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Effect of dyslipidemia, obesity, and atopic status on exhaled and alveolar nitric oxide in asthmatic children

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Abstract

Background: Dyslipidemia and obesity contribute to a pro-inflammatory state. Eosinophilic airway inflammation can be indirectly measured by fractional exhaled nitric oxide (FeNO) produced in the airways of asthmatic subjects.

Objective: To compare exhaled nitric oxide (NO) and alveolar NO in asthmatic children with and without dyslipidemia.

Methods: Asthmatic children (5–18 years old) had fasting serum low-density lipoprotein cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C) concentrations, and C-reactive protein (CRP) concentrations measured. FeNO was measured at constant flow rates of 20, 50, 100, and 300 ml/s by the chemiluminescence method. NO concentrations in tissue of the upper airways (CawNO) and the total flux of NO in the conducting airways (JawNO) were determined through FeNO at 20, 100, and 300 ml/s using a mathematical model. The atopic status was assessed using the skin prick test for aero-allergens.

Results: One hundred forty-one asthmatic children were enrolled with a mean (standard deviation) age of 11.82 (3.38) years. Sixty-four (45.4%) children had dyslipidemia and 20 (14.2%) were obese. Children with low HDL-C concentrations had significantly higher CawNO and JawNO than those with normal HDL-C concentrations (both $p = 0.03$). Asthmatic children with obesity had higher CRP concentrations than those with a normal weight ($p < 0.001$). Atopic children had a significantly higher FeNO, CawNO, and JawNO than non-atopic children (all $p < 0.05$).

Conclusion: This study suggests an effect of HDL-C on CawNO and JawNO in asthmatic children. An intervention that normalizes HDL-C concentrations may be beneficial for airway inflammation in asthmatic children.

Key words: high-density lipoprotein cholesterol (HDL-C), airway inflammation, asthma, lipid, nitric oxide, children, obesity, atopy

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Introduction

In children, asthma is one of the most common chronic respiratory conditions. However, evidence on the association between dyslipidemia and asthma is conflicting.[1,2] Pediatric dyslipidemia is defined as an elevation in low-density lipoprotein cholesterol (LDL-C) concentrations above the normal value of LDL-C ≥ 130 mg/dL,

triglycerides (TG) concentrations above the normal value of TG ≥ 100 mg/dL for children aged 0–9 years and ≥ 130 mg/dL for those aged 10–19 years, or below the normal value of high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL.[3] The prevalence of dyslipidemia in children has increased in recent years because of the global epidemic of childhood obesity. Approximately 20% of children and adolescents aged 8–17 years in the United States have abnormal lipid levels for at least one or more lipids.[4] However, a recent study showed a higher prevalence of dyslipidemia in asthmatic children.[5] A greater airway obstruction in infants with high LDL-C concentrations was reported in a prospective cohort study of children born to mothers who had a doctor's diagnosis of asthma.[1] Children with high HDL-C concentrations showed greater lung function and decreased bronchial reactivity. However, a recent epidemiological study in China showed no association between blood lipid concentrations and asthma in children.[2] Dyslipidemia and metabolic syndrome, which contribute to a pro-inflammatory state, have been proposed as potential causes of asthma.[6]

Several studies have highlighted nitric oxide (NO) as an inflammatory biomarker in the lungs and airways. NO can be measured noninvasively, and instruments and techniques for measuring fractional exhaled nitric oxide (FeNO) concentrations have been created and optimized.[7] The value of FeNO at the constant expiratory flow of 50 mL/s ($FeNO_{50}$) is widely used as a marker of eosinophilic airway inflammation in asthma.[8] Increased $FeNO_{50}$ concentrations are correlated with poor asthma control.[9] However, the measurement of $FeNO_{50}$ predominantly represents NO from the proximal airway.[10] FeNO measurements at multiple flow rates (low, medium, and high) are used to calculate alveolar NO (alvNO) concentrations, NO concentrations in tissue of the upper airways (CawNO), and the total flux of NO in the conducting airways (JawNO). alvNO is reflective of peripheral airway inflammation which is a characteristic of airway inflammation in asthma.[11] No previous studies have evaluated the association of $FeNO_{50}$, alvNO, CawNO, and JawNO with dyslipidemia in asthmatic children. We recently showed associations of HDL-C, LDL-C and respiratory resistance as assessed by the forced oscillation technique in asthmatic children.[11] We hypothesize that asthmatic children with dyslipidemia have peripheral airway inflammation, resulting in increased alvNO concentrations. This study aimed to compare $FeNO_{50}$, alvNO, CawNO, and JawNO in asthmatic children with and without dyslipidemia.

