High-density lipoprotein function is associated with atherosclerotic burden and cardiovascular outcomes in type 2 diabetes

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ABSTRACT

Background and aims: Measures of HDL function are emerging tools for assessing cardiovascular disease (CVD) event risk. HDL-apoA-I exchange (HAE) reflects HDL capacity for reverse cholesterol transport.
Methods: HAE was measured in 93 participants with type 2 diabetes (T2D) and at least one additional CVD risk factor in the Asker and Bærum Cardiovascular Diabetes study. At baseline and after seven years, the atherosclerotic burden was assessed by invasive coronary angiography. Major CVD events were registered throughout the study.
Results: Linear regression analysis demonstrated a significant inverse association between HAE and atherosclerotic burden. Cox proportional hazard regression analysis showed a significant association between HAE and a composite of major CVD events when controlling for waist-hip ratio, HR = 0.89, 95% CI = 0.80–1.00 and p=0.040.
Conclusions: Despite the relatively small size of the study population and the limited number of CVD events, these findings suggest that HAE provides valuable information in determining CVD risk.

1. Introduction

There is a well established inverse relationship between high-density lipoprotein cholesterol (HDL-C) levels and coronary heart disease events and mortality [1–3]. Nevertheless, almost 40% of men with coronary heart disease have HDL-C levels in the normal range [4]. Furthermore, merely raising plasma HDL-C does not necessarily reduce risk for cardiovascular events, as evidenced by recent drug trials of niacin and cholesteryl ester transfer protein (CETP) inhibitors that significantly elevated HDL-C levels but failed to reduce the risk of CVD events [5–7]. This suggests that circulating HDL-C levels do not directly reflect the atheroprotective properties of HDL. As a result, assays of HDL function have been proposed as more relevant and reliable measurements than HDL-C

levels in determining CVD risk [8].

HDL has both anti-inflammatory and anti-oxidative properties, but its primary means of atheroprotection has been attributed to its ability to mediate reverse cholesterol transport (RCT) [9]. Cholesterol efflux capacity (CEC) is the most widely used assay for quantifying HDL's role in RCT. CEC has been found to be inversely associated with CVD events and improves CVD risk prediction models when included [10,11].

HDL carries 1–4 molecules of apolipoprotein A-I (apoA-I), its primary protein component [12]. Lipid-poor apoA-I is the preferred substrate for the ATP-binding cassette transporter A1 (ABCA1) and is essential for *de novo* HDL biogenesis in the intimal layer of the arterial wall [13–15]. Because apoA-I is synthesized primarily in the liver, lipid-poor apoA-I arrives in the intima associated with HDL and must exchange off the HDL particle. The exchange/dissociation of apoA-I off HDL is a rate-limiting step of RCT [14]. ApoA-I undergoes significant conformational change as it exchanges between HDL-bound and lipid-free states, and this conformational change can be reliably quantified by electron paramagnetic resonance

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(EPR) [16,17]. Lipid-free apoA-I readily exchanges with apoA-I on HDL, and measuring the conformational change of exogenous spin-labeled apoA-I added to blood plasma is the basis of the HDL-apoA-I exchange (HAE) assay. HAE correlates strongly with CEC ($r = 0.69$, $p < 0.001$) in normolipidemic subjects [18].

Cardiovascular disease is the leading cause of death in patients with type 2 diabetes (T2D), and the presence of both CVD and diabetes decreases lifespan in these patients by several years compared with the general population [19]. Even when LDL-C is well managed, there remains a significant residual risk of CVD, particularly in T2D [20]. The randomized-controlled Asker and Bærum Cardiovascular Diabetes (ABCD) study showed that 2 years of structured multi-intervention significantly reduced the esti-mated 10-year absolute risk for coronary heart disease [21], driven by between-group differences in LDL-C, triglycerides, HbA1c, and systolic blood pressure, but with no long-term benefit on CVD events or death [22]. In the present analysis we measure HDL function at the 7-year follow-up of these patients and examine associations with study outcomes.

We hypothesize that loss of HDL function is a major contributor to residual risk of CVD in T2D patients, exhibited by an inverse association of HAE with atherosclerotic burden and CVD events.

2. Patients and methods

2.1. Study population

The design, intervention and results from the ABCD-study (clinicaltrials.gov NCT00133718) have been described previously [21]. Briefly, 120 patients with T2D and ≥ 1 additional CVD risk factor were randomized to two years of either standard care by their general practitioner or structured multi-intervention care at a hospital outpatient clinic comprising an initial 6-month lifestyle program followed by intensified treatment to reach guideline tar-gets for glycemic control, blood pressure and lipid profile [21]. Clinical, laboratory and cardiac assessments, including invasive coronary angiography were performed at baseline. Seven years after baseline, 93 patients were re-examined, 85 of which had completed the intervention. Major cardiovascular events were registered throughout the duration of the study and until May 29, 2012.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Norwegian Regional Committee for Medical and Health Research Ethics. All participants provided their written informed consent.

