in reverse cholesterol transport, is associated with both the prevalence and

incidence of cardiovascular disease, and is a better predictor than steady-state circulating HDL-C levels[12-14]. However, CEC measurement is not instantly applicable in clinical settings because the methods for CEC assay require radiolabeled cholesterol and cultured cells and the procedures are time consuming[15, 16].

In order to address these concerns, we have recently established a simple, high-throughput, cell-free assay system to evaluate "cholesterol uptake capacity (CUC)" as a novel concept for HDL functionality[17]. The procedure for measuring CUC involves the following steps. Serum is incubated with fluorescently labeled cholesterol and is subjected to HDL capturing using an anti-apolipoprotein A1 (apoA1) antibody immobilized on a microplate. Subsequently, fluorescence signals are detected from the labeled cholesterol incorporated into HDL.

Because of a cell-free system, CUC is different from CEC in that CUC does not reflect ATP-binding cassette transporter A1 (ABCA1)-mediated efflux[18]. In this context, the CUC assay is complimentary to the CEC assay in understanding the role of HDL in CVD. We previously reported that CUC was inversely associated with the recurrence rate of coronary lesions after revascularization in patients with optimal control of LDL-C concentrations suggesting that CUC also potentially be useful for cardiovascular risk stratification. [17]. In this study, we sought to clarify the cross-sectional

relationship between CUC and coronary plaque properties, including the extent of lipid-rich plaque referred to as lipid index and macrophage accumulation assessed by OCT.

2. Patients and Methods

2.1. Study Population

The Kobe Cardiovascular Marker Investigation (CMI) registry is a single-center registry of patients referred to Kobe University Hospital with cardiovascular disease, which is conducted to identify blood-based biomarkers that have utility in predicting cardiovascular disease. The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. The study was approved by the Ethics Review Committee at Kobe University (Japan) and was registered at the UMIN Clinical Trials Registry (identification number 000030297). Written informed consent was obtained from all patients prior to enrollment in the study.

We enrolled consecutive patients registered in the Kobe CMI registry who underwent OCT assessment for native coronary plaques between January 2010 and May 2015. The inclusion criteria were as follows: 1) a history of percutaneous coronary intervention (PCI), 2) the presence of residual intermediate stenosis (diameter: 30%–70%) in the native coronary artery, and 3) under guideline-directed medical management[19]. The exclusion criteria were: 1) no written informed consent, 2) OCT images of unsatisfactory quality, 3) a history of cancer in the previous 5 years, or 4) a history of inflammatory

conditions such as active infections, inflammatory arthritis, or connective tissue disease.

Sample size was calculated on the basis of previous study, which have reported that CEC was inversely associated with non-calcified coronary plaque burden measured by coronary computed tomography angiography (correlation coefficient; -0.38, $p<0.001$)[20]. We hypothesized that there was an inverse relationship between CUC and lipid index. Assuming an alpha probability of 0.05 and a power of 80%, the recommended sample size in the present study was 106 individuals.

2.2. Study design

Blood sample analysis was performed before cardiac catheterization after overnight fasting to evaluate serum levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, triglycerides (TG), apoA1, hemoglobin A1c (HbA1c), and high sensitivity C-reactive protein (hs-CRP). Serum samples for CUC measurement were collected when patients underwent OCT and were stored at −80°C. Angiographically intermediate lesions (diameter of stenosis: 30%–70%) in the native coronary artery were evaluated using OCT.

Hypertension was defined as a blood pressure of ≥140/90 mmHg or if the subject was treated with antihypertensive drugs. Diabetes mellitus was defined as subjects having hemoglobin A1c ≥6.5% and fasting serum glucose ≥126 mg/dL or

non-fasting serum glucose ≥200 mg/dL, or if the subject was treated with antidiabetic drugs. Dyslipidemia was defined according to the Japan Atherosclerosis Society Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases[19]. Dyslipidemia was also recorded for patients treated with anti-hyperlipidemic drugs.

2.3. Cholesterol uptake assay

The CUC assay was performed according to our previously described methods[17]. Briefly, apolipoprotein B-depleted serum was prepared from thawed serum samples by precipitation of apolipoprotein B-containing lipoproteins with polyethylene glycol[21]. Boron dipyrromethene difluoride (BODIPY)–cholesterol (Avanti Polar Lipids, Alabaster, AL, USA) were dissolved in dimethyl sulfoxide (DMSO) at 0.5mM. One microliter of the apoB-depleted serum sample, which was adjusted to an apoA1 concentration of 5 μg/mL in PBS, was incubated with 100 μL of 5 μM BODIPY–cholesterol (diluted 100-fold) in PBS containing 2% BSA and 1.1% liposome stock solution (for reduction of nonspecific binding of BODIPY–cholesterol to microtubes and microplates) at 37°C for 2 h. Subsequently, the apoB-depleted serum mixture was transferred into the wells of a 96-well black-bottomed microplate coated with the anti-apoA1 antibody (clone 1C5) (Sanbio B.V., Uden, Netherlands) and the plate was incubated at 25°C for 1 h. After the wells were washed with PBS five times,

100 μL of 20 mM cyclodextrin (Sigma-Aldrich, St. Louis, MO, USA) in PBS was added to the wells to enhance the fluorescence signal derived from the BODIPY–cholesterol and the plate was incubated at 25°C for 1 h. The fluorescence intensity was measured at 535 nm with excitation at 485 nm on an Infinite 200 PRO microplate reader (Tecan, Männedorf, Switzerland), followed by measurement of apoA-I captured on the microplate by ELISA method. The fluorescence intensity was divided by apoA-I signals for capture-efficiency adjustment. To correct for inter-plate variations, standardization using a calibration curve generated by serial dilutions of recombinant apoA1 (Sigma-Aldrich) for each microplate. A quality control (QC) sample was prepared from pooled apoB-depleted serum. The intra-assay coefficient of variation (CV) was 4.6% (determined by measuring five individual samples 8 times in a single batch) and the inter-assay CV was 6.7% (determined by measuring five individual samples on 5 different days, in separate batches).

2.4. Cholesterol efflux assay

The CEC assay was performed using J774A.1 macrophages (JCRB9108, JCRB Cell Bank, Osaka, Japan) treated with cyclic adenosine monophosphate (cAMP) to upregulate ABCA1 levels based on previously described methods[12]. Briefly, cells were dispensed into a 48-well plate at 75,000 cells per well and cultured at 37°C and 5% CO_2 in DMEM containing 10% FBS. The next day, the cells were

incubated with 2 μCi/ml of [^{3}H] cholesterol, 0.3 mM 8-CPT-cAMP (Abcam, Cambridge, UK), 0.2% BSA, and 2 μg/mL Acyl-CoA cholesterol acyltransferase (ACAT) inhibitor (Sandoz 58-035, Sigma-Aldrich, St. Louis, MO, USA) in DMEM containing 1% FBS. Subsequently, [^{3}H] cholesterol labeled cells were incubated with 2.8% apoB-depleted serum for 4 h. The percent of radioactive cholesterol from the cells effluxed into the media were quantified using liquid scintillation counting. The CEC was calculated as the radioactivity of [^{3}H] cholesterol in the medium (the radioactivity of [^{3}H] in mediums containing 2.8% apoB-depleted serum – the radioactivity of [^{3}H] in serum-free mediums) divided by the radioactivity of [^{3}H] cholesterol in the medium plus the cell fraction. All samples were measured in duplicate. To correct for inter-assay variation, we included QC samples on each plate for normalization. The intra-assay CV was 7.3 % and the inter-assay CV was 9.0%.

