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Total HDL cholesterol efflux capacity in healthy children – Associations with adiposity and dietary intakes of mother and child

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Abstract *Background and aims:* High-density lipoprotein (HDL) cholesterol efflux capacity in adults may be a measure of the atheroprotective property of HDL. Little however, is known about HDL cholesterol efflux capacity in childhood. We aimed to investigate the relationship between HDL cholesterol efflux capacity and childhood anthropometrics in a longitudinal study.
Methods and results: Seventy-five children (mean age = 9.4 ± 0.4 years) were followed from birth until the age of 9 years. HDL cholesterol efflux capacity was determined at age 9 by incubating serum-derived HDL-supernatants with ^{3}H-cholesterol labeled J774 macrophages and percentage efflux determined. Mothers provided dietary information by completing food frequency questionnaires in early pregnancy and then 5 years later on behalf of themselves and their children. Pearson's correlations and multiple regression analyses were conducted to confirm independent associations with HDL efflux.

There was a negative correlation between HDL cholesterol efflux capacity and waist circumference at age 5 ($r = -0.3$, $p = 0.01$) and age 9 ($r = -0.24$, $p = 0.04$) and BMI at age 5 ($r = -0.45$, $p = 0.01$) and age 9 ($r = -0.19$, $p = 0.1$). Multiple regression analysis showed that BMI at age 5 remained significantly associated with reduced HDL cholesterol efflux capacity ($r = -0.45$, $p < 0.001$).

HDL-C was negatively correlated with energy-adjusted fat intake ($r = -0.24$, $p = 0.04$) and positively correlated with energy-adjusted protein ($r = 0.24$, $p = 0.04$) and starch ($r = 0.29$, $p = 0.01$) intakes during pregnancy. HDL-C was not significantly correlated with children dietary intake at age 5. There were no significant correlations between maternal or children dietary intake and HDL cholesterol efflux capacity.
Conclusions: This novel analysis shows that efflux capacity is negatively associated with adiposity in early childhood independent of HDL-C.

Introduction

HDL-cholesterol (C) concentrations are inversely associated with cardiovascular disease independent of LDL-C [1–4]. Many properties of HDL contribute to resisting the development of atherosclerosis; it possesses anti-inflammatory properties [5] and preserves endothelial function [6]. Arguably the most important contribution of HDL to atheroprotection is through the reverse cholesterol transport (RCT) system that was first described by Glomset [7]. RCT is the only method by which cells can effectively reduce their cholesterol content. In RCT, cholesterol is released from cells

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onto HDL particles, which in turn deliver acquired cholesterol to the liver for excretion in the feces [7]. HDL cholesterol efflux capacity refers to the capacity of HDL particles in the blood to extract cholesterol from macrophages.

HDL-C concentrations are among the traditional markers of cardiovascular risk but may be insufficient when considered in isolation. Observational and genetic studies have shown variability in cardiovascular risk within groups that have similar HDL-C levels [8–10]. HDL efflux on the other hand is a stronger predictor of reduced carotid intimal thickness compared to static HDL-C levels [11]. Human studies have asserted that the quality and function of HDL particles are more predictive factors of cardiovascular disease, diabetes, and pro-inflammatory conditions [12–17].

There has been a growing interest in identifying determinants of efflux capacity [18]. Adolescent studies have emphasized the implications of altered HDL biology in states of metabolic significance such as dyslipidemia and diabetes [19–21]. Given that lipoprotein levels in children track into adulthood [22], it is worthwhile identifying childhood factors that may affect efflux capacity with a view to modify them.

Currently, there is a gap in the literature with regards to efflux capacity in healthy children [23,24]. Population studies have suggested that children's diet may be a factor that explains the variation in lipoprotein profiles between different countries [25]. There have been mixed results with regards to the effects of diet on HDL-C levels in children [26–31], however its effect on HDL cholesterol efflux capacity has yet to be assessed.

The in utero environment is a further factor for consideration, as maternal characteristics during pregnancy, such as obesity and gestational weight gain, are known to predict cardiovascular risk factors in the offspring [32–34]. Few studies have looked at the effect of diet during pregnancy on lipoprotein levels [35–38], while there are no studies that have explored maternal diet in relation to HDL cholesterol efflux capacity.

