

# Cholesterol efflux capacity of high-density lipoprotein was not associated with cognitive decline and brain structures in older people with diabetes mellitus

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## Keywords

Cholesterol efflux capacity, Dementia, High-density lipoprotein cholesterol

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## Clinical Trial Registry

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## ABSTRACT

**Aims/Introduction:** To examine the association between cholesterol efflux capacity (CEC) of serum high-density lipoprotein (HDL) and cognitive function and brain structures in older people with diabetes mellitus.

**Materials and Methods:** Participants of a randomized placebo-controlled trial of 27-month vitamin B<sub>12</sub> supplementation in older people with diabetes mellitus, which showed no effect on cognition, were further followed up at month 72. Cognitive tests included the Clinical Dementia Rating scale, Neuropsychological Test Battery in memory, executive function and psychomotor speed. Brain magnetic resonance imaging scans were carried out in a subset at baseline, month 27 and month 45. Fasting serum at baseline, month 9, month 27 and month 72 were analyzed for adenosine triphosphate-binding cassette transporter A1-mediated CEC of HDL and apolipoprotein A1 (ApoA1).

**Results:** Serum HDL cholesterol at baseline was associated with better executive and memory function at follow up. Serum ApoA1 was associated with a better memory Z-score at month 18. Serum CEC and ApoA1 were not associated with Clinical Dementia Rating scale, Neuropsychological Test Battery, hippocampal volume and white matter disease on magnetic resonance imaging at baseline and whole brain atrophy rates. They were also not associated with cognitive function at month 27 and 72 on multilevel modeling. CEC and ApoA1 decreased significantly from baseline to month 27. Faster decliners in CEC had a greater increase in brain peak width of skeletonized mean diffusivity.

**Conclusions:** Higher serum HDL cholesterol was associated with more favorable changes in memory and executive function in older people with diabetes mellitus. However, this was not due to CEC or ApoA1. A decline in CEC was associated with small vessel disease in the brain.

## INTRODUCTION

Older people with diabetes mellitus are at greater risk of cognitive decline and dementia<sup>1</sup>, because of the association of diabetes mellitus with cerebrovascular disease, white matter brain changes and Alzheimer's disease (AD)<sup>2–4</sup>.

There is epidemiological evidence that executive function is more impaired when AD co-exists with diabetes mellitus<sup>5</sup>. This renders older people with diabetes mellitus more susceptible to the disabling effects of AD. Furthermore, executive dysfunction might also hamper chronic disease management, leading to poor diabetic control<sup>6</sup>, and thus a downward spiral to poor health and dementia.

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Diabetes mellitus is known to impair the function of high-density lipoprotein (HDL), and serum HDL cholesterol (HDL-C) is lower in people with diabetes mellitus. Apart from the well-established anti-atherosclerosis effects of HDL<sup>7,8</sup>, there is an epidemiological link between lower serum HDL-C and dementia. In a population study in the Netherlands, older people with dementia had lower serum HDL-C and lower serum HDL-C was associated with a higher incidence of dementia (odds ratio 2.3, 95% confidence interval 1.2–4.3)<sup>9</sup>. In our previous negative trial of vitamin B<sub>12</sub> supplementation in older people with diabetes mellitus, higher baseline serum HDL-C was associated with less decline in executive function over a period of 2 years<sup>10</sup>.