2.2. Clinical and laboratory methods

Venous blood samples were drawn in the morning after an overnight fast and routine laboratory analyses performed by the local laboratory. All intra- and inter-assay coefficients of variations were <10%. Urinary albumin and creatinine concentrations were determined in timed overnight urine samples.

2.3. HAE assay

The HAE assay was performed on 93 samples from the 7-year follow-up. Freshly thawed plasma was apoB depleted by precipi-tation with polyethylene glycol 6000. The apoB-depleted plasma was combined with 3 mg/ml nitroxide spin-labeled apoA-I probe as previously described [17]. Samples were incubated for 15 min at 37 °C and scanned at 37 °C. The peak amplitude of the nitroxide signal from HAE probe in the sample (3462–3470 Gauss) was compared to the peak amplitude of a proprietary internal standard (3507–3515 Gauss). The internal standard is contained within the

eScan spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) cavity and does not contact the sample. The %HAE activity was calculated by comparison with a standard curve determined for each production lot of apoA-I probe. The final result is a measure of the relative efficiency of HDL-apoA-I exchange. All samples were read in duplicate and averaged. The intra-assay coefficient of vari-ation was 4%.

2.4. Invasive coronary angiography

At baseline and at the 7-year follow-up, invasive coronary angiography was performed according to standard procedures [23]. The angiograms were analyzed visually by an experienced radiol-ogist with the support of a quantitative coronary analysis program (Sectra Cardiology Package 1.0, Sectra Imtec, Sweden). They were divided into 16 segments and graded from 0 to 4. Grade 0: <25% stenosis, 1: 25–50% stenosis, 2: 50–75% stenosis, 3: >75% stenosis and 4: Occlusion. The atherosclerotic burden was described by the Severity score, the average grade of the diseased segments graded ≥ 1, and the Extent score, the number of coronary segments graded ≥ 1 adjusted to 16 coronary segments [24].

2.5. Outcome

The CVD outcome was characterized as time to CVD death, non-CVD death, stroke, acute myocardial infarction, hospitalization for unstable angina or congestive heart failure, amputation or revas-cularization procedures including percutaneous transluminal an-gioplasty. Revascularization procedures occurring in relation to the study angiography were excluded. All events underwent adjudi-cation by two of the authors (K.I.B., L.G.) blinded to treatment allocation.

2.6. Statistical analysis

Clinical and metabolic data are presented as either proportions, means with their standard deviations (SD) or medians with 25th and 75th percentiles. Student's t-test was applied to compare means between groups with normally distributed data, and Mann-Whitney U test to compare medians in case of non-normal distri-butions. Linear regression analysis was performed to identify as-sociations between HAE as the exposure variable and Severity score and Extent score as outcome variables. Potential confounding var-iables were required to be associated significantly with both the exposure and outcome variable with $p < 0.05$. Qualified con-founders were included in a multivariate regression analysis with a backward elimination procedure to determine a final model. All major CVD end points were combined in a composite variable. The association between HAE and the end point variable was deter-mined by Cox proportional hazard regression analysis. Potential confounders were identified as described for linear regression analysis. The proportional hazard assumptions were checked by Schoenfeld tests and found to be satisfied. The primary outcome was the composite endpoint. Follow-up data were censored with closing date May 29, 2012.

Statistical significance was assumed for $p < 0.05$. IBM SPSS Statistics for Macintosh, version 24 (Armonk, NY: IBM Corp) was applied for all statistical analyses.

3. Results

At inclusion, there were 25.6% and 19.0% females in the struc-tured care and standard care groups respectively. The clinical and metabolic characteristics of the participants were similar at base-line (Table 1).

Table 1
Clinical and metabolic characteristics.