Methods

This cross-sectional study was performed from January 2019 to December 2019. Asthmatic children (aged 5–18 years) who had clinically controlled asthma in the past 4 weeks were enrolled. The diagnosis of asthma was based on the Global Initiative for Asthma 2018 guideline. The definition of clinically controlled asthma was clinically well-controlled asthma according to the Global Initiative for Asthma 2018 guideline and having an asthma control test score of > 19. The exclusion criteria were children with other underlying chronic diseases (diabetes mellitus, chronic liver diseases, and chronic kidney diseases). Demographic data, atopic history, and medications were recorded. The Pediatric Asthma Control Test and the Pediatric Asthma Quality of Life Questionnaire were used to assess the control of asthma. Ethical approval was provided by the Human Rights and Ethics Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (ID: MURA2019/55). Written informed consent and assent were obtained for all participants and their parents.

Anthropometric measurements, such as height and weight, were performed. The body mass index (BMI) and BMI z-score were calculated. Children with obesity were defined as having a BMI z-score > 2.00 standard deviations (SDs) in accordance with the World Health Organization Report of the Commission on Ending Childhood Obesity.[12]

Blood samples were collected from the subjects after fasting for at least 8 hours. The definition of pediatric dyslipidemia was based on LDL-C concentrations ≥ 130 mg/dL, TG concentrations ≥ 100 mg/dL for individuals aged 0–9 years and ≥ 130 mg/dL concentrations for those aged 10–19 years, or HDL-C concentrations < 40 mg/dL.[3]

Skin prick pest

The atopic status of the enrolled children was assessed using the skin prick test to 13 commercial extracts of aeroallergen, namely Johnson grass, Bermuda grass, Careless weed, *Cladosporium sphaerospermum*, Alternaria, *Aspergillus fumigatus*, Curvularia, American cockroach, German cockroach, cat hair, dog pelt, *Dermatophagoides ptereonyssinus* (*Der p*), and *Dermatophagoides farini* (*Der f*) (ALK-albello Pharm., Inc., USA). The skin prick test was interpreted as positive when the mean wheal diameter was larger than the negative control by 3 mm.

NO measurement

NO concentrations were measured by the chemiluminescence method using a CDL 88 sp analyzer (ECO MEDICS, Duernten, Sweden) by the online single breath technique in accordance with the American Thoracic Society and the European Respiratory Society recommendations.[7] The analyzer had a 200 mL/min sampling rate and a detection rate of 0.1 to 5000 parts/billion (ppb). The measurement unit of NO is ppb. The chemiluminescence analyzer was calibrated daily using 100% nitrogen to zero and then using analyzed standard gas. This maneuver was performed three times, and the levels of exhaled NO were based on analysis of the plateau portion of the exhaled and nasal curves.

To perform $FeNO_{50}$ measurement, the children were asked to perform a single slow exhalation starting from the total lung capacity. The children exhaled using a mouthpiece and a one-way non-rebreathing valve against a resistance of 16 cm H_2O under a visual biofeedback to maintain a steady flow of 50 mL/s. The children were at rest, sitting down, and refrained from eating and exercising for at least 1 hour. They breathed filtered NO-free air (Denox 88; ECO MEDICS) with a nose-clip before the single exhalation maneuver. FeNO concentrations were measured at the plateau (> 2 s during an exhalation of > 6 s) of the end-exhaled reading. JawNO and alvNO were then determined by the multiple exhalation flow technique at 20, 100, and 300 ml/s. The children were instructed to exhale, starting from the level of maximum inspiration, at three constant expiratory flow rates (20, 100 and 300 ml/s). Initially, determinations were performed at a flow rate of 20 ml/s, followed by 100 ml/s, and finally at 300 ml/s. All measurements were made in accordance with the recommendations of the European Respiratory Society and the American Thoracic Society.[7] The coefficient of variability among the three determinations had to be within 10%. After NO determination was obtained at different flow rates, alvNO, CawNO, and JawNO were calculated using a mathematical model by Hogman and Merilainen.[13] When alvNO values were below zero, they were assigned a value of zero.[14]