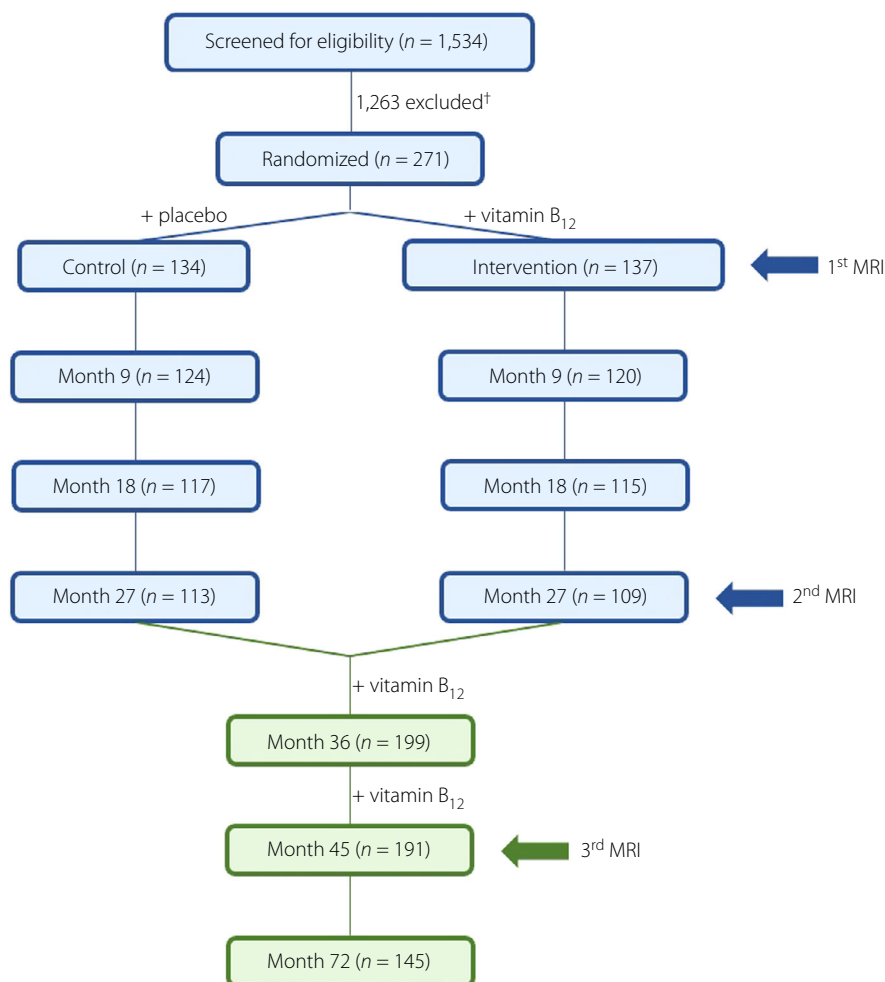
Although it is generally thought that there is no net transfer of cholesterol from the periphery into the central nervous system because of the blood–brain barrier, plasma HDL might affect the blood–brain barrier probably through its effects on endothelial function<sup>11</sup>. Serum HDL-C is a crude marker of HDL function. More direct measurements of HDL function

have been devised. The most well-validated method is cholesterol efflux capacity of HDL. This has been shown to have a robust association with cardiovascular outcomes<sup>12</sup>.

We further followed up the older individuals with diabetes mellitus who participated in the aforementioned randomized trial of vitamin B<sub>12</sub> supplementation. The objective was to ascertain if serum HDL function, as estimated by cholesterol efflux capacity, was associated with a lower risk of global cognitive decline and neurodegenerative changes in the brain over the 6-year period.

## MATERIALS AND METHODS

Participants of the present study were recruited from our previous randomized placebo-controlled trial of vitamin B<sub>12</sub> supplementation for 27 months to prevent cognitive decline in older people with diabetes mellitus and mild vitamin B<sub>12</sub> deficiency<sup>10,13</sup>, and they were followed up at month 72 (shown in Figure 1). The study protocol for the first 27-month period has been described elsewhere<sup>13</sup>. Briefly, a total of 271 participants



**Figure 1** | Participant flow of the randomized placebo-controlled trial (in blue color) plus the open-label extension study (in green color)<sup>†</sup>. MRI, magnetic resonance imaging.<sup>†</sup>Refer to our previous report<sup>13</sup> for a more detailed description during 0–27 months.

with diabetes aged  $\geq 70$  years with borderline low plasma vitamin B<sub>12</sub> 150–300 pmol/L were randomly assigned to either the placebo or active group (methylcobalamin 1,000  $\mu\text{g}/\text{day}$ ) for 27 months<sup>13</sup>. Those with a clinical diagnosis of dementia, clinical depression, disabling stroke, renal failure, peripheral neuropathy or anemia were excluded. During the 27-month randomized period, all study participants were followed up and had cognitive assessment repeated at 9-monthly intervals. A total of 222 participants (81.9%) completed the randomized trial. All of them received open-label oral methylcobalamin (1,000  $\mu\text{g}/\text{day}$ ) for another 18 months, and were followed up at months 36 and 45.