2.5. OCT imaging

OCT was performed using a commercially available frequency-domain OCT imaging system (ILUMIEN; St. Jude Medical Inc., St Paul, MN, USA). Under this system, a 0.014-inch guide wire was inserted distally from the target lesion, and a 2.7-Fr OCT imaging catheter (C7 and C8 Dragonfly TM; St. Jude Medical) was advanced to the distal end of the lesion; subsequently, automated pullback was initiated while replacing blood in the lumen area with an iodine contrast

agent through a continuous power injector. The target lesion was scanned at 20 mm/s, and all images were digitally stored.

2.6. OCT analysis

Analysis of OCT images was performed using an Off-line Review Workstation, as previously described[22]. The morphology of the lesion was evaluated according to the 2012 intravascular optical coherence tomography document in Journal of the American College of Cardiology[23]. The lesion length was defined as the region around the site with the smallest lumen area, where the lumen area was <50% of the largest reference lumen area. A lipid-rich plaque was defined as a diffusely bordered, signal-poor region (lipid pools)[24]. The lipid arc was measured at 1-mm intervals throughout the length of each lesion and the values were averaged. The lipid length was also measured on the longitudinal view. The lipid index was defined as the mean lipid arc multiplied by the lipid length[22, 25]. Calcification was also recorded when an area contained a signal-poor or heterogeneous region with a sharply delineated border (defined as a calcified plaque). The fibrous cap was defined as a distinct layer of connective tissue covering the lipid-rich plaque[26]. Macrophage accumulation regions can be identified as signal-rich, distinct, or confluent punctate regions that exceed the intensity of backscattering[27-29]. To quantify the extent of macrophage accumulation, we determined the macrophage score

according the methods described by a previous report[27]. The angle of macrophage accumulation sites was measured at 1.0-mm intervals and divided into five grades: Grade 0, no macrophages; Grade 1, localized macrophage accumulation <30°; Grade 2, clustered accumulation, ≥30° and <90°; Grade 3, clustered accumulation, ≥90° but <270°; and Grade 4, clustered accumulation, ≥270°. The macrophage score was evaluated by the sum of the scores of grades 0 to 4 (Figure 1A). Four independent investigators (YN, KK, YN, HO), who were blinded to the angiographic and clinical findings, analyzed the OCT images using the Off-line Review Workstation. When discordance in terms of qualitative plaque morphology arose among observers, a consensus was reached with the assistance of a fifth investigator (TS).

2.7. Statistical analysis

All statistical analysis was performed using Stata 14.2 (StataCorp, College Station, TX, USA). Categorical variables are expressed as numbers and percentages. Continuous variables were expressed as mean ± standard deviation (SD), unless otherwise specified. The P-value for differences between two groups was determined by unpaired Student's t-test or the Mann-Whitney test according to the data distribution, with or without normality. The relationships between two numerical variables were investigated using a simple linear regression analysis. Multiple regression models were used to explore the

influence of independent variables on lipid index as the dependent variable. A two-sided P value of 0.05 or less was considered statistically significant.

3. Results

3.1. Baseline patient characteristics

We enrolled 135 patients in the present study based on the inclusion and exclusion criteria (Figure 1B). The baseline patient characteristics, medications, and laboratory data are shown in Table 1A. Approximately 80% of the patients were receiving statin therapy, and a mean LDL-C level of less than 100 mg/dL was achieved, which is the goal for secondary prevention of coronary artery disease (CAD) in Japan[19]. The minimum, maximum, and mean CUC values were 0.16, 0.60, and 0.38 ± 0.08 arbitrary units (A.U.), respectively. The distribution of CUC was normally distributed (P=0.83, skewness/kurtosis tests for normality) (Supplemental Figure 1A). The plaque characteristics are shown in Table 1B. Lipid-rich plaque lesions were identified in 125 (92.6%) patients. Although the sample size was too small to draw any definite conclusions, the comparisons of CUC or conventional lipid profiles between patients with and without lipid-rich plaques are shown in Supplemental Table 1. The relationships between the CUC and baseline patient characteristics are also shown in Supplemental Table 2.

3.2. Inverse association between CUC and lipid-rich plaque burden

We investigated the relationship between lipid-rich plaque burden and the CUC. The CUC was inversely associated with the mean lipid arc and lipid length (Figure 2A, B), and consequently, with the lipid index (Figure 2C). Because the CUC was measured at a constant level of apoA1, we calculated serum-CUC by multiplying the CUC by the apoA1 concentrations in each apoB-depleted serum sample. There was also a statistically significant negative relationship between serum-CUC and the lipid index (Supplemental Figure 2A). Conversely, although HDL-C and apoA1 showed significant relationships with serum-CUC (Supplemental Figure 2B, C), neither had any association with the lipid index (Figure 2D, E). In addition, neither LDL-C, TG, nor non-HDL-C showed any significant relationship with the lipid index (Figure 2F, G, H). Sex, smoking, comorbidities such as hypertension, diabetes and dyslipidemia, or prescribed medications (statin, ezetimibe, and eicosapentaenoic acid) were also not associated with lipid index (Table 2A). A multivariate linear regression analysis showed that impaired CUC was associated with the lipid index independently of age, weight, body mass index, LDL-C, HDL-C, TG, apoA1, HbA1c, or hs-CRP (Table 2B).

3.3. OCT-detected macrophage accumulation and impaired CUC

Next, we assessed the relationship between CUC, and other indicators of plaque morphology and composition. With regard to calcification, we could not identify any significant difference in the CUC in patients with (n=85) or without (n=50) calcified plaque (Figure 3A; 0.38 ± 0.08 vs. 0.38 ± 0.07 A.U., p = 0.83). Similarly, there was no relationship between CUC and fibrous cap thickness (Figure 3B). Conversely, CUC was inversely associated with the OCT-detected macrophage score (Figure 3C), while HDL-C and apoA1 showed no significant relationships with the macrophage score (Figure 3D, E).

We also examined the relationship between CEC and the lipid index or macrophage score. CEC showed a left-skewed distribution (P<0.05) (Supplemental Figure 1B) and a significant correlation ship with HDL-C (Supplemental Figure 3). In contrast, there was no relationship between CUC and CEC (Supplemental Figure 4A, B). CEC showed no significant relationship with lipid index and macrophage score (Figure 4A, B).

4. Discussion

In the present study, we demonstrated that CUC, a novel indicator of HDL functionality, was inversely associated with lipid-rich plaque burden. In contrast, HDL-C did not show a similar relationship, indicating the deterioration of HDL function is more strongly implicated in the

pathophysiology of atherosclerosis than the reduction of HDL levels. Moreover, we also found that CUC was inversely associated with the extent of OCT-detected macrophage accumulation.