This study set out to investigate potential predictors of HDL cholesterol efflux capacity in children. The study used data from a cohort of healthy children with longitudinal data available from birth and early and late childhood. The main outcome measure of interest was the HDL cholesterol efflux capacity at age 9. The objectives of the study were to (a) examine the relationship between HDL cholesterol efflux capacity and other biochemical parameters; (b) investigate the impact of children anthropometrics at different time points on HDL cholesterol efflux capacity at age 9; (c) investigate the relation between children's dietary intake at age 5 and HDL cholesterol efflux capacity at age 9; (d) investigate the relation between maternal diet during pregnancy and HDL cholesterol efflux capacity at age 9.

Methods

Participants

This study used longitudinal data from the Lifeways Cross-Generation Cohort Study which was established in 2001. Expectant mothers were recruited through two maternity hospitals and followed up in repeated sweeps until 2012. The design for the study has been described previously [39]. Data from a sub-group of 75 children, who provided blood samples at 9–10 years of age, were used in the current analysis. The children included in this study did not have any metabolic disorders or type 1 diabetes, and their parents reported no personal history of dyslipidemia or myocardial infarction.

Blood samples

Mothers and children gave consent to provide blood samples when the children were 9 years old. Blood was drawn through standard venipuncture by a trained pediatric phlebotomist. Five ml of whole blood was collected in Serum gel Z/2.6 ml tube and spun at 3000 rpm for 15 min at 25 °C. Blood serum was separated through centrifugation at 4 °C. Aliquots of about 200 μl were kept at −80 °C and stored for future analysis.

Cholesterol efflux assay protocol

Total HDL was isolated from serum samples using Polyethylene Glycol (PEG) solution containing 20% PEG 8000 MW (Sigma) in 200 mM glycine buffer, pH 7.4. One hundred parts serum were incubated in 40 parts of PEG solution at room temperature for 20 min. The precipitate was removed by high-speed centrifugation (10,000 rpm, 30 min at 4 °C). Supernatant containing the HDL lipoprotein fraction was then recovered.

J774 macrophages were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Lonza) containing 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin in 5% CO_2 at 37 °C. Cells were plated onto a 96 well plate (70,000 cells/well) and labeled for 24 h with 1 μCi/ml [1,2-^{3}H] Cholesterol (Perkin Elmer) in Roswell Park Memorial Institute (RPMI) (Gibco) in 1% FBS. J774 macrophages were equilibrated in DMEM containing 0.2% BSA and were stimulated with cAMP (0.3 mM) to up-regulate ABCA1 in 0.2% BSA in DMEM for 16 h. Cells were subsequently incubated with Minimum Essential Media (MEM) (Gibco) containing 2.8% of PEG HDL for 4 h. The media was then removed from each well, centrifuged to remove cellular debris, and ^{3}H cholesterol quantified by liquid scintillation counting. Cell lipids were then extracted using isopropanol, and the proportion of ^{3}H cholesterol left in the lipid extract was quantified by liquid scintillation counting. Percentage efflux was determined as ^{3}H cholesterol in media divided by total cellular ^{3}H cholesterol.

All measures were repeated twice, and values were normally distributed according to standard pool measurements of adult serum. Previous studies in adults that have used the same protocol have demonstrated that HDL cholesterol efflux capacity is inversely related to cardiovascular disease [11,40].

Lipoprotein and glucose measurements at age 9

Non-fasting serum samples were analyzed using an automated chemistry analyzer, Randox Daytona (Randox

Laboratories limited, T. Claxton – Sept. 2011). Total cholesterol, HDL-cholesterol, triglycerides and glucose levels were measured. LDL-cholesterol was calculated using the Friedewald formula [41].