Cognitive function tests including the Clinical Dementia Rating scale (CDR) and Neuropsychological Test Battery (NTB) in memory, executive function, and psychomotor speed<sup>13</sup> were applied to all participant at each follow up. The CDR is a clinician-rated scale based on a semi-structured interview of both participants and informants; it covers six domains of cognitive functioning: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care, and reflects a clinical impression of global cognitive and functional impairment<sup>14</sup>. A family member or a caregiver with close contact with the participant also needs to complete the interview CDR rating ranges from 0 (normal) to 3 (severe dementia). The CDR-global score (CDR-global), computed by an algorithm, ranges from 0 to 3, with 0 indicating normal cognition, 0.5 indicating 'questionable dementia' or 'mild cognitive impairment' and  $\geq 1$  indicating 'clinical dementia'. The CDR sum of boxes score, ranging from 0 to 18, was obtained by summing each of the domain box scores. NTB included International Shopping List Test and Continuous Paired Associates Learning for memory; Controlled Oral Word Association Test and Category Fluency Test for executive function; and the simple reaction time test and choice reaction time test for psychomotor speed<sup>13</sup>. The score of each cognitive test was standardized to the Z-score, as previously suggested<sup>13</sup>, with higher scores reflecting better performance. For the Continuous Paired Associates Learning, simple reaction time test and choice reaction time tests, where higher scores showed poorer performance, the sign was reversed when calculating Z-scores. The domain Z-scores were the mean of the two tests in each domain<sup>13</sup>.

Brain volumetric magnetic resonance imaging (MRI) scans were carried out in a subset of 176 participants at baseline with a 1.5-T magnetic resonance system (Sonata; Siemens Medical Solutions, Erlangen, Germany) at Prince of Wales Hospital, Hong Kong, China. The MRI protocol included high-resolution T1-weighted acquisition, gradient echo (FLASH-Fast Low angle shot) 3-D acquisition with 1-mm isotropic voxels Flip angle 19° TR = 12 ms TE = 5.65 ms; 208 slices per slab with one slab acquired in coronal orientation, one average. A total of 70 participants had additional sequence for diffusion tensor imaging. Brain peak width of skeletonized mean diffusivity (PSMD) was computed from diffusion tensor imaging data following the

standard PSMD pipeline ([www.psm-d-marker.com](http://www.psm-d-marker.com))<sup>15</sup>. The scan was repeated at months 27 and 45. The serial MRI scans were analyzed for the annual rate of whole brain atrophy by SIENA<sup>16,17</sup>, and for hippocampal volume and white matter density (WMH) by 'ACCUBRAIN'<sup>18</sup>.

Fasting serum samples at baseline, and months 9, 27 and 72 were analyzed for adenosine -binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux capacity (CEC) of HDL and apolipoprotein A1 (ApoA1). Briefly, HDL efflux capacity was measured in apolipoprotein B (ApoB)-depleted serum after removing ApoB-containing lipoproteins by polyethylene glycol precipitation. RAW264.7 mouse macrophages (ATCC, Manassas, VA, USA) were seeded at 70,000 cells/well in 24-multiwell plates. Cells were maintained in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum and antibiotics in 5% CO<sub>2</sub> for 2 days to reach 70–80% confluence. Macrophages were then labeled for 24 h with 1  $\mu\text{Ci}/\text{mL}$  of [3H]-cholesterol in the presence of 5.0% fetal bovine serum. To upregulate ABCA1 in RAW264.7 cells, 0.3 mmol/L cyclic adenosine monophosphate (Sigma, St. Louis, MO, USA) in 0.2% bovine serum albumin/Dulbecco's modified Eagle's medium was added to the cell culture and incubated for another 16 h. Cells were then washed once with phosphate buffer before the addition of 2.5% ApoB-depleted serum as HDL fraction for 4 h incubation. Media were finally removed and cells were lysed in 0.1 mol/L NaOH. Samples of both cells and media were counted by liquid scintillation for radioactivity. The efflux of [3H]-cholesterol was calculated as the percentage of radiolabel in the media compared with that present in the media plus cells. Background efflux (measured in the absence of ApoB-depleted serum) was subtracted in all experiments.

