

Original Article

High Insulin in Early Childhood Is Associated with Subsequent Asthma Risk Independent of Body Mass Index

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What is already known about this topic? The relationship of asthma to obesity is complex, but strengthened among those with insulin resistance.

What does this article add to our knowledge? We found that, in 2 birth cohorts, high insulin in childhood is associated with increased risk of asthma development through adolescence and adulthood, independent of body mass index.

How does this study impact current management guidelines? This association signals an early-life metabolic pathway toward asthma development that may be a modifiable risk factor.

BACKGROUND: Asthma and obesity are major, interconnected public health challenges that usually have their origins in childhood, and for which the relationship is strengthened among those with insulin resistance.

OBJECTIVE: To determine whether high insulin in early life confers increased longitudinal risk for asthma independent of body mass index.

METHODS: The study used data from the Tucson Children's Respiratory Study (TCRS) and the Avon Longitudinal Study of Parents and Children (ALSPAC). Nonfasting insulin was measured in TCRS participants at age 6 years and fasting insulin in ALSPAC participants at age 8 years. Physician-diagnosed

active asthma was determined at baseline and at subsequent assessments up to age 36 years in TCRS and 17 years in ALSPAC. **RESULTS:** In TCRS, high insulin (upper quartile) at age 6 years was associated with increased odds of having active asthma from ages 8 to 36 years compared with low insulin (odds ratio, 1.98; 95% CI, 1.28-3.05; $P = .002$). Similarly, in ALSPAC, high insulin was associated with a significantly higher risk of active asthma from ages 11 to 17 years compared with low insulin (odds ratio, 1.59; 95% CI, 1.12-2.27; $P = .009$). These findings were independent of baseline body mass index in both cohorts, and were not related to other demographic and asthma risk factors nor other tested markers of systemic inflammation and metabolic syndrome.

CONCLUSIONS: In 2 separate birth cohorts, higher blood insulin level in early childhood was associated with increased risk of active asthma through adolescence and adulthood, independent of body mass index. High insulin indicates a novel mechanism for asthma development, which may be a target for intervention. © 2021 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2021;■:■-■)

Key words: Asthma; Insulin; Metabolic syndrome; Obesity

INTRODUCTION

Asthma is a major health challenge and the common label used to diagnose a heterogeneous set of conditions that most often begin in childhood and are associated with various genetic, environmental, and developmental risk factors.¹ In the last 2 decades, obesity has been consistently found to be associated with asthma, both in children² and in adults.³ The exact nature and direction of this association is unknown. Obesity is postulated to increase the risk of asthma by affecting lung mechanics,⁴ increasing type 2 inflammation,⁵ increasing type 1 inflammation,⁶ and inducing resistance to corticosteroid-based treatment⁷⁻⁹ and beta-agonists.¹⁰ However, patients with asthma are also more likely to become obese after diagnosis.^{11,12}

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Abbreviations used

ALSPAC- Avon Longitudinal Study of Parents and Children

BHR- bronchial hyperresponsiveness

BMI- body mass index

CRP- C-reactive protein

OR- odds ratio

TCRS- Tucson Childrens' Respiratory Study

VmaxFRC- maximal expiratory flow at functional residual capacity

zBMI- z-scored BMI

This risk has been attributed to a combination of factors, including the use of systemic corticosteroids and reduced physical activity levels.¹³ Weight loss variably benefits obese patients with asthma,¹⁴ suggesting that the relation between obesity and asthma may not result directly from increased adipose tissue but may be mediated by biological mechanisms that are common to both asthma and obesity, such as insulin resistance.

Insulin resistance is a metabolic disorder strongly linked with obesity,¹⁵ and there is robust evidence that systemic inflammation generated by adipose tissue may trigger insulin resistance.¹⁶ Data from the Avon Longitudinal Study of Parents and Children (ALSPAC) have previously shown that greater weight gain in the first 3 years of life is related to higher body mass index (BMI) and fasting insulin levels at age 8 years.¹⁷ However, genetic studies have also raised the possibility that carbohydrate-generated insulin secretion may play a causal role in obesity,¹⁸ suggesting that the association between obesity and high insulin may be bidirectional. Interestingly, a cross-sectional analysis of the National Health and Nutrition Examination Survey revealed that the relation of obesity to asthma prevalence was strongest among persons with insulin resistance.¹⁹ These results raise the possibility that insulin resistance may have an effect on asthma risk that is independent of obesity. However, the influence of insulin resistance in early life on the development of asthma is not yet known.

We hypothesized that increased insulin levels in early life contribute to the risk for the development of asthma, independent from BMI. To test this hypothesis, we used longitudinal data from 2 separate cohorts—the Tucson Children's Respiratory Study (TCRS) and ALSPAC—in which serum insulin levels were measured in childhood and longitudinal follow-up for asthma was performed.

