Published in final edited form as:

J Allergy Clin Immunol. 2022 October ; 150(4): 861–871. doi:10.1016/j.jaci.2022.04.033.

Endotyping pediatric obesity-related asthma: contribution of anthropometrics, metabolism, nutrients, and CD4+ lymphocytes, to pulmonary function

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Abstract

Background: Obesity-related complications including visceral fat, metabolic abnormalities, nutrient deficiencies, and immune perturbations are interdependent but have been individually associated with childhood asthma.

Objective: To endotype childhood obesity-related asthma by quantifying contributions of obesity-related complications to symptoms and pulmonary function.

Methods: Multi-omics analysis using Similarity Network Fusion followed by mediation analysis were performed to quantify prediction of obese asthma phenotype by different combinations of anthropometric, metabolic, nutrient, and Th cell transcriptome and DNA methylome datasets.

Results: Two clusters (n=28 and 26) distinct in their anthropometric (neck and midarm circumference, waist to hip ratio (WHR) and BMI z-score), metabolic, nutrient, and Th cell transcriptome and DNA methylome footprint predicted 5 or more pulmonary function indices across 7 different dataset combinations. Metabolic measures attenuated the association of neck, WHR and BMI z-score with FEV_1/FVC ratio and ERV, of neck, midarm, and BMI z-score with FRC, but only of WHR with IC. Nutrient levels attenuated the association of neck, midarm circumference, and BMI z-score with FRC, and of WHR with FEV_1/FVC ratio, ERV and IC. Th cell transcriptome attenuated the association of all four anthropometric measures with FEV_1/FVC ratio, but only of WHR with ERV and IC. The DNA methylome attenuated the association of all four anthropometric measures with FEV_1/FVC ratio and ERV, but only of WHR with IC.

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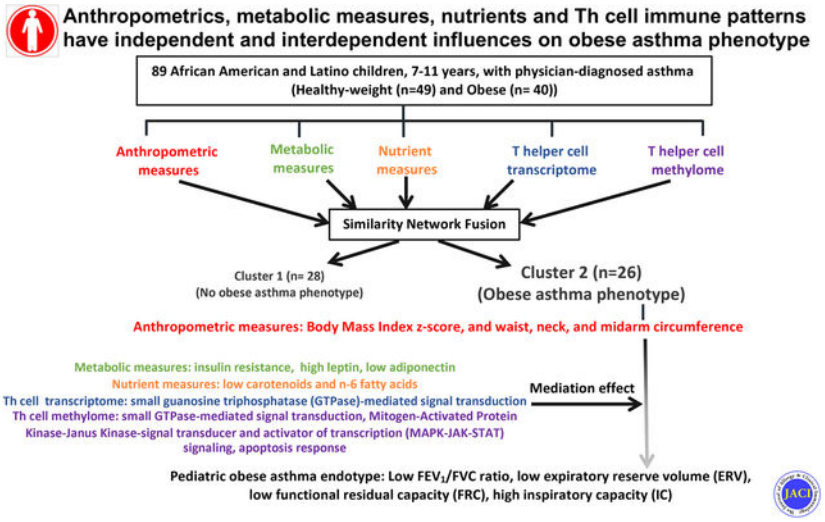
The authors do not have any conflict of interest.

Conclusion: Anthropometric, metabolic, nutrient, and immune perturbations have individual but interdependent contributions to obese asthma phenotype, with the most consistent effect of WHR, highlighting the role of truncal adiposity in endotyping childhood obesity-related asthma.

Graphical Abstract



Capsule summary

Obesity-related complications including visceral fat, metabolic abnormalities, nutrient deficiencies, and immune perturbations have individual as well as interdependent influences on lung function deficits contributing to the pediatric obese asthma endotype

Keywords

Obesity; asthma; fat distribution; metabolic abnormalities; nutrients; Th lymphocytes

Introduction

Childhood obesity and asthma are highly prevalent conditions that are inter-related and disproportionately affect minority populations, including those of African American and Hispanic descent.[1–3] Compared to healthy-weight children with asthma, obese children with asthma have more severe disease with worse pulmonary function deficits,[3] and are less responsive to currently available treatments for asthma.[4]

Early investigations into contribution of obesity-related complications to asthma emphasized the role of excess adipose tissue with a predominant truncal distribution.[5–7] Subsequent studies highlighted the interactive relationship between truncal fat and metabolic abnormalities including insulin resistance and dyslipidemia.[6,8,9] Recognizing the key role of diet in obesity, macro- and micronutrients have also been linked with obesity-related asthma. Higher levels of pro-inflammatory saturated fatty acids (SFA) and n-6/n-3 polyunsaturated fatty acid (PUFA) ratio,[10,11] and lower levels of carotenoids, the anti-oxidants found in fruits and vegetables,[12] as well as vitamin D, have been associated with asthma and obesity,[13]

including pulmonary function deficits.[11,14] In keeping with their interactive relationship with truncal fat, insulin resistance and dyslipidemia also correlate with fatty acid and micronutrient levels.[11]

In addition, obesity is associated with adipose tissue-mediated systemic non-atopic inflammation.[6,15] In keeping with this observation, obese children with asthma have non-atopic T helper 1 (Th1) polarized systemic inflammation that correlates with pulmonary function deficits.[3,15,16] Exploration of Th cell transcriptome to identify biological pathways underlying non-atopic responses revealed upregulation of genes in the Cell Division Cycle 42 (CDC42) pathway, a Rho-GTPase pathway, in Th cells from obese children with asthma as compared to healthy-weight children with asthma.[17] CDC42 expression was associated with expression of interferon gamma (IFNγ) and tumor necrosis factor (TNF), two cytokines linked with Th1 cells. The DNA methylome in obese asthmatic Th cells was also enriched for Rho-GTPase pathways.[18] Th cell transcriptome and methylome were both enriched in their association with pulmonary function deficits found in obesity-related asthma.[18]

Although these obesity-related complications are inter-related, the interdependence of their influences on the obese asthma phenotype are not well understood. To address this gap in knowledge, we used multi-omics analytic tools to quantify the independent and interdependent associations of anthropometric, metabolic, and nutrition measurements and Th cell immune patterns, defined by the Th cell transcriptome and DNA methylome, with the obese asthma phenotype, defined by symptom burden-based classification of asthma severity and control, and pulmonary function indices. We hypothesized that effects of obesity on anthropometrics, metabolic profile, nutrition, and Th cell immune patterns have individual as well as interdependent influences on childhood asthma.

