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Carotenoids, fatty acids, and disease burden in obese minority adolescents with asthma

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Abstract

Background: Pediatric obesity-related asthma causes high disease burden, is associated with metabolic abnormalities, has few therapeutic options, and disproportionately affects urban minority children. Although poor diet quality is linked to asthma, the association of nutritional status with disease burden among children with obesity-related asthma is not well understood.

Objective: To quantify nutritional status, defined as concentrations of serum carotenoids and n-3 fatty acids, and its association with pulmonary function and metabolic markers among obese asthmatic children.

Methods: We quantified serum carotenoids and fatty acids in a study cohort of 158 urban minority adolescents including 39 obese asthmatics, 39 healthy-weight asthmatics, 38 obese controls and 42 healthy-weight controls and compared between the groups. We correlated carotenoid and fatty acid levels with pulmonary function indices and with insulin resistance and dyslipidemia.

Results: Mean total carotenoids were lowest in obese asthmatic children (0.41 µg/ml), lower than healthy-weight asthmatics (0.52 µg/ml, $p < 0.05$) and healthy-weight controls (0.60 µg/ml, $p < 0.001$). n-6/n-3 polyunsaturated fatty acid (PUFA) ratio also differed between the groups ($p < 0.05$). Total carotenoids positively correlated with percent-predicted FEV₁ and inversely correlated with insulin resistance among obese asthmatics only. n-6/n-3 PUFA ratio inversely correlated with percent-predicted FEV₁ in obese asthmatics.

Conclusions & Clinical Relevance: Our findings suggest that carotenoids, which are lowest in obese asthmatic children, may have protective effects on metabolic health and pulmonary function among obese asthmatic children. Similarly, n-3 PUFA appear to be protective for pulmonary function.

Introduction

In recent decades, asthma and obesity have become two of the most common pediatric health problems that disproportionately affect minority populations, including African

American and Hispanic children.^{1, 2} Obesity is now known to be an independent risk factor for asthma.^{3, 4} Several mechanisms distinguish obesity-related asthma from normal-weight asthma,⁵ such as the distinct patterns of pulmonary function deficits, and lack of response to available treatments.⁶⁻⁹ Furthermore, obese-asthmatic children show evidence of non-atopic Th1 polarized systemic inflammation, which is associated with pulmonary function deficits.^{6, 7} Insulin resistance, mediated by obesity, is also associated with worse pulmonary function and mediates the relationship between Th1 polarization and pulmonary function.⁷ Vitamin D deficiency, more common among obese children, is also associated with pulmonary function deficits in obese asthmatics, but not among normal-weight asthmatics.¹⁰ These findings suggest that dietary intake and metabolic measures likely play a role in obesity-related asthma.

Dietary intake has been researched as a contributing factor to both obesity and asthma, and recent investigations have focused on its role in obesity-related asthma.¹¹ Obesity is characterized by an increased intake of high energy, processed foods.¹² This leads to increased levels of circulating fatty acids (FAs),¹³ which have been associated with obesity and poor asthma outcomes.¹¹ Dietary FAs are classified according to their degree of saturation, as saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), with PUFA further subdivided into n-6 and n-3 forms. SFA and many n-6 PUFA are pro-inflammatory, while n-3 PUFA have anti-inflammatory properties. As such, a high ratio of n-6 to n-3 PUFA indicates a diet with pro-inflammatory potential, which has previously been linked to asthma risk in children.¹⁴

High intake of processed foods results in a decreased intake of micronutrients such as vitamins, minerals and antioxidants.¹⁵ Of particular interest are the carotenoids, which are antioxidants predominantly found in fruits and vegetables.¹⁶ Carotenoids have been reported to lower the risk of chronic diseases including cancer, cardiovascular disease, asthma, and chronic obstructive pulmonary disease, by inhibiting the damaging effects of free radicals.^{17, 18} Low α -carotene, β -carotene, and β -cryptoxanthin have specifically been linked to both asthma and obesity in observational studies.¹⁹⁻²² Poor diet quality has been linked to low lung function in children with asthma.²³ Diet modification, including increased consumption of fruits and vegetables, was found to improve asthma outcomes in adults, with a reduction in exacerbation risk.²⁴ However, no studies thus far have objectively quantified carotenoids or FAs and investigated their association with disease severity in obese children with asthma.