### Statistical analysis

All statistical analyses were carried out using SPSS 26.0 (IBM Corp., Armonk, NY, USA). Log-transformation was used as appropriate when data were with skewed distribution (e.g., triglycerides, homocysteine). Independent-samples *t*-test (for continuous variables) and the  $\chi^2$ -test (for categorical variables) were used to compare the baseline difference between groups in descriptive characteristics and cognitive performance. The effect of CEC or ApoA1 on cognitive decline and brain atrophy or other MRI-based measures were examined by a general linear regression model with adjusting for age, sex, education years (for cognitive outcomes only) and group assignment. Partial correlation was applied for correlation analysis of cognitive decline and brain atrophy rate with adjustments. All statistical tests were two-sided, and significance was defined as a *P*-value  $< 0.05$ .

### RESULTS

As shown in Figure 1, 145 out of 271 (52.0%) older diabetes patients, who had participated in the randomized trial of vitamin B<sub>12</sub> supplementation, were further followed up at month 72. The reasons for dropouts were refusal (78), death

(38), frailty (6) and loss of contact (4). The 126 participants that were lost to follow-up at month 72 were of older age than those who completed the study, and they were less educated and had relatively worse cognitive function (Table 1). These differences were well considered in followed analyses. Brain volumetric MRI scans were carried out in a subset of 171 participants at baseline. Vitamin B<sub>12</sub> supplementation had no significant effect on CEC ( $P = 0.278$ ,  $0.964$  and  $0.260$ , respectively) or ApoA1 ( $P = 0.309$ ,  $0.785$  and  $0.957$ , respectively) at

months 9, 27 and 72, and our previous study showed no significant effect of vitamin B<sub>12</sub> supplementation on cognitive decline<sup>13</sup>. All subjects in the present study were, therefore, included as a whole in the analysis, and adjustment was made for random group assignment.

As shown in Table 2, serum HDL-C at baseline was associated with better executive function at 18, 27, 36 and 45 months ( $P = 0.014$ ,  $<0.001$ ,  $0.001$  and  $0.039$ , respectively), and also better memory function at 9, 27 and 36 months ( $P = 0.042$ ,  $0.012$

**Table 1** | Comparisons of baseline characteristics between trial participants followed up at month 72 and lost to follow up