Statistical analysis

Statistical analysis was performed using SPSS software, version 18. Differences between groups were examined using the chi-square test, Student *t*-test, or Mann–Whitney test as appropriate.

Results

We enrolled 141 asthmatic children with a mean (standard deviation) age of 11.87 ± 3.40 years. Eighty-eight (62.4%) of the children were boys, and 102 (72.3%) had atopic asthma. Only 20 (14.2%) of the children were obese. Sixty-four (46.7%) children met the dyslipidemia criterion. High LDL-C (65.6%) concentrations were the most common type of dyslipidemia. The baseline characteristics of the enrolled children are shown in **Table 1**.

Table 1. Baseline characteristics of the participants.

Patient features	N = 141
Age (years)	11.87 (3.40)
Gender: Male	88 (62.4)
Female	53 (37.6)
BW (kg)	47.00 (19.18)
Height (cm)	148.57 (17.07)
BMI (kg/m^2)	20.49 (5.15)
Obesity	20 (14.2)
Severity of asthma	
• Mild	103 (73)
• Moderate to severe	38 (37)
Atopic Asthma	102 (72.3)
Dyslipidemia	64 (45.4)
• High TC	37 (26.2)
• High LDL-C	42 (29.8)
• High TG	23 (16.3)
• Low HDL-C	10 (7.1)
• Combined dyslipidemia	31 (21.9)
CRP (mg/dL)	0.65 (0.23,1.77)
PACT* (score)	23.88 (1.93)
PAQLQ** (score)	6.60 (0.41)

Data present as mean (S.D.), median (IQR) or n (%); PACT*, Pediatric Asthma Control Test; PAQLQ**, Pediatric Asthma quality of life questionnaire; CRP, C- Reactive protein; HDL, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides;

Table 2. Comparison between asthmatic children with dyslipidemia and non-dyslipidemia group.

Parameters	Dyslipidemia N = 66	Non-dyslipidemia N = 77	*P*-value
Age (year)	11.55 (3.37)	12.14 (3.37)	0.302
Body weight (kg)	47.87 (21.52)	46.28 (17.12)	0.626
Height (cm)	146.68 (17.18)	150.14 (16.93)	0.231
Obesity	14 (21.9)	6 (7.8)	0.027
Atopic asthma	42 (65.6)	60 (77.9)	0.131
PACT (score)	23.89 (1.8)	23.88 (2.1)	0.779
PAQLQ (score)	6.61 (0.36)	6.58 (0.44)	0.791
CRP (mg/dL)	0.71 (0.28, 1.92)	0.45 (0.20, 1.48)	0.104

Table 2. (Continued)

Parameters	Dyslipidemia N = 66	Non-dyslipidemia N = 77	*P*-value
NO measurement			
• $FeNO_{50}$(ppb)	15.90 (6.48, 23.70)	14.00 (7.30, 28.65)	0.857
• alvNO (ppb)	0.00 (0.00, 0.00)	0.00 (0.00, 1.05)	0.133
• CawNO (ppb)	42.70 (21.83, 81.38)	45.35 (23.55, 107.43)	0.361
• JawNO (pl/s)	988.10 (559.50, 2025.83)	1015.55 (545.63, 1957.70)	0.745

Data are present as mean (SD), median (IQR) or n (%); alvNO, alveolar NO; CawNO, NO concentration in the tissue of the upper airways; JawNO, total flux of NO in the conducting airways;

Comparison of NO measurement between asthmatic children with dyslipidemia and those without dyslipidemia