METHODS**TCRS cohort**

TCRS is a nonselected birth cohort enrolled from 1980 to 1984, which was designed to assess early-life risk factors for subsequent respiratory outcomes, and which has had continuous follow-up for more than 36 years.²⁰ The enrollment process and study design have been presented previously.²¹ Participants of TCRS underwent the first in-depth study visit at age 6 years (6.1 years $\pm$ 0.7 years). BMI was assessed at age 6 years using standard methods in 303 of the participants included in these analyses; participant-reported height and weight was available for an additional 35 participants. BMI was calculated and z scored (zBMI) based on Centers for Disease Control growth chart reference tables.²² Blood was collected for serologic studies; participants were not required to be fasting at the time of blood collection. Physician-diagnosed active asthma at age 6 years and in subsequent surveys through age 36 years was defined as a

physician diagnosis plus active symptoms in the past year as previously reported.²³ Lung function testing was performed at age 6 years (maximal expiratory flow at functional residual capacity [VmaxFRC])²⁴ through spirometry as previously reported.²⁵⁻²⁷ VmaxFRC at age 6 years was adjusted for height and z scored. At age 6 years, bronchial hyperreactivity (bronchial hyperresponsiveness [BHR]) to cold, dry air was defined as a drop in VmaxFRC of greater than 41.1%, the 90th percentile for decline for reference children.^{28,29} At age 6 years, 189 participants underwent BHR testing, which was not performed in children with a history of active respiratory disease³⁰; 152 were not tested and had a higher prevalence of asthma (20.4%) than those tested (4.8%) and were included in the models as a third category for BHR. Allergy skin prick testing was performed at age 6 years. Atopy was defined as 1 or more positive skin test result.²⁹ Serum IgE was measured as previously reported.³¹

Nonfasting insulin, leptin, C-reactive protein (CRP), and IL-6 serum levels were measured by multiplex (Human Multi-Analyte Profile panel version 1.6, Myriad Rules Based Medicine, Austin, Tex) on all individuals for whom serum collected at the age 6 year visit was available for analysis (n = 383). Insulin analyses were limited to measurements in a single batch of serum samples measured over 7 plates (n = 342). There was no relation between plate and insulin level ($P = .69$ by Kruskal-Wallis). Results for insulin from Rules Based Medicine assays were corroborated by ELISA (R&D Systems, Minneapolis, Minn) in a randomly selected subset of the same samples used for the Rules Based Medicine assays (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). Insulin levels at age 6 years were divided into quartiles, separately by sex. In 98 of the 342 included samples (29%), insulin levels were lower than the minimum detectable concentration (0.67 uIU/mL). IL-6 levels were undetectable in 75% of the participants in which these were measured; thus, these participants were divided into detectable and undetectable groups for purposes of analysis.

This research was approved by the Institutional Review Board of the University of Arizona, and informed consent/assent was obtained from/for all subjects.

ALSPAC cohort

The ALSPAC is a birth cohort that recruited 14,541 pregnant woman from Bristol, UK, with expected dates of delivery between April 1991 and December 1992.³²⁻³⁴ A study website contains details of all the data that are available through a fully searchable data dictionary and variable search tool.³⁵ Additional information on the cohort is available in this article's Methods section in the Online Repository at www.jaci-inpractice.org.

Doctor-diagnosed active asthma was assessed by questionnaire at ages 8, 11, 14, 15, and 17 years.^{36,37} Body weight and height were measured using standardized methods.³⁸ Calculated BMI from these measurements at ages 7 and 9 years were averaged for each participant to approximate the child's BMI around age 8 years. If both measurements were not available, BMI at age 7 or age 9 years was included. zBMI based on the UK growth chart reference tables was used for analysis.²² Spirometry was performed according to American Thoracic Society and European Respiratory Society criteria at age 8 years.^{33,39} Methacholine challenge was assessed at age 8 years, the dose at which FEV₁ dropped by 20% was calculated,⁴⁰ and methacholine responsiveness was defined.⁴¹ From the transformed least squares dose-response slope calculated from the percent decline in FEV₁ versus cumulative dose, the participants were categorized

TABLE 1. Characteristics of study participants in the TCRS cohort by age 6 y high and low insulin groups

Characteristic	Group	Low insulin (N = 254)		High insulin (N = 88)		P
		%	n	%	n	
Sex	Male	48.0	122	46.6	41	.816
Ethnicity	Non-Hispanic White	61.4	156	67.0	59	
	Hispanic White	25.2	64	21.6	19	
	Other	13.4	34	11.4	10	.642
Maternal asthma	Yes	7.2	18 of 250	10.3	9 of 87	.352
Maternal smoking during pregnancy	Yes	14.9	37 of 249	15.1	13 of 86	.954
Maternal age at delivery (y)	Mean (95% CI)	27.4 (26.9 to 27.9)	254	28.1 (27.2 to 29.0)	88	.198
Ever breast-fed in the first 6 mo	Yes	85.3	215 of 252	84.9	73 of 86	.922
Day care first 6 mo	Yes	8.1	20 of 248	8.2	7 of 85	.960
Indoor dogs in infancy	Yes	34.0	86 of 253	27.3	24 of 88	.245
Maternal smoking first year after birth	Yes	17.9	45 of 252	15.9	14 of 88	.678
Characteristics, age 6 y						
BMI,* CDC z score (SD)	Mean (95% CI)	0.11 (−0.04 to 0.27)	251	0.45 (0.22 to 0.69)	87	.026
BHR*	Yes	14.2	21 of 148	29.3	12 of 41	.024
Asthma	Yes	11.1	28 of 253	13.6	12 of 88	.519
Skin test positivity	Yes	36.7	92 of 251	46.0	40 of 87	.125
Total IgE (IU/mL)	GM (95% CI)	36.7 (29.7 to 45.4)	252	45.3 (31.0 to 66.1)	88	.329
VmaxFRC (mL/s)	GM (95% CI)	1198 (1149 to 1249)	198	1102 (1029 to 1180)	64	.047

CDC, Centers for Disease Control.

