

Original Research

HDL remodeling and enrichment with apoCIII and apoE in Indigenous Australians



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KEYWORDS

HDL remodeling;
HDL function;
Indigenous populations

BACKGROUND: Indigenous Australians have an increased risk of type 2 diabetes mellitus (T2DM) and premature cardiovascular disease. Subpopulations of high-density lipoprotein (HDL) have been associated with increased cardiovascular risk, but HDL composition, size, or function have not been studied in Indigenous Australians.

METHODS: The study consisted of 86 non-Indigenous participants, 43 of whom had T2DM, and 75 Indigenous participants, 36 of whom had T2DM. HDL lipid and apolipoprotein content were determined using enzymatic assays and enzyme-linked immunosorbent assays, respectively, and HDL size and distribution were investigated using nuclear magnetic resonance spectroscopy. Transporter-independent, ATP-binding cassette transporter (ABC)A1- and ABCG1-specific cholesterol efflux capacity (CEC) were determined using cell lines stably expressing human ABCA1 or ABCG1.

RESULTS: Indigenous participants had significantly lower concentrations of large (10.3–12.0 nm), small (7.4–7.8 nm), and total HDL particles, which persisted after adjustment for serum triglyceride (TG), body mass index (BMI), and T2DM. HDL from Indigenous Australians was also highly enriched in TG, apolipoprotein (apo) E, and apoCIII (all $P < .001$). Transporter-independent and ABCG1-mediated CEC were not different between the populations. ABCA1-specific CEC per HDL particle was higher in Indigenous than in non-Indigenous subjects ($P < .001$), and persisted after adjustment for TG, BMI, and T2DM. Multivariable analysis identified that ABCA1-specific CEC was independently and positively associated with HDL-apoCIII and HDL-apoE levels.

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Submitted April 21, 2025. Accepted for publication August 5, 2025.

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CONCLUSIONS: Indigenous Australians demonstrate significant compositional, size, and functional changes in circulating HDL, which is only partially explained by BMI, hypertriglyceridemia, or T2DM. Remodeled HDL may serve as a biomarker of increased cardiovascular risk in Indigenous Australians. © 2025 The Authors. Published by Elsevier Inc. on behalf of National Lipid Association. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

Introduction

First Nations populations around the world have much higher rates of cardiovascular disease (CVD) and worse outcomes after cardiovascular events than non-Indigenous reference populations.¹ Social and economic disadvantage, structural impediments to primordial prevention, high-quality care, and treatment are all likely important contributors, and may manifest themselves in pathological biological processes which predispose to premature disease. Indigenous Australians in particular have a disproportionally increased risk of CVD compared to non-Indigenous Australians,^{2,3} with type 2 diabetes mellitus (T2DM) being a major contributing factor.^{2,4} Dyslipidemia is prevalent in this population and includes increased levels of circulating triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), particularly small LDL particles, and very low levels of high-density lipoprotein cholesterol (HDL-C),^{4,5} yet surprisingly little is known about the biochemical or functional significance of these changes.

Epidemiological studies have indicated that low circulating HDL-C levels are independently associated with an increased risk of CVD.⁶ However, the hypothesis of a causal relationship between HDL-C levels and CVD has been challenged by genetic⁷ and pharmacologic intervention studies,⁸ which showed that increased HDL-C was either not protective, or that HDL-C may demonstrate a biphasic response with both high and low concentrations being adverse.⁹ More recent studies emphasize the importance of functional and structural diversity of HDL species.¹⁰ For example, increased abundance of certain subpopulations of HDL containing apoCIII or apoE is evident in patients with obesity or coronary artery disease^{11,12} and confers an adverse prognosis.^{13,14} In some studies, large HDL species in particular have shown a negative correlation with cardiovascular risk.¹⁵

It has been proposed that HDL function may be a superior indicator of cardiovascular risk than is total HDL concentration. One of the main protective functions of HDL is its role in reverse cholesterol transport (RCT). In RCT, HDL removes cholesterol from macrophages within the arterial wall and delivers it to the liver for excretion via bile and the feces, thereby reducing atherosclerotic plaque formation and progression. Cholesterol efflux from foam cell macrophages is mediated by 3 main processes: (1) passive diffusion (transporter-independent), (2) ATP-binding cassette transporter A1 (ABCA1)-mediated efflux, and (3) ATP-binding cassette transporter G1 (ABCG1)-mediated efflux.¹⁶ ABCA1-mediated transport functions most efficiently with

lipid poor, small HDL species while the passive diffusion-driven pathway and ABCG1-mediated transport favor larger lipidated HDL species.^{17,18} In landmark studies, the capacity of HDL to stimulate cholesterol efflux from macrophages measured as cholesterol efflux capacity (CEC) was a better predictor of CVD risk than was HDL-C concentration.^{19,20} Subsequent studies have found that although reduced CEC generally associates with reduced CVD prevalence and incidence, in some populations with increased CVD risk, such as patients with diabetes, metabolic syndrome, chronic kidney disease, and schizophrenia, ABCA1-mediated efflux was unexpectedly increased or associated with higher risk.²¹⁻²³ The recent Aegis-II trial indicated that increased ABCA1-specific CEC is not necessarily linked to improved cardiovascular outcomes.²⁴ This suggests that the interpretation of CEC, and ABCA1 efflux in particular, may be context and population-specific.

