

Asthma: The Role of Low High-Density-Lipoprotein Cholesterol in Childhood and Adolescence

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Key Words

Asthma · Childhood · Lipid transfer protein · Pediatric allergy

Abstract

Background: Several studies have examined the relationship of asthma with serum dyslipidemia and reported positive, negative or no association. Most studies were limited by their cross-sectional design and the wide age range of the participants. In a cohort of children in Cyprus, we explored the association of asthma with serum high-density-lipoprotein cholesterol (HDL-C) at age 16–18 years (follow-up) independently of and in relation to HDL-C at age 11–12 years (baseline). **Methods:** In a case-control design, we recruited active asthmatics (AA; n = 68), current wheezers only (CWO; n = 123) and non-asthmatic controls (n = 660). Logistic regression models were used to evaluate associations of asthma with follow-up serum HDL-C and the role of baseline HDL-C. **Results:** At follow-up, mean HDL-C levels in AA and CWO patients were significantly lower than in the controls (47.9 and 49.7 vs. 53.4 mg/dl; p = 0.001 and p = 0.011). We

observed significant associations of AA patients with low HDL-C (<15th percentile; OR 2.32, 95% CI 1.16–4.47) that remained significant after further adjustment for baseline HDL-C (OR 2.14, 95% CI 1.06–4.14). Stratification by baseline HDL-C indicated that the association was significant only in those with high baseline HDL-C (OR 2.40, 95% CI 1.03–5.20). Stratification by IgE sensitization showed that the association was pronounced only in subjects who were sensitized (OR 3.41, 95% CI 1.12–9.88). **Conclusions:** Adolescent asthma is associated with low serum HDL-C independent of previous HDL-C levels in childhood. The association appears pronounced in those with a drop in HDL-C levels between childhood and adolescence and in those who have IgE sensitization.

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Introduction

Several studies have examined the relationship of asthma with serum dyslipidemia in adults and children and reported positive [1–5], negative [6–8] or no association

[9, 10]. Most studies were limited by their cross-sectional design and the wide age range of the participants. In a cohort of 3,982 children in Cyprus, we recently reported low levels of high-density-lipoprotein cholesterol (HDL-C) at age 11–12 years to be associated with active asthma (AA) in adolescence (age 16–18 years), suggesting a potential role of this lipoprotein in the pathogenesis of pediatric asthma or, at least, coexistence of these two conditions in early life [11].

The role of HDL and several cholesterol trafficking regulators has been studied in animal models of asthma. Mice sensitized with ovalbumin while taking a high cholesterol diet had significantly increased airspace eosinophils and interleukin (IL)-5 but not allergen-specific serum IgE [12]. In contrast, mice deficient in ATP binding cassette transporter G1, which plays a central role in cholesterol efflux from lung cells to HDL, displayed increased neutrophils and reduced airway eosinophils and T helper (Th) 2 inflammation after ovalbumin sensitization and challenge [13]. Apolipoprotein A-I mimetics, which recapitulate the structure and anti-inflammatory function of the HDL-associated protein apolipoprotein A-I, decrease eosinophil peroxidase activity, eosinophil counts and total IgE, which suggests a reduction in pulmonary inflammation and airway resistance [14]. No link of dyslipidemia to the allergic or non-allergic phenotype of asthma has been reported in human studies. In our 2012 study, after stratifying by family history of atopy, we were only able to report that the increased risk for asthma seemed to be particularly high in those children with a positive family history of atopy and low serum HDL [11].

In this study, we aimed to further explore the link between asthma and dyslipidemia in the Cyprus cohort of children by examining (i) the association of asthma with serum HDL at age 16–18 years independently of and in relation to serum HDL levels at age 11–12 years and (ii) the phenotypic features that are associated with low serum HDL.

Materials and Methods

Study Population

Participants were selected from the cohort of 3,982 children in Cyprus who at age 11–12 and 15–17 years participated in two large school-based surveys (fig. 1). The first survey in 2001–2003 involved all children ($n = 19,849$) attending the 6th form across all primary schools in Cyprus and focused on nutrition and physical fitness, and included measurements of serum total cholesterol, low-density-lipoprotein cholesterol (LDL-C), HDL-C, triglycerides and body mass index (BMI). The second survey in

2007 evaluated 3,982 subjects who were selected randomly from the subjects who took part in the first survey (20.1%) and focused on respiratory health (International Study of Asthma and Allergies in Childhood – ISAAC) and risk factors for asthma [11].