Anthropometric measures at birth, age 5, and age 9

Birth data for the children, including birth length and weight, were obtained from hospital medical records. At age 5, children's heights were measured by a trained researcher with a Leicester portable height scale (Chasmors Ltd, London) correct to the nearest 0.1 cm, and weights were measured with a Tanita digital weighing scale Model HD305 (Chasmors Ltd, London) correct to 0.1 kg. Children were dressed in light clothing and asked to remove shoes. Waist circumference was measured with a body tape and attached plastic slider (Chasmors Ltd, London) and was recorded to the nearest 0.1 cm. At age 9, children's height, weight and waist circumference measurements were taken by their general practitioner following the same protocol. BMI for each time point was calculated as the weight in kilograms divided by the square of the height in meters.

Dietary data

Mothers provided dietary information both at baseline and when children were 5 years old. Data was collected using a 147-item validated semi-quantitative food frequency questionnaire (FFQ) developed by the same group at the National Nutrition Surveillance Centre (NNSC), used in the European Prospective Investigation on Cancer (EPIC) study [42]. The FFQ has been validated and extensively used in the Irish national surveys on nutrition (SLAN) [43,44]. At baseline, the questionnaire responses were self-administered by mothers after their first antenatal visit. Mothers were asked to recall their average food and drink consumption since becoming pregnant. Dietary information for children was adapted from a 52-item FFQ used by the UK National Diet and Nutrition Survey (UKNDNS) conducted on 4.5 year old children [45]. At five years follow-up, mothers completed the FFQ reporting their child's food and beverage intake over the previous year. They also completed the adult FFQ reporting their own intakes.

Nutrient and energy intake

Food nutrient values were computed using McCance and Widdowson's food composition tables [46] and FFQ Software version 1.0 ©. This software uses standard food portion sizes for each food item and uses the food frequency data to estimate intakes of the food in grams per day. Standard food composition data are then applied (frequency weight x nutrient content) to estimate the nutrient intake per day [47]. Macronutrients included in the analysis were proteins, fat and carbohydrates. Macronutrients were expressed in absolute amounts and as a percentage of total energy. Skewed crude nutrient data were transformed to natural logarithm values.

Breastfeeding status

In this study, breastfeeding was adjusted for as a potential confounder variable. Breastfeeding status was identified from maternal hospital records (intention to breastfeed) and from self-administered questionnaires at five years follow-up. Mothers who retrospectively reported breastfeeding in the year-5 follow-up and mothers who had missing data at year-5 follow-up but intended to breastfeed at baseline were classified into the "breastfed" status. Mothers who report that they did not breastfeed in the year-5 follow-up, mothers who had missing data at year-5 but reported at baseline as having no intention to breastfeed, and mothers who had missing data at baseline and year-5 were classified as "did not breastfed".

Statistical analysis

The main outcome measure was the HDL cholesterol efflux capacity to total HDL at age 9 years. In addition, the secondary outcome measure was the HDL-C level. All statistics were applied using SPSS® Version 20 for Windows (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk test was conducted to test for normality of data. Scatterplots were used to test for a relationship between cholesterol efflux capacity and HDL-C and total cholesterol levels. Means and standard deviation were calculated for HDL cholesterol efflux.

The correlations of HDL cholesterol efflux capacity, lipoprotein and glucose levels with children's anthropometric measures at birth, age 5, and age 9 were examined using Pearson's correlation coefficient. Multiple regression analyses were run to investigate children's anthropometric measures as predictors of HDL cholesterol efflux capacity. Potential confounders were included in the regression models - sex, birth weight, breastfeeding status, HDL-C, total cholesterol and triglycerides.

R squared, the coefficient of determination was used as a measure of goodness of fit. Multicollinearity was measured by examining tolerance and the variance inflation factor (VIF).

A P value less than 0.05 was considered statistically significant. No correction was made for multiple testing.

Ethical approval

Ethical approval for the Lifeways Cohort and follow-up was granted by ethics committees in the National University of Ireland, Galway; The Coombe Women's Hospital, Dublin; University College Hospital, Galway; The Irish College of General Practitioners.

Results

The characteristics of the children at age 9 are presented in Table 1. At age 9, the mean HDL cholesterol efflux capacity was 16.25 ± 2.697% and the mean BMI was 18.6 kg/m^2 ± 3.1 (Table 1). No significant difference in HDL cholesterol efflux capacity was found between boys and girls (16.69% vs. 15.9%, p = 0.12). All other biochemical markers except

Table 1 Children characteristics stratified by sex.