	Completed follow-up ( <i>n</i> = 145)	Lost to follow up ( <i>n</i> = 126)	<i>P</i> -value
Demographic			
Age (years)	74.32 ± 3.74	76.20 ± 4.15	<0.001*
Female, <i>n</i> (%)	54 (37.2%)	59 (46.8%)	0.110
Education (years)	6.62 ± 4.66	4.70 ± 4.09	<0.001*
Ever smoking, <i>n</i> (%)	50 (37.3%)	35 (36.8%)	0.942
DM duration (≥10 years), <i>n</i> (%)	112 (78.9%)	98 (78.4%)	0.925
HBP (%)	125 (86.2%)	108 (85.7%)	0.907
Stroke, <i>n</i> (%)	5 (3.4%)	13 (10.3%)	0.024*
ApoE4 carriers, <i>n</i> (%)	23 (15.9%)	23 (18.4%)	0.580
Drug use			
Aspirin, <i>n</i> (%)	32 (22.1%)	35 (27.8%)	0.277
Insulin, <i>n</i> (%)	17 (11.7%)	13 (10.3%)	0.713
Metformin, <i>n</i> (%)	124 (85.5%)	106 (84.1%)	0.750
ACEI/ARB, <i>n</i> (%)	93 (64.1%)	81 (64.3%)	0.980
Statin, <i>n</i> (%)	83 (57.2%)	63 (50.0%)	0.233
Blood biochemistry			
HbA <sub>1c</sub> (%)	7.06 ± 0.95	7.17 ± 0.93	0.351
Triglyceride <sup>†</sup> (g/L)	1.10 (0.90, 1.60)	1.20 (0.90, 1.60)	0.212
Total-C (mmol/L)	4.25 ± 0.79	4.23 ± 0.84	0.816
LDL-C (mmol/L)	2.32 ± 0.65	2.33 ± 0.72	0.926
HDL-C (mmol/L)	1.33 ± 0.30	1.25 ± 0.33	0.062
Non-HDL-C (mmol/L)	2.90 ± 0.78	2.98 ± 0.81	0.509
Creatinine (μmol/L)	87.83 ± 22.18	92.03 ± 27.90	0.169
ACR (mg/mmol)	11.39 ± 19.97	18.75 ± 39.15	0.116
Hemoglobin (g/dL)	13.20 ± 1.30	13.02 ± 1.26	0.263
MCV (fL)	89.61 ± 8.13	90.12 ± 6.68	0.579
Cognitive function			
MMSE (max 30)	26.01 ± 3.26	24.43 ± 3.74	<0.001*
GDS (max 15)	3.13 ± 2.26	3.68 ± 2.34	0.050*
CDR-global = 0.5, <i>n</i> (%)	51 (35.2%)	68 (54.0%)	0.002*
CDR-SOB	0.74 ± 0.73	1.01 ± 0.85	0.006*
NTB <sup>‡</sup>			
Executive function	0.19 ± 0.83	-0.21 ± 0.85	<0.001*
Psychomotor speed	0.15 ± 0.90	-0.14 ± 0.81	0.009*
Memory	0.18 ± 0.78	-0.19 ± 0.79	<0.001*

Data were shown as mean ± standard deviation, median (quartile 1, quartile 3) or *n* (%), as appropriate. \* $P$ -value <0.05. <sup>†</sup>Use log-transformed data for comparison. <sup>‡</sup>Z-scores, as compared with the mean of all participants; higher score indicating better performance. ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; ACR, urine albumin-to-creatinine ratio (mg/mmol); ApoE, apolipoprotein E; CDR-global, Clinical Dementia Rating scale global score; CDR-SOB, CDR sum of boxes score; DM, diabetes mellitus; GDS, Geriatric Depression Scale; HbA<sub>1c</sub>, glycosylated hemoglobin; HBP, high blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MCV, mean corpuscular volume; MMSE, Mini-Mental State Examination; non-HDL-C, non-high-density lipoprotein cholesterol; NTB, Neuropsychological Test Battery; Total-C, total cholesterol.

**Table 2** | Baseline level of serum high-density lipoprotein cholesterol, adenosine-binding cassette transporter A1-mediated cholesterol efflux capacity and apolipoprotein A1 with cognitive function over 72 months