In this study we aimed to quantify serum carotenoid and FA concentrations as objective measures of nutritional status in urban minority adolescents and to investigate their association with disease burden in obese adolescents with asthma. We hypothesized that obese asthmatic adolescents have lower serum carotenoid concentrations, higher serum total FA concentrations, and a higher n-6/n-3 PUFA ratio compared to healthy-weight asthmatics and healthy-weight controls. Further, we hypothesize that carotenoid concentrations are inversely associated, while total FA and n-6/n-3 PUFA ratio are positively associated, with asthma burden in obese adolescents.

Methods

Study population and procedures

This cross-sectional, observational study was conducted in n=158 of 168 adolescents who were recruited for a prior study and on whom fasting serum samples were available for further analysis.⁷ Briefly, 168 Hispanic and African American adolescents, aged 13-18 years, were recruited from clinics at Children's Hospital at Montefiore, as previously described.⁷ The study cohort comprised of 42 obese adolescents with asthma, 42 healthy-weight adolescents with asthma, 40 obese controls without asthma and 44 healthy-weight control subjects without asthma. Obesity was defined as BMI>95th percentile and healthy-weight as BMI<85th percentile for age and sex.²⁵ Asthma classification was based on physician diagnosis, and confirmed by electronic medical records. To define disease severity and control, adolescents with asthma completed the Asthma Control Test (ACT) questionnaire,²⁶ and reported on symptom frequency, and medication use, which was utilized to calculate the Composite Asthma Severity Index (CASI).²⁷

Subjects underwent pulmonary function testing including spirometry and lung volume quantification by body plethysmography (SensorMedics, Yorba Linda, CA) as per the American Thoracic Society (ATS) criteria.²⁸ National Health And Nutrition Examination Survey (NHANES) reference values were used to calculate percent-predicted values of the spirometric indices.²⁹ Reference equations developed by ATS were used to calculate percent-predicted values of lung volume indices.³⁰ The percent predicted values of the following pulmonary function variables were included in the analysis: forced vital capacity (FVC), forced expiratory volume in 1st second (FEV₁), FEV₁/FVC ratio, mid-expiratory flow rates (FEF_{25-75%}), total lung capacity (TLC), residual volume (RV), RV/TLC ratio, expiratory reserve volume (ERV), functional residual capacity (FRC) and inspiratory capacity (IC).

Details of metabolic measures have been previously described.⁷ Briefly, measurements for HDL, total cholesterol and triglycerides were carried out in fasting serum by enzymatic immunoassay and measured by AU400 chemistry autoanalyzer (Beckman-Coulter Corporation) using commercially available enzymatic reagents. Triglycerides and total cholesterol were measured by direct enzymatic measurements, while HDL was separately measured using two reagent homogenous systems with selective detergents to homogenize the lipoprotein of interest. LDL levels were calculated using the Friedewald equation. Insulin was quantified by radioimmunoassay (Millipore Corporation) on a Wizard2 gamma counter (Perkin Elmer Corporation). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using glucose and insulin levels (glucose (mg/dl) × insulin (uU/ml) /405).

Participants whose samples were analyzed, and their guardians, provided written informed consent at the time of recruitment for future use of samples and the Institutional Review Board at Albert Einstein College of Medicine approved the study.