Using a case-control design, all children of the second survey who reported wheezing in the past 12 months (ISAAC questionnaire) were invited to participate in this study. Cases were further assigned to the AA group if they had also reported a diagnosis of asthma ever and to the current wheezers only (CWO) group if they did not. Controls were selected amongst the 3,685 adolescents who did not report either wheezing or asthma ever (never wheezers/never asthmatics – NWN; fig. 1). NWN were selected using a stratified random sampling approach in order to increase the probability of selection of children at the extremes of BMI change between childhood and adolescence, in line with the scope of another study on the relation of adiposity with asthma. The study was approved by the Cyprus National Bioethics Committee, and informed signed consent was obtained from the participants and their guardians.

Characteristics of the Study Population

Between November 2007 and May 2008, information was obtained from the adolescents on their nationality, parents' education level, and exposure to passive and active smoking. In the asthmatic group, information was also obtained regarding the age at the first asthma attack, the number of asthmatic attacks in the past 12 months, admissions ever to the emergency room or hospital for an asthma attack, and intake of any regular treatment for asthma at the time of the study.

Measurements

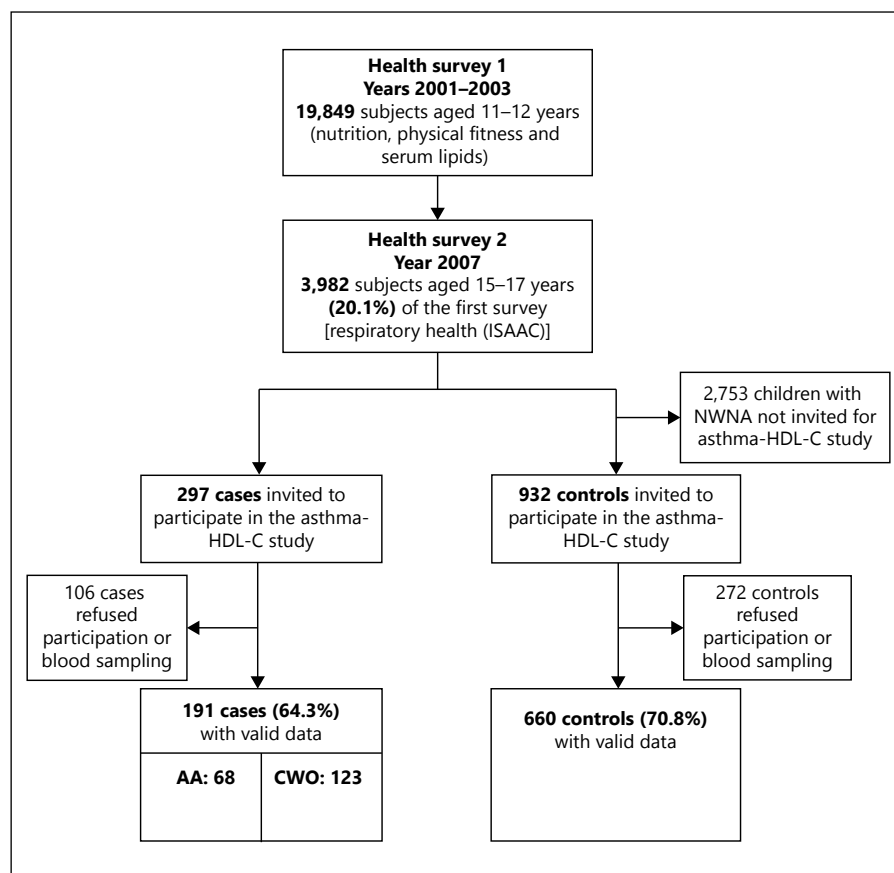
The adolescents had anthropometric measurements and assessments of serum total cholesterol, LDL-C, HDL-C and triglycerides after a 12-hour fast. We calculated BMI (kg/m^2) and expressed it as age- and gender-specific z-scores based on the US Centers for Disease Control 2000 growth charts [15]. The methodology for the baseline assessment of serum lipids was described in our previous paper [11]. Lipids at follow-up were determined with the ILab 600 analyzer using ILab chemistry systems (Instrumentation Laboratory SpA, Monza, Italy). IgE sensitization was assessed with skin prick tests (SPT) to 8 common aeroallergens (Greer, S.C., USA) performed and interpreted according to the GA2LEN recommendations [16]. Children with a positive SPT to any of the 8 allergens tested were considered sensitized to IgE.

