HDL particle size is increased and HDL-cholesterol efflux is enhanced in type 1 diabetes: a cross-sectional study

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Abstract

Aims/hypothesis The prevalence of atherosclerosis is increased in type 1 diabetes despite normal-to-high HDL-cholesterol levels. The cholesterol efflux capacity (CEC) of HDL is a better predictor of cardiovascular events than static HDL-cholesterol. This cross-sectional study addressed the hypothesis that impaired HDL function contributes to enhanced CVD risk within type 1 diabetes.

Methods We compared HDL particle size and concentration (by NMR), total CEC, ATP-binding cassette subfamily A, member 1 (ABCA1)-dependent CEC and ABCA1-independent CEC (by determining [³H]cholesterol efflux from J774-macrophages to ApoB-depleted serum), and carotid intima-media thickness (CMT) in 100 individuals with type 1 diabetes (37.6 ± 1.2 years; BMI 26.9 ± 0.5 kg/m²) and 100 non-diabetic participants (37.7 ± 1.1 years; 27.1 ± 0.5 kg/m²).

Results Compared with non-diabetic participants, total HDL particle concentration was lower (mean $\pm$ SD 31.01 ± 8.66 vs 34.33 ± 8.04 μ mol/l [mean difference (MD) -3.32 μ mol/l]) in participants with type 1 diabetes. However, large HDL particle concentration was greater (9.36 ± 3.98 vs 6.99 ± 4.05 μ mol/l [MD $+2.37$ μ mol/l]), resulting in increased mean HDL particle size (9.82 ± 0.57 vs 9.44 ± 0.56 nm [MD $+0.38$ nm]) ($p < 0.05$ for all). Total CEC ($14.57 \pm 2.47\%$ CEC/4 h vs $12.26 \pm 3.81\%$ CEC/4 h [MD $+2.31\%$ CEC/4 h]) was greater in participants with type 1 diabetes relative to non-diabetic participants. Increased HDL particle size was independently associated with increased total CEC; however, following adjustment for this in multivariable analysis, CEC remained greater in participants with type 1 diabetes. Both components of CEC, ABCA1-dependent ($6.10 \pm 2.41\%$ CEC/4 h vs $5.22 \pm 2.57\%$ CEC/4 h [MD $+0.88\%$ CEC/4 h]) and ABCA1-independent ($8.47 \pm 1.79\%$ CEC/4 h vs $7.05 \pm 1.76\%$ CEC/4 h [MD $+1.42\%$ CEC/4 h]) CEC, were greater in type 1 diabetes but the increase in ABCA1-dependent CEC was less marked and not statistically significant in multivariable analysis. CMT was increased in participants with type 1 diabetes but in multivariable analysis it was only associated negatively with age and BMI.

Conclusions/interpretation HDL particle size but not HDL-cholesterol level is independently associated with enhanced total CEC. HDL particle size is greater in individuals with type 1 diabetes but even after adjusting for this, total and ABCA1-independent CEC are enhanced in type 1 diabetes. Further studies are needed to understand the mechanisms underlying these effects, and whether they help attenuate progression of atherosclerosis in this high-risk group.

Keywords HDL particle size · HDL-cholesterol efflux capacity · Type 1 diabetes

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Research in context

What is already known about this subject?

- Increased atherosclerotic disease in type 1 diabetes is not fully explained by traditional cardiovascular risk factors
- HDL-cholesterol efflux capacity (CEC) is a better predictor of cardiovascular outcome than HDL-cholesterol levels
- HDL-cholesterol levels are increased in type 1 diabetes but there is some evidence that this increase is not associated with improved HDL function

What is the key question?

- Does HDL CEC differ in individuals with type 1 diabetes compared with similar people without diabetes, and are any observed changes related to HDL particle size?

What are the new findings?

- HDL particle size, but not HDL-cholesterol level, is independently associated with enhanced total CEC. HDL particle size is greater in type 1 diabetes but, even after adjusting for this, total and particularly ATP-binding cassette subfamily A, member 1 (ABCA1)-independent CEC are enhanced in type 1 diabetes

How might this impact on clinical practice in the foreseeable future?

- Measurements of HDL particle size and function might be more useful than HDL-cholesterol levels in evaluating cardiovascular risk in type 1 diabetes

Abbreviations

ABCA1	ATP-binding cassette subfamily A, member 1
ABCG1	ATP-binding cassette subfamily G, member 1
CEC	Cholesterol efflux capacity
CETP	Cholesteryl ester transfer protein
CIMT	Carotid intima-media thickness
JUPITER	Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin
MACE	Major atherosclerotic cardiac events
RCT	Reverse cholesterol transport
SAA	Serum amyloid A

Introduction

Despite many improvements in the management of type 1 diabetes, life expectancy for affected individuals remains approximately 12 years less than for the non-diabetic population [1, 2]. This is mostly attributable to an elevated risk of CVD that is not explained by conventional risk factors. In contrast to individuals with type 2 diabetes, lipid profiles in type 1 diabetes are normal or even apparently better than in the general population, with higher HDL-cholesterol, lower LDL-cholesterol and lower triacylglycerol levels [3]. However, these relatively simple lipid measurements potentially mask more subtle lipoprotein abnormalities, including disorders of lipoprotein function, that might contribute to residual atherosclerosis risk in type 1 diabetes.