Serum carotenoid analysis

High performance liquid chromatography (HPLC) was used to determine serum concentrations of α -carotene, β -carotene, lycopene, β -cryptoxanthin and lutein using an

established method.²⁴ Briefly, to isolate carotenoids from the serum sample, ethanol was first added to allow deproteinization followed by ethyl acetate containing an internal standard (cantaxanthin), then vortexed and centrifuged (3,000rpm for 5 min at 4°C). The supernatant was collected in separate tubes and stored on ice. This process was repeated three times, adding ethyl acetate twice, then hexane to the pellet. Milli-Q water was then added to the pooled supernatant and the mixture was vortexed and centrifuged. The supernatant was decanted and placed on a nitrogen evaporator until completely evaporated. The dried extract was reconstituted in dichloromethane:methanol (1:2 vol/vol). Chromatography was performed on Agilent 1200 series gas chromatograph (Agilent Technologies, United States, Part No. G1311-90011) including Chemstation data analysis software at a flow rate of 0.3 ml/min. Carotenoids were analyzed using a mobile phase of acetonitrile: dichloromethane: methanol 0.05% ammonium acetate (85:10:5) and a wavelength of 450 nm.

Serum fatty acid analysis

Total fatty acids were analyzed based on an established method using gas chromatography.³¹ Methanol:toluene (4:1 v/v) containing an internal standard (C19:0) was added to the serum sample. Fatty acids were methylated using acetyl chloride, then heated for 60 min at 100°C. The reaction was stopped by adding 6% potassium chloride (K₂CO₃). Samples were then centrifuged and the supernatant used for gas chromatograph analysis of the fatty acid methyl esters using a Hewlett-Packard 6890 Gas Chromatograph, with a 30m (0.25mm ID) carbon-silica capillary column and flame ionization detector and Chemstation version A.04.02 software.

Statistical analysis

Descriptive analysis was performed on the study cohort. Continuous variables were compared between the four study groups, obese adolescents with asthma, healthy-weight adolescents with asthma, obese controls without asthma and healthy-weight controls without asthma using Analysis of Variance (ANOVA) followed by post-hoc Bonferroni correction. Categorical variables were compared using X² test. The primary outcomes of interest were between-group comparison of serum carotenoids and total FA, SFA, MUFA, PUFA and n-6/n-3 PUFA ratio and their association with metabolic measures (insulin and lipid levels) and pulmonary function variables. Serum carotenoids and FA were log transformed to achieve normal distribution and were compared between the four study groups using ANOVA. The association of carotenoids and of n-6/n-3 PUFA ratio with metabolic measures and with pulmonary function was calculated using Pearson correlation. Since BMI z-score and HOMA-IR were associated with serum carotenoid levels, multivariable linear regression analysis was performed to elucidate the independent association of carotenoids with pulmonary function variables. Analysis was performed on STATA v.14 and significance was defined *a priori* as p-value of <0.05.

Results

Demographic and clinical characteristics of the study cohort

The demographic and clinical characteristics of the study population are summarized in Table 1. The study groups did not differ in regards to age, gender, or ethnicity. By definition,

participants in the obese groups had a higher BMI than participants in the healthy weight groups. There were several differences in pulmonary function indices between the study groups. Obese controls had higher FEV₁ in comparison to healthy-weight asthmatic participants and a higher FEF_{25-75%} in comparison to both healthy-weight asthmatic and control subjects. Obese and healthy-weight asthmatic participants had lower FEV₁/FVC and FEF_{25-75%} than healthy-weight control subjects. Both obese groups had lower RV, RV/TLC ratio, and FRC in comparison to healthy-weight asthmatic and control groups. Participants in the obese groups had a higher IC than those in the asthmatic and healthy control groups. No differences were observed in FVC, TLC, and ERV.

Analysis of metabolic measures identified differences in glucose, HDL, insulin, and HOMA-IR levels among the four groups. Glucose was higher in obese asthmatics as compared to healthy-weight asthmatic and healthy-weight controls. HDL was lower in obese asthmatics in comparison to healthy-weight control participants and lower in obese non-asthmatics in comparison to both healthy-weight control and healthy-weight asthmatic participants. Both obese groups had higher insulin and HOMA-IR levels compared to participants in the healthy-weight asthmatic and healthy-weight control groups. No differences were observed in cholesterol and LDL levels.