Methods

Study Participants

The data included in this analysis was collected from 120 African American and Hispanic children ages 7 and 11 years with a physician diagnosis of asthma, as previously reported and detailed in the online repository.[18] Children were classified as healthy-weight (body mass index (BMI) 5th to <85th percentile) (n=61) or obese (BMI>95th percentile for sex and age) (n=59) at recruitment using the CDC definition.[19] The study was approved by the Albert Einstein College of Medicine IRB where the study was conducted and by the Children's National Medical Center IRB for the current analysis of the de-identified data sets.

Study Measures (details for all measures are included in the online repository)

Asthma severity, asthma control and pulmonary function testing—Participant-reported data was used to calculate the Composite Asthma Severity Index (CASI) score,[20] a measure of severity, and Asthma Control Test (ACT) score,[21] a measure of control. Pulmonary function testing (spirometry and lung volume quantification using nitrogen washout) was performed on all participants according to the American Thoracic Society

guidelines.[22] Percent predicted values of pulmonary function indices[23,24] were included in the analysis.

Anthropometric Measurements—Participants underwent measurement of weight in kilograms, and of height, neck, midarm, waist, and hip circumference in centimeters. These measures, along with BMI z-score and waist to hip ratio (WHR), comprised the anthropometric dataset.

Metabolic Measures—Fasting serum from all participants was used to quantify measures of glucose and lipid metabolism and adipokines. The metabolic dataset included total cholesterol, HDL, LDL, triglyceride, insulin, glucose, leptin, and adiponectin levels, as well as the homeostatic measurement of insulin resistance (HOMA-IR) values, calculated as (glucose (mg/dl) × Insulin (uU/ml) /405).[25]

Nutrient Measures—Carotenoids, 25-hydroxy vitamin D, α-tocopherol, and SFA, MUFA, PUFA levels and n6/n3 PUFA ratio, also quantified in fasting serum, comprised the nutrition dataset.

Th-cell Transcriptome—RNA from unstimulated Th cells underwent directional RNA-sequencing (RNA-seq).[17] 103 of the 120 RNA-seq libraries passed quality control, including 48 from obese asthmatic and 55 from healthy-weight Th cells. Using DESeq2, differentially expressed genes between the two groups at false discovery rate (FDR (q value)) <0.05) were retained as the transcriptome dataset.

Quantification of the Th-cell DNA Methylome—The enzyme digestion-based HELP (Hpall Tiny Fragment Enrichment by Ligation-mediated PCR)-tagging assay was used to quantify the Th-cell DNA methylome.[26] 99 of the 120 libraries passed quality control criteria, including 45 from obese asthmatic and 54 from healthy-weight asthmatic Th cells. Multivariate analysis was performed to account for effects of technical covariates (batch of library preparation and of sequencing), and biological covariates. CpG sites where the model was statistically significant at an FDR (q value) <0.05, and between-group difference in methylation was significant (p value<0.05), were retained as the DNA methylome dataset.

Statistical Analysis (additional details in the online repository)

The analytic approach is summarized in Figure 1. Based on data availability on all variables in all datasets, the study cohort narrowed to 89 participants, including 49 healthy-weight and 40 obese children with asthma. Variables in the lower dimension predictive datasets (anthropometric, metabolics and nutrition) were compared between the study groups using Welch's two sample t-test and reported as means with standard deviation. Statistical significance was set *a priori* at p<0.05. Th cell transcriptome and DNA methylome subsets for the 89 samples were analyzed as detailed above.

Multi-omic Analysis through Similarity Network Fusion (SNF)—We performed multi-omic analysis using the SNFtool package[27] on R statistical software v.4.1.0 to quantify the individual and composite contribution of the predictive datasets to obese asthma phenotype (CASI and ACT scores, and pulmonary function). A total of twenty-one fusions

were performed for the twenty-one different combinations of the five predictive datasets. Samples were clustered using spectral clustering for each of these combinations and Welch's two sample t-tests were conducted to quantify the discriminating ability of the clusters within each combination for CASI and ACT scores and pulmonary function indices.

Downstream analysis on clusters identified by SNF tool—To verify the SNF findings, univariate analyses were conducted on the predictive datasets comparing their distribution between the two clusters from SNF analysis and their association with CASI, ACT, and pulmonary function. Given that more than one predictive dataset correlated with CASI, ACT, and pulmonary function, we conducted mediation analyses[28,29] to identify the independent contribution of anthropometric, nutrition and metabolic datasets and that of the Th cell transcriptome and methylome to outcomes of interest. Biological relevance of Th cell transcriptome and DNA methylome mediating the associations was derived using NetworkAnalyst software and reported as Gene Ontology (GO) pathways.[30] To elucidate the clinical relevance of predictor datasets, we quantified variance of FEV_1/FVC ratio, ERV, FRC and IC explained by neck circumference, midarm circumference, WHR and BMI z-score in the anthropometric dataset, leptin, adiponectin and HOMA-IR in the metabolic dataset, and total carotenoids and n-6 PUFA, that were significantly different ($p<0.05$) between clusters. Analysis was limited to these three datasets since they can be more readily quantified in a clinical setting at this time as compared to the Th cell transcriptome and DNA methylome.

Th cell transcriptome, DNA methylome, and patient characteristics are available at dbGAP study ID 33254.

Results

Characteristics of the study cohort

The 89 study participants were similar in their demographics but differed in several pulmonary function indices, including FVC, FEV_1/FVC ratio, RV/TLC ratio, ERV, FRC and IC [Table 1]. As expected, anthropometric measures were higher in obese children with asthma than healthy-weight children with asthma, except for height, which did not differ between the groups [Table 2]. Among the metabolic measures, HOMA-IR and leptin levels were higher, and adiponectin levels were lower, in obese children as compared to healthy-weight children with asthma [Table 2]. Fatty acid levels did not differ substantially between the two groups, while β-carotene and total carotenoid levels were lower in obese children relative to healthy-weight children with asthma [Table 2]. Gene expression of 365 genes and methylation at 497 CG sites also differed between these two groups [Table E1a **and** E1b].

Similarity Network Fusion analysis of predictive datasets and their combined association with the obese asthma phenotype

Figure 2 summarizes the predictive ability for CASI and ACT score, and pulmonary function indices [Figure 2, **x-axis**], of the sample clusters, identified by the SNF tool for each of the 21 combinations of the predictive datasets [Figure 2, **y-axis]**. Sample clusters

in 7 of the 21 combinations predicted 5 or more pulmonary function indices, of which FEV_1/FVC ratio, ERV, IC, and to a lesser degree, FRC, were most frequently predicted. Few clusters predicted CASI or ACT score. Among the 7 combinations, samples in the 2 clusters were identical for 6 combinations and included 54 of the 89 samples; 28 were in 'Cluster 1' and 26 in 'Cluster 2'. The 6 combinations included the transcriptome and anthropometric (TxA), transcriptome, anthropometric and nutrition (TxAxN), transcriptome, methylome, anthropometric and nutrition (TxMxAxN), anthropometric and nutrition (NxA), anthropometric and metabolic (AxMb), and methylome, anthropometric, and metabolic (MxAxMb) combinations.