There were no significant differences in baseline characteristics between children with dyslipidemia and those without dyslipidemia (normal blood lipid concentrations). There were no significant differences in $FeNO_{50}$, alvNO, CawNO, or JawNO values between children with dyslipidemia and those without dyslipidemia (**Table 2**). A subgroup analysis of the type of dyslipidemia showed no significant differences in $FeNO_{50}$, alvNO, CawNO, or JawNO values between children who had high TC, TG, and LDL-C concentrations and those who had normal TC, TG, and LDL-C concentrations. Only children with low HDL-C concentrations had a significantly higher median (interquartile range) CawNO (113.55 [31.38, 330.53] vs. 42.90 [22.13, 96.10] ppb; p = 0.03) and JawNO [2004.39 [761.78, 5190.60)] vs. 980.75 [521.44, 1906.97] pl/s; p = 0.03] than those with normal HDL-C concentrations (**Table 3**). There were no significant differences in baseline characteristics between children with low HDL-C concentrations and those with normal HDL-C concentrations, except for body weight (**Table 3**).

Table 3. Comparison between asthmatic children with low HDL-C and normal HDL-C group.

Parameters	Low HDL-C N = 10	Normal HDL-C N = 131	*P*-value
Age (year)	13.00 (3.33)	11.79 (3.40)	0.278
Body weight (kg)	73.36 (21.44)	44.99 (17.52)	< 0.001
Height (cm)	158.59 (15.33)	147.81 (17.01)	0.054
Obesity (n)	6 (60)	14 (10.7)	0.001
Atopic asthma	7.0 (70.0)	95.0 (72.5)	0.864
PACT (score)	24.0 (1.9)	23.9 (1.9)	0.848
PAQLQ (score)	6.7 (0.2)	6.6 (0.4)	0.386
CRP (mg/dL)	2.1 (1.14,5.16)	0.5 (0.22,1.58)	0.004
TG (mg/dL)	130.80 (78.34)	82.87 (33.66)	< 0.001
TC (mg/dL)	174.20 (51.57)	182.61 (29.05)	0.41
LDL-C (mg/dL)	126.70 (50.06)	112.95 (28.98)	0.17
HDL-C (mg/dL)	36.70 (1.94)	58.78 (14.26)	< 0.001
NO measurement			
• $FeNO_{50}$(ppb)	16.95 (8.63, 92.40)	14.40 (6.50, 26.20)	0.347
• alvNO (ppb)	0.00 (0.00, 0.88)	0.00 (0.00, 0.32)	0.451
• CawNO (ppb)	113.55 (31.38, 330.53)	42.90 (22.13, 96.10)	0.033
• JawNO (pl/s)	2004.39 (761.78, 5190.60)	980.75 (521.44, 1906.97)	0.033

Data are present as mean (SD), median (IQR) or n (%); alvNO, alveolar NO; CawNO, NO concentration in the tissue of the upper airways; JawNO, total flux of NO in the conducting airways;

Table 4. Comparison between obese asthmatic children and non-obese asthmatic children.

Parameters	Obese N = 20	Non-obese N = 121	*P*-value
Age: year	11.20 (3.82)	11.98 (3.33)	0.341
Body weight: kg	63.83 (21.92)	44.22 (17.27)	< 0.001
Height: cm	147.04 (16.94)	148.82 (17.15)	0.666
Atopic asthma: n	13 (65)	89 (73.6)	0.429
PACT: score	23.45 (1.79)	23.96 (1.95)	0.495
PAQLQ: score	6.66 (0.26)	6.59 (0.42)	0.278
Dyslipidemia: n	14 (70)	50 (41.3)	0.027
TG: mg/dL	123.30 (61.96)	80.15 (31.48)	< 0.001
TC: mg/dL	181.45 (34.41)	182.11 (30.52)	0.930
LDL-C: mg/dL	116.80 (39.41)	113.44 (29.41)	0.654
HDL-C: mg/dL	53.45 (26.69)	57.83 (11.95)	0.223
CRP (mg/dL)	1.85 (0.82, 5.26)	0.42 (0.21, 1.51)	< 0.001
NO measurement			
• $FeNO_{50}$(ppb)	12.75 (5.73, 25.25)	15.30 (7.10, 26.35)	0.487
• alvNO (ppb)	0.00 (0.00, 0.9)	0.00 (0.00, 0.29)	0.282
• CawNO (ppb)	64.99 (25.50, 150.23)	42.25 (22.28, 96.85)	0.204
• JawNO (pl/s)	875.40 (519.75, 2318.13)	1020.40 (563.78, 1970.70)	0.868