Recent studies have demonstrated that compared to HDL cholesterol levels, the CEC of HDL is a better predictor of CVD[12-14]. In contrast to the CEC, the CUC assay is a cell-free system, as described in the introduction. We recently demonstrated that an impaired CUC indicated the presence of a residual cardiovascular risk in patients with optimal LDL-C control independently of traditional risk factors including HDL-C[17]. In this study, the CUC was inversely associated with the lipid index, while neither apo A-I concentration nor HDL-C showed any relationship with the lipid index, implying that the functionality of HDL is more important rather than the quantity of HDL for cardiovascular risk stratification.

Conversely, there was no relationship between CUC and fibrous cap thickness in the present study. In contrast, a prospective study has recently revealed a strong and independent association between fibrous cap thickening and improved CEC among patients with CAD who were receiving intensive statin therapy[30]. The discrepancy between the previous and present findings might be attributed to the difference in the mechanisms evaluated on CEC and CUC analysis. Meanwhile, the discrepant findings might also be due to a difference in study design. The present study is a cross-sectional survey, while the

abovementioned study enrolled 85 patients with stable CAD, and assessed changes in CEC and intracoronary imaging after 4 to 8 weeks of intensive lipid lowering-therapy using rosuvastatin. Therefore, at present, we cannot conclude on the relationship between CUC and fibrous cap thickness without additional longitudinal observations.

Previous studies have reported the utility of OCT to detect macrophages the in coronary plaque and quantitatively evaluate the extent of macrophage accumulation[29, 31]. In this study, we revealed there was an inverse relationship between CUC and the macrophage score assessed according to previously described methods[27]. The present findings might indicate that HDL with impaired CUC is accompanied by a decrease in its anti-inflammatory properties. On the other hand, a recent study could not find a significant association between the anti-oxidative capacity of HDL and the CEC[32]. However, the removal of cholesterol from the plasma membrane by HDL might modulate the pro-inflammatory properties of the macrophage. Lipid rafts are cholesterol-rich membrane micro-domains, and function as platforms for signal transduction. Previous studies have demonstrated that disorganization of lipid rafts on macrophages can lead to an attenuation of its inflammatory activation[33, 34]. For instance, methyl-β-cyclodextrin disrupts lipid rafts through the depletion of cholesterol, leading to the reduction of Toll-like Receptor 4 expression on the macrophage[35-38]. It has also been reported that

reducing cholesterol from caveolae in endothelial cells leads to a reversal of the inhibitory endothelial nitric-oxide synthase/caveolin-1 interaction and suppression of inflammatory activation[39]. Further studies are required to elucidate the precise mechanisms underlying the inverse relationship between CUC and macrophage accumulation in coronary plaques.

Although a previous study has reported that the CEC was inversely correlated noncalcified plaque burden measured by coronary computed tomographic angiography in patients with psoriasis[20], we could not find any significant relationship between the CEC and the lipid index in the present study. In the present study, the CEC could not be studied in all participants, although the enrollment for CEC measurement was not biased. Therefore, the results may possibly be due to the low statistical power arising from limited samples. On the other hand, differences in concept between CEC and CUC might cause the discrepancy in their associations with coronary plaque properties.

Cholesterol-enriched macrophages can release cholesterol to HDL through several pathways[40]. ABCA1 is an important player in HDL biogenesis. Conversely, the aqueous diffusion pathway contributes to the maturation of HDL. Because the ABCA1 transporter is assumed to be a major mediator of cholesterol export from cell macrophages, most recent studies have determined the CEC using J774 cells treated with cAMP to upregulate ABCA1 expression[12-14]. Since the CUC was determined using a cell free assay, the

CUC appears to reflect the contribution of HDL to cholesterol removal via the aqueous diffusion pathway. In this study, we did not identify a significant relationship between the CUC and CEC assessed using ABCA1-upregulated cells. However, we previously demonstrated that CUC correlated with CEC determined using J774 cells without cAMP treatment (non-ABCA1-dependent CEC)[17], which was also inversely associated with the presence of atherosclerotic CVD in patients with familial hypercholesterolemia. On the other hand, the cell-free assay system to measure CUC will allow a high-throughput characterization of HDL functionality. We are currently developing a completely automated system to measure CUC. Further trials are required to confirm the clinical usefulness of the CUC measurement as a complementary assay for CEC in the clinical setting.

There are several limitations to the present study. First, this study should be considered exploratory because the number of participants was small. In addition, since the present study was a cross-sectional survey, larger prospective randomized studies are required to elucidate whether an impaired CUC is implicated in the progression of coronary atherosclerosis. Second, it is possible that the medications used by patients might have influenced the CUC. In this study, the CUC appeared to be preserved in patients treated with ezetimibe (Supplemental Table 2A). However, the sample size was too small to reach a definitive conclusion regarding the relationship between the CUC and

the prescribed medications. A prospective interventional trial is warranted to address this concern. Third, BODIPY-cholesterol was applied for CUC measurement instead of radiolabeled cholesterol toward the clinical application. We previously confirmed a significant correlation between the use of radiolabeled cholesterol and BODIPY-cholesterol in the CUC, suggesting that that the bulky BODIPY group has little effect on the behavior of cholesterol[17]. However, BODIPY-labeling might influence the biding of cholesterol to HDL particles. Fourth, DMSO was used to dissolve BODIPY-cholesterol, and the addition of albumin and liposome was required for reduction of nonspecific binding of BODIPY–cholesterol to microtubes and microplates. These agents might also affect the biding of cholesterol to HDL particles.

5. Conclusion

We demonstrated that CUC is inversely related to lipid-rich plaque burden and the extent of macrophage accumulation. Findings from the present study suggest that the impaired CUC could be a novel risk for the prevention of CVD.

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CRediT authorship contribution statement

Toshihiko Oshita: Data curation, Investigation, Formal analysis, Writing - original draft. Ryuji Toh: Conceptualization, Project administration, Methodology, Funding acquisition, Writing – review & editing. Yuichiro Nagano: Data curation. Koji Kuroda: Data curation. Yoshinori Nagasawa: Data curation. Amane Harada: Conceptualization, Methodology, Data curation, Writing - review & editing. Katsuhiro Murakami: Conceptualization, Methodology, Data curation, Writing - review & editing. Maria Kiriyama: Methodology, Data curation. Keiko Yoshikawa: Methodology, Data curation. Keiko Miwa: Methodology, Data curation. Takuya Kubo: Methodology, Data curation. Takuya Iino: Methodology, Data curation. Manabu Nagao: Data curation. Yasuhiro Irino: Conceptualization, Writing - review & editing, Funding acquisition. Tetsuya Hara: Data curation. Masakazu Shinohara: Data curation. Hiromasa Otake: Data curation. Toshiro Shinke: Data curation. Katsuyuki Nakajima: Writing - review & editing. Tatsuro Ishida: Conceptualization, Funding acquisition. Kenichi Hirata: Supervision.