	All (n = 75)	Boys (n = 33)	Girls (n = 42)	*P* value
	Mean ± SD	Mean ± SD	Mean ± SD	
Age (y)	9.4 ± 0.3	9.4 ± 0.3	9.4 ± 0.28	0.67
Bwt (g)	3440 ± 582.6	3622 ± 508	3301 ± 610	0.02*
BMI at age 5 (Kg/m^2)	16.96 ± 1.64	17.11 ± 1.96	16.83 ± 1.33	0.52
WC at age 5 yr (cm)	56.99 ± 4.08	57.31 ± 4.49	56.72 ± 3.77	0.58
BMI at age 9 yr (Kg/m^2)	18.6 ± 3.1	18.69 ± 3.37	18.5 ± 2.88	0.85
WC at age 9 yr (cm)	65.9 ± 8.1	66.01 ± 8.10	64.60 ± 10.82	0.52
Total cholesterol (mmol/L)	4.45 ± 0.68	4.34 ± 0.56	4.52 ± 0.75	0.19
HDL-C (mmol/L)	1.42 ± 0.37	1.50 ± 0.36	1.37 ± 0.37	0.13
Triglycerides (mmol/L)	1.15 ± 0.52	0.92 ± 0.40	1.30 ± 0.51	<0.01**
Glucose (mmol/L)	4.75 ± 0.73	4.49 ± 0.80	4.85 ± 0.79	0.05
LDL-C (mmol/L)	2.96 ± 0.57	2.78 ± 0.43	3.07 ± 0.63	0.02*
% cholesterol efflux capacity	16.25 ± 2.69	16.70 ± 3.01	15.92 ± 2.42	0.23
	n (%)	n (%)	n (%)	
Breastfed	46 (61)	20 (60.6)	25 (59.5)	0.92

Bwt: birth weight; BMI: body mass index; WC: waist circumference; P value derived from Student's T-Test.
* Statistical significance at $p < 0.05$.
** Statistical significance at $p < 0.01$.

LDL-C and triglycerides were significantly different between boys and girls. With regards to anthropometric measures, birth weight significantly differed between sexes, but all other measures at age 5 and 9 were comparable.

The representativeness of the children who provided blood samples and who were included in the current analysis was assessed. These children were compared to the original baseline cohort (n = 1026) in terms of mothers' age, baseline BMI, and education level. There were no significant differences in maternal education or weight status. However, mothers were older than the cohort at baseline (mean age 32·5 ± 5.2 years versus 29.6 ± 5.9 years, P < 0·001).

Table 2 presents the results of the correlation analyses between biochemical markers and children's anthropometric measures. At age 9, children's HDL cholesterol efflux capacity was positively correlated with HDL-C as well as total cholesterol ($r = 0.47$, $p < 0.01$ and $r = 0.39$, $p < 0.01$ respectively). There were no correlations with triglyceride or glucose levels. With regards to anthropometric measurements, no significant correlation was found between HDL cholesterol efflux capacity and birth weight. At age 5, HDL cholesterol efflux capacity was negatively correlated with waist circumference ($r = -0.32$, $p < 0.01$), and BMI ($r = -0.45$, $p < 0.01$). At age 9, the there was a negative correlation with waist circumference ($r = -0.24$, $p < 0.05$), but no correlation with BMI.

Table 3 shows the correlation between children's dietary intake at age 5 and HDL-C and HDL cholesterol efflux capacity at age 9. A trend towards negative correlation was noted between HDL-C and energy-adjusted carbohydrate and sugar intake ($r = -0.15$, $p = 0.2$ and $r = -0.17$, $p = 0.1$ respectively), however this did not reach statistical significance. A trend towards a positive correlation was noted

Table 2 Pearson's correlation between HDL cholesterol efflux capacity with lipoprotein and different indicators adiposity measures.