The importance of a more complete understanding of HDL biology is emphasised by the observation that although HDL-cholesterol levels are inversely associated with CVD [4], increasing HDL-cholesterol levels using cholesteryl ester transfer protein (CETP) inhibitors was found not to result in clinical benefits in high-risk individuals [5, 6]. HDL particles exert numerous atheroprotective functions including antioxidant, anti-inflammatory and cholesterol efflux promoting actions [7]. HDL particles play a critical role in the reverse cholesterol transport (RCT) pathway by promoting efflux of cholesterol from peripheral cells, including lipid-laden foam cells within atherosclerotic lesions, and delivering this acquired lipid back to the liver [8]. Non-esterified cholesterol can be shunted from the liver to the gallbladder via the ABCG5/8 heterodimer [9] or converted into bile acids and shuttled to the gallbladder via ABCB11 [10]. Macrophages efflux cholesterol to smaller HDL particles via the ATP-binding cassette subfamily A, member 1 (ABCA1) transporter while larger particles interact with ATP-binding cassette subfamily G, member 1 (ABCG1) and scavenger receptor class B member 1 (SR-B1) transporters [11]. Evidence that the potentially atheroprotective effects of HDL are clinically relevant is provided by a significant body of literature demonstrating cross-sectional and longitudinal associations between the cholesterol efflux capacity (CEC) of HDL and evidence of both atherosclerosis and cardiovascular events [12–14].

There are theoretical reasons why HDL function in individuals with type 1 diabetes might differ from that in non-diabetic individuals [3]. These include greater particle size,

reduced HDL triacylglycerol content, reduced cholesterol esters in small HDL3 particles, increased susceptibility to oxidation, increases in phospholipid transfer protein (PLTP), CETP and lecithin-cholesterol acyltransferase (LCAT), and changes in the HDL proteome (summarised in detail in [15]). Data from the Pittsburgh Epidemiology of Diabetes Complications Study demonstrate that the usual inverse association between HDL-cholesterol and coronary artery disease risk, although retained in men, is altered in women with type 1 diabetes; the women show little increased protection with concentrations above the range of 50–60 mg/dl (1.3–1.55 mmol/l), suggesting that at least in some individuals, HDL is dysfunctional [16]. Few studies to date have addressed HDL function in type 1 diabetes and none relate aspects of HDL function to early evidence of atherosclerosis.

The aims of this study were to compare total CEC, ABCA1-dependent CEC and ABCA1-independent CEC between individuals with type 1 diabetes and matched control participants to determine whether any observed between-group differences were associated with differences in HDL particle size. We also aimed to determine whether HDL particle size and/or CEC were associated with carotid intima-media thickness (CIMT), a marker of early atherosclerosis.

Methods

Study design and participants This was a cross-sectional study designed to compare HDL particle concentration, HDL particle size and CEC between 100 participants with type 1 diabetes and 100 non-diabetic participants matched for age ± 5 years, sex and BMI ± 3 kg/m².

Participants with type 1 diabetes were contacted either by phone or at the time of their scheduled clinic visit. Inclusion criteria were as follows: type 1 diabetes; duration of diabetes of at least 1 year; age 18–69 years; BMI 18–40 kg/m²; and HbA_{1c} ≤ 97 mmol/mol ($\leq 11\%$). Exclusion criteria were as follows: non-white ethnicity; pregnant or lactating; and recent illness or any chronic illness likely to influence results. Each participant with type 1 diabetes was pair-matched with a healthy volunteer on no medications, recruited from the general population. Inclusion criteria were as follows: non-diabetic; age 18–69 years; and BMI 18–40 kg/m². Exclusion criteria were as for participants with type 1 diabetes. Twenty-seven participants with type 1 diabetes and no non-diabetic participants were on statin treatment.

Ethical approval All participants gave written informed consent to the study, which was approved by the Research Ethics Committee of the Adelaide and Meath Hospital and St James' Hospital (Dublin, Ireland).

Study protocol and anthropometric data All participants were studied after they had fasted for 12 h, having avoided excessive exercise and alcohol for the previous 24 h. Height was measured with a Harpenden stadiometer. Weight was measured in a hospital gown. Data relating to previous medical history, medication use and smoking status were recorded. Alcohol consumption was recorded in all participants with type 1 diabetes and in 80 of 100 non-diabetic participants.

Laboratory methods Plasma levels of total cholesterol, triacylglycerols and HDL-cholesterol were measured by an enzymatic calorimetric method on the Roche P Module (Roche, Mannheim, Germany). Additional samples were centrifuged at 1500 g for 15 min at 4°C, and plasma and serum were stored at –80°C until the end of the study.