Declaration of Competing Interest

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Figure legends

Figure 1. Macrophage accumulation and enrollment of study population

(A) Semiquantitative macrophage accumulation scoring by optical coherence tomography (OCT) and (B) enrollment of study population. In (A), each image represents the accumulation of macrophages in the cross section of the OCT image from Grades 0 to 4.

Figure 2. Associations between cholesterol uptake capacity or conventional lipid profiles and coronary lipid-rich plaque burden.

Correlation between CUC in patients with lipid-rich plaque and (A) mean lipid arc (°), (B) lipid length (mm), and (C) lipid index (n=125). Relationship between lipid index and (D) HDL-C, (E) apoA1, (F) LDL-C, (G) TG and (H) non HDL-C.

CUC, cholesterol uptake capacity; HDL-C, high-density lipoprotein cholesterol; apoA1, apolipoprotein A1; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; A.U., arbitrary units.

Figure 3. Relationship between cholesterol uptake capacity and plaque features.

(A) The average of CUC was compared in patients with (n=85) and without (n=50) calcified plaque lesions (data presented as mean ± SD). Relationship

between the CUC and (B) fibrous cap thickness and (C) OCT-detected macrophage score (n=135). CUC, cholesterol uptake capacity; A.U., arbitrary units.

Figure 4. Associations between cholesterol efflux capacity, lipid index, and macrophage score.

Correlation between CEC in patients with lipid-rich plaques and (A) the lipid index (n=84) or (B) the OCT-detected macrophage score (n=84).
CUC, cholesterol uptake capacity; CEC, cholesterol efflux capacity; A.U., arbitrary units; OCT, optical coherence tomography.

Supplemental Figure 1. Distribution of Cholesterol uptake capacity and cholesterol efflux capacity.

(A) Distribution of CUC (n=135). (B) Distribution of CEC (n=84). CUC, cholesterol uptake capacity. CEC, cholesterol efflux capacity.

Supplemental Figure 2. Relationship between cholesterol uptake capacity per serum and lipid index, HDL-C or apoA1.

Relationship between serum-CUC and (A) the lipid index, (B) HDL-C and (C) apoA1 levels. CUC, cholesterol uptake capacity. HDL-C, high-density lipoprotein cholesterol. apoA1, apolipoprotein A1. A.U., arbitrary units.

Supplemental Figure 3. Relationship between cholesterol efflux capacity and HDL-C.

CEC, cholesterol efflux capacity. HDL-C, high-density lipoprotein cholesterol.

Supplemental Figure 4. Relationship between cholesterol efflux capacity and cholesterol uptake capacity or cholesterol uptake capacity.

Relationship between CEC and (A) CUC or (B) serum-CUC. CUC, cholesterol uptake capacity. CEC, cholesterol efflux capacity. A.U., arbitrary units.

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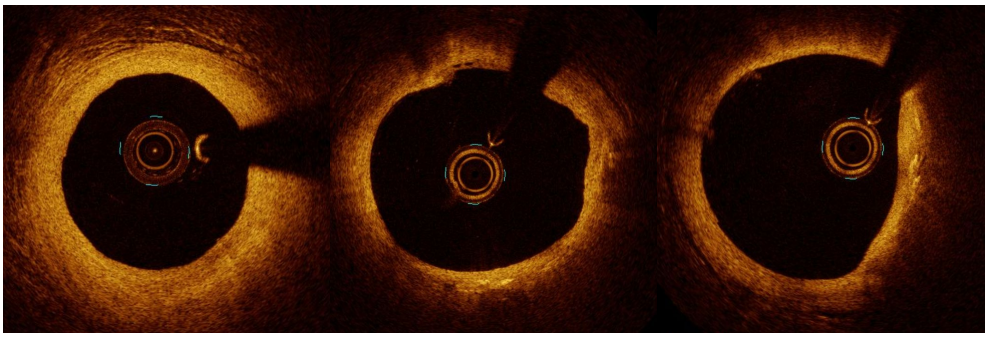
Figure 1

(A)



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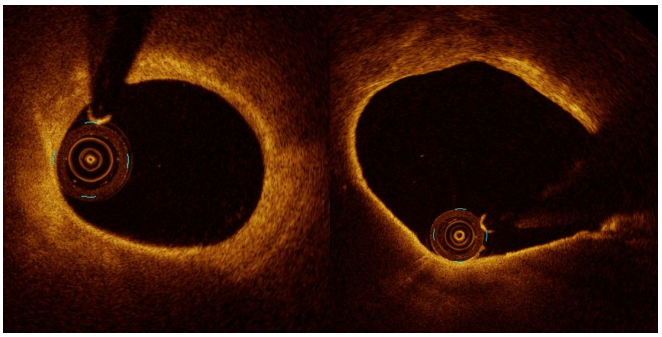


Grade 3 Grade 4

(B)