	Birth	Age 5			Age 9							
	Weight	BMI	SDS-BMI	WC	BMI	SDS-BMI	WC	Total CHOL	HDL-C	TRIG	Glucose	% efflux
Birth												
Weight	–											
Age 5												
BMI	0.06	–										
SDS-BMI	0.04	0.98**	–									
WC	0.21	0.78**	0.74**	–								
Age 9												
BMI	0.14	0.67**	0.64**	0.51**	–							
SDS-BMI	0.15	0.70**	0.68**	0.53	0.98**	–						
WC	−0.01	0.57**	0.57**	0.50	0.79**	0.82**	–					
Total CHOL	−0.02	−0.10	−0.16	−0.11	−0.09	−0.09	−0.20	–				
HDL-C	−0.03	−0.28*	−0.24	−0.33**	−0.26*	−0.31*	−0.33**	0.51**	–			
LDL-C	0.02	−0.01	−0.07	0.05	0.05	0.07	−0.04	0.85**	−0.01			
TRIG	−0.22	0.19	0.24	0.19	0.18	0.24	0.21	0.00	−0.36**	–		
Glucose	−0.15	0.17	0.14	−0.05	0.12	0.13	0.06	0.08	0.12	0.17	–	
% efflux	0.14	−0.45**	−0.50**	−0.32*	−0.19	−0.26*	−0.24*	0.39**	0.47**	−0.16	0.04	–

SDS-BMI: standard deviation score of body mass index.
* Statistical significance at $p < 0.05$.
** Statistical significance at $p < 0.01$.

Table 3 Pearson's correlation between HDL cholesterol efflux capacity and HDL-C at age 9 and children's dietary intake at year-5 follow-up (n = 72).

	HDL efflux		HDL-C	
	r	*p*	r	*P*
Mean intake[a]				
Energy (kcal)	−0.024	0.844	−0.096	0.420
Energy (kJ)	−0.025	0.839	−0.096	0.421
Protein (g)	−0.058	0.628	−0.063	0.600
Fat (g)	0.012	0.922	−0.020	0.864
Carbohydrate (g)	−0.037	0.756	−0.164	0.169
Saturated fatty acid	0.091	0.451	−0.013	0.912
Monounsaturated fatty acids (g)	0.024	0.842	0.001	0.997
Polyunsaturated fatty acids	−0.050	0.681	−0.122	0.308
Sugar (g)	−0.090	0.453	−0.198	0.096
Starch (g)	0.061	0.615	−0.026	0.831
Energy adjusted dietary intake				
Fat	0.087	0.473	0.165	0.166
Carbohydrate	−0.042	0.729	−0.147	0.216
Protein	−0.094	0.435	0.154	0.197
Saturated fatty acid	0.103	0.391	0.208	0.080
Monounsaturated fatty acid	0.087	0.469	0.151	0.207
Polyunsaturated fatty acid	−0.035	0.775	−0.076	0.524
Sugar	−0.104	0.387	−0.169	0.157
Starch	0.127	0.291	0.060	0.616

[a] Log transformed data.

between HDL-C and energy-adjusted total fat (r = 0.17, p = 0.17), MUFA (r = 0.15, p = 0.21), SFA (r = 0.21, p = 0.08) and total protein (r = 0.15, p = 0.2) but this also did not reach significance. There was no association between children's diet and HDL cholesterol efflux capacity.

Table 4 shows the correlations of maternal dietary intake during pregnancy with children's HDL-C and HDL cholesterol efflux capacity. HDL-C was negatively correlated with energy-adjusted intakes of fat (r = −0.24, p = 0.04), and monosaturated fatty acid (r = −0.27, p = 0.02). HDL-C positively correlated with energy-adjusted intakes of protein (r = 0.24, p = 0.04) and starch (r = 0.29, p = 0.01). These correlations were independent of maternal pre-pregnancy BMI (data not presented). There were no significant associations between matern diet during pregnancy and HDL cholesterol efflux capacity. There was no association between maternal diet at 5 years follow-up and HDL-C or HDL cholesterol efflux capacity.

Table 5 Multiple regression analyses of the relationship between HDL cholesterol efflux capacity at age 9 and children's anthropometric measurements at age 5 and 9.