	Baseline			2-year follow-up			7-year follow-up		
	Structured care N = 43	Standard care N = 43	p-value	Structured care N = 43	Standard care N = 42	p-value	Structured care N = 43	Standard care N = 42	p-value
Age (years)	58.5 (7.1)	57.3 (10.0)	0.526	60.6 (7.0)	59.1 (10.0)	0.429	65.7 (7.1)	64.4 (9.9)	0.491
Diabetes duration (years)[a]	4.0 (2.0, 9.0)	3.5 (1.0, 12.0)	0.849	6.1 (4.6, 11.2)	5.5 (3.7, 14.5)	0.854	11.4 (9.7, 16.3)	10.6 (8.8, 19.6)	0.930
BMI (kg/m^2)	30.6 (4.8)	29.0 (5.0)	0.140	30.8 (5.2)	29.4 (4.9)	0.196	30.9 (4.9)	28.4 (4.3)	**0.015**
Waist-hip ratio	0.99 (0.08)	0.97 (0.07)	0.193	1.00 (0.08)	0.99 (0.08)	0.196	1.00 (0.07)	0.97 (0.07)	**0.047**
Systolic blood pressure (mmHg)	141 (18)	141 (17)	0.995	136 (21)	140 (21)	0.316	138 (16)	138 (17)	0.908
Diastolic blood pressure (mmHg)	83 (10)	82 (9)	0.794	78 (9)	80 (8)	0.453	79 (10)	82 (10)	0.341
HbA1c (%)	7.5 (1.6)	7.8 (1.7)	0.467	6.8 (0.8)	8.0 (1.6)	**<0.001**	7.1 (1.0)	7.5 (1.1)	0.056
Total cholesterol (mmol/l)	4.9 (1.0)	4.9 (0.9)	0.831	4.2 (0.7)	4.6 (0.9)	**0.021**	4.3 (1.1)	4.2 (0.9)	0.623
HDL cholesterol (mmol/l) [a]for 7-year follow-up	1.30 (0.39)	1.31 (0.42)	0.934	1.36 (0.37)	1.32 (0.43)	0.680	1.30 (1.0, 1.7)	1.20 (1.0, 1.4)	0.534
LDL cholesterol (mmol/l)	2.9 (0.9)	2.8 (0.8)	0.857	2.2 (0.6)	2.6 (0.9)	**0.032**	2.4 (0.9)	2.2 (0.8)	0.487
Triglycerides (mmol/l)*	1.6 (1.0, 2.5)	1.3 (1.1, 2.3)	0.499	1.3 (0.9, 1.7)	1.7 (1.0, 2.1)	0.060	1.2 (1.0, 1.8)	1.2 (0.9, 1.9)	0.944

*Mean (standard deviation).
Bold indicates significant p-values.
[a] Median (25th and 75th percentile).

Two years of intervention in the structured care group resulted in significant changes in CVD risk factors, with significantly lower HbA1c, total cholesterol and LDL cholesterol than in the control group, as reported previously [21]. These differences were no longer present at the 7-year follow-up, with the possible exception of HbA1c, which was near significantly lower in the structured care group (mean between group difference = −0.44, 95% CI = −0.90 - 0.01 and p = 0.056). BMI and waist-hip ratio were similar in both groups at baseline and after the intervention, but after seven years, both were significantly higher in the structured care group.

At the 7-year follow-up, 85% of the participants were treated with statins and 91% with anti-diabetic medication, with no significant differences between the groups. The median number of anti-diabetic drugs was two in both groups. The median number of drugs to treat hypertension was two in the structured care group and one in the standard care group, p = 0.012. There was no significant difference between the two groups in median (25th, 75th percentile) albumin-creatinine ratio, 0.9 (0.4, 4.0) in the structured vs. 0.8 (0.5, 2.7) in the standard care group.

The Severity score and the Extent score were assessed in 72 participants at baseline and 69 at the 7-year follow up. Their distributions are shown in Fig. 1.

The atherosclerotic burden in all participants assessed by the Severity score and the Extent score at baseline and after seven years of follow-up.

Throughout the present study, from January 17. 2002 to May 29. 2012, twelve major CVD events occurred (Table 2). Seven in the structured care group, four in the standard care group and one in a participant that did not complete the intervention.

There was no significant difference between the groups (p = 0.354). The mean follow-up time was 8.7 (SD = 2.0) years.

At the 7-year follow-up, %HAE was 46.8 (SD = 8.9) in the structured care group compared with 46.6 (SD = 7.4) in the standard care group (p = 0.914). A linear regression analysis showed a significant inverse association between HAE and Severity score (B = −0.028, p = 0.043 and R^2 = 0.431), controlling for Severity score at baseline and age. There was no significant association between HAE and Extent score. A Cox regression model, adjusted for waist-hip ratio, showed a significant association between %HAE and CVD outcome (HR = 0.89, 95% CI = 0.80−1.00 and p = 0.040).

Similarly, serum levels of HDL-C at the 7-year follow-up were significantly and inversely associated with Severity Score (B = −0.520, p = 0.039 and R^2 = 0.433), controlling for Severity score at baseline and age. There was no significant association between HDL-C and Extent score. For comparison, serum levels of LDL- C at the 7-year follow-up were significantly associated with Extent score (B = 0.413, p = 0.014 and R^2 = 0.548), controlling for Extent score at baseline. There was no significant association between LDL-C and Severity score. In Cox regression models, HDL-C was significantly associated with CVD outcome (HR = 0.48, 95% CI = 0.004−0.54 and p = 0.014) adjusted for HbA1c, total cholesterol and diabetes duration, while there was no association between LDL and CVD outcome.