Data are present as mean (SD), median (IQR) or n (%); alvNO, alveolar NO; CawNO, NO concentration in the tissue of the upper airways; JawNO, total flux of NO in the conducting airways;

Comparison of NO measurement between asthmatic children with obesity and those without obesity

There were no significant differences in $FeNO_{50}$, alvNO, CawNO, or JawNO values between children with obesity and those without obesity (**Table 4**). Asthmatic children with obesity had a considerably higher median (interquartile range) CRP concentration than those with a normal weight (1.85 [0.82, 5.26] vs. 0.42 [0.21, 1.51] mg/dL; $p < 0.001$). No significant differences in age, height, Pediatric Asthma Control Test scores, or Pediatric Asthma Quality of Life Questionnaire scores were observed between the two groups of children (**Table 4**).

Comparison of NO measurement between atopic children and non-atopic children

There were no significant differences in baseline characteristics between atopic and non-atopic children (children with a negative skin prick test). Atopic children had significantly higher median (interquartile range) $FeNO_{50}$ (17.75 [9.45, 33.70] vs. 7.20 [5.88, 11.20] ppb; $p < 0.001$), CawNO (47.35 [26.23, 99.93] vs. 27.85 [13.12, 59.08] ppb; $p = 0.027$), and JawNO (1296.45 [729.70, 2478.83] vs. 567.95 [376.08, 933.58] pl/s; $p < 0.001$) values than non-atopic children (**Table 5**).

Table 5. Comparison between atopic asthmatic children and non-atopic asthmatic children.

Parameters	Atopic N = 102	Non-atopic N = 39	*P*-value
Age: year	12.07 (3.23)	11.36 (3.80)	0.269
Body weight: kg	47.49 (19.99)	45.73 (17.07)	0.628
Height: cm	149.24 (17.02)	146.82 (17.31)	0.453
Obesity: n	13 (12.7)	7 (17.9)	0.429
PACT: score	23.74 (1.98)	24.28 (17.31)	0.134
PAQLQ (score)	6.58 (0.43)	6.65 (0.33)	0.390
Dyslipdemia	42 (41.2)	22 (65.4)	0.131

Table 5. (Continued)

Parameters	Atopic N = 102	Non-atopic N = 39	*P*-value
TG: mg/dL	83.32 (36.13)	93.97 (48.33)	0.158
TC: mg/dL	180.20 (31.38)	186.74 (29.740	0.260
LDL-C: mg/dL	112.94 (29.62)	116.48 (34.22)	0.544
HDL-C: mg/dL	56.17 (12.31)	59.94 (20.05)	0.178
CRP (mg/dL)	0.46 (0.23, 1.79)	1.05 (0.33, 1.89)	0.241
NO measurement			
• $FeNO_{50}$(ppb)	17.75 (9.45, 33.70)	7.20 (5.88, 11.20)	< 0.001
• alvNO (ppb)	0.00 (0.00, 0.64)	0.00 (0.00, 0.00)	0.191
• CawNO (ppb)	47.35 (26.23, 99.93)	27.85 (13.12, 59.08)	0.027
• JawNO (pl/s)	1296.45 (729.70, 2478.83)	567.95 (376.08, 933.58)	< 0.001

Data are present as mean (SD) or median (IQR); alvNO, alveolar NO; CawNO, NO concentration in the tissue of the upper airways; JawNO, total flux of NO in the conducting airways;

Subgroup analysis on atopic and, obesity status, dyslipidemia status, and HDL-C status

Subgroup analyses were performed to examine the associations between atopic status and different factors, including obesity status, dyslipidemia status, and HDL-C status. Both obese and non-obese children with atopic asthma exhibited significantly higher levels of $FeNO_{50}$ and JawNO compared to non-atopic children ($p < 0.001$). Additionally, $FeNO_{50}$ and JawNO levels were significantly elevated in both dyslipidemic and non-dyslipidemic children with atopic asthma in comparison to non-atopic individuals. ($p < 0.001$) (**Table 6**).