Obese asthmatics did not differ from healthy-weight asthmatics in their disease severity, based on the CASI score (3.46 ± 2.10 vs. 3 ± 1.93 , $p=0.32$) or their asthma control, based on the ACT score (19.39 ± 4.77 vs. 20.22 ± 3.91 , $p=0.41$).

Comparison of carotenoids and FAs between the study groups

Comparison of serum carotenoids and FAs between the study groups is summarized in Figure 1. Serum levels of total carotenoids inversely associated with BMI z-score ($r = -0.38$, $p < 0.001$) for the entire cohort, and were lower in obese asthmatic participants compared to those in the healthy-weight asthmatic and healthy-weight control groups (Figure 1a). There was a trend of decreased n-3 PUFA levels in both obese groups compared to the healthy-weight groups, but these differences did not reach statistical significance (Figure 1f). In keeping with lower n-3 PUFA, the n-6/ n-3 PUFA ratio was different across groups, with means being higher in the obese groups, although posthoc testing did not identify between group differences (Figure 1g), and correlation analysis did not identify a significant association with BMI z-score ($r = 0.12$, $p = 0.14$). No differences were observed in total FA, SFA, MUFA or n-6 PUFA levels between the study groups (Figures 1b-e). Since total carotenoids and the n-6/n-3 PUFA ratio differed between the study groups, we focused further analysis on their association with pulmonary function variables and metabolic measures.

Association of carotenoids and FAs with pulmonary function for each study group.

Total carotenoid levels positively correlated with FEV₁/FVC, RV, RV/TLC and IC for the entire cohort (Table 2a). Among the individual groups, total carotenoid levels positively correlated with FEV₁ only in obese asthmatic participants (Table 2a, Figure 2a). The n-6/n-3 PUFA ratio was inversely correlated with FEV₁/FVC ratio for the entire cohort a finding that approached statistical significance (Table 2b). The n-6/n-3 PUFA ratio inversely correlated

with FEV₁ only in obese asthmatic participants (Figure 2b) and with FEV₁/FVC and FEF_{25-75%} in obese controls (Table 2b). There was no association between the n-6/n-3 PUFA ratio and pulmonary function in the healthy-weight asthmatic or healthy-weight controls (Table 2b).

Association of carotenoids and FAs with metabolic measures for each study group

Total serum carotenoids negatively correlated with HOMA-IR levels for the entire cohort, a finding that was observed only among the obese asthmatic subgroup (Table 2a, Figure 3a). Additionally, serum carotenoids positively correlated with HDL for the entire cohort, and also among obese asthmatic and obese control participants (Table 2a, Figure 3b). The association between the n-6/n-3 PUFA ratio and HOMA-IR approached statistical significance for the whole study cohort but did not correlate with HOMA-IR levels for any individual study group or with HDL levels (Table 2b).

Having identified an association between carotenoids and both FEV₁ and HOMA-IR in obese asthmatics, and that between carotenoids and BMI z-score, we conducted multivariable analysis to investigate whether the association of carotenoids with FEV₁ was mediated by HOMA-IR and/or BMI z-score. When adjusted for HOMA-IR alone, the association between carotenoids and FEV₁ (β 22.1 (95% CI 2.4-41.9), $p=0.03$) was attenuated (β 19.4 (95% CI -2.6-41.4), $p=0.08$) suggesting that HOMA-IR is a mediating factor in the relationship between carotenoids and FEV₁ in obese asthmatics. Inclusion of BMI z-score in the model did not further influence the association between carotenoids and FEV₁ (β 19.2 (95% CI -3.7-42.0), $p=0.09$).