Cognitive function	Time point	HDL-C		CEC		ApoA1		
		$\beta \pm SE$	<i>P</i> -value	$\beta \pm SE$	<i>P</i> -value	$\beta \pm SE$	<i>P</i> -value	
CDR-SOB	Month 9	-0.182 ± 0.103	0.080	0.005 ± 0.004	0.250	-0.002 ± 0.001	0.074	
	Month 18	0.111 ± 0.177	0.533	-0.006 ± 0.007	0.405	0.000 ± 0.002	0.985	
	Month 27	-0.134 ± 0.181	0.460	-0.003 ± 0.007	0.663	-0.002 ± 0.002	0.321	
	Month 36	-0.114 ± 0.235	0.628	0.007 ± 0.009	0.427	-0.001 ± 0.002	0.686	
	Month 45	-0.350 ± 0.233	0.135	0.011 ± 0.009	0.221	-0.001 ± 0.002	0.697	
	Month 72	-0.633 ± 0.574	0.273	0.017 ± 0.021	0.429	-0.002 ± 0.006	0.786	
NTB <sup>†</sup>	Executive function	Month 9	0.170 ± 0.114	0.137	0.009 ± 0.005	0.061	0.001 ± 0.001	0.278
		Month 18	0.330 ± 0.134	0.014*	0.006 ± 0.006	0.272	0.003 ± 0.001	0.020*
		Month 27	0.444 ± 0.124	<0.001*	0.004 ± 0.005	0.488	0.004 ± 0.001	0.001*
		Month 36	0.496 ± 0.141	0.001*	0.002 ± 0.006	0.712	0.005 ± 0.001	0.002*
		Month 45	0.323 ± 0.156	0.039*	0.006 ± 0.006	0.328	0.003 ± 0.002	0.083
		Month 72	0.009 ± 0.212	0.966	0.006 ± 0.008	0.457	0.000 ± 0.002	0.874
	Memory	Month 9	0.283 ± 0.138	0.042*	0.003 ± 0.006	0.621	0.003 ± 0.001	0.032*
		Month 18	0.266 ± 0.136	0.052	-0.002 ± 0.006	0.722	0.005 ± 0.001	<0.001*
		Month 27	0.384 ± 0.151	0.012*	0.000 ± 0.006	0.981	0.003 ± 0.002	0.052
		Month 36	0.374 ± 0.165	0.025*	0.011 ± 0.007	0.107	0.004 ± 0.002	0.013*
		Month 45	0.292 ± 0.164	0.077	0.002 ± 0.007	0.758	0.003 ± 0.002	0.099
		Month 72	0.386 ± 0.209	0.067	0.006 ± 0.008	0.464	0.003 ± 0.002	0.180
Psychomotor speed	Month 9	0.189 ± 0.167	0.261	0.005 ± 0.007	0.530	-0.001 ± 0.002	0.589	
	Month 18	0.317 ± 0.198	0.111	-0.003 ± 0.008	0.710	0.001 ± 0.002	0.743	
	Month 27	0.268 ± 0.186	0.151	0.009 ± 0.008	0.254	0.000 ± 0.002	0.830	
	Month 36	0.737 ± 0.238	0.002*	0.000 ± 0.010	0.959	0.004 ± 0.003	0.108	
	Month 45	0.609 ± 0.255	0.018*	0.000 ± 0.010	0.999	0.003 ± 0.002	0.287	
	Month 72	0.051 ± 0.277	0.855	-0.011 ± 0.011	0.323	0.001 ± 0.003	0.655	

\**P*-value <0.05. †*Z*-scores, as compared with the mean of all participants; higher score indicating better performance. ApoA1, apolipoprotein A1; CDR-SOB, Clinical Dementia Rating scale sum of boxes score; CEC, cholesterol efflux capacity; HDL-C, high-density lipoprotein cholesterol; NTB, Neuropsychological Test Battery.

and 0.025, respectively) and of borderline significance at 18 months (*P* = 0.052). Similarly, serum ApoA1 was associated with better executive function at 18, 27 and 36 months (*P* = 0.020, 0.001 and 0.002, respectively), and also better memory function at 9, 18 and 36 months (*P* = 0.032, <0.001 and 0.013, respectively) and of borderline significance at 27 months (*P* = 0.052). Neither serum HDL-C nor ApoA1 was associated with whole brain atrophy rates, hippocampal volume, WMH or PSMD (data not shown).

Cholesterol efflux capacity correlated significantly with female sex, serum HDL-C, ApoA1 and sodium, and mean corpuscular volume (*R*<sup>2</sup> = 0.256, 0.177, 0.253, 0.204, -0.168; *P* < 0.01). CEC was not associated with cognitive function, hippocampal volume and white matter disease (i.e., WMH, PSMD) at baseline.