Asthmatic children had measurements of forced expiratory volume in 1 s (Vitalograph, UK) before and after inhalation of 200 μg of salbutamol, and values were expressed as percent of predicted [17]. Details of the methods employed in assessing population characteristics and performing the measurements can be derived from the online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000368405).

Statistical Analysis

The main study outcomes were AA, CWO and NWN. Primary exposures were the serum HDL-C levels at follow-up and baseline. HDL-C was used as a binary variable using either a clinically relevant cutoff point in line with the consensus statement from the International Diabetes Federation (i.e. $\leq 40 \text{ mg}/\text{dl}$ for boys

Fig. 1. Course of the study: from the 19,849 participants (11–12 years) in survey 1, a random sample of 3,982 adolescents (15–17 years) participated in survey 2. All (n = 297) subjects from the 3,982 sample who reported wheezing in the last 12 months were invited to participate as cases. A sample of 932 children with no diagnosis of asthma and no wheezing in the past 12 months were invited to participate as controls.



and ≤ 50 mg/dl for girls) [18] or gender-specific (15th, 25th and 50th) percentiles at both time points. Baseline and follow-up serum lipid levels were also calculated after dividing each of the three study groups into subgroups by IgE sensitization status. Comparisons between groups were assessed using either the χ^2 test (for binary comparisons) or Mann-Whitney U test (in case of continuous variables due to skewness in the data). Descriptive statistics of other independent variables were presented as means (SD) or frequency distribution, as appropriate.

Logistic regression models were used to evaluate the associations of the study groups with the main exposure adjusted for age, gender, nationality, BMI z-score, parental education, passive and active smoking, and IgE sensitization. Due to the sampling procedure for the selection of controls (see paragraph on the study population) normalized sampling weights were incorporated in all models based on the inverse probability of selection from the original population. Since the initial models revealed that categorization of HDL-C at follow-up based on the 15th gender-specific percentile provided the most robust association between asthma and low HDL-C, this classification was used in further analyses. Post hoc power calculations are presented in the online supplementary material.

Models were further expanded adjusting for baseline serum HDL-C, IgE sensitization or age, as well as stratifying the analyses by each of these variables. The potential modifying effects of baseline serum HDL-C levels, IgE sensitization or age were examined

by including interaction terms of follow-up HDL-C levels with each of these variables in the models. Analyses were performed with R statistical software (version 3.0.1) [19].

Results

A total of 191 patients with current wheezing (68 AA and 123 CWO) and 660 NANA participated in the study. This corresponds to response rates of 64.3 and 70.9%, respectively, of the targeted populations for recruitment (fig. 1). Mean participant age was 17 years (SD 0.6) and the three groups did not differ significantly in terms of age, gender and nationality. CWO had marginally lower BMI (mean z-score 0.25 vs. 0.37 in NANA; $p = 0.047$). Active smoking was higher in AA and CWO (26.1 and 30.3 vs. 14.0% in NANA; $p = 0.009$ and $p < 0.001$, respectively), whereas CWO were also more exposed to passive smoking (58.2 vs. 46.7%, $p = 0.023$) than the controls. AA had a significantly higher rate of IgE sensitization (51 vs. 27.3%, $p < 0.001$) than the controls (table 1).

Table 1. Descriptive characteristics of the three study groups

Characteristics	NWNA n = 660	CWO n = 123	AA n = 68
Mean age, years	17.0	17.1	16.9
SD	0.6	0.6	0.6
Gender, n			
Boys	284	52	30
Girls	376	71	38
Mean BMI z-score	0.37	0.25*	0.32
SD	1.07	0.96	1.06
Nationality, n			
Cyprus	624	117	63
Other	35	6	5
Parental education, n			
Primary	19	10	4
Secondary	389	70	38
Tertiary	221	40 [†]	26
Passive smoking, n			
No	352	51	28
Yes	306	71 [†]	40
Active smoking, n			
No	568	85	50
Yes	91	37 ^{†††}	18 ^{††}
Atopic sensitization, n			
No	453	73	24
Yes	170	41	25 ^{†††}

Mann-Whitney U test: * $p < 0.05$ vs. NWNA. BMI z-scores of NWNA were normalized with sampling weights based on the inverse probability of selection from the original population. χ^2 test: [†] $p < 0.05$, ^{††} $p < 0.01$, ^{†††} $p < 0.001$, vs. NWNA.