CEC J774 murine macrophages, derived from BALB/c mice (European Collection of Authenticated Cell Cultures), were seeded (7×10^5 cells/ml) onto 96-well plates for 24 h, and cultured at 37°C. Cells were subsequently labelled with [³H]cholesterol (1 μ Ci/ml) for 24 h before being equilibrated for 18 h in DMEM containing 0.2% BSA with or without cAMP (0.3 mmol/l) to drive ABCA1 expression. J774 macrophages do not express ABCA1 basally, and stimulation with cAMP specifically drives ABCA1 protein expression. ApoB-containing lipoproteins were removed from serum by polyethylene glycol precipitation, leaving an HDL-enriched supernatant fraction. Ex vivo efflux from labelled macrophages to 2.8% HDL-enriched supernatant fraction in minimal essential media (MEM) was measured over 4 h. Percentage efflux was determined by liquid scintillation counting. The difference in efflux to HDL from cells stimulated in the presence and absence of cAMP was taken to represent ABCA1-dependent CEC while ABCA1-independent CEC was calculated from unstimulated cells.

NMR HDL particle concentration and size were measured in serum samples using 400 MHz NMR spectroscopy (LipoScience, Raleigh, NC, USA). Each lipoprotein subclass emits a distinctive lipid methyl group NMR signal and the amplitudes of the measured signals are directly proportional to the number of subclass particles, which in turn provides the estimated mol/l concentration for the three HDL particle subclasses (large 8.9–13 nm, medium 8.3–8.8 nm, small 7.3–8.2 nm) [17]. Weighted-average HDL particle sizes in nanometres were calculated from the subclass levels, and the diameters were assigned to each subclass.

CIMT CIMT was measured as previously described [18] in all participants using a single ultrasonographic machine (Sonosite Micromaxx Ultrasound System, Sonosite, Japan). Measurements were taken from each common carotid artery,

within 1 cm of the carotid bulb, with three views of each artery measured, and averaged to form a composite measurement.

Statistical analysis Results in tables are presented as mean $\pm$ SD unless otherwise specified. Data in graphs are presented as mean $\pm$ SD. Data were tested for normality using the Shapiro–Wilk test, as well as inspected visually using histograms. Statistical significance for categorical variables was calculated using the χ^2 test. For continuous variables, the paired Student's *t* test was used to assess statistical significance. Correlations were evaluated using the Pearson correlation or Spearman test as appropriate. Generalised estimating equations with robust Huber–White (sandwich) SE estimation was used to examine the joint effect of all predictors while accounting for the non-independence of matched pairs. IBM SPSS Statistics (Version 24.0. Armonk, NY) and GraphPad Prism (Version 8.4.3. San Diego, CA) software were used for data analysis. Statistical significance is presented as $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results

Clinical and biochemical characteristics of 100 participants with type 1 diabetes and 100 healthy non-diabetic participants are shown in Table 1. There were no significant differences in total cholesterol or triacylglycerol levels between type 1 diabetes and control groups. LDL-cholesterol was lower, HDL-cholesterol and HbA_{1c} were higher, and CIMT was greater in participants with type 1 diabetes than in non-

diabetic participants (Table 1, Fig. 1). Mean alcohol consumption was greater in the non-diabetic participants, although the difference did not reach statistical significance.

HDL particle size and subclass distribution Total HDL particle concentration was significantly lower in participants with type 1 diabetes (Fig. 1a) than in non-diabetic participants despite HDL-cholesterol concentrations being higher (Table 1). This reduction was specifically evident in women (mean $\pm$ SD for type 1 diabetes vs non-diabetic group: 28.42 ± 9.64 vs 34.91 ± 9.22 $\mu\text{mol/l}$ [mean difference -6.49 $\mu\text{mol/l}$]) with no effect evident in men (34.79 ± 5.07 vs 33.77 ± 5.99 $\mu\text{mol/l}$ [mean difference 1.02 $\mu\text{mol/l}$]) (Fig. 1a). Mean HDL particle size was greater in participants with type 1 diabetes than in non-diabetic participants, with similar effects evident in men and women (Fig. 1b). Consistent with an increase in mean HDL particle size, the concentration of large HDL particles was increased in participants with type 1 diabetes, with similar patterns evident in men (7.09 ± 3.34 vs 4.56 ± 2.15 $\mu\text{mol/l}$ [mean difference $+2.53$ $\mu\text{mol/l}$]) and women (10.92 ± 3.64 vs 8.68 ± 4.25 $\mu\text{mol/l}$ [mean difference $+2.24$ $\mu\text{mol/l}$]) (Fig. 1c). By contrast, the concentration of medium and small HDL particles was significantly reduced in participants with type 1 diabetes relative to non-diabetic participants (Fig. 1d,e). These effects were particularly pronounced in women: mean $\pm$ SD HDL particle concentration for type 1 diabetes vs non-diabetic groups was 10.08 ± 6.91 vs 13.64 ± 7.77 $\mu\text{mol/l}$ (mean difference -3.56 $\mu\text{mol/l}$, $p > 0.05$) for medium HDL particles and 7.44 ± 5.77 vs 12.53 ± 7.03 $\mu\text{mol/l}$ (mean difference -5.09 $\mu\text{mol/l}$, $p < 0.001$) for small HDL particles. A non-