Significant differences were observed in the levels of $FeNO_{50}$, CawNO, and JawNO among individuals with different atopic and high-density lipoprotein cholesterol (HDL-C) statuses. Specifically, atopic asthmatic children with low HDL-C exhibited the highest levels of CawNO and JawNO compared to the other groups ($p = 0.023$ and $p < 0.001$, respectively). Although there was a trend towards higher alvNO in atopic children with low HDL-C compared to other groups, statistical significance was not reached due to the limited number of atopic children with low HDL-C (**Table 6**).

Table 6. Subgroup-comparison among atopic and non-atopic asthmatic children with obesity status, dyslipidemia status and HDL-C status.

NO Measurement	Atopic - Obesity N = 13	Atopic - Non Obesity N = 89	Non Atopic - obesity N = 7	Non Atopic - Non obesity N = 32	*P* value
$FeNO_{50}$(ppb)	17.40 (9.10,5.40)	17.80 (9.25, 32.75)	6.00 (4.80, 10.70)	7.60 (5.95,11.40)	<0.001
alvNO (ppb)	0.00 (0.00,1.35)	0.00 (0.00, 0.45)	0.00 (0.00, 0.30)	0.00 (0.00,0.00)	0.364
CawNO (ppb)	66.00 (35.75,230.65)	44.18 (24.60, 99.65)	53.73 (10.50, 152.10)	25.50 (13.40,56.10)	0.075
JawNO (pl/s)	1308.57 (768.17,3723.80)	1290.70 (728.80, 2470.30)	522.28 (382.60, 769.10)	573.80 (356.50,942.20)	< 0.001
NO Measurement	**Atopic - Dyslipidemia N = 42**	**Atopic - Non Dyslipidemia N = 60**	**Non Atopic - Dyslipidemia N = 22**	**Non Atopic - Non Dyslipidemia N = 17**	***P* value**
$FeNO_{50}$(ppb)	17.85 (13.63, 34.18)	16.80 (8.50, 33.08)	6.50 (6.65, 11.25)	8.30 (5.70,11.15)	< 0.001
alvNO (ppb)	0.00 (0.00, 0.39)	0.10 (0.00, 1.05)	0.00 (0.00, 0.00)	0.00 (0.00,1.3)	0.266
CawNO (ppb)	46.06 (28.68, 94.80)	51.15 (23.85, 107.43)	26.90 (11.84, 58.55)	29.55 (19.68,120.78)	0.142
JawNO (pl/s)	1380.80 (882.65, 2662.28)	1183.27 (611.10, 2338.10)	598.84 (406.35, 1025.23)	567.95 (363.03,867.98)	< 0.001

Table 6. (Continued)

NO Measurement	Atopic - LowHDL N = 7	Atopic -Normal HDL N = 95	Non Atopic - LowHDL N = 3	Non Atopic - Normal HDL N = 36	*P* value
$FeNO_{50}$(ppb)	17.60 (16.50, 102.0)	17.70 (9.00, 32.10)	6.90 (4.80, 11.16)	7.20 (5.83, 11.03)	< 0.001
alvNO (ppb)	0.50 (0.00, 1.70)	0.00 (0.00, 0.40)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.227
CawNO (ppb)	144.60 (32.10, 316.70)	44.72 (24.40, 98.00)	82.50 (48.85, 227.25)	25.50 (12.29, 56.10)	0.023
JawNO (pl/s)	2700.20 (1290.80, 5786.40)	1290.35 (724.80, 2320.10)	769.10 (739.80, 2250.84)	553.20 (356.50, 930.70)	< 0.001

Data are median (IQR); alvNO, alveolar NO; CawNO, NO concentration in the tissue of the upper airways; JawNO, total flux of NO in the conducting airways.