4. Discussion

This study reports the observation that HDL function, as measured by HAE, is significantly inversely associated with atherosclerotic burden, as assessed by coronary angiography. HAE was also significantly inversely associated with CVD end-points. These relationships emerge despite the relatively low number of patients and few registered CVD events in this study.

Inverse relationships between HDL function and both atherosclerotic burden and CVD end points have previously been demonstrated for CEC [10,11]. Confirmation in large clinical studies
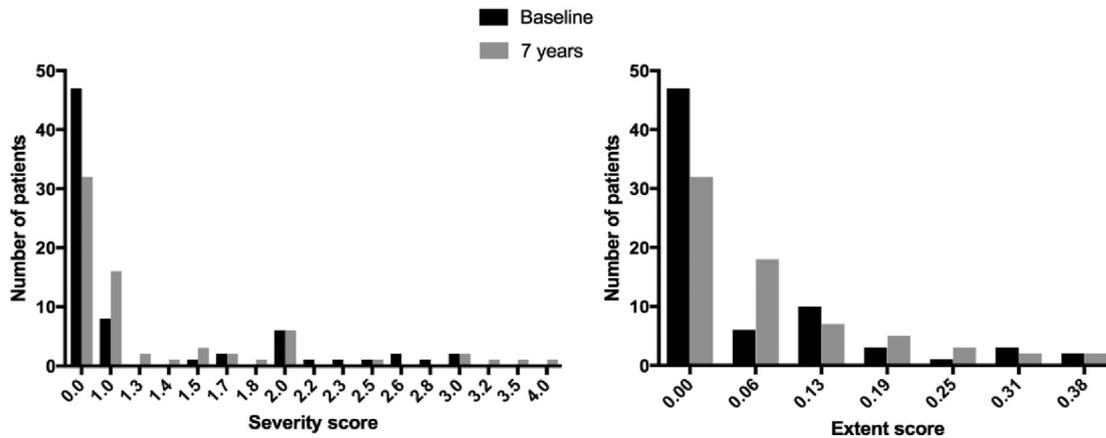
Fig. 1. The distribution of Severity score and Extent score.

Table 2
Major cardiovascular events.

CV death	0
Non-CV death	2
Stroke	1
Acute myocardial infarction	4 (3)
Unstable angina	2 (1)
Congestive heart failure	0
Revascularization	3 (2)

N (N before the 7-year follow-up).

is certainly needed, but the present study indicates for the first time that HAE may be similarly discriminating. This is important for two reasons. First, measures of HDL function, both CEC and HAE, are emerging as valuable tools for the assessment of residual risk after LDL-C reduction. Second, the simplicity and precision of the HAE assay makes HDL function analysis more convenient and accessible, facilitating its clinical application.

Clinical intervention in the ABCD study significantly modified the CVD risk factor profile at two years, but these differences were no longer present at seven years. Similarly, there was no difference in HAE levels between the groups. Despite a substantial reduction in LDL cholesterol after two years, the intervention did not reduce mortality [22]. This suggests the presence of a residual risk in T2D potentially involving reduced HDL function.

There are several limitations to this study, particularly the relatively low number of participants and clinical events. Serial HAE measurements from baseline and after the clinical intervention were unfortunately not available to construct prediction models. Previous work with HAE has shown an increased discriminatory ability of HAE-apoA-I ratio compared with HAE alone [18,25], but apoA-I was not measured in this study. Half of the registered major CVD events occurred before HAE measurement at the 7-year follow-up. This precludes any assessment of the predictive value of HAE. This timing might also attenuate the relationship between HAE and atherosclerotic burden, as most patients after a CVD event will be provided intensified treatment and potentially improved HDL function.

In conclusion, this is the first study to demonstrate the inverse relationship between HAE, as a measure of HDL function, and atherosclerotic burden and major CVD events in T2D.

Conflicts of interest

APO and OEJ are presently employed by Boehringer Ingelheim

Norway.

MNO is a founder of and owns a significant stake in Seer Biologics, Inc. Potential benefit in no way influenced the thoroughness, stringency, interpretation and presentation of this manuscript's content.

KIB has received grants to his institution for lectures and consulting from Astra Zeneca, Novo Nordisk, Sanofi, Lilly, Boehringer Ingelheim, Roche and Merck Sharp & Dohme.

Author contributions

MH performed the HAE assay, conducted statistical analyses and wrote the manuscript. MSB and MNO analyzed data and provided technical support. MNO provided reagent and equipment resources. OEJ, KIB and LG designed the study. KE performed and analyzed the coronary angiographies. APO and OEJ collected the data. All authors reviewed and edited the manuscript.

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