Table 1 Baseline characteristics of type 1 diabetes and non-diabetic cohorts

Characteristic	T1DM (<i>n</i> =100)	Non-diabetic (<i>n</i> =100)	Mean difference	95% CI	<i>p</i> value
Sex, <i>n</i> male/ <i>n</i> female	40/60	40/60	NA	NA	1.0
Age, years	37.6 ± 11.9	37.7 ± 11.0	-0.1	-0.6, 0.5	0.8
BMI, kg/m^2	26.9 ± 4.9	27.1 ± 5.0	-0.2	-0.4, 0.1	0.13
Smoker, %	20	18	N/A	N/A	0.68
Alcohol intake, units/week	2 (0–8)	6 (0–12)	N/A	-4.9, -0.1	0.04*
Systolic BP, mmHg	128 ± 16	127 ± 15	1	-3, 5	0.58
Diastolic BP, mmHg	77 ± 9	82 ± 11	-6	-8, -3	<0.0001***
CIMT, mm	0.590 ± 0.084	0.556 ± 0.071	0.034	0.003, 0.047	0.03*
HbA _{1c} , mmol/mol	70 ± 16	35 ± 3	35	32, 39	<0.0001***
HbA _{1c} , %	8.5 ± 1.5	5.3 ± 0.3	3.2	2.9, 3.6	<0.0001***
Total cholesterol concentration, mmol/l	4.5 ± 0.9	4.7 ± 0.9	-0.2	-0.4, 0.1	0.32
Triacylglycerol concentration, mmol/l	0.9 (0.7–1.3)	0.9 (0.7–1.3)	N/A	-0.1, 0.3	0.37
LDL-cholesterol concentration, mmol/l	2.3 ± 0.9	2.7 ± 0.8	-0.4	-0.6, -0.1	0.004**
HDL-cholesterol concentration, mmol/l	1.6 ± 0.4	1.5 ± 0.4	0.1	0.1, 0.3	0.002**

Data are displayed as mean $\pm$ SD, median (IQR) or %

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ for type 1 diabetes vs non-diabetic cohort

T1DM, type 1 diabetes

Fig. 1 NMR analysis of HDL particles in individuals with type 1 diabetes and non-diabetic participants (control), showing results for total cohort combined ($n=100$) and separately for men ($n=40$) and women ($n=60$). Bar charts show total HDL particle concentration (a), mean HDL particle diameter (b), large HDL particle concentration (c), medium HDL particle concentration (d) and small HDL particle concentration (e). Data are presented as mean $\pm$ SD. * $p<0.05$, ** $p<0.01$ *** $p<0.001$ for type 1 diabetes vs non-diabetic control. HDL-P, HDL particle; T1D, type 1 diabetes

significant reduction in medium HDL particle concentration (11.1 ± 4.8 vs 13.11 ± 6.12 $\mu\text{mol/l}$ [mean difference -2.01 $\mu\text{mol/l}$]), and a non-significant increase in small HDL particle concentration (16.6 ± 4.95 vs 16.1 ± 5.66 $\mu\text{mol/l}$ [mean difference $+0.5$ $\mu\text{mol/l}$]) was evident in men with type 1 diabetes relative to non-diabetic participants (Fig. 1d,e).

HDL CEC Total CEC was significantly greater in participants with type 1 diabetes than in non-diabetic participants (Fig. 2a, Table 2). A significant increase in both ABCA1-dependent and ABCA1-independent efflux was evident in participants with type 1 diabetes relative to non-diabetic participants (Fig. 2b,c). Interestingly, total CEC and ABCA1-independent CEC were increased to a greater extent relative to non-diabetic participants in men ($14.48 \pm 2.51\%$ CEC/4 h vs $11.02 \pm 4.03\%$ CEC/4 h [mean difference $+3.46\%$ CEC/4 h] and $9.16 \pm 1.65\%$ CEC/4 h vs $6.43 \pm 2.06\%$ CEC/4 h [mean

difference $+2.73\%$ CEC/4 h], respectively) than in women ($14.63 \pm 2.46\%$ CEC/4 h vs $13.16 \pm 3.46\%$ CEC/4 h [mean difference $+1.47\%$ CEC/4 h] and $8.01 \pm 1.74\%$ CEC/4 h vs $7.44 \pm 1.42\%$ CEC/4 h [mean difference $+0.57\%$ CEC/4 h], respectively) (Fig. 2a,c). In parallel, CIMT burden was a significantly greater in men with type 1 diabetes relative to non-diabetic men (0.618 ± 0.09 vs 0.566 ± 0.07 mm [mean difference $+0.052$ mm], $p < 0.01$) with no significant difference evident in women (Fig. 2d).