Serum Lipids at Follow-Up and Baseline

At age 16–18 years, mean serum HDL-C levels in AA and CWO were significantly lower than in the controls (47.9 and 49.7 vs. 53.4 mg/dl; $p = 0.001$ and $p = 0.011$; table 2). The mean serum HDL-C levels of AA patients at age 11–12 years were also significantly lower than in controls (56.0 vs. 59.7 mg/dl; $p = 0.033$). Mean serum levels of total cholesterol, LDL-C and triglycerides were similar in the three groups at age 16–18 and 11–12 years (table 2). Stratification of each group by IgE sensitization indicated that SPT-positive AA patients had much lower HDL-C than the non-IgE-sensitized AA patients (44.4 vs. 51.8 mg/dl; $p = 0.008$). No difference in HDL-C levels was observed between the SPT-positive and -negative CWO and controls (table 2).

The 15th, 25th and 50th percentiles of HDL-C were estimated in the NWNA (controls) group and were similar in boys and girls at baseline. At follow-up, HDL-C values were overall reduced in both genders, with the re-

duction being more profound in boys than in girls (15th percentile: 36 vs. 40 mg/dl, 25th percentile: 40 vs. 47 mg/dl, 50th percentile: 47.5 vs. 55 mg/dl; table 3). At both time points, 4.6, 11.8 and 33.6% of children were below the 15th, 25th and 50th HDL-C percentiles, respectively (table 3).

Associations of AA with Different Cutoff Levels of Low HDL-C at Follow-Up and Baseline

In table 4, we present associations of AA with low HDL-C at 16–18 years as a binary variable using either an absolute cutoff value (≤ 40 mg/dl for boys and ≤ 50 mg/dl for girls) as well as the 15th, 25th and 50th percentiles of HDL-C.

In the unadjusted logistic regression models, the strongest association appeared with the 15th percentile of HDL-C (OR 2.49, 95% CI 1.28–4.61) although significant associations were also observed with the 25th percentile (OR 2.28, 95% CI 1.28–3.96) and the absolute HDL-C cutoff (OR 2.06, 95% CI 1.18–3.54). There was no association of AA with low HDL-C when using the 50th percentile as cutoff (OR 1.69, 95% CI 0.99–2.90). After adjusting for gender, age, BMI z-score, nationality, parental education, and passive and active smoking (model 1), the associations with AA remained grossly unchanged, with the stronger estimate again appearing at the 15th percentile cutoff (OR 2.32, 95% CI 1.16–4.47). After further adjusting for IgE sensitization (model 2), the association of AA with low HDL-C attenuated (OR 1.95, 95% CI 0.84–4.22), suggesting an important confounding effect of this variable.

Significant associations of AA were also observed with low HDL-C at age 11–12 years. Associations were statistically significant when using the 15th percentile at least before adjusting for IgE sensitization, as previously. While not always statistically significant, the OR estimates were close to 2 with all the low baseline HDL-C cutoff levels we used (table 4).

Role of Baseline HDL-C, IgE Sensitization and Gender in the Association of AA with Low HDL-C at Follow-Up

The observed associations between AA and low HDL-C (≤ 15 th percentile cutoff) at 16–18 years were explored further by adjusting for baseline HDL-C, IgE sensitization and gender in logistic regression models (table 5).

Baseline HDL-C. After further adjustment of model 1 for baseline HDL-C as a categorical variable (≤ 15 th percentile), the association of AA with follow-up low

Table 2. Comparison of baseline and follow-up serum lipid levels between AA, CWO and NWNA groups and within each group by atopic sensitization