Predictor variables	B	95% CI	*P* value
Age 5 yr (n = 61)			
BMI	−0.62	(-0.97,-0.26)	<0.001**
WC	−0.14	(-0.30,0.03)	0.07
Age 9 yr (n = 73)			
BMI	−0.11	(-0.31,0.08)	0.24
WC	−0.03	(-0.09,0.03)	0.31

Adjusted for sex, birth weight, breastfeeding status, total cholesterol, HDL-C, LDL-C and TRIG; BMI: body mass index; WC: waist circumference.
** Statistical significance at p < 0.01.

Table 5 shows the results of the multiple regression analyses for HDL cholesterol efflux capacity at age 9. There was a significant association between BMI at age 5 and efflux capacity at age 9 (p < 0.001). The association with waist circumference at age 5 observed in the linear regression model became non-significant on adjusting for confounding factors.

Table 4 Pearson's correlation between HDL cholesterol efflux capacity and HDL-C at age 9 and mothers 'dietary intake during pregnancy and year-5 follow-up (n = 72).

	During pregnancy				Year-5 follow-up			
	HDL efflux		HDL		HDL efflux		HDL	
	r	*P*	r	*p*	r	*p*	r	*p*
Mean intake[a]								
Energy (kcal)	−0.004	0.973	−0.037	0.750	0.122	0.309	−0.003	0.983
Energy (kJ)	−0.005	0.969	−0.033	0.774	0.122	0.312	−0.002	0.984
Protein (g)	−0.014	0.904	0.106	0.363	0.070	0.560	0.142	0.234
Fat (g)	−0.004	0.707	−0.125	0.281	0.176	0.142	−0.064	0.595
Carbohydrate (g)	−0.053	0.650	0.014	0.903	0.059	0.625	−0.027	0.821
Saturated fatty acid	−0.036	0.760	−0.088	0.450	0.183	0.115	−0.075	0.519
Monounsaturated fatty acids (g)	−0.074	0.528	−0.152	0.189	0.171	0.151	−0.064	0.594
Polyunsaturated fatty acids	0.014	0.903	−0.103	0.378	0.130	0.280	0.006	0.961
Sugar (g)	0.041	0.729	−0.106	0.364	0.121	0.314	−0.037	0.760
Starch (g)	0.065	0.580	0.114	0.328	−0.017	0.886	−0.025	0.837
Energy adjusted dietary intake								
Fat	−0.123	0.292	−0.236	0.040	0.167	0.164	−0.152	0.204
Carbohydrate	0.134	0.251	0.048	0.684	−0.118	0.326	−0.068	0.568
Protein	−0.024	0.839	0.236	0.040	−0.092	0.445	0.196	0.099
Saturated fatty acid	−0.090	0.444	−0.198	0.089	0.168	0.163	−0.107	0.369
Monounsaturated fatty acid	−0.192	0.098	−0.274	0.017	0.135	0.261	−0.109	0.362
Polyunsaturated fatty acid	0.001	0.998	−0.061	0.600	0.073	0.545	−0.011	0.930
Sugar	0.041	0.725	−0.126	0.279	0.059	0.623	−0.045	0.710
Starch	0.109	0.354	0.290	0.011	−0.202	0.091	−0.060	0.614

[a] Log transformed data.

Discussion

Childhood obesity has been known to be associated with adverse outcomes in adulthood including cardiovascular risk [48]. This study demonstrated that HDL cholesterol efflux capacity is associated with measures of adiposity in childhood. The strength of his longitudinal cohort is the ability to track children over time and determine changes in lifestyle (dietary intake and anthropometrics) that might be associated with reduced HDL cholesterol function. This investigation may help guide research on interventions and management of dyslipidemia.

This study investigated the HDL cholesterol efflux capacity of HDL particles isolated from the serum samples of 75 children. This method of isolating HDL is a more insightful measure of the atheroprotective properties of HDL as it excludes the effects of lipid-rich, apo-B-containing lipoproteins (such as LDL and VLDL) on efflux [49,50].