The correlations between baseline CEC and those at follow up were significant, but weak (correlation coefficients 0.219, 0.195 and 0.165, and *P* < 0.001, *P* = 0.004 and 0.049 at months 9, 27 and 72, respectively). As shown in Table 3, CEC declined significantly in the first 27 months (-7.46 ± SD 8.15%

**Table 3** | Serum level high-density lipoprotein cholesterol, adenosine-binding cassette transporter A1-mediated cholesterol efflux capacity and apolipoprotein A1 over 72 months

Time point	HDL-C (mmol/L)	CEC (%)	ApoA1 (mg/dL)
Baseline	1.29 ± 0.32	30.5 ± 7.2	160.2 ± 29.9
Month 9	1.32 ± 0.37	23.0 ± 5.5	155.8 ± 29.8
Month 27	1.29 ± 0.36	17.0 ± 4.4	153.9 ± 27.0
Month 72	1.34 ± 0.36	20.8 ± 6.2	151.6 ± 25.8
<i>P</i> -value	>0.05	<0.001* <sup>†</sup>	<0.001*

\**P*-value <0.05. †Not for cholesterol efflux capacity (CEC) between month 27 and 72. ApoA1, apolipoprotein A1; HDL-C, high-density lipoprotein cholesterol.

at month 9; -13.11 ± 7.91% at month 27; *P* < 0.0001 on paired *t*-test), but not between month 27 and month 72. In contrast, serum ApoA1 decreased significantly at each follow up (-5.16 ± 28.57; -7.47 ± 23.60; -11.40 ± 24.50 mg/L at months 9, 27 and 72, respectively; *P* < 0.0001 on paired *t*-test),

but serum HDL-C did not change significantly at follow up. No clinical factors at baseline correlated significantly with CEC decline in the first 27 months.

To examine the potential association between CEC and changes in cognitive function and brain structures in the first 27 months, all participants were categorized by tertiles of CEC at baseline. CEC tertiles were not significantly associated with CDR-global score or sum of boxes score, executive and memory function, and psychomotor speed, hippocampal volume, WMH, PSMD and whole brain atrophy rate in the first 27 months. Multilevel modeling of data at baseline, month 27 and month 72 showed that neither CEC nor ApoA1 was associated with cognitive function.

The fast decliners in CEC ( $-12.5\%$  or more), when compared with slower decliners in the first 27 months, had a significantly greater increase in PSMD ( $0.641 \pm 0.313$  vs  $-0.314 \pm 0.281$ ;  $P = 0.025$ ). There were no significant group differences in changes in cognitive function, whole brain atrophy rate, WMH, serum ApoA1 or HDL-C.

## DISCUSSION

The present study showed that higher serum HDL-C was associated with more favorable changes in executive and memory function in older people with diabetes mellitus. HDL function, as estimated by ABCA1-mediated CEC and serum ApoA1, declined significantly with time in older people with diabetes mellitus. However, CEC was not significantly associated with cognitive function and brain structures both cross-sectionally and prospectively. Those with faster decline in CEC in the first 27 months, when compared with those with stable CEC, had greater increases in small vessel disease, as estimated by PSMD on MRI. However, this was not associated with other changes in brain structures and cognitive function.

The present more in-depth analysis showed that higher serum HDL-C was associated with more favorable changes in memory, as well as executive function, in older people with diabetes mellitus over a 6-year period. This is consistent with the previously reported association between higher serum HDL-C and lower incidence of dementia in older people<sup>10,19,20</sup>. It is noteworthy that serum HDL-C was not significantly associated with brain atrophy and CDR, suggesting that HDL might not protect against Alzheimer's disease.