Discussion

Our study is the first to examine the associations between serum carotenoid and FA concentrations, metabolic markers and pulmonary function in pediatric obesity-related asthma. We found that serum levels of total carotenoids were the lowest in obese asthmatic adolescents, lower than both healthy-weight asthmatics and obese controls. Total carotenoids positively correlated, and the n-6/n-3 PUFA ratio inversely correlated, with FEV₁, only in the obese asthmatic participants. These findings suggest that total carotenoids and n-3 fatty acids may have a protective effect on airway obstruction in adolescents with obesity-related asthma. We also found an inverse association between serum levels of total carotenoids and insulin resistance, again in obese asthmatic children only. Furthermore, there was a direct positive association between total carotenoids and HDL in both obese study groups, suggesting that carotenoids are additionally protective against metabolic dysregulation in pediatric obesity. Insulin resistance mediated the association between carotenoids and FEV₁, suggesting that insulin resistance may influence pulmonary function in obese children with asthma by modulating both immune responses and serum carotenoids. Overall, our findings suggest that poor nutritional status, reflected in low carotenoid and n-3 fatty acid levels are correlated with airway obstruction and metabolic dysregulation in pediatric obesity-related asthma, together suggesting a role for targeted dietary intervention to decrease disease morbidity in urban minority adolescents with obesity-related asthma.

Low serum carotenoid levels have been associated with both asthma^{19, 21, 32, 33} and obesity^{20, 34, 35} in several pediatric studies. However, the specific mechanism by which carotenoids are related to obesity-related asthma are currently unknown. Carotenoids are antioxidants, primarily found in fruit and vegetables.¹⁶ As both obesity³⁶ and asthma³⁷ are characterized by increased oxidative stress in children, the antioxidant effect of carotenoids is likely to be central to the protective effects of carotenoids in the airways. Fresh fruit and vegetable intake has been associated with higher FEV₁ and FVC in children, particularly in those with wheeze/asthma^{23, 38, 39} suggesting that carotenoids may link with asthma via altered pulmonary function. While several of these studies used dietary recall to quantify carotenoid intake,^{23, 40} here we show a direct association between serum carotenoids and FEV₁, findings that were observed specifically in obese children with asthma and not among healthy-weight children. We speculate that the protective effect of carotenoids was only observed in the obese asthma subgroup, as this group is most susceptible to oxidative stress⁴¹ and thus has most potential to benefit from the anti-oxidative properties of carotenoids.

Our findings are supported by randomized trials of carotenoid supplementation, specifically that of lycopene, in improving FEV₁ and exercise tolerance.^{42, 43} Since some, but not all, studies have found an association between serum carotenoids and asthma,^{44, 45} we speculate that inclusion of both normal-weight and obese children with asthma in a study cohort may potentially mask the association, which we report is restricted to obese children. As carotenoids are well described to have anti-oxidant effects, we hypothesize that their local antioxidant activity may modulate airway inflammation,⁴⁶ a hypothesis that is supported by earlier reports of a strong correlation between serum and airway carotenoid levels,²¹ suggesting that carotenoids may potentially influence airway physiology via its local concentration.

Our observed association between carotenoids and insulin resistance has been previously reported in healthy adults⁴⁷ and differs from that observed in obese children in atopic asthma.⁴⁸ Unlike findings reported by Ferreira et.al,⁴⁸ we found that obese children with asthma had lower levels of serum carotenoids than their obese non-asthmatic counterparts and insulin resistance was associated with serum carotenoids. Based on our findings of the mediating effect of insulin resistance on the association of carotenoids with FEV₁, we speculate that metabolic and dietary factors are interrelated. Thus dietary intervention rather than weight loss alone may be a feasible option for obese children with asthma. A recent study did provide evidence supporting this hypothesis, wherein obese adolescents that had the highest weight loss had the largest improvement in both anti-oxidant and vitamin levels and cardiometabolic profile.⁴⁹