The number of participants in each group is noted in the table when it differs from the overall insulin groups.

*BMI included as z scores from CDC reference tables; BHR included as positive and negative response to cold air challenge³⁰; VmaxFRC included as z scores of height-adjusted values.²⁴

into less than or equal to 0 slope and the greater than 0 slope divided into tertiles. Allergy skin prick testing was performed at age 7.5 years using a panel of extracts.⁴² Cohort-specific cutoffs were used, defined as a skin test wherein the sum of the longest diameter plus the diameter of the orthogonal was at least 1 mm was considered positive. Atopy was defined as 1 or more positive skin test result.

At age 8 years (mean age, 8.2 ± 0.1 years; range, 8.0–8.5 years), a randomly selected subsample attended the research clinic in the morning while fasting from at least midnight the previous day. A venous blood sample was collected.³⁸ Methods used for serologic studies are as previously published.¹⁷ Insulin was measured by ELISA immunoassay (DSL, London, UK) with no cross-reactivity with proinsulin up to 1000 pmol/L. Fasting insulin levels at age 8 years were divided into quartiles, separately by sex. Of these, 9.6% (82 of 857) had undetectable levels of insulin (minimum detectable concentration, 0.26 uIU/mL).

Ethical approval for ALSPAC was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Signed consent was obtained from a parent, and verbal assent was obtained from the child.

Statistical methods

Analyses were performed using Stata (Version 16, StataCorp, College Station, Tex). Demographic variables were compared using χ^2 test or ANOVA. Fisher exact test was used when appropriate. Longitudinal risk for asthma was determined using generalized estimating equations. For each cohort, we grouped insulin levels into quartiles separately for males and females. The lower 3 quartiles were combined for males and for females to create a dichotomous insulin variable for each sex, highest quartile versus lower 3 quartiles. The sex-specific groups were then combined to create a dichotomous insulin variable: highest quartile, hereafter called “high insulin,” and lower 3 quartiles combined, hereafter called “low insulin.”

Interaction terms were tested by using continuous z scores of BMI and testing for interaction with the dichotomous insulin variable. For TCRS, because of the number of time points, the exchangeable structure had the lowest quasilielihood under the independence model criterion. The lowest quasilielihood under the independence model criterion for ALSPAC was with unstructured correlation structure. In TCRS, cold air BHR was included in the model as category 0 for no BHR, 1 for BHR positive, and 2 for those who did not do BHR testing. In the model, category 1 was compared with category 0 and category 2 was compared with category 0.

RESULTS

Analyses in TCRS

In the TCRS, levels of nonfasting insulin were measured in serum samples collected at the age 6 year visit in 342 subjects who had subsequent asthma assessments through age 36 years. None of these participants were using inhaled or systemic steroids at the time of the age 6 year visit (circa 1988). Participants included in this analysis (n = 342) were more likely to have non-Hispanic White and Hispanic White ethnicity and less likely to have maternal asthma history than those excluded for lack of insulin measurement or asthma information (n = 904) (see Table E1 in this article's Online Repository at www.jaci-inpractice.org).

Nonfasting insulin levels were higher in females (n = 179) than in males (n = 163) (median [interquartile range], 1.4 [0.67–2.8] uIU/mL vs 1.1 [0.33–2.60] uIU/mL, respectively; $P = .045$ by Kruskal-Wallis). As described in the Methods section, we defined groups with high insulin, defined as the top quartile of insulin for each sex, and low insulin, representing the lower 3 quartiles. Maternal characteristics and early-life exposures were similar among participants with high insulin and low insulin (Table 1).

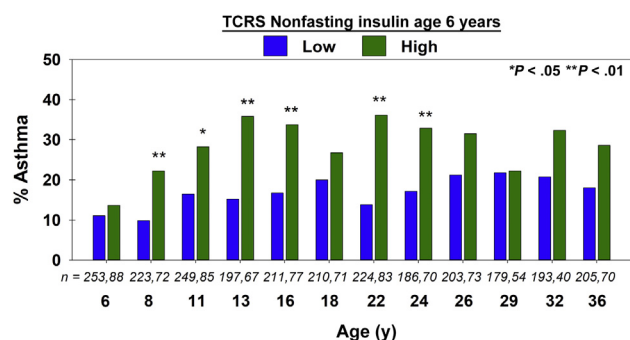


FIGURE 1. The proportion of participants with asthma at each age, by low vs high nonfasting insulin, in the TCRS cohort. Number of participants in the low and high insulin groups is shown in italics.