	NWNA group			CWO group			AA group		
	whole group	SPT–	SPT+	whole group	SPT–	SPT+	whole group	SPT–	SPT+
Baseline, n	590	411	152	105	63	35	61	23	21
Follow-up, n	660	453	170	123	73	41	68	24	25
Total cholesterol									
Baseline	165.7 (28.7)	165.8 (29.0)	166.6 (29.3)	170.9 (32.5)	165.8 (29.0)	178.3 (40.9)	161.0 (27.7)	161.0 (22.9)	158.8 (26.4)
Follow-up	162.8 (32.5)	162.1 (32.5)	165.3 (32.9)	162.5 (34.0)	159.3 (31.4)	170.9 (39.0)	160.7 (33.1)	163.1 (23.2)	152.2 (30.0)
HDL-C									
Baseline	59.7 (12.5)	59.5 (12.3)	60.3 (12.6)	59.6 (12.1)	60.6 (12.7)	57.6 (11.4)	56.0 (11.9)*	54.2 (11.7)	56.5 (12.7)
Follow-up	53.4 (13.9)	53.7 (14.1)	53.4 (14.0)	49.7 (10.9)*	51.1 (11.0)	48.2 (10.7)	47.9 (10.6)**	51.8 (10.2)	44.4 (11.0)††
LDL-C									
Baseline	92.2 (25.3)	92.4 (25.8)	92.8 (24.8)	97.4 (29.7)	92.5 (24.2)	105.5 (36.7)†	92.1 (22.4)	93.0 (22.1)	91.4 (18.6)
Follow-up	105.1 (28.1)	104.3 (28.0)	107.3 (28.1)	105.5 (29.6)	101.3 (27.5)	114.6 (33.3)	105.0 (29.6)	106.7 (21.4)	96.5 (22.4)
Triglycerides									
Baseline	69.3 (37.0)	70.7 (34.3)	67.3 (45.2)	68.2 (26.3)	66.0 (22.4)	72.2 (32.0)	68.9 (30.0)	69.2 (30.6)	69.0 (27.5)
Follow-up	70.0 (31.4)	69.0 (31.6)	71.5 (30.2)	72.7 (32.3)	71.3 (31.1)	76.0 (35.5)	76.8 (33.6)	71.1 (36.2)	73.9 (28.8)

Mann-Whitney U test: * $p < 0.05$, ** $p < 0.01$, vs. NWNA; † $p < 0.05$, †† $p < 0.01$, vs. SPT-negative patients in the respective group.

Table 3. The 15th, 25th and 50th percentiles¹ of HDL-C in patients aged 11–12 (baseline) and 16–18 years (follow-up) and categorization of the study population by persistently low HDL-C at the two time points

Percentile	HDL-C at baseline, mg/dl		HDL-C at follow-up, mg/dl		Subjects with HDL-C below the respective percentile at both time points (n = 746 boys and girls)	
	boys	girls	boys	girls	n	%
≤15th	48	48	36	40	34	4.6
≤25th	51	51	40	47	88	11.8
≤50th	59	58	47.5	55	251	33.6

¹ The gender-specific (15th, 25th and 50th) percentiles at both time points were estimated in the NWNA group (controls).

Table 4. Associations (OR, 95% CI) of AA patients with low HDL-C aged 16–18 (follow-up) and 11–12 years (baseline) as categorical variable using different cutoff points

Low HDL-C	n	HDL-C <40 (boys) or <50 mg/dl (girls)	HDL-C <15th percentile	HDL-C <25th percentile	HDL-C <50th percentile
<i>16–18 years</i>					
Unadjusted	637	2.06 (1.18–3.54)	2.49 (1.28–4.61)	2.28 (1.28–3.96)	1.69 (0.99–2.90)
Model 1	607	1.98 (1.10–3.51)	2.32 (1.16–4.47)	2.10 (1.15–3.75)	1.54 (0.88–2.71)
Model 2	568	1.64 (0.81–3.22)	1.95 (0.84–4.22)	1.96 (0.96–3.86)	1.33 (0.69–2.58)
<i>11–12 years</i>					
Unadjusted	637	1.71 (0.84–3.27)	1.94 (1.01–3.57)	1.79 (0.99–3.13)	1.90 (1.11–3.33)
Model 1	607	1.98 (0.91–4.10)	2.00 (1.02–3.77)	1.83 (1.00–3.29)	1.92 (1.10–3.43)
Model 2	568	1.93 (0.75–4.57)	2.11 (0.98–4.31)	1.87 (0.92–3.67)	2.21 (1.14–4.50)

Model 1 was adjusted for gender, follow-up age, follow-up BMI z-score, nationality, parental education, passive smoking at follow-up and active smoking at follow-up. Model 2 was adjusted for all model 1 variables plus atopic sensitization.