Association of clusters with the obese asthma phenotype and variables in predictive datasets

To confirm the prediction ability of SNFtool-based clustering, we compared CASI, ACT and pulmonary function between the two clusters and found that the clusters were predictive of pulmonary function but not of CASI or ACT score. Percent predicted $FEF_{25–75\%}$, ERV, FRC, and percent FEV_1/FVC ratio were lower, and IC was higher in Cluster 2 relative to Cluster 1 [Figure E1a]. Between-cluster comparison of individual variables within each predictive dataset revealed that all variables in the anthropometrics dataset were higher in Cluster 2 as compared to Cluster 1 [Figure E1b]. In the metabolic dataset, leptin, insulin and HOMA-IR were higher and adiponectin was lower, with no difference in lipids, in Cluster 2 as compared to Cluster 1 [Figure E1c]. In the nutrition dataset, n-6 PUFA, lutein, β-cryptoxanthin, lycopene, β-carotene, and total carotenoids were lower in Cluster 2 relative to Cluster 1 [Figure E1d]. In addition, 195 genes were differentially expressed and 286 CGs were differentially methylated between Cluster 1 and Cluster 2 [Table E2a **and** E2b]. Distribution of variables in lower dimension datasets in the samples that clustered and those that did not cluster are summarized in Table E3. Furthermore, variables within the anthropometric, nutrient, metabolic, Th cell transcriptome, and Th cell methylome datasets that distinguished the clusters correlated with ERV, FRC and IC, and percent FEV_1/FVC ratio [Figure E2]. These findings verify those of SNF analysis regarding the contribution of each predictive dataset to clustering of samples associated with the obese asthma phenotype.

Mediation analysis to identify independent effects of predictive datasets on obese asthma phenotype

Since the anthropometric dataset was present in each of the 6 combinations with identical sample clusters, we quantified the effect of metabolic, nutrition, Th cell transcriptome and methylome datasets on the association of the anthropometric measures (neck, midarm, WHR and BMI z-score) with the 4 pulmonary function indices (FEV_1/FVC ratio, ERV, IC, and FRC) that were discriminated by the 2 clusters. The association of FEV_1/FVC ratio with neck, WHR, and BMI z-score was rendered non-significant by the metabolic dataset [Figure 3a], that with WHR was rendered non-significant by the nutrition dataset [Figure 3a], and the association with neck, midarm, WHR and BMI z-score were attenuated by the Th cell transcriptome and methylome [Figure 3b]. The association of ERV with neck, WHR, and BMI z-score was rendered non-significant by the metabolic dataset [Figure 3c], and that with WHR, and BMI z-score, was rendered non-significant by the nutrition dataset [Figure 3c]. Although the Th cell transcriptome only attenuated the association of ERV with WHR

[Figure 3d], the methylome attenuated the association with neck, midarm, WHR, and BMI z-score [Figure 3d]. FRC was associated with neck, midarm and BMI z-score but not with WHR; all these associations were rendered non-significant by both metabolic and nutrition datasets [Figure 3e]. The transcriptome only attenuated the association with neck and WHR [Figure 3f] while the methylome attenuated the association of FRC with WHR [Figure 3f]. IC correlated with neck, midarm, WHR and BMI z-score, but its association with WHR was the only one rendered non-significant by both metabolic and nutrition datasets [Figure 3g] as well as by the Th transcriptome and methylome [Figure 3h].

Given the findings of mediation analysis, to define the clinical relevance of variables within predictor datasets that were different between clusters, we quantified the predictive ability of neck, midarm, WHR, BMI z-score from the anthropometric dataset, HOMA-IR, leptin and adiponectin from the metabolic dataset, and total carotenoids and n-6 PUFA from the nutrient dataset for FEV_1/FVC ratio, ERV, FRC, and IC. These variables together predicted 20.2% variance of FEV_1/FVC ratio, 41.7% variance of ERV, 22.6% variance of FRC, and 51.2% variance of IC.

Pathway analysis of differentially expressed and methylated genes that mediated the association of anthropometric measures with pulmonary function

The highest number of differentially expressed genes (n=64) mediated the association of anthropometrics with FEV_1/FVC ratio with a 93% overlap between those mediating the association of neck, WHR and BMI-z-score [Table E4a]. Although fewer genes mediated the association of anthropometrics with ERV and FRC, they overlapped by 63% to 90% with those mediating the association with FEV_1/FVC ratio [Table E4b]. The overlapping differentially expressed genes were enriched for coagulation pathways, small GTPase mediated signal transduction, transmembrane receptor protein tyrosine kinase signaling, epidermal growth factor receptor signaling, and cell migration [Figure 4a, b].

Unlike the transcriptome, a higher number of differentially methylated genes mediated the association of neck, BMI z-score and WHR, with ERV, with 73% overlap between them. Furthermore, only 20% of CG sites mediating the association of anthropometrics with ERV overlapped with those mediating the association with FEV_1/FVC ratio or IC. The enrichment of differentially methylated genes also differed; those mediating the association of anthropometrics with ERV were enriched for RNA processing, Janus kinase (JAK)-signal transducer and activator of transcription (STAT) (JAK-STAT) and mitogen-activated protein kinase (MAPK) signaling, actin polymerization, and negative regulation of apoptosis [Figure 4c, d], while those mediating the association with FEV_1/FVC ratio were enriched for small GTPase-mediated signal transduction, transforming growth factor (TGF) receptor signaling, and RNA processing [Figure 4e, f], and those mediating the association with IC were enriched for cell cycle regulation, DNA damage/ apoptosis response, protein modification, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) regulation [Figure 4g, h].

Discussion

Our -omics approach including anthropometrics, metabolic measures, nutrient levels, Th cell transcriptome, and Th cell DNA methylome, identified a subset of participants that clustered into two distinct groups that were predictive of pulmonary function deficits consistently associated with the pediatric obese asthma phenotype.[15,16] As compared to Cluster 1, participants in Cluster 2 had lower pulmonary function indices including FEV_1/FVC ratio, ERV, FRC and higher IC. Participants in Cluster 2 had higher truncal and peripheral body fat, metabolic abnormalities, including insulin resistance, high leptin and low adiponectin levels, lower carotenoid and n6 PUFA levels, and differential Th cell gene expression and DNA methylation. These obesity-related complications, including the mechanical load of fat, metabolic abnormalities, nutrient deficiencies, and immune perturbations, were predictive of obese asthma phenotype independently as well as in an interdependent manner, but not predictive of symptoms-based classification of asthma severity and control. Although pulmonary function indices are inter-related, we identify differences in the predictive abilities of obesity-related complications for individual pulmonary function indices. While FEV_1/FVC ratio was influenced by all four obesity-related complications, IC correlated with anthropometrics with minimal influence of metabolic abnormalities, nutrient levels, and immune perturbations.