We have analyzed HDL particle size, composition, transporter-specific (ABCA1- and ABCG1-mediated) and transporter-independent CEC in Indigenous Australians from the Predicting Renal Ophthalmic and Heart Events in the Aboriginal Community (PROPHET) Study (AHREC 04-13-545) and compared these to non-Indigenous Australians. We here report altered HDL size distribution and composition, pathway specific changes in CEC in Indigenous Australians, and investigate their relationship to pre-existing T2DM, body mass index (BMI), and hypertriglyceridemia.

Materials and methods

Participants

We recruited a total of 161 participants: 43 non-Indigenous participants without T2DM, 43 non-Indigenous participants with T2DM, and 75 Indigenous Australians (38 without T2DM and 36 with T2DM). Participants were between 20 and 80 years old. Participants with major surgery in the last month, a diagnosis of cancer within the last 5 years, or intercurrent infections were excluded.

Laboratory analyses

Total cholesterol (TC), TG, and HDL-C were determined using enzymatic tests on a Roche Cobas 8000. LDL-C was calculated using the Friedewald formula or determined by direct measurement. BMI was calculated by dividing participant's weight in kilograms by height in meters squared. Measurements of high-sensitivity C reactive protein (hsCRP)

were performed using an immuno-turbidimetric method on an autoanalyser (Cobas 8000). Glucose was measured by enzymatic assay (Cobas 8000) whilst glycosylated hemoglobin (HbA1c) was measured by high-pressure liquid chromatography (BioRad D10). T2DM was defined by plasma glucose ≥ 7.0 mmol/L (fasting), ≥ 11.1 mmol/L (nonfasting), or HbA1c $\geq 6.5\%$, or by self-reported medical history or medication status. Dyslipidemia was defined by pre-existing clinical diagnosis, lipid-lowering therapy, or plasma LDL-C > 4 mmol/L and/or TG > 2.0 mmol/L. Hypertension was defined by self-reported medical history, being on antihypertensive medication or diastolic blood pressure ≥ 140 mm Hg and/or systolic blood pressure ≥ 90 mm Hg. ApoB-depletion from the serum was achieved as described previously using polyethylene glycol (PEG) precipitation.²⁵ HDL-free cholesterol (FC), HDL-TC, HDL-phospholipid (PL) and HDL-TG were determined in PEG sera by commercial enzymatic assays (Novachem). HDL-cholesteryl ester (CE) was calculated by subtracting HDL-FC from HDL-TC. HDL-apoCIII and HDL-apoE levels were determined in PEG and total sera using a commercial (ThermoFisher), and an in house enzyme-linked immunosorbent assay,²⁶ respectively. Cholesteryl ester transfer protein (CETP) activity was determined using a CETP activity assay kit (MAK106; Roar Biomedical Inc).

Nuclear magnetic resonance spectroscopy

HDL lipoprotein size distributions were measured using nuclear magnetic resonance (NMR) spectroscopy. NMR spectra were acquired using EDTA plasma samples on the Vantera Clinical NMR Analyzer platform at Labcorp. Lipoprotein particle profiles were quantified using the most recently developed LP4 algorithm which provides quantitation of total HDL particle numbers (7.5–12.0 nm), overall HDL size, large-HDL (10.3–12.0 nm), medium-HDL (8.7–9.5 nm), and small-HDL (7.4–7.8 nm). Specific HDL subspecies H7 (12 nm), H6 (10.8 nm), H5 (10.3 nm), H4 (9.5 nm), H3 (8.7 nm), H2 (7.8 nm), and H1 (7.4 nm) concentrations were also determined.²⁷ Circulating ApoA-I levels and triglyceride-rich lipoprotein TG content (TRL-TG) were also determined by NMR.²⁷ Percent coefficients of variation (%CV) for ApoA-I were determined to be 1.4% to 1.8% for intra-assay imprecision and 1.7% to 2.2% for interassay imprecision for low, intermediate, and high concentration pools. In total, NMR data were analyzed from 76 non-Indigenous and 73 Indigenous Australians.

Cholesterol efflux capacity

Chinese hamster ovary (CHO) cells stably expressing tetracycline-inducible ABCA1 were used to determine ABCA1-specific cholesterol efflux as previously described.²⁵ ABCA1-dependent efflux was calculated as the difference in efflux rates between cells incubated with (ABCA1-expressing) and without tetracycline (transporter-independent efflux). ABCG1-mediated efflux was determined using an ABCG1-CHO cell line which expresses hu-

man ABCG1 constitutively and compared to that from the parental CHO cell line, in which endogenous ABCG1 expression is not detectable. ABCG1-specific efflux was calculated as the difference in efflux rates between ABCG1-CHO cells and the nontransfected control parental cell line. Efflux was determined to 1% (v/v) apoB-depleted serum over a 4-hour period without standardizing for HDL or protein concentration as previously reported.²⁵ A standard reference serum was included on every plate to adjust for intra and interassay variability. Efflux to individual participant samples was normalized to the efflux rate of this standard serum. Intra and interassay variability were 3.9% and 6.9%, 4.8% and 11.2%, 7.5% and 14.7%, for transporter-independent, ABCA1-CHO, and ABCG1-CHO efflux, respectively. Transporter-independent CEC values from the ABCA1-CHO and ABCG1-CHO cells without induced transporter expression were highly correlated ($r = 0.88$, $P < .001$, $n = 161$) and were averaged for each individual subject. Ranges (min-max) for CEC in healthy normolipidemic individuals were 0.54% to 1.45%, 0.95% to 1.84%, and 0.37% to 1.33% for nontransporter-, ABCG1- or ABCA1-specific CEC, respectively. Data summarizing individual data and averaged data for transporter-independent CEC are shown in Supplementary Table 1.