Table 5. OR of AA in terms of low HDL-C levels (≤ 15 th percentile) at the age of 16–18 years (follow-up) as estimated in logistic regression models before and after adjusting for baseline HDL-C and/or atopic sensitization and stratified by levels of baseline HDL-C or atopic sensitization

Low HDL-C	Before and after adjusting for baseline HDL-C				Stratified by baseline HDL-C levels				p value for interaction ¹
	before		after		low baseline HDL-C		high baseline HDL-C		
	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	
Unadjusted	637	2.49 (1.28–4.61)	637	2.25 (1.14–4.23)	99	1.50 (0.43–4.76)	538	2.69 (1.20–5.60)	0.416
Model 1	607	2.32 (1.16–4.47)	607	2.14 (1.06–4.14)	95	1.66 (0.46–5.47)	512	2.40 (1.03–5.20)	0.620
Model 2	568	1.95 (0.84–4.22)	568	1.78 (0.76–3.88)	91	0.55 (0.08–2.53)	477	2.82 (1.08–6.82)	0.093

Low HDL-C	Before and after adjusting for atopic sensitization				Stratified by atopic sensitization at follow-up				p value for interaction ²
	before		after		no atopic sensitization		atopic sensitization		
	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	
Unadjusted	637	2.49 (1.28–4.61)	594	2.08 (0.92–4.31)	426	1.24 (0.28–3.80)	168	3.17 (1.09–8.71)	0.257
Model 1	607	2.32 (1.16–4.47)	568	1.95 (0.84–4.22)	405	1.01 (0.22–3.28)	163	3.41 (1.12–9.88)	0.156
Model 3	607	2.14 (1.06–4.14)	568	1.78 (0.76–3.88)	405	0.94 (0.21–3.08)	163	3.07 (1.00–9.02)	0.172

Model 1 was adjusted for gender, follow-up age, follow-up BMI z-score, nationality, parental education, and passive and active smoking at follow-up. Model 2 was adjusted for all the variables in model 1 plus atopic sensitization. Model 3 was adjusted for all the variables in model 1 and also for baseline HDL-C as categorical variable (≤ 15 th percentile).

¹ p value for interaction between follow-up HDL-C and baseline HDL as categorical variable (≤ 15 th percentile).

² p value of interaction between follow-up HDL-C and atopic sensitization at follow-up.

HDL-C attenuated slightly but remained statistically significant (OR 2.14, 95% CI 1.06–4.14). Inclusion of interaction terms between baseline HDL-C and follow-up HDL-C did not yield statistically significant effects either before or after adjusting for other covariates (model 1), including IgE sensitization (model 2; p values for interaction 0.416, 0.620 and 0.093, respectively). However, stratification by baseline HDL-C indicated that the associations of low HDL-C at follow-up with AA were particularly pronounced among the subgroup of participants with high baseline HDL-C. This appeared consistent across the unadjusted model (OR 2.69, 95% CI 1.20–5.60), model 1 (OR 2.40, 95% CI 1.03–5.20) and model 2 (OR 2.82, 95% CI 1.08–6.82), suggesting that the association with AA was particularly pronounced in those who had dropped from higher HDL-C levels at age 11–12 years into the low HDL-C category by age 16–18 years. In subjects with persistently low HDL-C between baseline and follow-up, OR estimates were weaker and not statistically significant, indicating a smaller contribution of this subgroup in the observed association.

IgE Sensitization. In addition to the confounding effect of IgE sensitization, as shown above, we explored

the potential effect modification by this covariate (table 5). Use of an interaction term with follow-up HDL-C did not yield statistically significant results [p values 0.257, 0.156 and 0.172 in models before and after adjusting for other covariates (model 1) including baseline HDL-C (model 3), respectively]. Nevertheless, after stratification by IgE sensitization, it was evident that the association of low HDL-C with AA was particularly pronounced among IgE subjects with estimated OR >3 in all three models [unadjusted model: OR 3.17, 95% CI 1.09–8.71, model 1: OR 3.41, 95% CI 1.12–9.88 and model 3: OR 3.07, 95% CI 1.00–9.02]. In contrast, the estimates in the subgroup with no IgE sensitization were close to unity (table 5).

Gender. Using the same approach to explore the effect of gender, no significant confounding or interaction effect was observed (results not shown in detail).

Comparison of Phenotypic Characteristics of AA Patients Stratified by HDL-C Levels at Follow-Up

Phenotypic characteristics of AA patients with low versus high HDL-C levels were not statistically different (online suppl. table S1).

Discussion

In this community-based study, we have shown that AA adolescents aged 16–18 years have more than twofold increased odds to have lower HDL-C levels compared to their nonasthmatic peers, independent of BMI and other covariates. Interestingly, this association was not confounded by HDL-C levels at age 11–12 years. In our previous report [11] on the same cohort of children, we found an association of low HDL-C at age 11–12 years with asthma in adolescence. We have confirmed this finding in this case-control design, although the magnitude of the association of asthma with HDL-C at follow-up appears to be stronger than the one with HDL-C at baseline.