Similar to carotenoids, serum n-3 PUFA have been demonstrated to have anti-inflammatory actions⁵⁰ and low levels have been linked to asthma^{51, 52} and obesity.^{53, 54} In contrast, many n-6 PUFA have been shown to have pro-inflammatory effects, largely due to their role in leukotriene production, which has been postulated to increase the risk of allergy and asthma.⁵⁰ As such, the n-6/n-3 PUFA ratio has been found to be a risk factor for asthma.¹⁴ The n-6/n-3 PUFA ratio was different across the four groups in our study, with higher mean ratios in the two obese groups as compared to the healthy-weight groups. There was also an

association between the n-6/n-3 PUFA ratio and FEV₁ in obese asthmatic children and with FEV₁/FVC ratio in obese non-asthmatic children, findings that have previously been reported in adults.⁵⁵ While the associations that we have observed suggest a potential role for n-3 PUFA supplementation in asthma, previous studies using dietary supplementation of n-3 fatty acids in children to mediate asthma severity have had conflicting results.⁵⁶ One study reported that n-3 fatty acid supplementation improves pulmonary function and decreases asthma severity in environments with controlled allergen exposure.⁵⁷ Other studies have not observed a benefit of n-3 fatty acid supplementation, showing no effect on asthma severity or lung function.^{51, 58} Since we observed associations between the n-6/n-3 PUFA ratio and lung function specifically in obese asthmatics, and not in healthy-weight asthmatics, we speculate that obesity may be a confounding factor among asthmatic children, which thereby complicates the outcomes of intervention studies. None of the previous studies in asthmatic children have examined the effects of supplementation in relation to BMI; it is not known if this would have produced differing results. Thus, further sub-analysis of existing studies and future studies are needed, factoring in BMI as a modulating factor in the association between asthma and n-3 or n-6 PUFA.

We recognize that our study has several limitations. It is a secondary analysis of previously collected samples. Moreover, we do not have simultaneous measures of airway samples that would allow us to further elucidate whether systemic measures correlate with airway markers as previously observed.²¹ Further, it is a single-center study with the study population comprised only of African American and Hispanic children, limiting the generalizability of our findings. However, in light of the higher disease burden among these children,^{1, 2} our findings are pertinent and provide direction for modified management of obese adolescents with asthma.

In summary, we found that in obese asthmatic children, serum carotenoids were positively correlated with pulmonary function and metabolic measures, while the n-6/n-3 PUFA ratio was negatively associated with pulmonary function. These results suggest that improving nutritional status may improve clinical outcomes among obese children with asthma. Thereby, these findings open an avenue of diet modification as another approach to address the burden of pediatric obesity-related asthma. The results also highlight the need for future studies to elucidate the mechanisms that underlie the links between obesity-related asthma, carotenoids and n-3 fatty acids.

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Figure 1. Comparison of serum carotenoids and fatty acids between the study groups. a) Total carotenoids, b) total FA, c) SFA, d) MUFA, e) n-6 PUFA, f) n-3 PUFA and g) n-6/n-3 PUFA ratio were compared between the study groups

Figure 2.

Association of percent predicted FEV₁ with a) carotenoids and b) n-6/n-3 ratio among the study groups.

Figure 3.
Association of carotenoids with a) HOMA-IR and b) HDL levels among the study groups.

Table 1.