Statistical analysis

Categorical variables were summarized as frequencies and percentages, whereas continuous variables were presented as mean $\pm$ SD or median [first and third quartiles], as appropriate. Comparison between 2 groups was based on unpaired t-test for parametric distribution or a Mann-Whitney test for nonparametric distribution, whilst a one-way analysis of variance with Holms-Sidak correction (parametric) or Kruskal-Wallis test with Dunn's correction (nonparametric) were used for comparisons between multiple groups. Based on previous findings,^{25,28} power calculation (80% power with $\alpha = 0.05$) indicated group sizes of 24, 37, and 28 samples for transporter-independent, ABCA1-, and ABCG1-specific samples were necessary. Associations between CEC pathways and clinical or biochemical parameters were investigated using univariate and multivariable linear regression models (generalized linear model) with estimated marginal means determination. For all analyses, skewed data were log-transformed and a 2-tailed P value of $< .05$ was considered as the cut-off for statistical significance. Analyses were performed using Graphpad Prism 9 and IBM SPSS version 2.7.

Results

Participant baseline characteristics are shown in Table 1. Indigenous participants were younger (44 vs 54 years; $P < .001$) and were substantially more likely to be current smokers (41% vs 6%, $P < .001$). Indigenous participants had significantly higher BMI, TG, LDL-C, non-HDL-C, and hsCRP levels, whilst HDL-C and apoA-I levels were significantly

Table 1. Clinical and biochemical parameters.

	Non-Indigenous (n = 86)	Indigenous (n = 75)	P
Age (yr)	54 ± 14	44 ± 14	<.001
M/F (%M)	40/46 (47)	23/52 (40)	ns
BMI	25.0 [22.0-28.1]	31.7 [27.7-39.2]	<.001
TC (mmol/L)	4.5 ± 1.1	5.1 ± 1.4	.003
LDL-C (mmol/L)	2.2 [1.6-0.2.9]	3.0 [2.3-3.8]	<.001
TG (mmol/L)	1.4 [0.9-2.3]	1.8 [1.3-3.1]	.001
HDL-C (mmol/L)	1.4 [1.1-1.7]	1.2 [0.9-1.4]	<.001
Non-HDL-C (mmol/L)	3.0 ± 1.0	3.9 ± 1.4	<.001
apoA-I (mg/dL)	135 ± 24	114 ± 21	<.001
Creatinine (μmol/L)	71 [61-79]	69 [56-78]	ns
hsCRP (mg/L)	0.7 [0.4-1.6]	4.0 [2.0-9.5]	<.001
Risk factors for CVD (%)			
Diabetes	43 (50)	36 (48)	ns
Hypercholesterolemia	34 (40)	42 (56)	ns
Hypertension	34 (40)	35 (47)	ns
Smoking	5 (6)	31 (41)	<.001
HbA1c (%)	6.0 [5.2-7.9]	6.1 [5.4-8.0]	ns
No DM	5.2 [5.1-5.6]	5.4 [5.3-5.7]	.03
DM	8.0 [6.7-8.8]	8.2 [6.7-9.9]	ns

Abbreviations: apoA-I, apolipoprotein A-I; BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; HbA1c, hemoglobin A1c; hsCRP, high sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; M/F, male/female; ns, not significant; TC, total cholesterol; TG, triglycerides; yr, years.

Data presented are mean ± SD for normally distributed data or median [first and third quartiles] for nonparametrically distributed data.

Table 2. Large and small HDL particle concentrations are decreased in Indigenous Australians.

	Non-Indigenous (n = 76)	Indigenous (n = 73)	P
HDL size (nm)			
Unadjusted	9.0 ± 0.1	9.1 ± 0.1	ns
Adjusted for TG, BMI, and T2DM	9.0 ± 0.1	9.1 ± 0.1	ns
Total HDL-P (μmol/L)			
Unadjusted	20.5 ± 2.7	17.6 ± 3.4	<.001
Adjusted for TG, BMI, and T2DM	20.4 ± 0.4	17.7 ± 0.4	<.001
Large HDL-P (10.3-12.0 nm; μmol/L)			
Unadjusted	2.2 ± 0.2	1.4 ± 0.2	<.001
Adjusted for TG, BMI, and T2DM	2.1 ± 0.2	1.5 ± 0.2	<.05
Medium HDL-P (8.7-9.5 nm; μmol/L)			
Unadjusted	4.2 ± 0.3	4.4 ± 0.3	ns
Adjusted for TG, BMI, and T2DM	4.0 ± 0.3	4.4 ± 0.3	ns
Small HDL-P (7.4-7.8 nm; μmol/L)			
Unadjusted	14.0 ± 3.4	11.9 ± 3.4	<.001
Adjusted for TG, BMI, and T2DM	14.2 ± 0.5	11.7 ± 0.4	<.001

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HDL-P, HDL particle concentration; ns, not significant; T2DM, type 2 diabetes mellitus; TG, triglycerides.

All parameters were determined by nuclear magnetic resonance spectroscopy as described in the methods section.

Values are estimated marginal means determined using linear regression adjusted for TG, BMI, and T2DM.

lower. HbA1c levels were higher in Indigenous participants without known T2DM compared to non-Indigenous participants without diabetes (5.2% vs 5.4% $P = .03$). Insulin and sodium-glucose cotransporter-2 inhibitor treatment was less frequent in Indigenous participants with a diagnosis of diabetes (Supplement Table 2).