When compared with children with low insulin, those with high insulin were more likely to have BHR to cold air (29.3% [$n = 12$ of 41] vs 14.2% [21 of 148]; $P = .024$) at age 6 years (Table I). There was no difference in age 6 year asthma prevalence ($P = .519$), positive skin testing to aeroallergens ($P = .125$), or total IgE ($P = .329$) between the high and low insulin groups. Compared with the low insulin group, the high insulin group had a lower VmaxFRC (1102 mL/s [1029-1180] $n = 64$ vs 1198 mL/s [1149-1249] $n = 198$; $P = .047$) and a higher mean BMI (16.6 [16.0-17.1] vs 15.8 [15.6-16.0], $P = .002$) at age 6 years.

High insulin at age 6 years was associated with a higher prevalence of active asthma at ages 8 to 36 years compared with low insulin (Figure 1). In a longitudinal model, high insulin nearly doubled the odds of having active asthma from ages 8 to 36 years compared with low insulin (odds ratio [OR], 1.98 [95% confidence interval (CI), 1.28-3.05]; $P = .002$, $n = 342$; Table II). The relation between high insulin and active asthma remained significant after adjustment for age 6 year zBMI and sex (OR, 1.84 [1.18-2.87]; $P = .007$, $n = 338$), whereas that between age 6 year zBMI and asthma was not significant in this model (OR, 1.08 [0.90-1.30]; $P = .419$). There was no interaction between zBMI and insulin for asthma risk. A model including age 6 year zBMI, BHR, and z-scored VmaxFRC did not appreciably change the relation between high insulin and subsequent asthma (OR, 2.44 [1.50-3.97]; $P < .001$, $n = 262$). Serum leptin, CRP, and IL-6 at age 6 years were not related to asthma risk, and adjustment for these markers had no effect on the insulin-asthma association (see Table E2 in this article's Online Repository at www.jaci-inpractice.org).

To exclude the possibility for baseline asthma diagnosis to influence the outcome of interest, we performed a sensitivity analysis limited to individuals who did not have a diagnosis of asthma at age 6 years. This did not affect the relationship between high insulin and subsequent asthma (Table II).

Analyses in ALSPAC

Fasting insulin levels were measured at age 8 years in serum samples for 857 subjects who had at least 1 asthma assessment between ages 8 and 17 years. Participants included in this analysis ($n = 857$) were similar to those excluded for lack of insulin measurement and asthma information ($n = 37$) except that included participants had slightly older mothers (see

TABLE II. Odds of subsequent asthma for ages 8-36 y, comparing high nonfasting insulin with low insulin at age 6 y, in the TCRS cohort

Model	OR	95% CI	P	n	Observations
1					
All participants					
High insulin	1.98	1.28-3.05	.002	342	3080
Female	0.91	0.60-1.37	.646		
2					
All participants with BMI					
High insulin	1.84	1.18-2.87	.007	338	3046
Female	0.94	0.62-1.41	.756		
zBMI	1.08	0.90-1.30	.419		
3					
Adjusted for age 6 y BHR*					
High insulin	1.70	1.09-2.64	.018	338	3046
BHR positive	2.17	1.11-4.26	.024		
BHR, not tested	1.86	1.19-2.90	.006		
Female	0.98	0.65-1.47	.921		
zBMI	1.08	0.90-1.29	.396		
4					
Adjusted for age 6 y VmaxFRC*					
High insulin	2.44	1.50-3.97	<.001	262	2374
VmaxFRC* (SD)	0.74	0.59-0.94	.012		
Female	0.78	0.49-1.24	.294		
zBMI	1.13	0.90-1.41	.293		
5					
Limited to those with no asthma at age 6 y					
High insulin	1.80	1.12-2.92	.016	298	2678
Female	1.25	0.79-1.98	.337		
zBMI	1.26	1.01-1.58	.045		

All models adjusted for sex; models 2-5 adjusted for z-score BMI* at age 6 y. Models 1 and 2 include all participants, models 3 and 4 are limited to those who underwent the specified testing at age 6 y, and model 5 is limited to those with no asthma at age 6 y.

*BMI included as z scores from Centers for Disease Control reference tables; BHR included as positive and negative response to cold air challenge³⁰; VmaxFRC included as z scores of height-adjusted values.²⁴

Table E3 in this article's Online Repository at www.jaci-inpractice.org.

Fasting insulin levels at age 8 years were higher in females ($n = 403$) than in males ($n = 454$) (median [interquartile range] [in uIU/mL], 5.3 [3.5-8.5] vs 4.1 [2.9-7.1], respectively; $P < .0001$ by Kruskal-Wallis). We defined high insulin and low insulin groups for ALSPAC as described in the Methods section. There were no differences in maternal characteristics or early-life exposures between participants with high insulin and those with low insulin at age 8 years (Table III).