Effect of alcohol consumption To ensure the observed differences were not due to the finding that mean alcohol consumption was (non-significantly) greater in the non-diabetic individuals, comparisons were repeated in participants with an alcohol intake ≤ 10 units/week (79/100 participants with type 1 diabetes; 59/80 non-diabetic participants). There was no difference in findings (mean $\pm$ SD values for participants with

Table 2 CEC and lipoprotein particle analysis in type 1 diabetes and non-diabetic cohorts

Variable	T1DM (<i>n</i> =100)	Non-diabetic (<i>n</i> =100)	Mean difference	95% CI
Total CEC, %CEC/4 h	14.57 ± 2.47	12.26 ± 3.81	+2.31	1.36, 3.35 ***
ABCA1-dependent CEC, %CEC/4 h	6.10 ± 2.41	5.22 ± 2.57	+0.88	0.19, 1.66 *
ABCA1-independent CEC, %CEC/4 h	8.47 ± 1.79	7.05 ± 1.76	+1.42	0.90, 1.97 ***
Mean HDL-P size, nm	9.82 ± 0.57	9.44 ± 0.56	+0.38	0.24, 0.49 ***
Total HDL-P concentration, µmol/l	31.01 ± 8.66	34.33 ± 8.04	−3.32	−5.57, −0.29 *
Large HDL-P concentration, µmol/l	9.36 ± 3.98	6.99 ± 4.05	2.37	1.48, 3.41 ***
Medium HDL-P concentration, µmol/l	10.49 ± 6.13	13.45 ± 7.02	−2.96	−5.01, −0.48 *
Small HDL-P concentration, µmol/l	11.16 ± 7.06	13.90 ± 6.7	−2.74	−4.63, −0.62 *

Data are displayed as mean±SD

p*<0.05 and **p*<0.001 for type 1 diabetes vs non-diabetic cohort

HDL-P, HDL particle; T1DM, type 1 diabetes

type 1 diabetes vs non-diabetic participants: HDL particle size 9.84 ± 0.59 vs 9.43 ± 0.55 nm, *p* < 0.0005; total CEC $14.52 \pm 2.49\%$ CEC/4 h vs $12.32 \pm 4.01\%$ CEC/4 h *p* < 0.0005; ABCA1-dependent CEC $6.19 \pm 2.47\%$ CEC/4 h vs $5.24 \pm 2.70\%$ CEC/4 h, *p* < 0.05; ABCA1-independent CEC $8.32 \pm 1.84\%$ CEC/4 h vs $7.07 \pm 1.83\%$ CEC/4 h, *p* < 0.0005).

Univariable and multivariable associations In univariable analysis, total CEC was positively associated with type 1 diabetes status (greater in type 1 diabetes vs no diabetes), total cholesterol concentration and HDL particle size, and negatively associated with age (Table 3). In multivariable analysis, total CEC was positively associated with type 1 diabetes

status, total cholesterol concentration, HDL particle size and statin treatment (greater with vs without statin treatment), and negatively associated with age (Table 3).

In univariable analysis, ABCA1-dependent CEC was positively associated with total cholesterol and triacylglycerol concentration (Table 4). In multivariable analysis, ABCA1-dependent CEC was positively associated with total cholesterol concentration and negatively associated with age (Table 4).

In both univariable and multivariable analysis, ABCA1-independent CEC was positively associated with type 1 diabetes status, HDL particle concentration and statin treatment (Table 5).

Fig. 2 HDL CEC and CIMT measurements in participants with type 1 diabetes and non-diabetic participants (control), showing results for total cohort combined (*n*=100) and separately for men (*n*=40) and women (*n*=60). Bar charts show total CEC (a), ABCA1-dependent CEC (b), ABCA1-independent CEC (c) and CIMT measurements (d). Data are presented as mean ± SD. Statistical significance is presented as **p*<0.05, ***p*<0.01 and ****p*<0.001 for type 1 diabetes vs non-diabetic control. T1D, type 1 diabetes

Table 3 Univariable and multivariable association of clinical and biochemical variables with HDL CEC

Variable	Univariable model		Multivariable model	
	β coefficient	95% CI	β coefficient	95% CI
Age (years)	−0.061	−0.116, −0.007	−0.061	−0.114, −0.008
Sex (1 male, 2 female)	0.540	−0.696, 1.776	0.624	−1.882, 0.634
BMI (kg/m ²)	0.006	−0.094, 0.106	0.002	−0.082, 0.085
Type 1 diabetes (1 no, 2 yes)	1.507	0.318, 2.695	1.465	0.265, 2.666
Total cholesterol (mmol/l)	0.885	0.195, 1.576	0.870	0.195, 1.545
Triacylglycerols (mmol/l)	0.586	−0.249, 1.421	0.629	−0.351, 1.608
HDL-cholesterol (mmol/l)	0.804	−1.581, 3.189	0.600	−2.206, 3.406
HDL-P concentration (μ mol/l)	0.055	−0.033, 0.144	0.050	−0.039, 0.138
HDL-P size (nm)	2.426	0.442, 4.409	2.173	0.183, 4.163
Large HDL-P concentration (μ mol/l)	−0.189	−0.477, 0.098	−0.142	−0.420, 0.136
Statin treatment (1 no, 2 yes)	1.988	−0.012, 3.988	2.093	0.625, 3.561