HDL subclasses and composition were analyzed in detail by NMR. As hypertriglyceridemia, obesity, and T2DM are known to affect HDL subclass distribution and com-

position,^{29,30} the differences between Indigenous and non-Indigenous participants were also analyzed after adjustment for TG, BMI, and T2DM (Table 2) and by stratifying according to BMI < 25 or ≥ 25 kg/m² (Supplementary Table 3) or the presence or absence of T2DM (Supplementary Table 4). Total HDL particle concentration was significantly lower in Indigenous Australians and this was associated with significantly decreased numbers of both large and small HDL particles (Table 2). These findings persisted after adjustment for

Table 3. HDL lipid, apoE, and apoCIII composition.

Parameter	Non-Indigenous	Indigenous	P
HDL-FC			
(mg/mL)	7.3 ± 0.3	5.7 ± 0.4	<.001
(mol/mol)	12.9 ± 0.6	11.8 ± 0.6	ns
(mol/mol)*	12.0 ± 0.7	12.5 ± 0.6	ns
HDL-CE			
(mg/mL)	32.5 ± 0.8	26.4 ± 0.8	<.001
(mol/mol)	56.8 ± 1.3	54.6 ± 1.4	ns
(mol/mol)*	55.4 ± 1.7	55.0 ± 1.5	ns
HDL-TG			
(mg/mL)	20.8 ± 0.4	20.6 ± 0.4	ns
(mol/mol)	16.2 ± 0.5	18.7 ± 0.5	<.001
(mol/mol)*	16.6 ± 0.6	18.2 ± 0.5	.06
HDL-PL			
(mg/mL)	70.5 ± 1.9	60.6 ± 2.0	<.001
(mol/mol)	60.4 ± 1.4	61.9 ± 2.0	ns
(mol/mol)*	59.5 ± 1.8	61.4 ± 2.9	ns
HDL-apoA-I			
(mg/mL)	135 ± 3	116 ± 2.7	<.001
(mol/mol)	2.3 ± 0.03	2.3 ± 0.03	ns
(mol/mol)*	2.3 ± 0.03	2.3 ± 0.03	ns
HDL-apoE			
(mg/mL)	1.3 ± 0.2	2.2 ± 0.2	<.001
(mol/mol)	0.03 ± 0.003	0.05 ± 0.003	<.001
(mol/mol)*	0.03 ± 0.004	0.05 ± 0.003	.001
HDL-apoCIII			
(mg/mL)	4.6 ± 0.3	6.3 ± 0.4	.001
(mol/mol)	0.34 ± 0.02	0.52 ± 0.03	<.001
(mol/mol)*	0.38 ± 0.03	0.49 ± 0.02	<.001

HDL-FC, HDL-free cholesterol; HDL-CE, HDL-cholesteryl ester; HDL-TG, HDL-triglyceride; HDL-PL, HDL-phospholipid; HDL-apoE, HDL-apolipoprotein E; and HDL-apoCIII, HDL-apolipoprotein CIII were all determined in polyethylene glycol-precipitated serum. HDL-apoA-I, apolipoprotein A-I was determined in whole plasma by nuclear magnetic resonance.

Values are estimated marginal means ± SD determined using linear regression.

*Adjusted for TG, BMI and T2DM.

TG, BMI, and T2DM, suggesting that they were not driven by hypertriglyceridemia, increased BMI, or T2DM in Indigenous participants (Table 2). This was confirmed when stratifying participants according to BMI or T2DM which indicated that HDL particle number was significantly lower in Indigenous participants regardless of BMI or T2DM status (Supplementary Tables 3 and 4). Interestingly, in non-Indigenous subjects with either BMI ≥ 25 kg/m² or T2DM, large HDL concentrations were reduced to levels seen in Indigenous subjects, whereas BMI and T2DM had no effect on large HDL concentrations in the Indigenous group. Analysis of specific HDL particle subspecies indicated that 10.3 nm particle concentrations were 54% lower in Indigenous subjects ($P < .001$) and the concentration of 7.8 nm particles was 19% lower ($P < .001$) (Supplement Table 5). Because both large and small particles were reduced in concentration, mean HDL particle size did not differ between the Indigenous and the non-Indigenous cohorts (Table 2).

Determination of HDL lipid levels revealed that HDL-FC, HDL-CE, and HDL-PL levels were all lower in Indigenous participants (Table 3). After accounting for dif-

ferences in HDL particle concentrations, by expressing results per mol HDL, HDL-FC, HDL-CE, and HDL-PL were similar between Indigenous and non-Indigenous subjects, whereas HDL-TG was increased (Table 3). As the apoE and apoCIII content of HDL has been associated with metabolic syndrome and adverse cardiovascular outcomes^{13,14} HDL-associated apoE and apoCIII levels were also determined. Both HDL-apoE (2.5-fold increase, $P < .001$) and HDL-apoCIII (1.5-fold increase, $P < .001$) were increased in Indigenous subjects. There was a significant redistribution of both apolipoproteins from the non-HDL to the HDL fraction in Indigenous Australians (Supplementary Table 6). The proportion of apoE in HDL was 32.8% vs 25.6%, and the proportion of apoCIII in HDL was 38.2% vs 22.9% in Indigenous and non-Indigenous participants, respectively. There was no difference in apoA-I content in HDL of non-Indigenous and Indigenous participants. Obesity and T2DM both increased the enrichment of HDL with TG, apoE, and apoCIII in Indigenous participants (Supplementary Tables 7 and 8). To investigate whether TG enrichment was related to altered CETP activity, CETP activity was determined and was found

Table 4. Correlations between HDL size, composition, and CEC with TG, TRL-TG, and HbA1c.