Our observed association of FEV_1/FVC ratio with neck and WHR, measures of truncal fat distribution, mid-arm circumference, a measure of peripheral fat distribution, and with BMI z-score, a measure of generalized fat distribution, validates several prior studies that examined the association of one or more of these anthropometric measures with FEV_1/FVC ratio as a measure of airflow obstruction in obese children with asthma.[3,31] We build on this literature by demonstrating the contribution of the interactive relationship of body fat with metabolic abnormalities, nutrient levels, and altered Th cell immune profiles, on its links with FEV_1/FVC ratio. The attenuating effect of each of these obesity-related complications on the association of FEV_1/FVC ratio with neck and mid-arm circumference, WHR, and BMI z-score, identified their individual contribution to airflow obstruction in pediatric obesity-related asthma. While each variable within each obesity-related complication can serve as a potential biomarker, our study highlights their co-existence and cumulative contribution to the disease phenotype.

ERV is another pulmonary function index that is reduced in the context of obesity and obesity-related asthma.[15,16,32] However, unlike the attenuating effect of metabolic measures, nutrient levels, and Th cell transcriptome and methylome on all associations of FEV_1/FVC ratio with anthropometric measures, they only attenuated the association of ERV with WHR. The association of ERV with BMI z-score was attenuated by metabolic measures, nutrient levels and Th cell methylome, but not the Th cell transcriptome, while Th cell methylome was the only predictive dataset that influenced the association of ERV with mid-arm circumference. These observations suggest that metabolic and nutrient levels influence the association of truncal and generalized body fat distribution but not that of peripheral body fat with the obese asthma phenotype. These findings also highlight the discriminating association of obesity-related complications with the obese asthma phenotype.

Intriguingly, FRC, another pulmonary function index influenced in obesity and obesity-related asthma, was predicted by neck and BMI z-score but not by WHR. Similar to their discriminating effects on ERV, metabolic measures and nutrient levels attenuated the association of neck and BMI z-score with FRC, with minimal contribution of Th cell profiles. Lastly, IC, one of the least well described aspects of the obese asthma phenotype, was associated with neck and WHR, mid-arm, and BMI z-score. Metabolic measures, nutrient levels and Th cell immune profiles only attenuated the association of WHR with IC and did not influence any other association, identifying IC as the one pulmonary function index that was most independently associated with body fat distribution.

The association of WHR with pulmonary function indices was most consistently influenced by metabolic abnormalities, nutritional deficiencies, and Th cell immune profiles. These observations are highly clinically relevant and novel for pediatric pulmonology because they identify the association of WHR, metabolic syndrome, and pulmonary disease, reported in the adult population,[33] as early as pre-adolescent years. Given the high prevalence and increasing incidence of pediatric obesity,[1] these findings highlight the need for routine inclusion of WHR as a measure of truncal adiposity during clinic visits. Given existent pediatric predictive cutoffs,[34] and the changing visceral fat deposition for the same body weight in US children,[35] partly driven by racial influences on fat distribution,[36] inclusion of truncal adiposity as an additional vital sign will facilitate early identification of children at-risk to develop obesity-related asthma. Although dietary carotenoid and fatty acids are not measured routinely, each of the carotenoids included in the analysis have potent anti-oxidant properties, including β-carotene, which comprises 20% of total carotenoids. Given that leptin, adiponectin, HOMA-IR, and carotenoids and n6-PUFA, along with anthropometric measures including WHR, explain up to 50% of the variance of pulmonary function indices, their quantification in obese children or healthy-weight children with truncal adiposity will directly inform endotyping as well as risk stratification, bringing precision medicine into the realm of childhood obesity-related asthma.[37] Our findings of the association between metabolic abnormalities with the obese asthma phenotype support their quantification, which is more routine, particularly among children with truncal adiposity, followed by consideration of metformin and lipid lowering agents in addition to nutrient management, that have been effective in adult disease management,[38–42] as novel therapeutic options for pediatric obese asthma endotype. Studies are needed to investigate the effectiveness of these interventions in pediatric obesity-related asthma.

There are few known therapeutic targets for the immune perturbations found in pediatric obesity-related asthma. We found a substantial effect of Th cell transcriptome and DNA methylome on the association of anthropometric measures with pulmonary function indices, with marked overlap between differentially expressed genes but not between differentially methylated genes that mediated the association. The overlapping differentially expressed genes were enriched for small GTPase mediated signaling pathways and cell migration, while the Th cell DNA methylome was enriched for JAK-STAT, MAPK, NFkB, and TGF receptor signaling, as well as apoptosis response, cell cycle regulation, actin polymerization and cell migration. We have previously linked upregulation of small GTPases with non-allergic Th cell responses,[18] which are also known to influence cell migration, apoptosis and MAPK-NFkB signaling.[43–45] These observations suggest that small GTPase upregulation in

Th cells may influence the obese asthma phenotype through increased Th cell viability, migration, and activation of JAK-STAT-MAPK-NfkB signaling. These novel biological mechanisms, once confirmed in functional studies, will identify small GTPase pathways and their corresponding proteins as biomarkers as well as novel therapeutic targets for the pediatric obese asthma endotype.

We recognize that our study cohort did not include obese and healthy-weight participants without asthma which precludes our ability to quantity the distinct as well as overlapping effects of obesity-related complications on pulmonary function deficits among those with obesity-related asthma as compared to those with obesity alone. Our findings provide the foundation to further investigate the discriminating roles of these obesity-related complications between obese children with and without asthma. Furthermore, our sample size was small and was additionally limited by the quantification of all variables in the five predictive datasets. Yet, our identification of a subset of samples that clustered together and were similar for several variables in each of the predictive datasets, highlights that discriminating features of obesity tend to co-occur. These associations also suggest that samples that did not cluster either did not have the requisite number of obesity-related complications or these features were not abnormal enough to meet the threshold. Clinically, this suggests that the obese asthma endotype is associated with either higher intensity or number of obesity-related complications. Comparative analyses, like those conducted in this study, between obese children with and without asthma, are needed to address this question. Since these variables were quantified in a cross-sectional manner among children with clinically stable disease at recruitment, their relationship during disease exacerbation could not be ascertained. Having included only pre-adolescent children, and those of African American and Hispanic ethnicities, further influences the generalizability of our findings. However, these findings are highly relevant because of the racial disparities that exist in the burden of pediatric asthma and obesity.[1,2] We are also unable to validate our findings in another cohort since there is none other where all these obesity-related complications along with measures that define the obese asthma phenotype have been quantified together.