β coefficient was derived from linear regression and generalised estimating equation

HDL-P, HDL particle

In univariable analysis, CIMT was positively associated with ABCA1-dependent CEC and ABCA1-independent CEC and negatively associated with age and BMI (data not shown). In multivariable analysis, CIMT was negatively associated with age and BMI.

Discussion

We have demonstrated reduced total HDL particle concentration in type 1 diabetes, but greater large HDL particle concentration, resulting in greater mean HDL particle size. Increased HDL particle size was independently associated with

increased total CEC; however, even following adjustment for increased HDL-cholesterol and HDL particle size in multivariable analysis, CEC remained greater in participants with type 1 diabetes compared with closely matched participants without diabetes. Both components of CEC, ABCA1-dependent and ABCA1-independent CEC, were greater in type 1 diabetes but the increase in ABCA1-dependent CEC was less marked and not statistically significant in multivariable analysis.

This is the first study in type 1 diabetes to determine ABCA1-dependent and ABCA1-independent contributions to CEC, and the first to address associations between CEC, HDL particle size and HDL particle concentration, and

Table 4 Univariable and multivariable association of clinical and biochemical variables with ABCA1-dependent CEC in participants with type 1 diabetes and participants without diabetes

Variable	Univariable model		Multivariable model	
	β coefficient	95% CI	β coefficient	95% CI
Age (years)	−0.040	−0.081, 0.001	−0.041	−0.077, −0.004
Sex (1 male, 2 female)	0.731	−0.201, 1.664	0.827	−1.745, 0.092
BMI (kg/m ²)	−0.011	−0.086, 0.65	−0.017	−0.086, 0.053
Type 1 diabetes (1 no, 2 yes)	0.073	−0.824, 0.969	0.078	−0.965, 0.810
Total cholesterol concentration (mmol/l)	0.700	0.179, 1.221	0.686	0.151, 1.222
Triacylglycerol concentration (mmol/l)	0.698	0.068, 1.328	0.739	−0.036, 1.515
HDL-cholesterol concentration (mmol/l)	0.572	−1.227, 2.371	0.432	−1.974, 2.838
HDL-P concentration (μ mol/l)	−0.055	−0.121, 0.012	−0.056	−0.125, 0.012
HDL-P size (nm)	1.447	−0.049, 2.944	1.349	−0.184, 2.882
Large HDL-P concentration (μ mol/l)	−0.119	−0.336, 0.097	−0.108	−0.336, 0.121
Statin treatment (1 no, 2 yes)	0.887	−0.622, 2.396	0.973	−2.086, 0.140

β coefficient derived from linear regression and generalised estimating equation

HDL-P, HDL particle

Table 5 Univariable and multivariable association of clinical and biochemical variables with ABCA1-independent CEC in participants with type 1 diabetes and participants without diabetes

Variable	Univariable model		Multivariable model	
	β coefficient	95% CI	β coefficient	95% CI
Age (years)	−0.021	−0.049, 0.007	−0.021	−0.046, 0.004
Sex (1 male, 2 female)	−0.191	−0.823, 0.441	−0.198	−0.384, 0.781
BMI (kg/m ²)	0.017	−0.035, 0.068	0.017	−0.026, 0.061
Type 1 diabetes (1 no, 2 yes)	1.434	0.827, 2.041	1.420	0.835, 2.004
Total cholesterol concentration (mmol/l)	0.185	−0.168, 0.007	0.186	−0.155, 0.527
Triacylglycerol concentration (mmol/l)	−0.112	−0.539, 0.314	−0.115	−0.529, 0.299
HDL-cholesterol concentration (mmol/l)	0.233	−0.986, 1.452	0.215	−1.001, 1.431
HDL-P concentration (μ mol/l)	0.110	0.065, 0.155	0.109	0.069, 0.149
HDL-P size (nm)	0.975	−0.039, 1.989	0.927	−0.046, 1.899
Large HDL-P concentration (μ mol/l)	−0.070	−0.217, 2.041	−0.058	−0.202, 0.086
Statin treatment (1 no, 2 yes)	1.102	0.079, 2.124	1.103	0.213, 1.993

β coefficient was derived from linear regression and generalised estimating equation