	Non-Indigenous (n = 73)			Indigenous (n = 75)		
	TG	TRL-TG	HbA1c	TG	TRL-TG	HbA1c
HDL						
Size	-0.50**	-0.66**	-0.27*	-0.38**	-0.47**	-0.14
Large-HDL	-0.46**	-0.64**	-0.29*	-0.39**	-0.47**	-0.10
Medium-HDL	-0.27*	-0.36*	-0.26*	-0.30**	-0.29*	-0.38**
Small-HDL	0.26*	0.36	0.15	0.17	0.18	0.14
Composition						
FC content	-0.58**	-0.47**	-0.30**	-0.56**	-0.56**	-0.41**
CE content	-0.49**	-0.38**	-0.33**	-0.42**	-0.40**	-0.45**
TG content	0.40**	0.31**	0.12	0.53**	0.44**	0.30*
PL content	-0.31**	-0.19	-0.20	-0.17	-0.20	-0.29*
apoE content	-0.07	-0.09	0.02	0.23*	0.25*	0.15
apoCIII content	-0.06	0.07	0.09	0.51**	0.51**	0.23
CEC						
Transporter independent	-0.45**	-0.32**	-0.30**	-0.29*	-0.29*	-0.34**
ABCG1-specific	-0.05	-0.04	-0.12	-0.17	-0.11	-0.23
ABCA1-specific	-0.10	-0.10	.19	.10	.09	-0.11

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; apoE, apolipoprotein E; apoCIII, apolipoprotein CIII; CEC, cholesterol efflux capacity; CE, cholesteryl ester; FC, free cholesterol; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; HDLP, HDL particle concentration; PL, phospholipid; TG, triglyceride; TRL, triglyceride-rich lipoprotein; TG, triglyceride.

Spearman's correlation coefficients.

* $P < .05$.

** $P < .01$.

Table 5. Correlations between HDL lipid and apolipoprotein composition.

	HDL-FC	HDL-CE	HDL-PL	HDL-TG	HDL-apoE	HDL-apoCIII
HDL size						
Indigenous	0.83***	0.39***	0.35**	0.19	-0.17	-0.09
Non-Indigenous	0.74***	0.63***	0.63***	0.11	0.31**	0.11
HDL-FC						
Indigenous	-	0.90***	0.63***	0.02	-0.03	-0.05
Non-Indigenous		0.83***	0.82***	0.15	0.34**	0.34**
HDL-CE						
Indigenous		-	0.66***	0.03	0.06	0.02
Non-Indigenous			0.83***	0.10	0.37***	0.37***
HDL-PL						
Indigenous			-	0.33**	0.19	0.28*
Non-Indigenous				0.31**	0.40***	0.40***
HDL-TG						
Indigenous				-	0.23	0.63***
Non-Indigenous					0.27*	0.27**
HDL-apoE						
Indigenous					-	0.36**
Non-Indigenous						0.41***

Abbreviations: HDL, high-density lipoprotein; HDL-FC, HDL-free cholesterol; HDL-CE, HDL-cholesteryl ester; HDL-PL, HDL-phospholipid; HDL-TG, HDL-triglyceride; HDL-apoE, HDL apolipoprotein E; HDL-apoCIII, HDL-apolipoprotein CIII. Spearman correlation coefficients between HDL-parameters for Indigenous (n = 86) and non-Indigenous subjects (n = 75).

* $P < .05$, ** $P < .01$, *** $P < .001$.

not to be different between the 2 groups (Supplementary Fig 1). After adjustment for TG, BMI, and T2DM, HDL-TG content was not significantly increased, whilst HDL-apoE and HDL-apoCIII content remained significantly higher in Indigenous participants (Table 3).

The relationships between HDL parameters and TG, TRL-TG and HbA1c were investigated (Table 4). Plasma

TG, TRL-TG, and HbA1c were negatively correlated with HDL size, and with the concentration of large and medium sized HDL particles. Small HDL particles behaved differently, showing a positive correlation with TRL-TG in the non-Indigenous group only. HDL-FC and CE content were also negatively correlated with TG, TRL-TG, and HbA1c. HDL-TG was in contrast positively correlated with TG and

Table 6. Transporter-independent, ABCG1-specific, and ABCA1-specific CEC in Indigenous and non-Indigenous subjects.

	Transporter-independent CEC			ABCG1-specific CEC			ABCA1-specific CEC		
	Non-Indigenous		Indigenous	Non-Indigenous		Indigenous	Non-Indigenous		Indigenous
		<i>P</i>			<i>P</i>			<i>P</i>	
Unadjusted	1.00 ± 0.01		0.93 ± 0.02	1.02 ± 0.01		0.99 ± 0.02	0.87 ± 0.02		0.90 ± 0.02
Adjusted for HDLP	0.96 ± 0.01	.004	0.98 ± 0.01	1.00 ± 0.01	ns	1.02 ± 0.01	0.83 ± 0.01	ns	0.94 ± 0.02
Adjusted for HDLP, TG, BMI, T2DM	0.95 ± 0.02	ns	0.98 ± 0.01	1.00 ± 0.02	ns	1.02 ± 0.02	0.84 ± 0.02	ns	0.92 ± 0.02
Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; BMI, body mass index; CEC, cholesterol efflux capacity; HDLP, high-density lipoprotein particle number; ns, not significant; T2DM, type 2 diabetes mellitus; TG, triglyceride.									
Non-transporter and transporter-specific CEC in non-Indigenous and Indigenous subjects.									
Data are presented as mean ± SEM.									
Estimated marginal means in non-Indigenous and Indigenous subjects were compared using linear regression with HDLP, TG, BMI, and T2DM.									

TRL-TG. HDL-apoE and HDL-apoCIII were positively correlated with TG and TRL-TG in Indigenous subjects.