Conclusion

In summary, this is the first study to conduct a multi-omics analysis and identify the independent and inter-dependent roles of body fat distribution, metabolic, and nutrition abnormalities, and Th cell immune profiles in airflow obstruction and lung volume deficits in obesity that are distinct measures of obese asthma phenotype, narrowing down on the contribution of waist to hip ratio, insulin resistance and leptin/adiponectin levels, perturbations in micronutrients, including carotenoids and dietary fatty acids, as well as a subset of genes in Th cells, to pediatric obesity-related asthma. Confirmation of these findings and distinguishing the contribution of these obesity-mediated complications to lung function in obesity without asthma will define these variables as biomarkers of the obese asthma endotype. Inclusion of these variables in routine evaluation of obese children would identify those at-risk for obesity-related asthma and suggest potential therapeutic strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to acknowledge the National Institutes of Health (Grants HL118733 and HL141849) for funding this study.

This research was funded by the National Institutes of Health (HL118733 and HL141849)(D.R.).

Abbreviations

SFA	Saturated fatty acids
PUFA	Polyunsaturated fatty acids
MUFA	Monounsaturated fatty acids
Th	T helper
CDC42	Cell division cycle 42
BMI	Body Mass Index
CASI	Composite Asthma Severity Index
ACT	Asthma Control Test
WHR	Waist to hip ratio
FVC	Forced vital capacity
FEV_1	Forced expiratory volume in 1^{st} second
$FEF_{25–75\%}$	Forced expiratory flow at 25–75% of FVC
TLC	Total lung capacity
RV	Residual volume
ERV	Expiratory reserve volume
FRC	Functional residual capacity
IC	Inspiratory capacity
FDR	False discovery rate
JAK-STAT	Janus kinase *(*JAK*)*-signal transducer and activator of transcription *(*STAT*)*
MAPK	Mitogen-activated protein kinase
TGF	Transforming growth factor

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Key messages

- Obesity-related complications including visceral fat, metabolic abnormalities, nutrient deficiencies, and immune perturbations have interdependent influences on lung function contributing to the pediatric obese asthma endotype
- Metabolic abnormalities, nutrient deficiencies, and immune perturbations most consistently attenuate the association of waist to hip ratio, a measure of visceral fat, with pulmonary function deficits.
- Evaluation of waist to hip ratio in obese children may identify those at-risk for obesity-related asthma

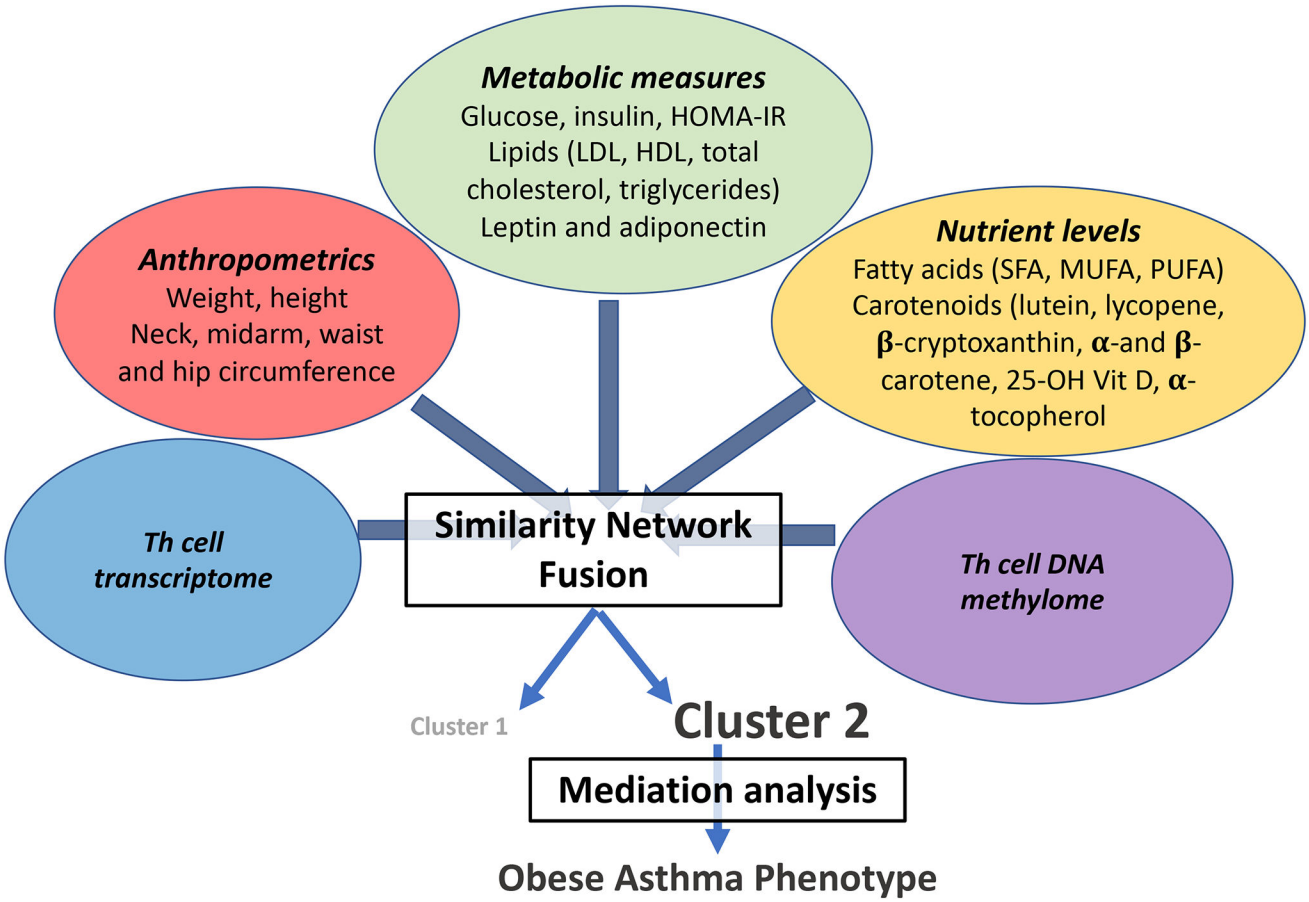

Figure 1. Summary of statistical analysis plan.

Similarity network fusion was performed on predictive datasets, including anthropometric and metabolic measures, nutrient levels, and Th cell transcriptome and DNA methylome to identify their association with the obese asthma phenotype. The individual contribution of each predictive dataset to the obese asthma phenotype was quantified by mediation analysis.