HDL-P, HDL particle

evidence of early atherosclerosis (CIMT). The study includes the following strengths: (1) comparison between a cohort of 100 type 1 diabetic participants and carefully matched non-diabetic participants; (2) profiling of HDL particle size by NMR and corresponding measurement of particle CEC; and (3) measurement of CIMT, an established marker of early atherosclerosis. Limitations include the fact that this was a cross-sectional study and was not designed to determine the longitudinal effect of the measured variables on progression of atherosclerosis and major atherosclerotic cardiac events (MACE). Furthermore, the study was performed within a population of white ethnicity; it is likely that the variables tested will be influenced by ethnicity, which should be an important consideration for future studies. Finally measures of HDL CEC do not capture information regarding the influence of type 1 diabetes on macrophage/cellular efflux capacity; Daffu et al [19] showed previously that murine diabetic macrophages exhibit impaired ABCG1-mediated efflux in an AGE receptor-dependent mechanism while Mauldin et al [20] similarly demonstrated that monocyte-derived macrophages from individuals with type 2 diabetes exhibit reduced CEC. To date, little is known about macrophage efflux capacity in type 1 diabetes.

Most of the studies on HDL-cholesterol levels in type 1 diabetes reported a significant increase in levels compared with non-diabetic control populations (summarised in [15]). Previous smaller studies investigating HDL function in type 1 diabetes have reported conflicting findings. De Vries et al demonstrated increased total CEC in 14 individuals with type 1 diabetes compared with healthy controls [21]. In contrast, Manjunatha et al demonstrated reduced HDL CEC in individuals with type 1 diabetes who had poor glycaemic control and

good glycaemic control (15 in each group), relative to matched non-diabetic counterparts [22]; notably differences in CEC were small and plasma HDL-cholesterol concentration was equivalent across groups. The current study confirms that CEC is increased in type 1 diabetes independent of HDL-cholesterol, and that this increase is contributed to particularly by ABCA1-independent CEC.

We demonstrated sex-specific differences in HDL biology in type 1 diabetes with preservation of HDL particle concentration and increased ABCA1-independent efflux evident in men but not in women relative to non-diabetic participants. In contrast, there was a profound reduction in the concentration of small HDL particles in women with type 1 diabetes compared with non-diabetic women, with no effect evident in men. Notably, however, division of type 1 diabetes participants by sex resulted in relatively small cohorts of 40 men and 60 women; these observations will require verification and further analysis in larger type 1 diabetes populations.

The observed increase in CEC in type 1 diabetes occurred in parallel with increased HDL-cholesterol concentration, HDL particle size and large HDL particle concentration. Although not previously demonstrated in populations with type 1 diabetes, the association between larger HDL particles and total CEC has been reported in older adults [23] and perimenopausal women [24]. In the current study, consistent with increased particle size, the increase in total CEC was predominantly attributable to increased ABCA1-independent CEC [11], with a lesser increase in ABCA1-dependent CEC. Interestingly, despite the reduction in concentration of small- and medium-sized HDL particles in type 1 diabetes, ABCA1-dependent CEC was enhanced relative to non-diabetic participants. ABCA1-dependent efflux typically correlates with

lipid-poor pre- β -1 HDL and not with large HDL particles [25] and thus these findings were unexpected; they indicate that the larger-sized HDL particles in type 1 diabetes might facilitate greater storage of non-esterified cholesterol, causing this unexpected change in association between ABCA1-dependent efflux and particle size. It is also important to note that NMR is limited in its ability to detect lipid-poor pre- β -1 HDL [26] as it specifically measures cholesterol on lipoprotein particles. These lipid-poor pre- β -1 HDL particles are the primary acceptor of ABCA1-dependent CEC [27] and thus we potentially did not capture the true association between ABCA1-dependent CEC and small HDL particles in the current study.

We previously demonstrated increased association of the acute-phase inflammatory protein serum amyloid A (SAA) on HDL2 and HDL3 subfractions in type 1 diabetes [28]. Enrichment of HDL with SAA has been reported to reduce CEC [29, 30], particularly in an acute-phase setting [31, 32], and impair the anti-inflammatory properties of the HDL particles [33]. The degree of enrichment of HDL with SAA during the acute-phase response (~1000-fold) [34], however, is considerably higher than that seen in type 1 diabetes (~1.7 fold) and therefore differences observed in type 1 diabetes might not be clinically relevant.

The observed increase in HDL particle size in type 1 diabetes has possible important implications for RCT through the lymphatic vasculature [35]. The level and particle size of HDL within the interstitial fluid that bathes peripheral cells is arguably more important for RCT than the size of particles within serum. Approximately 50% of all ApoA-I circulates within the interstitial fluid of peripheral organs [36], where pre- β particles predominate and CETP activity is low relative to plasma [36]. Recent findings by Apro et al have demonstrated that the CEC of HDL derived from interstitial fluid was significantly lower than that derived from plasma [37]. Furthermore, HDL CEC was significantly reduced in individuals with type 2 diabetes relative to healthy controls and this effect was more pronounced in HDL from interstitial fluid compared with plasma [37]. A limitation of the current study is that we have specifically measured serum CEC and not interstitial fluid CEC. Thus, while serum CEC is enhanced in type 1 diabetes, the shift towards larger particle size might in fact reduce access to the interstitial fluid and reduce this important route of RCT. In particular, the remarkable reduction in small HDL particle number in women may lead to loss of cholesterol flux through the lymphatic RCT pathway. This requires further investigation as it potentially contributes to residual cardiovascular risk in individuals with type 1 diabetes.