Strengths and Limitations

The main strength of this study is the availability of longitudinal data on HDL-C in childhood and adolescence in a cohort with a narrow age range recruited from a large community sample of children. Nevertheless, there are also several limitations. First, asthma definition was not based on clinical data but on information obtained from a young population describing a recent asthma history; we think, however, that any recall bias, which could have affected our results, is unlikely to have been influenced by HDL-C levels. Also, the lack of baseline asthma morbidity data precludes inferences on the effect of HDL-C on persistence or incidence of asthma between the two time points. Nevertheless, this limitation does not affect the validity of the relationship of low HDL-C at baseline and follow-up with asthma in adolescence. Second, the use of different assays to measure HDL-C at the two time points makes absolute HDL-C values not entirely comparable. We think that we solved this problem by using gender- and time point-specific percentiles of lipid values, especially in the models which compared the effects of HDL-C at baseline and follow-up. Finally, although we adjusted the HDL-C-asthma relationship for many covariates, we cannot exclude residual confounding. For example, we have not controlled for adherence to the Mediterranean diet, which is associated with HDL-C levels and is also high in antioxidants and n–3 fatty acids, which may reduce the risk of asthma independently [20, 21].

Generalization of the Data

This is the first study that confirms an association of asthma with low HDL-C measured at two time points: in childhood and adolescence. The reduction in HDL-C levels between childhood and adolescence has been described before [22, 23] and seems to have a particular effect on the observed

association. The stronger magnitude of the association among those who had a drop in HDL-C levels between the two time points may underline the importance of aging in the association of serum lipids with asthma. It is very interesting that large population studies in adults have shown an increased incidence of cardiovascular events in adults with bronchial asthma [24–28]. Our findings suggest that the clustering of asthma and cardiovascular disease in adulthood may have its origins in early life, and aging may have a particular role in the appearance of this association. It is clear that longitudinal cohort studies are needed to reveal the natural course of determinants such as HDL-C levels and their interactions with disease-causing mechanisms for both conditions that cluster together in advanced age [29].

In this study, we observed that the association between asthma and low HDL-C appears particularly pronounced in IgE-positive adolescents, suggesting an enhanced role of HDL-C in asthma development in those who have a Th2-type adaptive immunity profile. In general, there are many unanswered questions in our understanding of how IgE sensitization translates into clinical manifestations and appearance of asthma [30]. Several reports and a recent review [31] suggest that inflammation of the lung is influenced by lipoproteins and/or defects in lipoprotein metabolism. However, even data from experimental models do not yet provide clear evidence whether functions similar to those of low HDL-C promote eosinophilic [12, 14] or neutrophilic [13] inflammation in the lung. Asthma is a systemic inflammation, and multiple mechanisms and biomarkers such as high-sensitivity C-reactive protein [32], tumor necrosis factor- α , IL-6, IL-8 [33] and fibrinogen [34] have been associated with this disease, which are not necessarily specific to the Th2 immunity profile and atopy. Another possible explanation for the link between asthma and HDL-C is the share of a common genetic influence between low HDL-C and allergic sensitization, as suggested in a report in 1,187 Chinese twins [35]. In the setting of a genetic link between low HDL-C and allergic sensitization, common adverse environmental influences (e.g. unhealthy diet) can interact with both of these two determinants and have a particularly important role in asthma development.

In summary, asthma is associated with low serum HDL-C in adolescence independent of HDL-C levels in childhood. The association of adolescent asthma with low HDL-C appears to be particularly pronounced in those who have a drop in their HDL-C levels between childhood and adolescence, and in those with IgE sensitization. Longitudinal cohort studies are needed to reveal the interactions of HDL-C changes with aging and mechanisms causing asthma and other comorbidities.

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Online Supplementary Material

Material and Methods

Characteristics of study population

All participants attended the pediatric department of their nearest local hospital between November 2007 and May 2008. Information was obtained from the adolescents on the following characteristics: nationality (operationalized as both parents Cypriot vs. at least one parent not Cypriot), parents' education level (allocated into primary, secondary and tertiary according to the highest level attained by either parent), exposure to passive smoking (i.e. report of regular smoking by any person in the household in last 12 months) and active smoking (i.e. report of ever smoking for a period of one year). In the case of the asthmatic group, information was also obtained regarding the age of first asthma attack, number of asthmatic attacks they had experienced in the past 12 months, admissions ever in emergency room or hospital for an asthma attack and intake of any regular treatment for asthma at the time of the study.