Demographic and clinical characteristics of the study groups

	All participants (n=158)	Healthy-weight Controls (n=42)	Healthy-weight Asthmatics (n=39)	Obese Controls (n=38)	Obese Asthmatics (n=39)	p-value
Demographics						
Age (years)	16.1±1.6	16.4±1.7	15.9±1.6	16.3±1.3	15.8±1.8	0.21
Male (n(%))	69 (43.6)	14 (33.3)	20 (51.3)	14 (36.4)	21 (53.8)	0.16
Hispanic (n(%))	92(58.6)	23 (54.8)	22 (57.9)	24 (63.2)	23 (59)	0.9
BMI (kg/m ²)	27.3±8.2	21.3±2.2	22.4±2.5	32.7±8.8 ^{*†}	33.3±7.9 ^{*†}	<0.001
Pulmonary Function Indices[^]						
FVC	95.1±14.2	93.0±12.0	94.4±12.3	98.9±12.4	94.2±18.9	0.26
FEV ₁	89.2±12.2	90.0±11.8	85.9±13.3 [*]	93.4±12.6 [*]	87.4±10.2	0.04
FEV ₁ /FVC ratio	82.2±7.6	85.6±6.9 ^{†, **}	80.1±7.8 ^{**}	83.3±7.4	79.8±7.1 [†]	<0.001
FEF _{25-75%}	78±21.1	83.1±19.9	70.6±23.0 [†]	85.7±19.7 [*]	72.4±18.0 [‡]	0.001
TLC	97.9±13.4	99.1±13.9	99.1±14.0	97.7±13.9	95.8±12.0	0.68
RV	105±39.1	121.6±39.5 ^{#†}	118.9±39.1 ^{*, **}	85.5±35.0 ^{#*}	93.5±30.4 ^{†***}	<0.001
RV/TLC ratio	23.8±6.6	27.3±6.5 ^{#†}	26.5±5.9 ^{*, **}	19.8±6.0 ^{#*}	21.7±5.0 ^{†***}	<0.001
FRC	88.2±20.9	97.0±18.9 ^{#†}	96.6±20.9 ^{*, **}	79.2±18.5 ^{#*}	80.0±18.5 ^{†***}	<0.001
ERV	80.4±22.9	85.4±24.5	85.0±25.4	77.5±19.4	73.6±20.5	0.06
IC	92.1±17.2	84.9±15.3 ^{#†}	86.6±13.2 ^{*, **}	98.7±15.9 ^{#*}	98.2±19.5 ^{†***}	<0.001
Metabolic Measure^{^^}						
Glucose	89.7±8.3	87.6±6.1 [†]	87.8±9.9 ^{**}	90.9±8.6	92.8±7.6 ^{†**}	0.007
Cholesterol	147.5±30	145.5±29.4	152.5±36.4	147.2±31.0	144.9±21.8	0.93
HDL	48.7±11.3	54.8±11.2 ^{#†}	50.9±12.1 ^{**}	46.3±9.3 [#]	42.2±8.1 ^{†***}	<0.001
LDL	82.1±23.2	77.8±24.2	81.0±21.7	83.4±27.5	86.4±18.4	0.39
Insulin	25±17.7	16.5±7.6 ^{#†}	18.0±7.3 ^{*, **}	31.2±22.8 ^{#*}	35.3±20.0 ^{*†}	<0.001
HOMA-IR	5.6±4.2	3.6±1.7 ^{#†}	3.9±1.8 ^{*, **}	7.1±5.5 ^{#*}	8.1±4.7 [†]	<0.001

[^] Pulmonary function indices are reported as percent predicted for age, sex and height, other than FEV₁/FVC ratio and RV/TLC ratio which are reported as percentages.

^{^^} Metabolic measures are reported in mg/dl other than insulin that is reported in mU/ml.

Post-hoc Bonferroni analysis:

^{*} differs between obese control and healthy-weight asthmatic groups

[†] differs between obese asthmatic and healthy-weight control groups

[‡] differs between obese asthmatic and obese control groups

^{**} differs between healthy-weight asthmatic and healthy-weight control groups

[#] differs between healthy-weight control and obese control groups

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Table 2a.

Univariate association of total carotenoids with pulmonary function and metabolic measures.