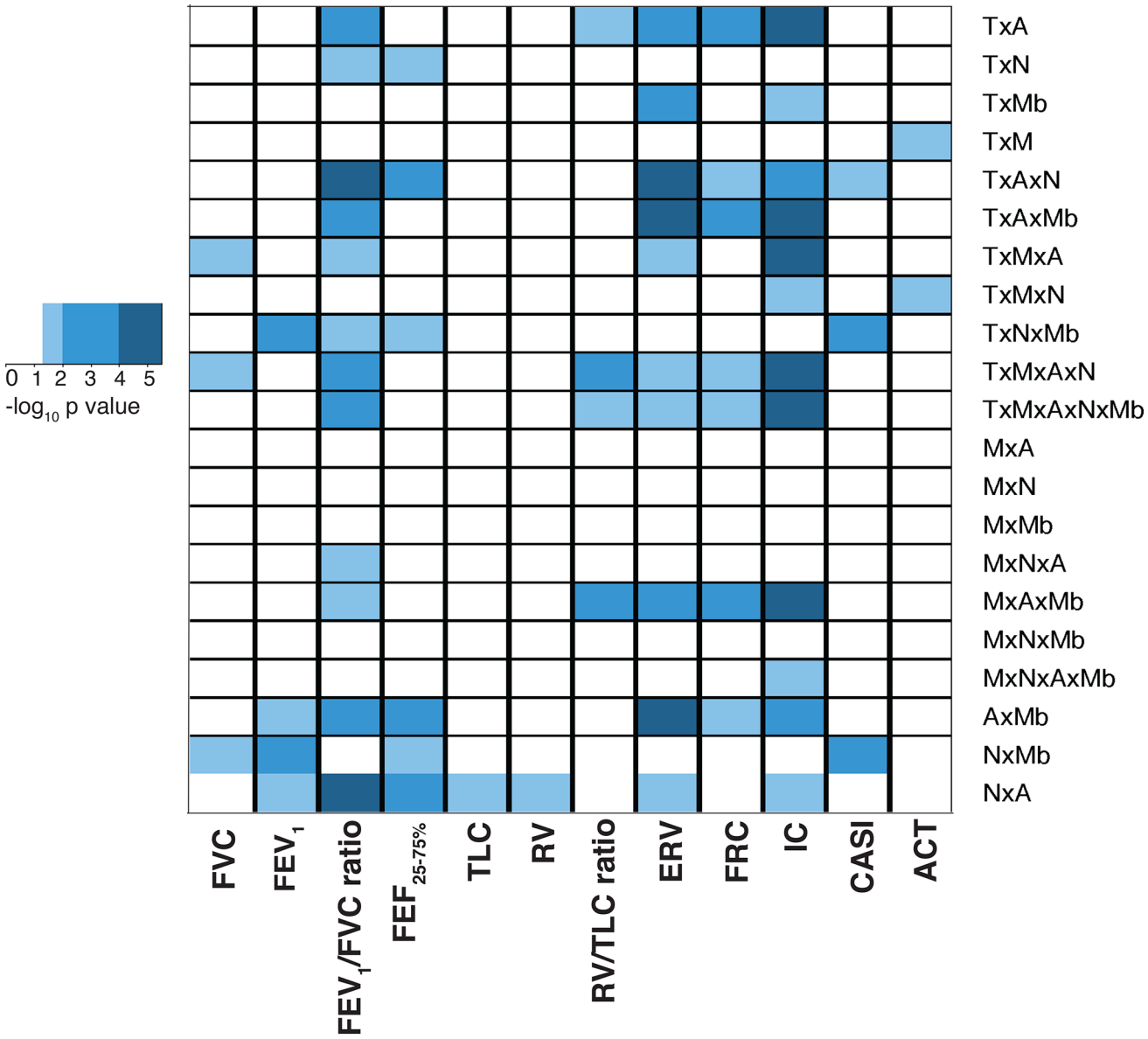

Figure 2. Summary of SNF analysis.

The heatmap reports the signficance of prediction of CASI, ACT and pulmonary function indices, plotted on the x-axis, by the sample clusters, determined by SNF tool, for each of the 21 different combinations of the 5 predictive datasets, including anthropometric measures (A), metabolic measures (Mb), nutrient levels (N), and Th cell transcriptome (T) and DNA methylome (M), plotted on y-axis. The color key summarizes the significance of the predictive ability of the sample clusters within each combination, for CASI, ACT or pulmonary function index. White denotes an association that did not reach statistical significance (p=>0.05); light blue denotes associations with p<0.05 and ≥0.01, intermediate shade of blue denotes associations with p<0.01 and ≥0.0001, and dark blue denotes associations with p<0.0001.