The current study demonstrates both potentially protective (increased HDL particle size and CEC) and harmful (reduced HDL particle number) effects of type 1 diabetes on HDL biology. Similar to CEC, measuring the number of HDL

particles by NMR has been shown in numerous trials (including the Veterans Affairs High-Density Lipoprotein Intervention Trial [VA-HIT] and the primary prevention Multiple Risk Factor Intervention Trial [MRFIT]) to be a better predictor of future cardiovascular events than circulating HDL-cholesterol or ApoA-I levels [38, 39] (reviewed in reference [40]). Recent subanalysis of findings from Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) has suggested that NMR-measured HDL particle number is superior to CEC in predicting CVD events [41]. Levels of large HDL particle subpopulations, as determined by NMR, are inversely associated with CVD risk while levels of small HDL particles are often positively correlated with risk [40, 42]. Circulating levels of large α -1 HDL, as determined by 2D gel electrophoresis, are also associated with reduced cardiovascular risk while levels of small HDL particles are elevated in individuals with CHD [43–45]. The Epidemiology of Diabetes Complications (EDC) study demonstrated prospectively that HDL particle size could predict the development of cardiovascular events in type 1 diabetes [46]. Similar to our findings, Colhoun et al demonstrated more large HDL particles and fewer small HDL particles in type 1 diabetes, and greater mean HDL particle size [47]; however, they did not find a relationship between particle size and computer tomography-defined coronary artery calcification and in the current study we did not demonstrate associations between any components of HDL structure and function and CIMT.

We observed a positive correlation between both ABCA1-dependent and ABCA1-independent CEC and CIMT, although these effects did not persist in multivariable analysis. While this was unexpected it is noteworthy that the study participants were relatively young (mean age 37 years) and predominantly female (60%) with low lesion burden. Furthermore, the association between CEC and CVD is controversial. The cornerstone study by Khera et al demonstrated an association between HDL CEC and CVD burden in an older population (mean age 57 ± 9 years) with angiographically confirmed CVD [12]. Khera et al have more recently demonstrated superiority of measuring HDL particle number compared with HDL CEC as a predictor of incident CVD, finding no association between baseline CEC and incident CVD within JUPITER [41]. However, apparently contrary to these findings, Li et al demonstrated a positive association between CEC and cardiovascular events in non-diabetic individuals (1.66-fold increase in MACE), findings that remained similar (1.85-fold increase) after adjustment for traditional cardiovascular risk factors [48]. Furthermore, in the Multi-ethnic Study of Atherosclerosis [14], CEC was inversely associated with incident coronary artery disease but positively associated with carotid plaque progression. Taken together, these findings suggest significant complexity in the

association between CEC and progression of atherosclerosis at different sites. Larger studies including type 1 diabetes participants only are needed to clarify whether and to what extent changes in both particle size and CEC influence atherosclerosis risk in this condition.

An important consideration in future studies will be investigation of the effects of statin treatment and alcohol intake on HDL structure and function in diabetes. Although statin treatment was independently and positively associated with CEC in multivariable analysis, it is difficult to separate this apparent effect from the fact that statins were predominantly used in older male participants, and only in the type 1 diabetes group. Alcohol intake was greater in non-diabetic participants and statistically significant correlations were identified between alcohol intake and certain components of CEC. Limiting analysis, however, to participants from both groups with weekly alcohol intake not greater than ten units did not change our findings. Future studies in larger cohorts will help explore potential contributions of statins and alcohol to HDL structure and function in diabetes.

In conclusion, we have demonstrated potentially important differences in CEC, particularly its ABCA1-independent component, in type 1 diabetes. These differences remain statistically significant following adjustment for differences in HDL-cholesterol concentration and HDL particle size and concentration. Future studies are needed to determine the mechanism underlying these differences and their role in the atherosclerotic complications of type 1 diabetes.

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Contribution statement MOA co-designed the study, recruited the participants, measured CIMT, analysed clinical data and prepared the first draft of paper. FCM and JG co-designed the study: JG managed the clinical recruitment and CIMT analysis; and FCM led the laboratory-based analysis of HDL. REB performed the HDL function assays, analysed data, prepared figures and co-wrote the first draft of the paper. WG, AP, AG, IF, MS, KSA, AM and KM were involved in study design, recruitment of participants into the study, clinical measurements of CIMT and reviewed and edited the final manuscript. GB collaborated on study design, sample analysis, data acquisition and reviewed the manuscript. RS planned the re-analysis of data for the revised manuscript, oversaw data analysis and presentation, and description of the new statistical methods and results throughout the text, and helped draft the final manuscript. FCM and JG co-edited the final paper and all authors approved the final draft. JG is responsible for the integrity of the work as a whole.

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