	All participants	Healthy-weight controls	Healthy-weight asthmatics	Obese Controls	Obese asthmatics
Pulmonary function indices[*]					
FEV ₁ [^]	0.05, 0.54	-0.07, 0.67	-0.02, 0.89	-0.003, 0.99	0.35, 0.03
FEV ₁ /FVC ratio	0.17, 0.04	0.26, 0.10	-0.09, 0.61	0.13, 0.42	0.08, 0.62
FEF _{25-75%} [^]	0.11, 0.16	0.11, 0.48	-0.05, 0.75	0.10, 0.57	0.20, 0.22
RV [^]	0.20, 0.01	0.001, 1.00	0.15, 0.35	0.19, 0.26	0.07, 0.66
RV/TLC ratio	0.27, 0.001	0.07, 0.66	0.05, 0.75	0.22, 0.19	0.27, 0.10
ERV [^]	0.07, 0.39	0.20, 0.21	-0.19, 0.24	-0.22, 0.18	0.11, 0.51
IC [^]	-0.17, 0.04	-0.23, 0.17	-0.24, 0.14	-0.03, 0.84	0.22, 0.17
Metabolic measures[*]					
HOMA-IR ^{^^}	-0.28, <0.001	0.011, 0.50	0.12, 0.47	-0.09, 0.60	-0.42, 0.01
HDL ^{^^}	0.33, <0.001	0.03, 0.84	0.24, 0.14	0.38, 0.02	0.31, 0.05

* Results are reported as correlation coefficient r, followed by its p value (r, p value). Those that are statistically significant are in bold font.

[^] Percent predicted values of FEV₁, FEF_{25-75%}, RV, ERV and IC, and percent values of FEV₁/FVC and RV/TLC ratios were included in the Pearson correlation analysis with serum carotenoids.

^{^^} HDL measured in mg/dl and HOMA-IR, calculated using insulin measured in mU/ml and glucose measured in mg/dl, were included in the Pearson correlation analysis with serum carotenoids.

Table 2b.

Association of n-6/n-3 PUFA ratio with pulmonary function and metabolic measures

	All participants	Healthy-weight controls	Healthy-weight asthmatics	Obese controls	Obese asthmatics
Pulmonary function indices *					
FEV ₁ [^]	-0.08, 0.33	-0.16, 0.32	-0.07, 0.69	-0.27, 0.11	-0.34, 0.04
FEV ₁ /FVC ratio	-0.16, 0.05	-0.14, 0.40	-0.11, 0.51	-0.40, 0.01	0.04, 0.82
FEF _{25-75%} [^]	-0.14, 0.08	-0.21, 0.19	-0.11, 0.52	-0.35, 0.03	-0.06, 0.74
RV [^]	-0.04, 0.67	0.02, 0.93	-0.09, 0.58	0.17, 0.30	0.10, 0.56
RV/TLC ratio	-0.09, 0.29	-0.01, 0.97	-0.16, 0.35	0.05, 0.79	0.19, 0.25
ERV [^]	-0.10, 0.25	0.04, 0.83	-0.18, 0.29	-0.29, 0.09	0.16, 0.34
IC [^]	-0.002, 0.98	-0.11, 0.50	0.06, 0.72	0.04, 0.79	-0.25, 0.13
Metabolic measures *					
HOMA-IR ^{^^}	0.15, 0.06	0.21, 0.18	-0.22, 0.20	0.27, 0.11	-0.04, 0.83
HDL ^{^^}	0.02, 0.79	0.12, 0.46	0.20, 0.23	0.02, 0.89	-0.18, 0.29

* Results are reported as correlation coefficient r, followed by its p value (r, p value). Those that are statistically significant are in bold font.

[^] Percent predicted values of FEV₁, FEF_{25-75%}, RV, ERV and IC, and percent values of FEV₁/FVC and RV/TLC ratios were included in the Pearson correlation analysis with serum carotenoids.

^{^^} HDL measured in mg/dl and HOMA-IR, calculated using insulin measured in mU/ml and glucose measured in mg/dl, were included in the Pearson correlation analysis with serum carotenoids.