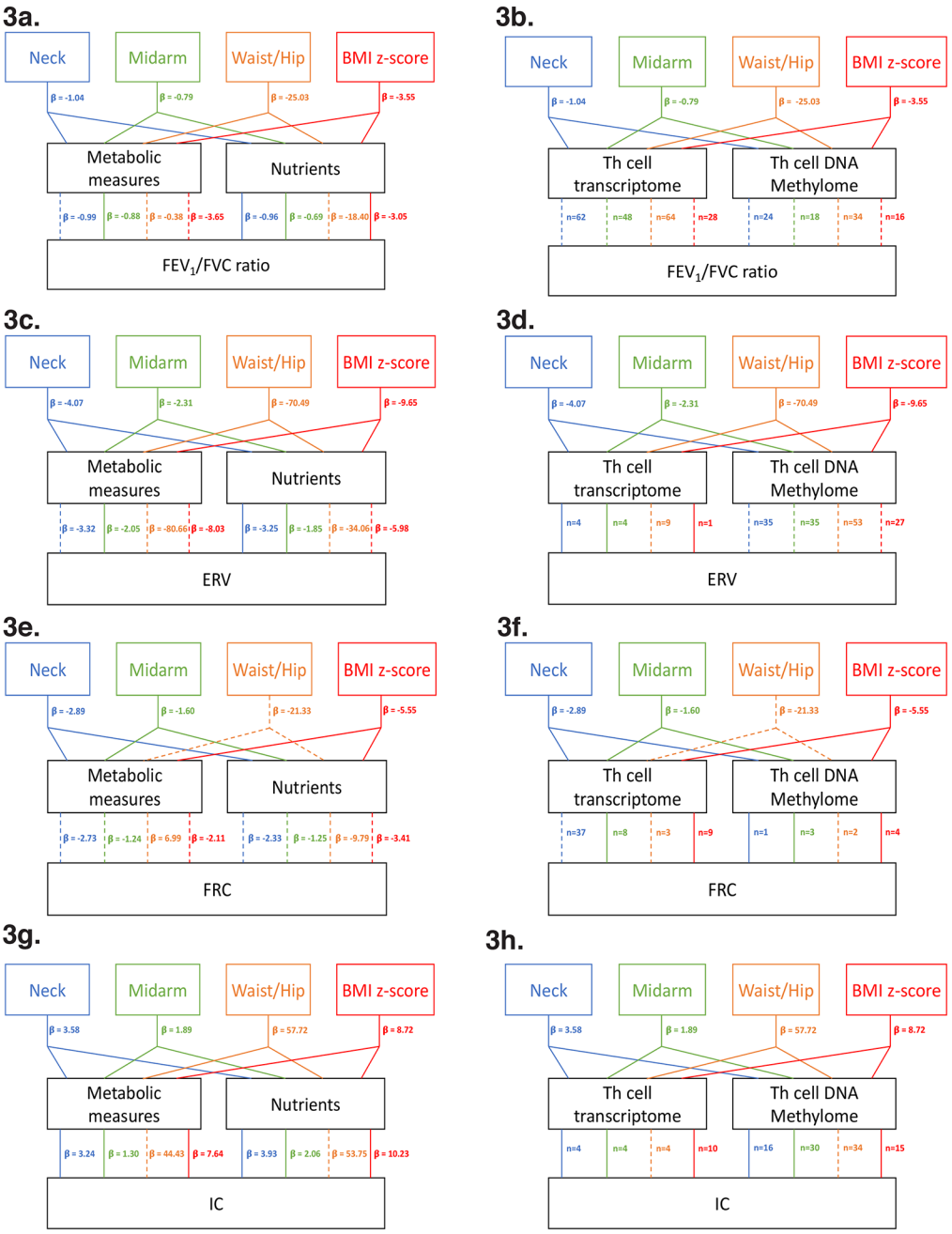

Figure 3. Mediation effects of metabolic and nutrient datasets, and Th cell transcriptome and DNA methylome, on the association of anthropometric measures with pulmonary function.

The mediation effects of metabolic and nutrient datasets are reported as change in beta (β) value of the association while that of the transcriptome and DNA methylome are reported as the number of genes mediating the effect, since the large number of genes precluded biologically meaningful interpretation of change in β value of the association. Statistically significant associations between anthropometric measures (neck, midarm, WHR, and BMI z-score), and pulmonary function indices (FEV_1/FVC ratio, ERV, FRC and IC) are depicted with solid lines, while significant associations of anthropometric measures with the pulmonary function index that were rendered non-significant by the predictor

dataset, or were not significant even prior to mediation analysis (e.g. WHR with FRC), are shown as dashed lines. Statistical significance was set *a priori* at $p<0.05$. **a**. summarizes the mediation effect of metabolic and nutrition datasets, and **b**. the mediation effect of Th cell transcriptome and methylome, on the association of FEV_1/FVC ratio with neck, midarm, WHR, and BMI z-score. **c**. summarizes the mediation effect of metabolic and nutrition datasets, and **d**. the mediation effect of Th cell transcriptome and methylome, on the association of ERV with neck, midarm, WHR, and BMI z-score. **e**. summarizes the mediation effect of metabolic and nutrition datasets, and **f**. the mediation effect of Th cell transcriptome and methylome, on the association of FRC with neck, midarm, WHR, and BMI z-score. **g**. summarizes the mediation effect of metabolic and nutrition datasets, and **h**. the mediation effect of Th cell transcriptome and methylome, on the association of IC with neck, midarm, WHR, and BMI z-score.

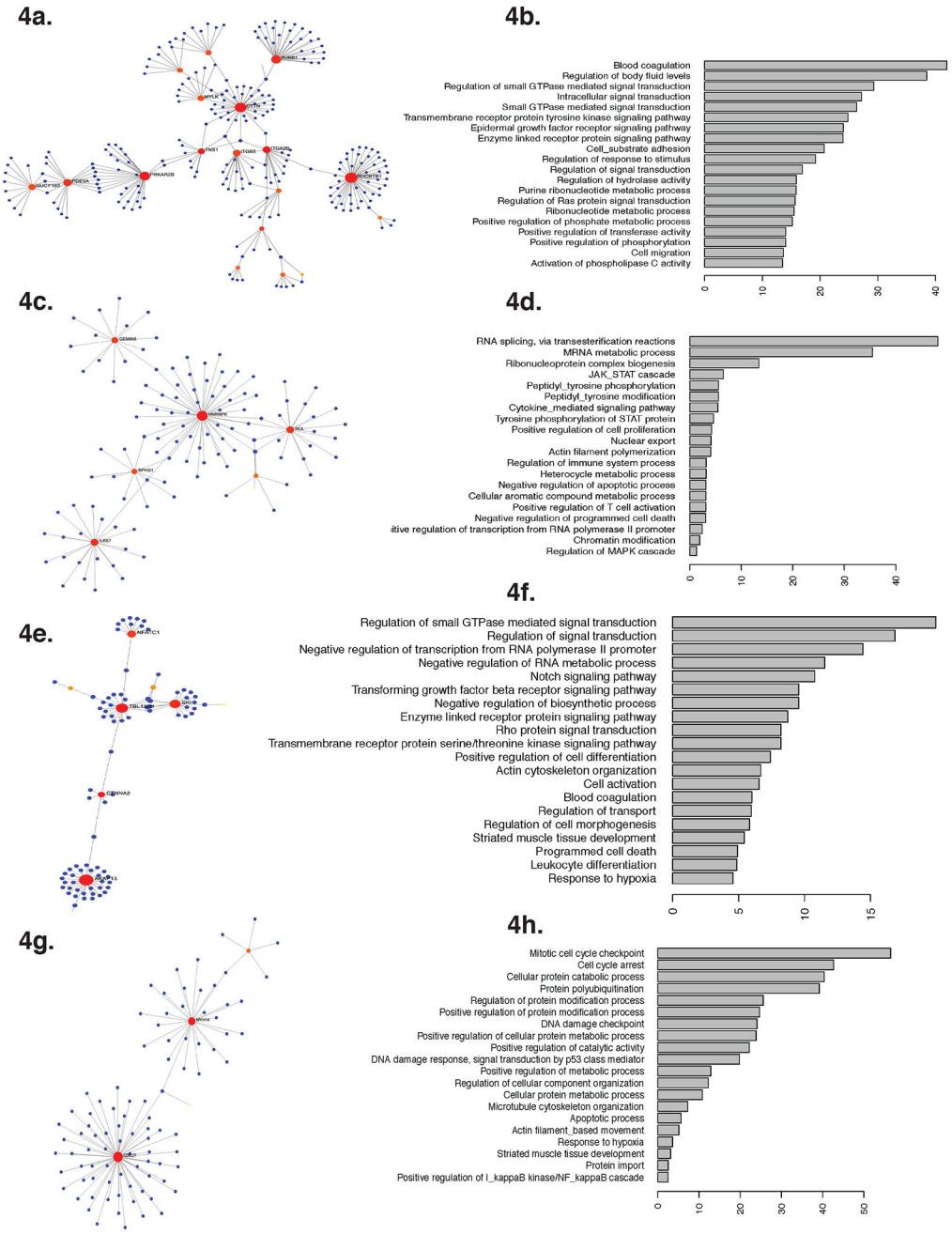

Figure 4. Pathway analysis of Th cell transcriptome and DNA methylome that mediated the association of anthropometrics with pulmonary function.