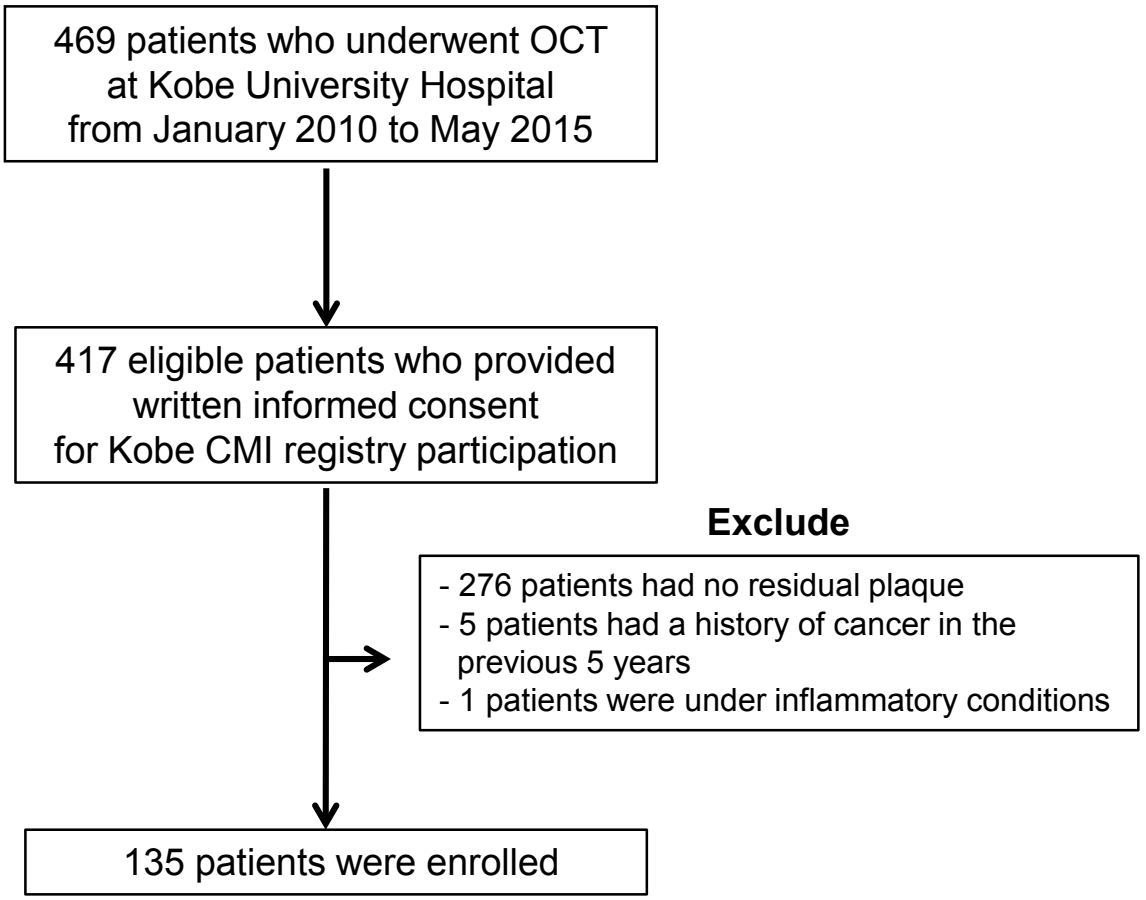


Figure 2

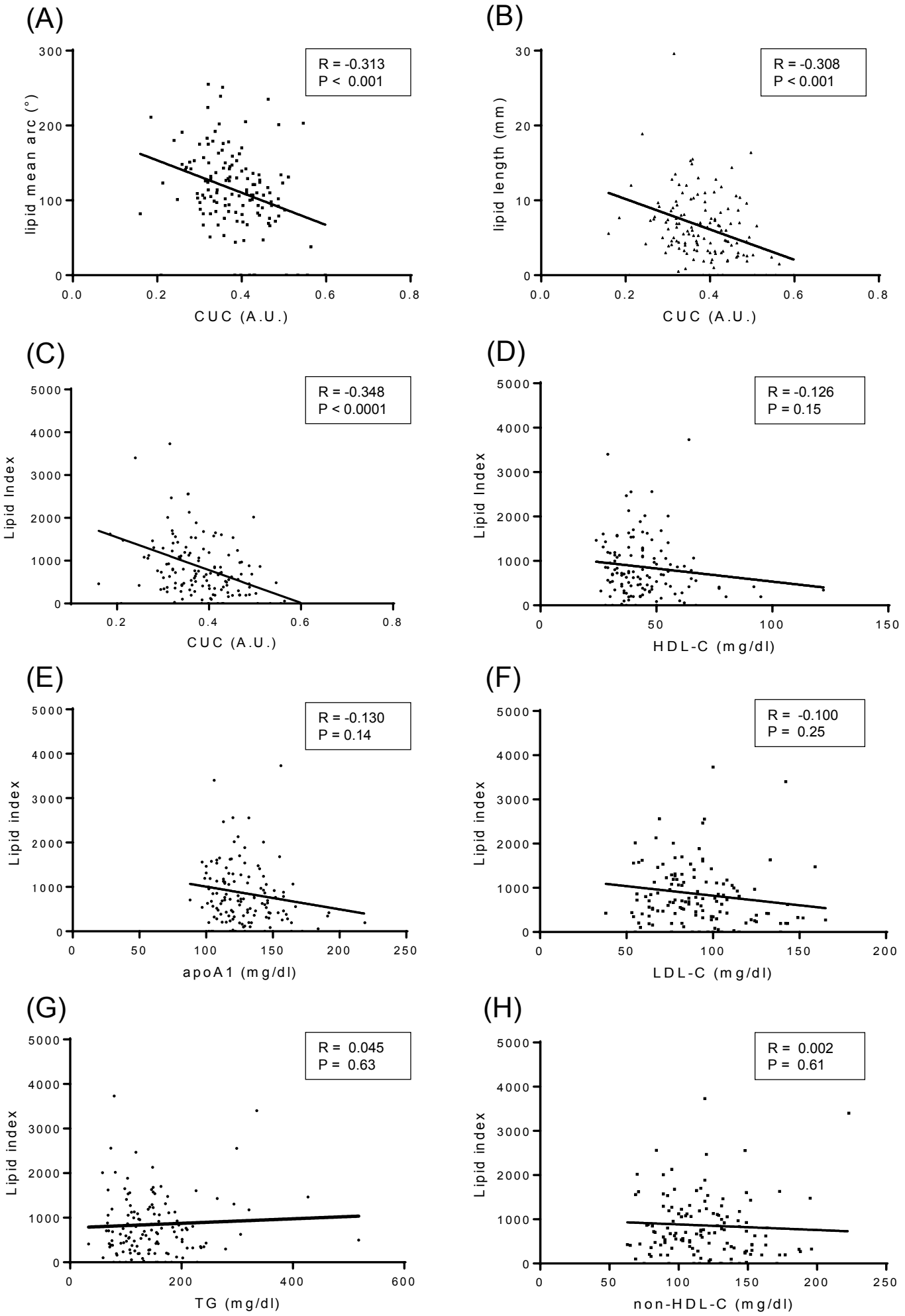

(A)
R = -0.313
P < 0.001
lipid mean arc (°)
CUC (A.U.)
(B)
R = -0.308
P < 0.001
lipid length (mm)
CUC (A.U.)
(C)
R = -0.348
P < 0.0001
Lipid Index
CUC (A.U.)
(D)
R = -0.126
P = 0.15
Lipid Index
HDL-C (mg/dl)
(E)
R = -0.130
P = 0.14
Lipid index
apoA1 (mg/dl)
(F)
R = -0.100
P = 0.25
Lipid index
LDL-C (mg/dl)
(G)
R = 0.045
P = 0.63
Lipid index
TG (mg/dl)
(H)
R = 0.002
P = 0.61
Lipid index
non-HDL-C (mg/dl)