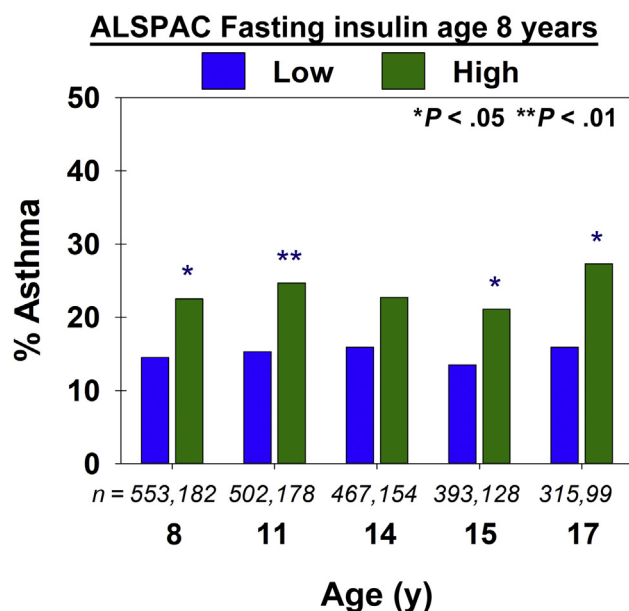
Children in the high insulin group were more likely to have asthma at age 8 years (22.5% [$n = 41$ of 182] vs 14.5% [$n = 80$ of 553]; $P = .011$), and to have a higher mean BMI compared with those with low insulin (18.1 [95% CI, 17.7-18.6] vs 16.7 [16.6-16.9]; $P < .001$) (Table III). Fasting insulin levels at age 8 years were unrelated to total IgE ($P = .303$), positive skin testing result at age 7 years ($P = .922$), or methacholine responsiveness ($P = .946$).

TABLE III. Characteristics of study participants in the ALSPAC cohort by age 8 y high and low insulin groups

Characteristic	Group	Low insulin (N = 641)		High insulin (N = 216)		P
		%	n	%	n	
Sex	Male	53.0	340	52.8	114	.946
Ethnic background	White	97.1	606 of 624	98.1	205 of 209	.619
	Non-White	3.0	18 of 624	2.0	4 of 209	
Maternal asthma	Yes	14.6	91 of 624	14.4	30 of 208	.955
Maternal smoking, last 2 mo of pregnancy	Yes	11.8	74 of 630	13.2	28 of 211	.557
Maternal age at delivery	<20 y	1.4	9 of 641	1.4	3 of 216	
	20-36 y	92.0	590 of 641	92.6	200 of 216	
	>36 y	6.6	42 of 641	6.0	13 of 216	.967
Ever breast-fed in the first 6 mo	Yes	84.3	525 of 623	83.9	177 of 211	.895
Day care attendance during the first year	Yes	8.9	55 of 616	7.0	14 of 201	.385
Pets ownership during the first year	Yes	67.3	417 of 620	70.8	143 of 202	.349
Maternal smoking at 8 mo after birth	Yes	16.4	102 of 622	15.9	33 of 207	.877
Characteristics, age 8 y						
BMI,* UK z score (SD)	Mean (95% CI)	0.21 (0.13-0.28)	637	0.73 (0.59-0.88)	216	<.001
BHR*	Yes	15.3	94 of 613	14.4	29 of 201	.946
Asthma	Yes	14.5	80 of 553	22.5	41 of 182	.011
Skin test positivity	Yes	23.0	131 of 569	22.7	44 of 194	.922
Total IgE (IU/mL)	GM (95% CI)	72.0 (62.0-83.6)	483	84.4 (65.1-109)	151	.303

The number of participants in each group is noted in the table when it differs from the overall insulin groups.

*BMI included as z scores from UK reference tables; BHR included as the highest tertile with positive methacholine challenge.⁴¹

**FIGURE 2.** The proportion of participants with asthma at each age, by low vs high fasting insulin, in the ALSPAC cohort. Number of participants in the low and high insulin groups is shown in italics.

High insulin at age 8 years was significantly associated with active asthma between ages 8 and 17 years compared with low insulin, when assessed in cross-sectional analyses (Figure 2). In a longitudinal model, high insulin was associated with a significantly higher risk of active asthma from ages 11 to 17 years compared with low insulin (1.59 [1.12-2.27]; $P = .009$, $n = 814$; Table IV). The relation between high insulin and active

asthma remained significant after adjustment for zBMI and sex (OR, 1.51 [1.05-2.17]; $P = .026$, $n = 811$), whereas that between zBMI and asthma was not significant (OR, 1.10 [0.94-1.29]; $P = .243$). High insulin was also associated with active asthma in a sensitivity analysis limiting the model to those with no asthma at age 8 years (OR, 2.14 [1.10-4.17]; $P = .026$, $n = 584$) and adjusted for zBMI and sex. Effect estimates were not substantially changed in models adjusted for serum triglycerides, leptin, and CRP (see Table E4 in this article's Online Repository at www.jaci-inpractice.org).

Fasting insulin at 8 years tended to be weakly correlated with fasting insulin at age 13 years ($n = 448$, $r = 0.20$) and at age 17 years ($n = 425$, $r = 0.12$). There was no relation between fasting insulin measured at age 13 years and subsequent asthma through age 17 years, nor was there a relation between fasting insulin measured at age 17 years and asthma at age 17 years (see Table E5 in article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

In 2 independent birth cohorts, higher serum insulin levels at ages 6 or 8 years were associated with active asthma in childhood, adolescence, and adulthood. The effect was independent of concurrent BMI and was not related to other demographic and asthma risk factors nor other tested markers of systemic inflammation and metabolic syndrome. However, there was no relation between fasting insulin during puberty and concurrent or subsequent asthma. These data suggest that during the pre-pubertal years, insulin may contribute to asthma risk, possibly via pathways directly related to insulin function.