There was over 63% overlap between differentially expressed genes that mediated the association of anthropometric measures with FEV_1/FVC ratio, ERV and IC. Functional interpretation of these differentially expressed genes are visualized as **a**. Pathway analysis, and **b**. top 20 Gene Ontology pathways for biological process in which the differentially expressed genes were enriched. Unlike the transcriptome, there was minimal overlap among the differentially methylated genes that mediated the association of anthropometric measures with different pulmonary function indices. Functional interpretation of the differentially methylated genes that mediated the association of anthropometric measures are reported

as pathway analyses and their top 20 Gene Ontology pathways enriched for biological processes for ERV **[Fig. c, d]**, for FEV_1/FVC ratio **[Fig. e, f]** and for IC **[Fig. g, h]**.

Table 1.

Demographic and clinical characteristics of the study groups

	Healthy-Weight (n=49)	Obese (n=40)	P value
Demographics			
Age (years)	8.79 ± 1.27	8.93 ± 1.37	NS
Male (n (%))	27 (55.10)	24 (60.00)	NS
Hispanic (n (%))	29 (59.18)	27 (67.50)	NS
Pulmonary function indices *			
FVC	95.02 ± 13.01	103.23 ± 12.00	<0.01
FEV_1	91.41 ± 15.57	94.18 ± 16.55	0.42
FEV_1/FVC ratio	84.24 ± 7.46	79.75 ± 8.33	0.01
$FEF_{25–75\%}$	79.27 ± 29.38	74.51 ± 29.05	0.48
TLC	93.36 ± 11.78	95.85 ± 13.99	0.38
RV	113.49 ± 31.49	100.43 ± 38.14	0.09
RV/TLC ratio	29.38 ± 5.90	24.80 ± 6.77	<0.01
ERV	88.36 ± 26.54	71.13 ± 19.15	<0.01
FRC	106.41 ± 22.91	90.38 ± 22.83	<0.01
IC	81.36 ± 13.14	98.73 ± 14.91	<0.01
Measures of asthma severity and control			
CASI	7.95 ± 3.47	8.58 ± 3.20	0.38
ACT	19.58 ± 4.60	19.62 ± 4.53	0.97

All variables are reported as mean ± SD other than sex and ethnicity which are reported as proportions (n(%).

*Pulmonary function indices are reported as percent-predicted values other than the ratios which are reported as percentages. A CASI score of greater than 3 is suggestive of higher asthma severity and an ACT score of 19 and higher is suggestive of good asthma control.

Table 2.

Comparison of anthropometric, metabolic and micronutrient measures between healthy-weight and obese children with asthma

	Healthy-Weight (n=49)	Obese (n=40)	P value
Anthropometric measures			
Neck (cms)	27.52 ± 2.20	30.89 ± 2.50	<0.001
Midarm (cms)	20.12 ± 2.80	25.80 ± 3.90	<0.001
Waist (cms)	61.20 ± 6.10	77.90 ± 11.07	<0.001
Hip (cms)	74.14 ± 7.20	87.34 ± 13.98	<0.001
Waist/Hip Ratio	0.83 ± 0.07	0.90 ± 0.08	<0.001
Height (cms)	136.65 ± 9.43	139.43 ± 9.61	0.17
Weight (kgs)	32.05 ± 7.17	47.11 ± 12.44	<0.001
BMI (kg/m^2)	16.95 ± 1.87	23.86 ± 3.95	<0.001
BMI z-score	0.10 ± 0.87	1.84 ± 0.46	<0.001
Metabolic measures			
Glucose (mg/dl)	88.03 ± 7.71	91.24 ± 9.03	0.10
Cholesterol (mg/dl)	154.46 ± 29.73	155.55 ± 31.85	0.87
HDL (mg/dl)	52.23 ± 12.76	50.25 ± 9.01	0.39
LDL (mg/dl)	92.45 ± 25.75	97.13 ± 30.65	0.44
Triglycerides (mg/dl)	71.97 ± 38.74	70.98 ± 30.05	0.89
Insulin (mU/ml)	11.88 ± 6.93	16.11 ± 12.02	0.06
HOMA-IR *	2.56 ± 1.49	3.71 ± 3.03	0.03
Leptin (ng/ml)	7.67 ± 5.98	18.99 ± 9.67	<0.001
Adiponectin (μg/ml)	17.90 ± 7.84	14.84 ± 6.16	0.04
Nutrient measures			
Total FA (mg/dl)	3372.39 ± 654.38	3274.27 ± 625.57	0.48
SFA (mg/dl)	1165.83 ± 340.15	1130.76 ± 280.53	0.60
MUFA (mg/dl)	673.99 ± 166.59	635.31 ± 165.33	0.28
PUFA (mg/dl)	1528.59 ± 247.81	1505.16 ± 266.03	0.67
n6 PUFA (mg/dl)	1445.18 ± 227.39	1418.71 ± 241.73	0.59
n3 PUFA (mg/dl)	83.41 ± 27.42	87.45 ± 38.34	0.58
n6/n3 Ratio	18.87 ± 5.40	17.73 ± 5.68	0.34
Lutein (μg/ml)	0.20 ± 0.05	0.19 ± 0.07	0.60
β-Cryptoxanthin (μg/ml)	0.11 ± 0.03	0.10 ± 0.05	0.41
Lycopene (μg/ml)	0.52 ± 0.18	0.48 ± 0.16	0.30
α-Carotene (μg/ml)	0.04 ± 0.03	0.04 ± 0.02	0.48
β-Carotene (μg/ml)	0.28 ± 0.17	0.20 ± 0.09	<0.001
Total Carotenoids (μg/ml)	1.13 ± 0.34	0.99 ± 0.26	0.03
Vitamin D (ng/ml)	1.42 ± 0.13	1.38 ± 0.12	0.11

	Healthy-Weight (n=49)	Obese (n=40)	P value
α-Tocopherol (μg/ml)	8.83 ± 2.15	8.28 ± 2.21	0.25

* Calculated as (glucose (mg/dl) × insulin (uU/ml) /405