The relationships between HDL particle size and composition in non-Indigenous and Indigenous participants were explored (Table 5). HDL size correlated positively with HDL-FC, HDL-CE, and HDL-PL concentrations in both populations. HDL-TG was not correlated with HDL size or cholesterol content, but was strongly associated with HDL-apoCIII ($r = 0.63, P < .001$) in Indigenous participants, and more weakly with HDL-apoCIII ($r = 0.27, P < .01$) in non-Indigenous participants. HDL-apoE and HDL-apoCIII were positively correlated in both populations. HDL-size, FC, CE, and PL content correlated positively with HDL-apoE content only in the non-Indigenous group, suggesting larger apoE-containing HDL particles may be present in this population. There was no relationship with HDL size for HDL-apoCIII in either population.

Functional analysis of HDL indicated that transporter-independent CEC was significantly lower in Indigenous compared to non-Indigenous participants (Table 6). BMI ≥ 25 kg/m² or the presence of T2DM was associated with decreased transporter-independent CEC in the nonindigenous group but had little effect in the Indigenous group (Supplementary Tables 9 and 10). When adjusted by linear regression for HDL particle concentration, transporter-independent CEC was not significantly different between non-Indigenous and Indigenous cohorts, indicating that the difference was related to circulating HDL concentration (Table 6). ABCG1-specific CEC was not significantly different between non-Indigenous and Indigenous cohorts (Table 6) and was not significantly different after adjustment. ABCA1-specific efflux was significantly greater ($P < .001$) in Indigenous participants compared to non-Indigenous participants after adjustment for HDL particle number, and after adjustment for TG, BMI, and T2DM (Table 6). BMI > 25 was notably associated with increased ABCA1-specific CEC in the Indigenous group (Supplement Table 9) and was independent of T2DM (Supplementary Table 10).

Remodeling and compositional changes in HDL in patients with T2DM or coronary heart disease have been linked to changes in CEC.^{12,29,31} In the present study, large HDL particles correlated significantly with transporter-independent and to a lesser extent with ABCG1 and ABCA1-specific efflux in both groups. Medium HDL particles correlated with transporter-independent efflux, whilst small HDL correlated with all CEC pathways in the Indigenous population only (Table 7). The strongest correlations with CEC in Indigenous participants were observed between HDL particle number and transporter-independent ($r = 0.67, P < .001$) and ABCA1-specific CEC ($r = 0.60, P < .001$). Transporter-independent CEC was negatively correlated with TG, TRL-TG, and HbA1c in both groups (Table 4).

All 3 efflux pathways correlated strongly with HDL-PL, HDL-FC, and HDL-CE content in both Indigenous and non-Indigenous participants (Table 8). ABCA1-specific efflux correlated positively with HDL-TG, but only in Indigenous subjects ($r = 0.41 P < .001$). HDL-apoE and HDL-apoCIII

Table 7. Correlations between HDL NMR particle parameters and pathway-specific CEC.

	Non-Indigenous		Indigenous	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Transporter-independent CEC				
HDLP (μmol/L)	0.57	<.001	0.67	<.001
Large HDLP (μmol/L)	0.62	<.001	0.40	<.001
Medium HDLP (μmol/L)	0.34	.003	0.42	<.001
Small HDLP (μmol/L)	-0.09	ns	0.30	.002
ABCG1 CEC				
HDLP (μmol/L)	0.30	.009	0.32	<.001
Large HDLP (μmol/L)	0.28	.01	0.26	.007
Medium HDLP (μmol/L)	0.05	ns	0.11	ns
Small HDLP (μmol/L)	0.05	ns	0.20	.04
ABCA1 CEC				
HDLP (μmol/L)	0.41	<.001	0.60	<.001
Large HDLP (μmol/L)	0.34	.002	0.24	.01
Medium HDLP (μmol/L)	0.02	ns	0.20	.04
Small HDLP (μmol/L)	0.12	ns	0.42	<.001

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; CEC, cholesterol efflux capacity; HDL, high-density lipoprotein; HDLP, HDL particle concentration; NMR, nuclear magnetic resonance; ns, not significant.

Table 8. Correlations between HDL biochemical parameters and pathway-specific CEC.

	Non-Indigenous		Indigenous	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Transporter-independent CEC				
HDL-FC (mg/dL)	0.83	<.001	0.79	<.001
HDL-CE (mg/dL)	0.87	<.001	0.80	<.001
HDL-PL (mg/dL)	0.86	<.001	0.80	<.001
HDL-TG (mg/dL)	0.23	.03	0.21	.03
HDL-apoE (mg/dL)	0.37	<.001	0.14	ns
HDL-apoCIII (mg/dL)	0.41	<.001	0.19	ns
ABCG1-specific CEC				
HDL-FC (mg/dL)	0.34	.001	0.33	<.001
HDL-CE (mg/dL)	0.43	<.001	0.40	<.001
HDL-PL (mg/dL)	0.34	.001	0.24	.01
HDL-TG (mg/dL)	0.13	ns	0.09	ns
HDL-apoE (mg/dL)	0.13	ns	-0.07	ns
HDL-apoCIII (mg/dL)	0.16	ns	-0.01	ns
ABCA1-specific CEC				
HDL-FC (mg/dL)	0.48	<.001	0.29	.002
HDL-CE (mg/dL)	0.54	<.001	0.35	<.001
HDL-PL (mg/dL)	0.48	<.001	0.48	<.001
HDL-TG (mg/dL)	0.13	ns	0.41	<.001
HDL-apoE (mg/dL)	0.33	.002	0.29	.002
HDL-apoCIII (mg/dL)	0.36	<.001	0.50	<.001

Abbreviations: CEC, cholesterol efflux capacity; HDL, high-density lipoprotein; HDL-FC, HDL-free cholesterol content; HDL-CE, HDL-cholesteryl ester content; HDL-PL, HDL phospholipid content; HDL-TG, HDL triglyceride content; HDL-apoE, HDL apolipoprotein E content; HDL-apoCIII, HDL apolipoprotein CIII content; ns, not significant.

correlated significantly with ABCA1-specific efflux in Indigenous and non-Indigenous participants.