Measurements

The adolescents had anthropometric measurements and assessments of serum TC, LDL-C, HDL-C, TG after a 12-h fast. Height and weight were measured twice and the mean value of the two measurements was used in the analyses. We calculated BMI (kg/m^2) and expressed it as age- and gender-specific z-scores based on the United States' Centers for Disease Control 2000 growth charts [15]. The methodology for the baseline assessment of serum lipids was described in our previous paper [11]. Lipids at follow-up were determined with the ILab 600 analyzer using ILab Chemistry systems (Instrumentation Laboratory SpA, Monza, Milano, Italy). The measurements were reproducible and validated by internal (SeraChem Control Level 1 and Control Level 2, Instrumentation Laboratory SpA, Monza, Milano, Italy) and external quality control (Randox International Quality Assessment Scheme). The assays' coefficients of variation were 2.08% for TC, 1.53% for HDL-C, 1.15% for LDL-C and 2.45% for TG. IgE sensitization was assessed with skin prick tests (SPT) to 8 common aeroallergens (Greer, USA) Derp 1 & 2, cat, Bahia grass, mixed grasses, mugwort, mold mix, olive, weeds and negative (50% glycerinated saline) and positive (histamine, 1.0 mg/mL) controls. SPT were performed on the volar surfaces of the arms on normal skin and the largest wheal diameter was measured 15 minutes after application. Their interpretation followed the GA2LEN recommendations [16].

Children with a positive SPT to any of the 8 allergens tested were considered sensitized to IgE.

Asthmatic children had measurements of forced expiratory volume in one second (FEV1) (Vitalograph, UK) pre and post inhalation of 200 mcg of salbutamol via a spacer (Volumatic, GSK UK) and values were expressed as percent of predicted [17].

Statistical Analysis

Power Calculation

Post hoc power calculations suggested that the final achieved sample (123 vs 660; allocation ratio 1:5) was sufficient to detect an odds ratio for the association between low HDL (i.e. below the 15th percentile among controls) and current wheeze of the magnitude of 2 at the 5% statistical significance level with 80% statistical power. Similarly for 80% power, an association of the magnitude of an odds ratio of 2.4 was needed when the analysis was restricted to active asthmatics (68 vs 660; allocation ratio 1:9).

Results

Comparison of phenotypic characteristics of active asthmatics stratified by HDL-C levels at follow-up

The age at first asthma attack (6.6 Vs 6.5 years, $p=0.820$) was similar between AA with low and high HDL-C at follow-up (Table S1). FEV1 pre and post-bronchodilation and FEV1 change with bronchodilation were also similar between AA with low and high HDL-C. The frequency of IgE sensitization (66.7% Vs 45.9%, $p=0.360$), number of asthma attacks in past 12 months (6.1 Vs 2.7, $p=0.828$), hospital or emergency room admissions ever (66.7% Vs 45.9%, $p=0.528$) and intake of regular anti-asthma medication (42.9% Vs 37.0%, $p=0.122$) were all elevated among AA with low HDL-C compared to those with higher HDL-C levels but were not statistically significant.

Discussion

In our asthmatic population, there were some indications for a more severe clinical course in those with low HDL-C, which did not reach statistical significance. Further studies in larger asthmatic populations are needed to clarify the effect of low HDL-C on the asthmatic phenotype and course of disease.

Table S1. Comparisons of phenotypic features of children with active asthma stratified by HDL-C at follow-up

	Normal* HDL at follow-up		Low* HDL at follow-up		p-value
	n	mean(SD)	n	mean(SD)	
Age at first asthma attack	36	6.5(4.6)	13	6.6(5.0)	0.820
Number of asthma attacks in last 12 months	21	2.7(2.0)	7	6.1(9.0)	0.828
Pre FEV1	40	100.0(13.4)	12	102.5(12.4)	0.656
Post FEV1	42	103.5(15.2)	14	103.1(13.1)	0.828
FEV1 change post bronchodilator	38	3.8(3.8)	11	2.3(4.6)	0.458
	n	percent	n	percent	p-value
Regular intake of any asthma treatment	17	37.0	6	42.9	0.122
Skin prick testing sensitization	37	45.9	12	66.7	0.360
Ever in hospital or ER for an asthma attack	21	41.2	9	52.9	0.528

* Cutoff level of low–normal HDL-C at follow-up was set at the 15th gender-specific percentile.