The children in the analysis (mean age = 9.4 years) had lipoprotein levels and BMI values similar to those of fasting school-aged children (mean age = 12.2 years) from a larger cohort study [51]. In the correlation analysis of traditional lipoprotein markers with HDL cholesterol efflux capacity, HDL-C did not entirely explain the variance in HDL cholesterol efflux capacity in agreement with other studies [8,9]. HDL-C and total cholesterol levels showed a positive association with HDL cholesterol efflux capacity, accounting for 22% and 15% variance respectively while LDL-C and triglyceride levels did not predict HDL cholesterol efflux capacity similar to previous findings [13,52].

The children's HDL-C levels at age 9 were negatively correlated with both their current BMI and waist circumference as well as their measures at age 5. It can be interpreted that HDL-C is strongly related to weight status. HDL cholesterol efflux capacity, on the other hand, had no association with current BMI but was inversely correlated with BMI at age 5. Furthermore, there was no correlation with weight change between 5 and 9 years (data not shown). One possible explanation for this finding is that HDL particle number did not reduce that significantly by age 5 but reduced by age 9.

An analysis that compared lipoprotein levels from surveys in Japan, Australia, and America [53] found HDL-C levels to differ greatly between Japanese and other school children. It was suggested that this difference might be the result of large dietary variance among these countries. Studies so far on the effects of childhood diet on HDL-C have shown inconsistent results. For example, some studies have shown children's sugar intake to relate to HDL-C [26,30] while others have found no association [27,31]. In the current study, energy-adjusted sugar had no association with HDL-C. Study results are also mixed with regards to fat intake [28–30]. We found intake of energy-adjusted saturated fat to be positively associated with HDL-C, different to Sanchez-Bayle et al. [28] who found high saturated fat intake to be associated with low HDL-C in children.

In the literature, HDL cholesterol efflux capacity has not yet been explored in relation to children's dietary intake. In this study, HDL cholesterol efflux capacity was not associated with any crude or energy-adjusted dietary intake at age 5. Adult studies have generally found no effect of diet on HDL cholesterol efflux capacity [54,55] confirming the likely resistance of HDL cholesterol efflux capacity to environmental influence.

In the study, the effects of maternal diet on HDL-C and HDL cholesterol efflux capacity were also explored. It is known that certain cardiovascular risk markers such as blood pressure, insulin resistance and atherosclerosis can be related to maternal factors during pregnancy [56–58]. Men born during the Dutch famine had altered lipoprotein levels (although this observation did not reach statistical significance) setting the question whether dietary intake in pregnancy can affect offsprings' lipoprotein profiles [59]. The current study revealed that intake during pregnancy was associated with HDL-C levels in children 9 years later. Energy-adjusted fat, SFA, and MUFA were inversely related to HDL-C, while energy-adjusted protein and starch had a positive correlation with HDL-C. However, we did not find any significant association between maternal diet and HDL cholesterol efflux capacity. Previous studies [35–38] found no relation between maternal diet and HDL-C levels in the offspring, although these studies did not directly measure intake values; rather they assessed for dietary quality or the effect of dietary intervention. Animal studies have shown that maternal high fat intake during pregnancy is associated with low HDL-C in their offsprings in later life [60].

Study limitations

The current study was limited due to its small sample size and variable response rates across the study period. Nevertheless, our subjects were comparable to the original cohort which in turn is nationally representative. Another limitation was the use of a FFQ which underestimate the intake of energy and nutrients [61]. It is also difficult to directly obtain dietary data from preschool children. Parental reporting using a FFQ has been suggested to be a reliable method to assess children's food intake [62]. Furthermore, the children's FFQ had been validated during the pilot stage of the cohort study by comparing with a 7-day food diary in a sub-sample of children [63]. A further limitation is that data on children's physical activity was not considered in the analysis although this is an additional lifestyle factor that could potentially affect lipoprotein profiles.

Conclusion

This novel analysis shows that efflux capacity is associated with physical measurements in early childhood independent of HDL-C levels. The atheroprotective quality of HDL appears to be determined in early but not late childhood. Diet in pregnancy and childhood may affect HDL-C levels in childhood, but they did not affect HDL cholesterol efflux capacity and do not appear to predict functionality.

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Conflicts of interest

The authors have no conflict of interest to report.

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