Discussion

We found that children with low HDL-C concentrations had higher CawNO and JawNO than children with normal HDL-C concentrations, but there was no significant difference in the median $FeNO_{50}$ concentrations. However, we did not find a difference in alvNO concentrations between children with low HDL-C concentrations and those with normal HDL-C concentrations. Concentrations of alvNO are increased in severe uncontrolled asthma.[15] All of the enrolled children in this study had controlled asthma, which could explain the lack of a difference in alvNO concentrations. Several studies have addressed the potential role of high HDL-C concentrations and better pulmonary lung function. A recent study in children showed associations between high HDL concentrations and an improvement in specific airway resistance and decreased bronchial responsiveness.[1] An association of HDL concentrations and FEV_1% predicted was also found in adult atopic asthma.[16] We recently showed an association between HDL-C concentrations and respiratory resistance as assessed by the forced oscillation technique.[5] In an in vitro study, it has been demonstrated that HDL-C can exert an inflammatory effect on the lungs.[17] Additionally, a murine model of asthma treated intranasally with a synthetic substance that enhanced HDL function showed a reduction in pulmonary inflammation and airway resistance.[18] In our present study, children with low HDL-C concentrations exhibited significantly higher levels of CRP, a marker of systemic inflammation, compared to children with normal HDL-C concentrations. On the other hand, children with high levels of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) did not display elevated CRP levels compared to those with normal TG, TC, and LDL-C levels. This suggests that children with low HDL-C concentrations may experience higher levels of chronic inflammation, leading to increased CawNO and JawNO, indicative of greater airway inflammation. However, it is worth noting that in our study, although obese children demonstrated significantly higher CRP levels than non-obese children, there were no significant differences observed in CawNO and JawNO. This finding can possibly be explained by the fact that CRP is primarily synthesized by hepatocytes, but it is also produced by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes.[19]

Previous studies have shown an association of obesity or BMI with $FeNO_{50}$ in non-asthmatic subjects.[20,21] However, we did not find any differences in NO measurement between asthmatic children with obesity and those without obesity. This finding is in line with a previous report that showed no difference in $FeNO_{50}$ concentrations between asthmatic children with obesity and those without obesity.[20] In addition, we recently reported an association between dyslipidemia, independent to obesity, and airflow obstruction as assessed by spirometry and respiratory resistance accessed by the force oscillation technique.[5,22] This evidence suggests that dyslipidemia and obesity have an effect on the respiratory function test through different mechanisms. Further molecular investigations are required to examine this finding.

Atopic children have higher $FeNO_{50}$ concentrations than non-atopic children and this is enhanced by asthma.[23] Similar to $FeNO_{50}$, atopic subjects show higher CawNO and JawNO than non-atopic subjects.[11,24] However, alvNO concentrations are not affected by the atopic status.[11,24] These findings can be explained by the fact that all of these three parameters ($FeNO_{50}$, CawNO, and JawNO) represent proximal airway inflammation, but alvNO may represent small airway or alveolar inflammation.[15] In the subgroup analysis based on the atopic status and type of abnormal lipid, a notable finding emerged indicating that children with both atopy and low HDL-C had the highest levels of $FeNO_{50}$, CawNO, and JawNO. This intriguing observation suggests a potential synergistic effect between low HDL-C and atopy, which could have an influence on the levels of these three parameters. It is important to note that this study serves as a preliminary exploration, and further investigations that specifically target asthmatic children with low HDL-C are necessary to establish and confirm the existence of this potential synergistic effect. Conducting such studies would provide a more comprehensive understanding of the interplay between atopy, lipid abnormalities, and airway inflammation in asthmatic children.