Stepwise linear regression was performed to determine which parameters were independently associated with ABCA1-specific CEC in Indigenous participants. Regression analysis identified apoA-I as most strongly associated followed by HDL-apoCIII, age, TC, and HDL-apoE. Incorporating these parameters in a multivariable model provided an adjusted R^2 of 0.40, and estimated marginal means analysis adjusting for these variables indicated that ABCA1-specific efflux remained significantly higher in Indigenous participants (0.85 ± 0.02 vs 0.93 ± 0.01 , $P < .001$).

Discussion

We report the first detailed characterization of HDL composition, particle size, and transporter-independent and transporter-specific (ABCA1 and ABCG1-mediated) CEC in Indigenous Australians. We identify distinctive HDL remodeling in this population, with decreased concentrations of large and small HDL particles, HDL enrichment with TG, apoCIII, and apoE, and increased ABCA1-specific CEC.

Total HDL-C, apoA-I, and HDL particle concentrations were significantly lower in Indigenous participants and this was associated with a decrease in both large and small HDL particle concentrations. Several studies suggest that HDL subpopulations of specific sizes may be better predictors of CVD risk than total HDL-C,^{32,33} and low concentrations of large HDL particles, as seen in the present study, are associated with a high risk.^{15,34} However, these relationships are inconsistent between studies and the biological mechanisms underlying them are unclear. For example, large HDL particles are reported to be less anti-inflammatory and have reduced antioxidative capacity than small HDL particles,³⁵ but are also better inhibitors of platelet activation and are better substrates for the delivery of cholesterol to the liver via the scavenger receptor class B type 1 receptor.³⁶ In our study the lower concentrations of large HDL in Indigenous participants was observed even in those without T2DM, and was independent of BMI and serum TG levels. This suggests that in Indigenous Australians some aspects of adverse HDL remodeling may precede, or be independent of, the development of overt T2DM or obesity. Previous studies have observed a loss of large HDL particles and increased small TG-rich HDL in T2DM that may be due to increased CETP activity.²⁹ CETP activity, however, was not different between Indigenous and non-Indigenous participants in our study.

Our findings that Indigenous Australians had markedly increased levels of apoE and apoCIII associated with HDL, and that HDL-apoCIII and HDL-apoE were independently associated with ABCA1-mediated CEC are both novel. As the average HDL particle size was not different in Indigenous and non-Indigenous participants, the increase in HDL-apoE and apoCIII content observed in Indigenous participants is unlikely due to increases in HDL surface area and indicates a quantitative enrichment of apoE and apoCIII. This

differs from what is observed after CETP inhibition.³⁷ ApoE- and/or apoCIII-containing HDL represent approximately 7% of total HDL and a previous report indicated that they are more abundant in obese participants compared to healthy control participants.¹¹ In our study, the presence of obesity or T2DM were both associated with increased enrichment of HDL with apoE and apoCIII. However, the fact that HDL-apoE and apoCIII content were increased in Indigenous participants without T2DM and after adjustment for TG, BMI, and T2DM (Table 3) suggests other factors also contribute to HDL remodeling in this population. It should be noted that HDL containing apoCIII is associated with higher cardiovascular risk,^{13,14} and increased levels of HDL-apoCIII are observed in patients with coronary disease.^{12,38} Although HDL containing apoE is reportedly associated with lower CVD risk,^{38,39} HDL enrichment with apoE is not necessarily protective. HDL containing apoE in healthy participants is rapidly cleared and can stimulate RCT, however, the presence of apoCIII on HDL containing apoE attenuates its protective effects,¹³ just as the presence of apoCIII on HDL abolishes HDL-mediated anti-inflammatory and antiapoptotic actions.³⁸

The overall assessment of CEC combining transporter-independent and transporter-specific CEC could obscure more subtle effects of HDL subpopulations which have opposite effects on different pathways. We observed decreased transporter-independent CEC in indigenous participants, and that obesity and T2DM were associated with decreased transporter-independent CEC in the non-Indigenous group. Decreased transporter-independent CEC was predictably explained by lower HDL concentration (and HDL particle number), and support that impaired transporter-independent CEC is a feature of an adverse metabolic phenotype. As transporter-independent CEC was strongly related to the concentration of HDL particles (Table 6), therapeutics increasing circulating HDL levels, especially larger HDL particle concentrations, may be beneficial in populations with decreased transporter-independent CEC. ABCG1-specific efflux was more weakly correlated with the concentration of large HDL, and might also increase under conditions of increased HDL particle concentration. Unexpectedly, we observed higher ABCA1-specific efflux in Indigenous Australians after adjusting for HDL particle number, especially in participants with $\text{BMI} \geq 25 \text{ kg/m}^2$. Increased CEC, especially ABCA1-specific efflux has previously been observed in other cohorts at high risk of CVD indicating that increased ABCA1-specific efflux is not unique to Indigenous Australians.^{21-23,25} Increased ABCA1-dependent efflux in some of these studies has been linked to increased levels of pre β - or small HDL particle concentrations.^{22,25} In mice, a positive correlation between ABCA1-specific efflux and HDL-apoE content is observed.⁴⁰ An inverse relationship between CEC and HDL-apoCIII has been reported in patients with coronary artery disease,¹² noting that in this study CEC was measured as total cholesterol efflux, which combines transporter-independent, ABCA1- and ABCG1-specific efflux, making direct comparisons between studies difficult. Our finding that HDL con-