The analyses presented herein compared the highest quartile to lower 3 quartiles of insulin because no further dose response for insulin was evident in either cohort (see Figures E2 and E3 in this article's Online Repository at www.jaci-inpractice.org). In ALSPAC, insulin levels were measured after participants fasted

TABLE IV. Odds of subsequent asthma ages 11-17 y, comparing high fasting insulin with low fasting insulin at age 8 y, in the ALSPAC cohort

Model	OR	95% CI	P	n	Observations
1					
All participants					
High fasting insulin	1.59	1.12-2.27	.009	814	2236
Female	1.11	0.80-1.54	.521		
2					
All participants with BMI					
High fasting insulin	1.51	1.05-2.17	.026	811	2236
Female	1.11	0.80-1.53	.538		
zBMI	1.10	0.94-1.29	.243		
3					
Limited to those with no asthma at age 8 y					
High fasting insulin	2.14	1.10-4.17	.026	584†	1729
Female	1.55	0.82-2.95	.180		
zBMI	0.99	0.72-1.36	.942		

All models adjusted for sex; models 2 and 3 adjusted for z-score BMI* at age 8 y. Models 1 and 2 include all participants, but model 3 is limited to those with no asthma at age 8 y.

*BMI included as z scores from UK reference tables.

†584 = 811 – 106 (with diagnosis of asthma at age 8 y) – 121 (missing asthma information at age 8 y).

overnight, consistent with the most commonly used method of identifying insulin resistance in children.⁴³ In TCRS, as for most birth cohort studies, nonfasting blood samples were drawn. Although Hancox and Landhuis⁴⁴ showed that fasting and nonfasting serum insulin levels show only a moderate correlation ($r = 0.44$) in children, the fact that similar associations were found between high serum insulin and asthma risk in ALSPAC and TCRS strongly vouches for the validity of our findings. However, the potential utility of nonfasting testing for metabolic analytes should be acknowledged, for ease of sampling, participant safety, and reflection of actual daily state.^{45,46}

A strong association between obesity and asthma is now well established,^{10,47,48} but the mechanisms that explain it remain obscure. Obesity is associated with chronic low-grade systemic inflammation characterized by increased serum levels of TNF- α , IL-1 β , and IL-6,⁴⁹ but there is no current evidence that antagonists for any of these mediators are effective in treating obesity-associated asthma. Obesity increases the risk for various adverse long-term health consequences, but asthma outcomes appear to be more strongly associated with metabolic dysfunction than with BMI or body fat mass.³ Among the known markers of metabolic dysfunction, insulin resistance seems to be the most relevant: In the aforementioned National Health and Nutrition Examination Study citation,¹⁹ the interaction between obesity and insulin resistance as determinants of asthma prevalence in adults was robust to adjustments for other metabolic syndrome markers, including hypertriglyceridemia and levels of CRP. Bronchial epithelial cells from patients with asthma show evidence of insulin resistance,⁵⁰ and in a retrospective study in Taiwan involving more than 50,000 persons, the incidence of asthma was 30% higher among subjects with a diagnosis of diabetes than among those without diabetes, suggesting the importance of insulin in asthma risk.⁵¹ Moreover, in a general population cohort of adults in

Copenhagen, insulin resistance was associated with increased odds of subsequent development of wheezing or asthma-like symptoms.⁵² Recently, a cross-sectional analysis of the UK Biobank data supported elevated blood glucose levels as measured by hemoglobin A1C as a risk for asthma-related hospitalizations.⁵³ We extend these observations herein, showing that merely increased levels of insulin in early childhood predict subsequent asthma into adulthood. These data imply that the mechanism underlying insulin-associated asthma risk is neither solely mediated by obesity nor dependent on the metabolic syndrome, and instead may be due to a direct, still undefined biological effect of insulin itself.

Age-related effects are notably important in the relation of high insulin to asthma. The relation of age 6 year insulin to asthma was not present at age 6 years in TCRS, but was evident by age 8 years in both TCRS and ALSPAC cohorts. Furthermore, the relationship between increased serum insulin and risk for asthma was limited to insulin measured in the prepubertal period. These findings are in agreement with previous reports indicating that insulin measured during adolescence is unrelated to asthma.^{54,55} The discrepancy between asthma risk related to measurements of prepubertal versus peripubertal insulin may reflect the marked changes in insulin serum concentrations subsequent to concurrent growth hormone surges and decreased insulin sensitivity during puberty.^{56,57}

Animal and *ex vivo* human studies have indicated potential mechanisms by which excessive insulin may directly contribute to asthma development. In a murine model, exogenous intranasal insulin administration to mice increased lung beta-catenin levels, airway hyperreactivity, and collagen deposition in the lungs, with associated proliferation of procontractile and profibrotic smooth muscle cells.⁵⁸ Perhaps most relevant to our findings are experiments performed by Nie et al.⁵⁹ They showed that obesity increased bronchial responsiveness elicited by stimulation of the vagus nerve, but not by administration of a parasympathetic stimulant, acetylcholine. Furthermore, this effect was blocked by the drug streptozocin, which reduces insulin secretion from beta cells in the pancreas, and the effect was restored by insulin administration. Evidence was also presented suggesting that increased insulin mediates bronchial responsiveness by inducing a loss of presynaptic M₂ muscarinic (inhibitory) receptor function in both obesity-prone and obesity-resistant rats. Interestingly, we found a significant correlation between insulin levels and BHR induced by cold, dry air challenge in TCRS, which has a neural component,⁶⁰ but not by methacholine in ALSPAC. This observation suggests that the mechanisms through which insulin may elicit BHR may be different from those present in allergic forms of asthma associated with positive responses to methacholine, a speculation consistent with the lack of association between insulin levels and markers of an allergic diathesis in both cohorts.