ABCA1-mediated CEC is an important function of HDL and has been associated with cardiovascular outcomes<sup>12</sup>, and ApoA1 is an important lipoprotein component of HDL which influences its ABCA1-mediated CEC. Systemic HDL cannot cross the blood-brain barrier, but its well-documented protective effects against atherosclerosis might improve cognitive function by preserving blood-brain barrier function<sup>21,22</sup>. That is why we hypothesized that the CEC of serum HDL-C might be associated with cognitive function and brain structures in older people with diabetes mellitus. The results of the present analysis showed that CEC of HDL or serum ApoA1 could not account for the apparent cognitive benefits of higher serum HDL-C in

older people with diabetes mellitus. HDL has many lipid and protein components with functions other than reverse cholesterol transport (e.g., anti-oxidative, anti-inflammatory effects). Future studies might examine the effects of other HDL functions on cognitive decline in older people with diabetes mellitus.

It is important to point out that both CEC and ApoA1 decreased significantly with time in this older cohort with diabetes mellitus. There is a cross-sectional association between diabetes mellitus and the ABCA1-mediated CEC of the small HDL fraction<sup>23</sup>, and CEC predicted incident diabetes mellitus among renal transplant recipients<sup>24</sup>. More studies are required to confirm that diabetes mellitus is associated with a greater decline in CEC, as aging itself is also associated with decline in ABCA1-mediated CEC<sup>25</sup>. If confirmed, a decline in CEC might account for the propensity to cardiovascular events in diabetes mellitus patients.

Furthermore, the present study suggested that the decline in CEC might lead to an increase in small vessel disease in the brain, which could lead to vascular dementia and unsteady gait in older people<sup>26</sup>. Cholesterol ester transfer protein inhibitor has been shown to improve CEC in ischemic heart disease patients<sup>27</sup>. Although a trial of cholesterol ester transfer protein inhibitor has failed to prevent cardiovascular events<sup>28</sup>, this class of drugs might potentially be useful in slowing the progression of small vessel disease in the brain.

Relative to CEC and ApoA1, serum HDL-C was a relatively stable trait. It has been known that 40–60% of variations in serum HDL-C is due to genetic factors<sup>29</sup>. It would be interesting to explore if genetic factors of serum HDL-C are associated with cognitive decline in older people with diabetes mellitus. In addition, HDL is rich in phospholipids, which have important influences on AD, and variations in serum HDL-C might be accompanied by structural and qualitative changes in the lipid contents of HDL<sup>30</sup>. More in-depth analysis of the phospholipid contents of HDL might help to explain why serum HDL-C could predict cognitive decline in older people with diabetes mellitus<sup>31</sup>.

Diabetes mellitus is associated with lower serum HDL-C, but its effect on CEC has not been investigated. There is recent evidence that diabetes mellitus only affects the ABCA1-mediated CEC of the small HDL fraction<sup>23</sup>. Future studies on the biochemical, lifestyle or clinical factors of CEC decline in older people with diabetes mellitus might shed light on potential interventions to prevent small vessel brain disease, which is associated with stroke and dementia in older people with diabetes mellitus. Subgroup analysis on the small HDL fraction might provide additional insights.

The strength of the present study was the serial measurements of ABCA1-mediated CEC, detailed cognitive function and MRI data on multiple time points over a 6-year period. The limitations were the dropout of participants with time, introducing survival bias. ABCA1-mediated CEC is an important pathway in reverse cholesterol transport. However, there

are other pathways for example, ABCG1<sup>32</sup>, which have not been measured in the present study.

We concluded that higher serum HDL-C was associated with more favorable changes in executive and memory function in older people with diabetes mellitus. However, HDL function, as estimated by ABCA1-mediated CEC, was not significantly associated with cognitive function or structural brain changes in older people with diabetes mellitus. Those with more rapid decline in CEC had a greater increase in small vessel brain disease.

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## DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The trial was approved by the medical ethics committee of Chinese University of Hong Kong and New Territories East Cluster of Hospital Authority of Hong Kong.

Informed consent: All study participants provided informed consent.

Registry and the registration no. of the study/trial: This trial was registered at the Clinical trial registry of the US ([ClinicalTrials.gov](https://clinicaltrials.gov): NCT02457507) on 29 May 2015.

Animal studies: N/A.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1** | Comparisons of baseline characteristics between fast and slow decliners of adenosine triphosphate-binding cassette transporter A-mediated cholesterol efflux capacity.