taining apoE and/or apoCIII is associated with increased ABCA1-mediated CEC in Indigenous participants may help explain why ABCA1-CEC correlates with adverse metabolic profiles in some populations. The positive correlation between small HDL particle concentration and CEC in Indigenous subjects but not in non-Indigenous subjects, suggests that the small HDL species are compositionally and functionally distinct. As increased ABCA1-specific CEC in Indigenous Australians was incompletely explained by clinical or biochemical parameters there are likely to be other unidentified factors contributing to increased ABCA1-specific efflux in this population.

Our results support contemporary studies indicating the functional and biochemical heterogeneity of HDL sub-species, and identify considerable changes to HDL size, composition and function in Indigenous Australians. Extension of these studies will help uncover novel biomarkers for risk prediction and may identify novel targets for early intervention in our most vulnerable populations.

CRedit authorship contribution statement

Maaïke Kockx: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jeffrey Wang:** Writing – review & editing, Formal analysis, Data curation. **Natasha J. Howard:** Writing – review & editing, Project administration, Investigation, Data curation. **Avinash Suryawanshi:** Writing – review & editing, Resources, Data curation, Conceptualization. **Stephen J. Nicholls:** Writing – review & editing, Conceptualization. **Alex Brown:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Leonard Kritharides:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Ethical approval

This study was conducted according to the principles of the Declaration of Helsinki for experiments involving humans. Non-Indigenous participants were recruited with approval of the Sydney Local Health District Human Research Ethics Committee (2019/ETH11885) whilst Indigenous participants were from the PROPHECY Study (approved by Aboriginal Health Research Ethics Committee South Australia, AHREC 04-13-545). Written informed consent was obtained from all participants.

Funding

The PROPHECY Cohort Study was supported by funding from NHMRC Project Grant ([APP1086386](#)), NHMRC Clin-

ical Trials and Cohort Grant ([APP2006883](#)), MRFF Indigenous Health Round ([MRFF APP1200005](#)) and The Hospital Research Foundation (S-01-RSRCH). AB was supported by a NHMRC Senior Research Fellowship ([APP1137563](#)) and by a Viertel Senior Research Fellowship. The Multi-omic signatures of CVD in Aboriginal Australians (OSCA) Study was supported by the Australian Stroke and Heart Research Accelerator ASHRA Centre (ASHRA). ASHRA was established through MRFF funding from the Australian Government's Targeted Translation Research Accelerator program, delivered by MTPConnect. LK was supported by a NSW Health CVD Clinician Scientist grant.

Declaration of competing interest

LK has participated in clinical trials sponsored by Amgen and Novartis; he has given lectures and received consulting fees from Seqiris (CSL), Amgen and Novartis. SJN has received research support from Amgen, Anthera Pharmaceuticals, AstraZeneca, Cerenis Therapeutics, CSL Behring, Eli Lilly and Company, Esperion Therapeutics, Infraredx, New Amsterdam Pharma, Novartis, Resverlogix, and Sanofi Regeneron; and is a consultant for Akcea Therapeutics, Amgen, AstraZeneca, Boehringer Ingelheim, CSL Behring, Eli Lilly and Company, Esperion Therapeutics, Kowa Pharmaceuticals, Merck, Novo Nordisk, Pfizer, Sanofi Regeneron, Daichii Sankyo, Vaxxinity, Cyclarity, CSL Sequirus and Takeda. There are no competing interests for other authors.

Acknowledgments

We acknowledge Aboriginal people as the traditional custodians of the lands and waters on which this work was conducted and pay respect to their commitment, guidance, leadership and sharing of knowledge to strengthen and enable this project. The authors gratefully acknowledge the generosity and support of PROPHECY (Aboriginal Diabetes Study) participants, partner organizations and participating health and community services across Aboriginal communities of South Australia. This work was managed from Kaurua Country (Adelaide), but was conducted across the traditional lands of many Aboriginal peoples throughout South Australia. Study data and biospecimens are cared for within the Wardlipringga Aboriginal Health Equity Research Group at the South Australian Health and Medical Research Institute based on Kaurua Country. We acknowledge the guidance and wisdom of all members of the South Australian Aboriginal Governance Committee who have supported the PROPHECY Study throughout its development, planning, implementation and translation. This study would not have been possible without the tireless efforts of a large team of community-based, field, clinical and laboratory staff, Aboriginal and non-Aboriginal, who have contributed to the PROPHECY study. We thank Dr Raaj Kishore Biswas from the Sydney Local Health District for assistance with statisti-

cal analysis and Dr Margery Connelly for helpful advice and discussion on the nuclear magnetic resonance analysis.

Data statement

The data in this article are presented in the tables and supplementary data. Dr Maaïke Kockx had full access to all the data in the study and takes responsibility for its integrity and the data analysis. Additional data are available upon reasonable request from the corresponding author L.K. (Atherosclerosis Laboratory, The ANZAC Research Institute, Concord Repatriation General Hospital, and the University of Sydney, Sydney, Australia leonard.kritharides@sydney.edu.au).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jacl.2025.08.006](https://doi.org/10.1016/j.jacl.2025.08.006).

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