The present study did not reveal any significant associations between lipid profiles, apart from HDL-C, and the measured NO parameters. The existing body of research on the association between lipids and asthma has yielded conflicting findings. For example, a prospective cohort study focusing on infants born to mothers with physician-diagnosed asthma demonstrated that infants with elevated LDL-C levels experienced greater airway obstruction. In contrast, children with higher levels of HDL-C exhibited improved lung function and reduced bronchial hyperresponsiveness.[1] Similarly, in adults, low HDL-C levels and high TG levels have been linked to wheezing symptoms.[6] However, up to the author's knowledge, no prior investigation has specifically examined the relationship between blood lipid levels and NO measured parameters in asthmatic children. Thus, this preliminary study provides valuable insights into the potential significance of dyslipidemia as a comorbidity to be taken into account when aiming to enhance asthma outcomes in children.

Despite the valuable insights gained from this study, several limitations should be acknowledged. Firstly, the sample size of asthmatic children with low HDL-C was relatively small, comprising only 10 participants. This small sample size may limit the generalizability of our findings and could potentially affect the statistical power of the analyses. Therefore, caution should be exercised when interpreting the results related to the subgroup analysis of asthmatic children with low HDL-C. Furthermore, it is important to acknowledge that the number of obese children was observed in only 14% of the enrolled asthmatic children. This limited sample size may have restricted the ability to discern the independent effects of obesity and dyslipidemia on NO measurements in asthmatic children. Consequently, caution should be exercised when interpreting the findings related to the impact of obesity and dyslipidemia on NO levels, and further studies with larger sample sizes are warranted to provide more definitive conclusions. Therefore, a larger sample size may be necessary before advocating for universal screening of blood HDL-C in asthmatic children who do not possess other risk factors for metabolic syndrome, such as obesity. Secondly, the study design was cross-sectional, which hinders the establishment of causal relationships between lipid abnormalities, atopy, and NO parameters. Future longitudinal studies are warranted to better understand the temporal associations and potential mechanisms underlying these relationships. Lastly, it is important to note that our study lacked a non-asthmatic and non-dyslipidemia control group. The inclusion of such a control group would have allowed for a direct comparison and better assessment of the impact of dyslipidemia on NO measurements in asthmatic children. However, recent meta-analyses and studies have consistently demonstrated an association between dyslipidemia and the prevalence of asthma.[25-27] Despite the limitations mentioned, our study provides valuable insights into the potential influence of dyslipidemia and its interaction with atopy on airway inflammation in asthmatic children, as reflected by the levels of CawNO and JawNO. By examining these NO parameters, we were able to gain a better understanding of the inflammatory processes occurring in the airways of asthmatic children with dyslipidemia and atopy (**Figure 1**). Further research with larger sample sizes, longitudinal designs, and comprehensive assessments of confounding factors is warranted to confirm and expand upon these preliminary findings.

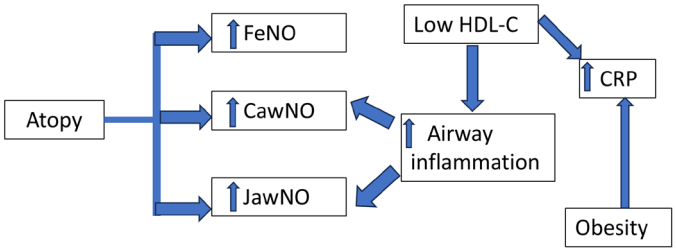

Figure 1. Proposed mechanism for HDL-C and asthma.

In conclusion, our preliminary findings suggest a potential association between HDL-C concentrations and CawNO and JawNO levels in asthmatic children, indicating a possible role of HDL-C in modulating airway inflammation. These results imply that interventions aimed at improving HDL-C concentrations may have a beneficial effect in reducing airway inflammation in asthmatic children. However, due to the limitations of our study, including the small sample size, further controlled research with a larger and more diverse sample size is needed to validate and strengthen our findings.

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Conflict of interest

All authors have no conflict of interest to declare.

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Author contributions

- PNC, WK, and WM designed the study.
- PNC, WJ, and PK contributed to data collection.
- PNC, AS, and WM performed the statistical analysis and interpretation of the results.
- WM drafted and edited the final manuscript.
- All authors read and approved the final manuscript.

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