Our results suggest the tantalizing possibility that therapeutic strategies that decrease insulin levels or address insulin resistance in early life may contribute to the prevention of asthma. Nutritional approaches that limit weight gain or support healthy metabolism between birth and 3 years could decrease the incidence of insulin resistance and thus of asthma.⁶ Interestingly, Chen et al.⁵¹ showed that Taiwanese diabetic patients treated with metformin were less likely to develop asthma than those treated with insulin. A claims-based analysis supported these data, showing that metformin therapy is associated with lower hazard for severe asthma exacerbations.⁶¹ It is therefore tempting

to speculate that treatment with metformin may decrease the incidence of asthma in young children with high insulin levels, while acknowledging that antidiabetic drug trials have previously failed to show benefit for asthma control.⁶²

Strengths of this study include the remarkable reproducibility and consistency of our findings in 2 extensively phenotyped, longitudinally studied independent populations. Limitations of these cohorts include the relative paucity of Black/African-American participants, to whom these findings may not be generalizable. In addition, there were a few differences observed between TCRS and ALSPAC subjects included versus excluded from the analyses that could indicate additional threat to generalizability, as described in the text. The Rules Based Medicine measurement of insulin used in TCRS is not a clinically used method and, therefore, should not be compared with published clinical reference ranges. We also acknowledge the lack of fasting insulin and abdominal obesity measurement in the TCRS cohort and lack of glucose measurement from which homeostatic model assessment of insulin resistance could be calculated as an alternative marker of insulin resistance. Longitudinal assessment of insulin measurements on asthma risk was limited by lack of insulin data throughout childhood and into adulthood. However, this analysis is likely to be of limited utility in light of the significant effect of puberty on insulin measurements. The definition of atopy by skin testing in both cohorts could contribute to measurement bias through including a limited panel of locally relevant allergens and a conservative wheal size cutoff. Finally, the potential effect of intercurrent asthma therapy is difficult to determine in these cohorts.

CONCLUSIONS

Higher blood insulin in early childhood is associated with an increased risk for asthma up to adult life, independent of BMI, and reduced lung function in adulthood. The possible pathophysiologic mechanisms of this association are not yet fully understood but warrant additional study. Higher serum insulin may be a pharmacologic target for prevention of asthma, particularly in early childhood.

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ONLINE REPOSITORY

METHODS

ALSPAC cohort study numbers

Pregnant women resident in Avon, UK, with expected dates of delivery from April 1, 1991, to December 31, 1992, were invited to take part in the study. The initial number of pregnancies enrolled was 14,541 (for these at least 1 questionnaire has been returned or a “Children in Focus” clinic had been attended by July 19, 1999). Of these initial pregnancies, there were a total of 14,676 fetuses, resulting in 14,062 live births and 13,988 children who were alive at age 1 year. When the oldest children were aged approximately 7 years, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. As a result, when considering variables collected from the age of 7 years onwards (and potentially abstracted from obstetric notes), there are data available for more than the 14,541 pregnancies mentioned above. The number of new pregnancies not in

the initial sample (known as phase I enrollment) that are currently represented on the built files and reflecting enrollment status at the age of 24 years is 904 (452, 254, and 198 recruited during phases II, III, and IV, respectively), resulting in an additional 811 children being enrolled. The phases of enrollment are described in more detail in the cohort profile article.^{E1} Please note that phase 4 enrollment (age 18-24 years) is not currently included in the cohort profile. The total sample size for analyses using any data collected after the age of 7 years is therefore 15,247 pregnancies, resulting in 15,458 fetuses. Of this total sample of 15,656 fetuses, 14,973 were live births and 14,899 were alive at age 1 year. A 10% sample of the ALSPAC cohort, known as the Children in Focus group, attended clinics at the University of Bristol at various time intervals between age 4 and 61 months. The Children in Focus group were chosen at random from the last 6 months of ALSPAC births (1432 families attended at least 1 clinic). Excluded were those mothers who had moved out of the area or were lost to follow-up, and those partaking in another study of infant development in Avon.