Figure 3

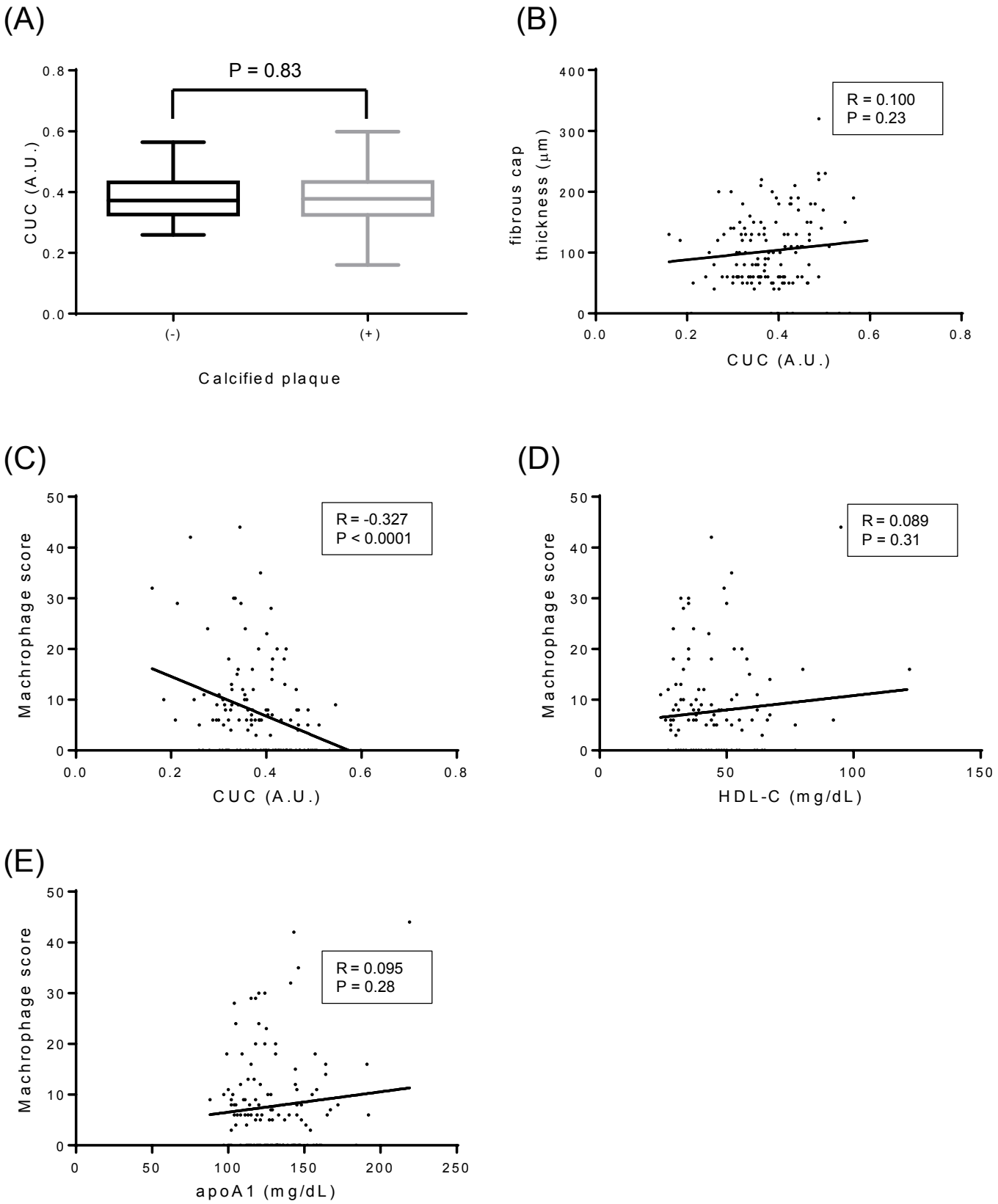
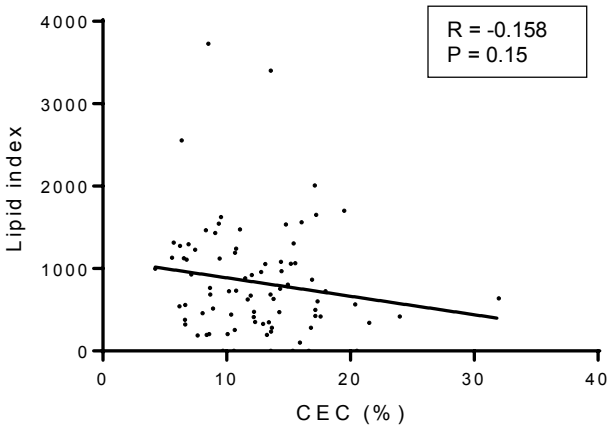

(A)
P = 0.83
CUC (A.U.)
0.0
0.2
0.4
0.6
0.8
(-)
(+)
Calcified plaque
(B)
fibrous cap thickness (μm)
0
100
200
300
400
R = 0.100
P = 0.23
0.0
0.2
0.4
0.6
0.8
CUC (A.U.)
(C)
Machrophage score
0
10
20
30
40
50
R = -0.327
P < 0.0001
0.0
0.2
0.4
0.6
0.8
CUC (A.U.)
(D)
Machrophage score
0
10
20
30
40
50
R = 0.089
P = 0.31
0
50
100
150
HDL-C (mg/dL)
(E)
Machrophage score
0
10
20
30
40
50
R = 0.095
P = 0.28
0
50
100
150
200
250
apoA1 (mg/dL)

Figure 4

(A)


R = -0.158
P = 0.15
Lipid index
4000
3000
2000
1000
0
0
10
20
30
40
CEC (%)

(B)

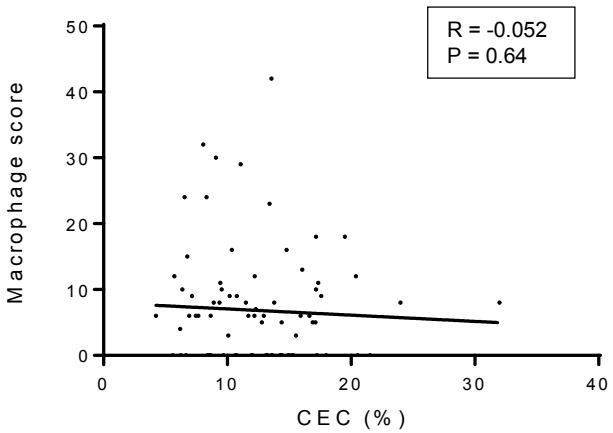

R = -0.052
P = 0.64
Macrophage score
50
40
30
20
10
0
0
10
20
30
40
CEC (%)

Table 1. Patient characteristics and plaque characteristics

(A). Patient characteristics

Variables	n=135
Male (%)	105 (77.8)
Age	68.9±9.8
Weight (kg)	65.3±11.9
BMI	24.9±3.5
Coronary risk factors	
Hypertention, n (%)	114 (84.4)
Dyslipidemia, n (%)	116 (85.6)
Diabetes Mellitus, n (%)	63 (46.7)
Smoking, n (%)	93 (70.0)
Medication	
Statin, n (%)	104 (77.0)
Ezetimibe, n (%)	8 (5.9)
EPA, n (%)	13 (9.6)
Fibrate, n (%)	0 (0)
Laboratory data	
CUC (A.U.)	0.38±0.08
Total cholesterol (mg/dl)	164.6±33.4
LDL cholesterol (mg/dl)	91.1±25.3
HDL cholesterol (mg/dl)	44.6±14.8
non-HDL cholesterol (mg/dl)	118.7±31.1
TG (mg/dL)	148.0±71.2
apoA1 (mg/dL)	128.6±22.1
HbA1c (%)	6.4±1.0
hs-CRP (mg/dl)	0.14 (0.09-0.19)

[a]. Values are presented as mean ± SD, geometric mean (95% confidence interval) or absolute numbers (%). BMI, body mass index; EPA, purified eicosapentaenoic acid ethyl ester; DES, drug-eluting stent; BMS, bare-metal stent; CUC, cholesterol uptake capacity; LDL, low-density lipoprotein; HDL,

high-density lipoprotein; TG, triglycerides; apoA1, apolipoprotein A1; HbA1c, hemoglobin A1c; hs-CRP, high sensitivity C-reactive protein.

(B). Plaque characteristics evaluated by OCT

Variables	n=135
Lesion length (mm)	11.5 (10.2-12.9)
Lipid-rich plaque, n (%)	125 (92.6)
Lipid length (mm)	6.5 (5.6-7.4)
Lipid max arc (°)	167.4 (153.4-181.4)
Lipid mean arc (°)	114.4 (105.2-123.6)
Lipid index	853.3 (706.5-1000.0)
Fibrous cap thickness (μm)	111.0 (110.1-112.1)
Calcificated plaque, n (%)	85 (63.4)
Macrophage score	7.5 (5.9-9.1)

[a]. Values are presented as geometric mean (95% confidence interval) or absolute numbers (%). OCT, optical coherence tomography.

Table 2. Relationship between patient characteristics and lipid index

(A). categorical variables

		n	Lipid index	*p*-value
Sex	M	105	855.4±934.1	0.34
	F	30	845.6±550.8	
Hypertension	+	114	879.2±880.3	0.18
	−	21	712.4±758.0	
Diabetes mellitus	+	63	906.2±770.8	0.27
	−	72	806.9±937.3	
Dyslipidemia	+	116	861.7±877.9	0.93
	−	19	904.1±847.2	
History of smoking	+	93	785.9±705.5	0.12
	−	42	1036.4±1148.2	
Statin	+	104	852.1±881.6	0.85
	−	31	857.3±806.1	
Ezetimibe	+	8	712.2±597.5	0.71
	−	127	862.1±876.8	
EPA	+	13	1045.8±724.5	0.17
	−	122	832.7±875.3	

[a]. Values are presented as mean ± SD. P value for two groups was determined by the Mann-Whitney test for lipid index. CUC, cholesterol uptake capacity; M, male: F, female; EPA, purified eicosapentaenoic acid ethyl ester

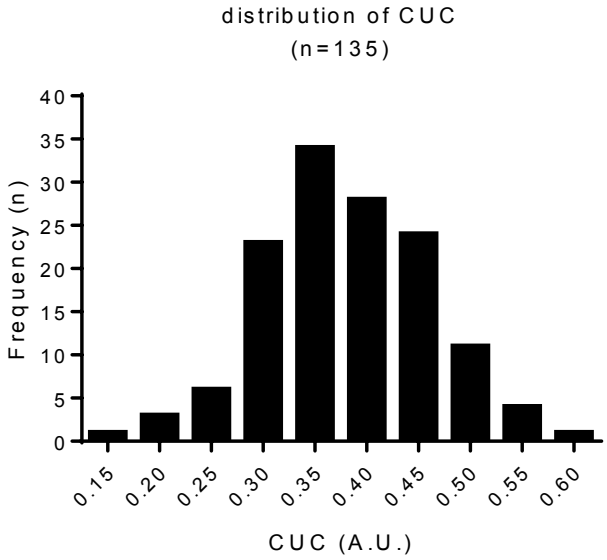
(B). numerical variables

	Univariate		Multivariate	
	R^2	*p*-value	Beta (standardized coefficients)	*p*-value
Age	0.01	0.25	2.66	0.79
Weight (kg)	0.002	0.58	-1.38	0.91
BMI	0.003	0.54	-10.68	0.79
CUC (A.U.)	0.121	<0.0001	-4038.89	<0.0001
LDL cholesterol (mg/dl)	0.010	0.25	-4.72	0.15
HDL cholesterol (mg/dl)	0.016	0.15	-4.17	0.74
TG (mg/dl)	0.002	0.63	0.02	0.99
apoA1 (mg/dl)	0.017	0.14	-1.12	0.89
HbA1c (%)	0.002	0.67	39.25	0.63
hs-CRP (mg/dl)	0.005	0.44	-494.23	0.14

[a]. Univariate and multivariate regression analysises demonstrating factors showing correlation with lipid index. CUC, cholesterol uptake capacity; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; apoA1, apolipoprotein A1; HbA1c, hemoglobin A1c; hs-CRP, high sensitivity C-reactive protein.

Supplemental Figure 1

(A)



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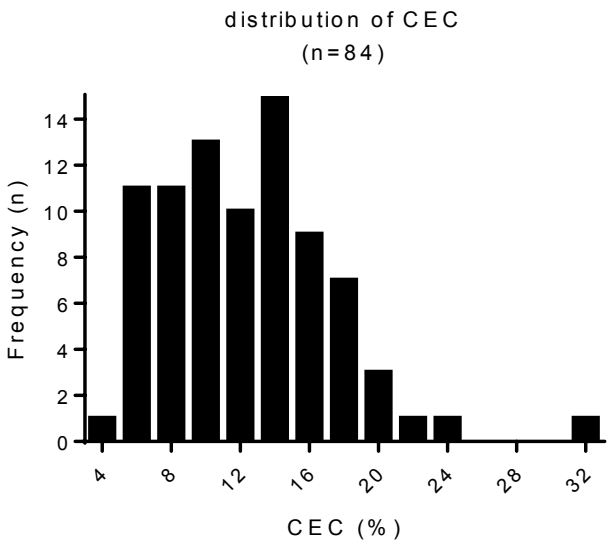
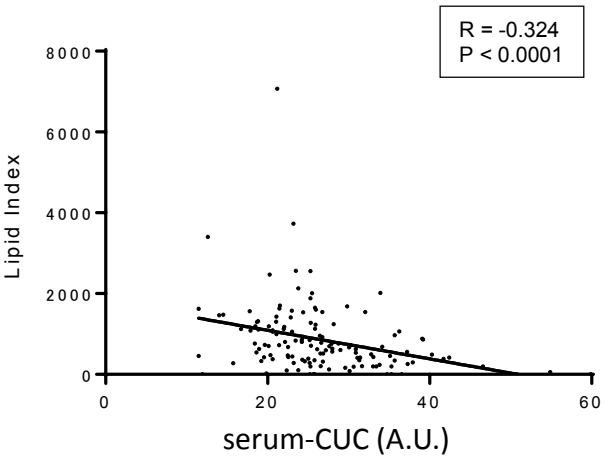
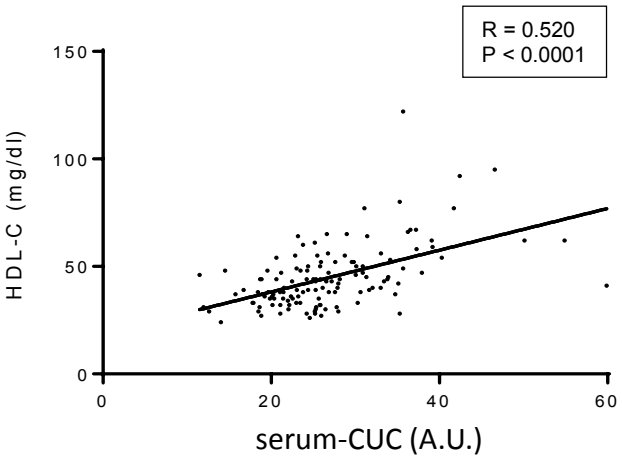


Supplemental Figure 2

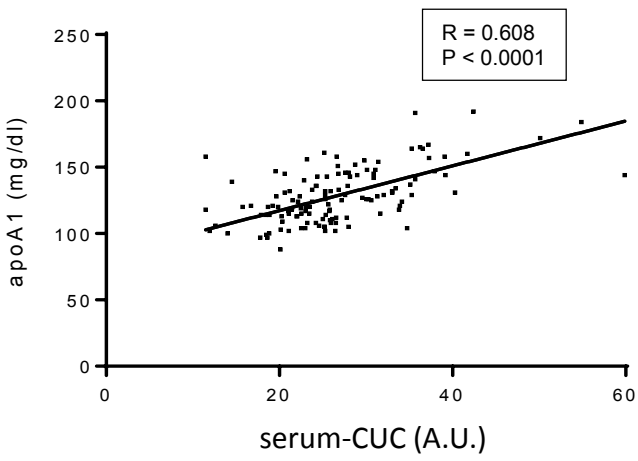
(A)



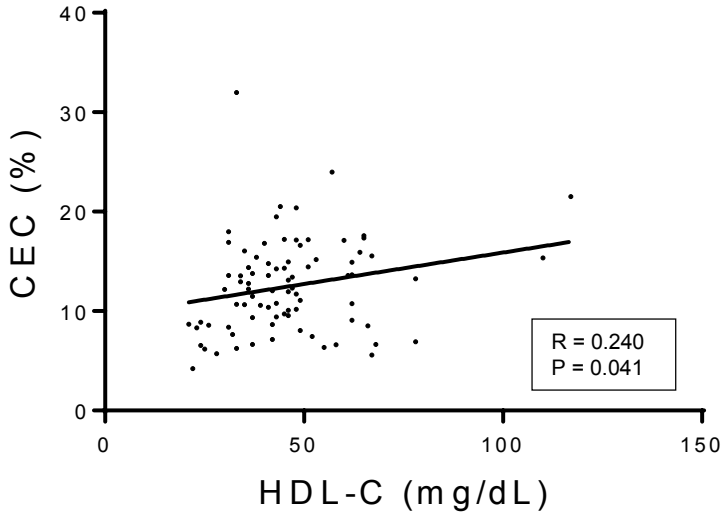
(B)



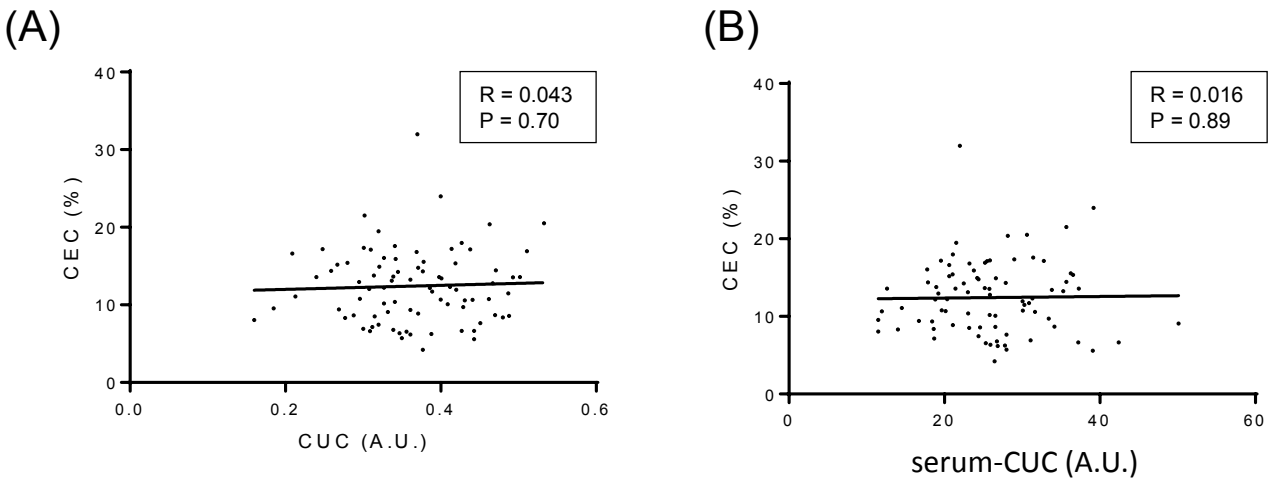
(C)



Supplemental Figure 3



Supplemental Figure 4



Supplemental table 1.

	Lipid-rich plaque (+)	Lipid-rich plaque (−)	*p*-value
	n=125	n=10	
CUC (A.U.)	0.38±0.07	0.45±0.11	<0.01
Total cholesterol (mg/dl)	163.8±33.1	173.8±37.1	0.36
LDL cholesterol (mg/dl)	90.7±25.5	96.3±23.6	0.52
HDL cholesterol (mg/dl)	44.8±14.9	42.1±14.5	0.60
non-HDL cholesterol (mg/dl)	118.4±31.3	123.1±28.5	0.66
TG (mg/dl)	148.7±72.9	139.1±47.8	0.68

[a]. Values are presented as mean ± SD. P value for two groups was determined by unpaired Student's t-test. CUC, cholesterol uptake capacity; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides.

Supplemental table 2

(A). categorical variables

		n	CUC (A.U.)	*p*-value
Gender	M	105	0.38±0.08	0.95
	F	30	0.38±0.08	
Hypertension	+	114	0.38±0.08	0.90
	−	21	0.38±0.10	
Diabetes mellitus	+	63	0.39±0.08	0.32
	−	72	0.38±0.07	
Dyslipidemia	+	116	0.38±0.09	0.59
	−	19	0.39±0.07	
History of smoking	+	93	0.38±0.08	0.95
	−	42	0.38±0.09	
Statin	+	104	0.38±0.08	0.17
	−	31	0.40±0.07	
Ezetimibe	+	8	0.45±0.05	<0.01
	−	127	0.38±0.08	
EPA	+	13	0.38±0.07	0.88
	−	122	0.38±0.08	

[a]. Values are presented as mean ± SD. P value for two groups was determined by unpaired Student's t-test for CUC. CUC, cholesterol uptake capacity; M, male: F, female; EPA, purified eicosapentaenoic acid ethyl ester.

(B). numerical variables

	CUC			
	R^2	*p*-value	95% CI lower	95% CI Upper
Age	0.0012	0.69	-0.0016	0.0011
Weight (kg)	0.0014	0.67	-0.0009	0.0014
BMI	0.0082	0.30	-0.0018	0.0058
Total cholesterol (mg/dl)	0.0080	0.31	-0.0006	0.0002
LDL cholesterol (mg/dl)	0.0062	0.37	-0.0008	0.0003
HDL cholesterol (mg/dl)	0.0005	0.80	-0.0008	0.001
non-HDL cholesterol (mg/dl)	0.0154	0.16	-0.0008	0.0001
TG (mg/dL)	0.0193	0.11	-0.00034	0.00004
apoA1 (mg/dL)	0.0034	0.51	-0.0004	0.0008
HbA1c (%)	0.0054	0.42	-0.0083	0.0201
hs-CRP (mg/dl)	0.0104	0.24	-0.0763	0.0193

[a]. Univariate regression analysis demonstrating factors showing correlation with CUC. BMI, body mass index; CUC, cholesterol uptake capacity; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; apoA1, apolipoprotein A1; HbA1c, hemoglobin A1c; hs-CRP, high sensitivity C-reactive protein.