TABLE E1. TCRS: Comparison of participants who were included in the study with those not included for lack of age 6 y serum insulin and subsequent asthma assessment

Characteristic	Group	Included (n = 342)		Not included (n = 904)		P
		%	n	%	n	
Sex	Male	47.7	163	49.7	449	.528
Race and ethnicity	Non-Hispanic White	62.9	215	57.4	519	
	Hispanic White	24.3	83	20.7	187	
	Other	12.9	44	21.9	198	.001
Ever maternal asthma	Yes	8.0	27	12.2	100	.037
Maternal smoking during pregnancy	Yes	14.9	50	16.5	120	.508
Ever breast-fed in the first 6 mo	Yes	85.2	288	85.2	688	.980
Day care first 6 mo	Yes	8.11	27	6.5	43	.355
Indoor dogs in infancy	Yes	32.3	110	32.2	287	.987
Maternal smoking first year after birth	Yes	17.3	59	18.9	155	.547
Maternal age at delivery (y)	Mean $\pm$ SD	27.6 $\pm$ 4.3	342	27.1 $\pm$ 4.9	903	.119

TABLE E2. TCRS: Odds of asthma for age 8-36 y, longitudinal model, for high nonfasting insulin compared with low insulin at age 6 y, adjusted for biomarkers of interest at age 6 y

Model	OR	95% CI	P	n	Observations
1					
All participants					
High insulin	1.98	1.28-3.06	.002	342	3080
Female	0.91	0.60-1.37	.646		
2a					
Limited to those with age 6 y BMI* information					
High insulin	1.88	1.21-2.92	.005	338	3046
Female	0.93	0.62-1.41	.747		
2b					
Adjusted for age 6 y BMI*					
High insulin	1.84	1.18-2.87	.007	338	3046
Female	0.94	0.62-1.41	.756		
zBMI	1.08	0.90-1.30	.419		
2c					
Adjusted for age 6 y leptin,* BMI					
High insulin	1.80	1.15-2.82	.010	338	3046
Leptin (SD)	1.09	0.84-1.41	.493		
Female	0.88	0.56-1.38	.567		
zBMI	1.04	0.85-1.27	.703		
2d					
Adjusted for age 6 y CRP,* BMI					
High insulin	1.85	1.19-2.87	.007	338	3046
CRP (SD)	0.93	0.69-1.27	.666		
Female	0.95	0.63-1.44	.818		
zBMI	1.09	0.90-1.31	.386		
2e					
Adjusted for age 6 y IL-6,* BMI					
High insulin	1.84	1.18-2.87	.007	338	3046
Detectable IL-6	1.10	0.69-1.76	.676		
Female	0.93	0.62-1.41	.734		
zBMI	1.08	0.89-1.30	.441		

Model 1 includes all participants; model 2 is limited to those with age 6 y BMI* and biomarkers of interest. All models adjusted for sex; models 2b through 2e additionally adjusted for z-score BMI.*

*BMI included as z scores from CDC reference tables; leptin and CRP were log₁₀ transformed and z scored; IL-6 was coded as detectable or undetectable.

TABLE E3. ALSPAC: Comparison of participants who were included in the study with those not included for lack of age 8 y serum insulin and subsequent asthma assessment

Characteristic	Group	Included (n = 857)		Not included (n = 37)		P
		%	n	%	n	
Sex	Male	53.0	454	62.2	23	.273
Race	White	97.4	811	94.1	32	
	Non-White	2.6	22	5.9	2	.242
Ever maternal asthma	Yes	14.5	121	25.8	8	.117
Maternal smoking during the last 2 mo of pregnancy	Yes	12.1	102	20.6	7	.178
Ever breast-fed in the first 6 mo	Yes	84.2	702	84.4	27	.999
Day care attendance during the first year	Yes	8.5	69	0	0	.159
Pet ownership during the first year	Yes	68.1	560	80.0	24	.229
Maternal smoking at 8 mo after birth	Yes	16.3	135	20.6	7	.482
Maternal age at delivery (y)	Mean $\pm$ SD	29.7 $\pm$ 4.4	857	27.8 $\pm$ 4.8	35	.011

TABLE E4. ALSPAC: Odds of asthma for ages 11-17 y, longitudinal model, for high fasting insulin compared with low insulin at age 8 y, adjusted for biomarkers of interest at age 8 y

Model	OR	95% CI	P	n	Observations
1					
All participants					
High fasting insulin	1.59	1.12-2.27	.009	814	2236
Female	1.11	0.80-1.54	.521		
2a					
Limited to those with age 8 y BMI* information					
High fasting insulin	1.59	1.12-2.26	.010	811	2231
Female	1.10	0.80-1.53	.551		
2b					
Adjusted for age 8 y BMI					
High fasting insulin	1.51	1.05-2.17	.026	811	2231
Female	1.11	0.80-1.53	.538		
zBMI	1.10	0.94-1.29	.243		
3					
Adjusted for leptin, BMI					
High fasting insulin	1.53	1.04-2.23	.030	786	2162
Leptin (SD)	1.02	0.81-1.29	.839		
Female	1.11	0.78-1.58	.559		
zBMI	1.07	0.87-1.33	.527		
4					
Adjusted for age 8 y CRP, BMI					
High fasting insulin	1.69	1.10-2.60	.017	565	1578
CRP (SD)	0.89	0.71-1.11	.304		
Female	1.13	0.76-1.68	.535		
zBMI	1.18	0.96-1.46	.118		
5					
Adjusted for age 8 y TG, BMI					
High fasting insulin	1.54	1.06-2.23	.022	787	2165
Triglycerides (SD)	0.98	0.86-1.16	.849		
Female	1.14	0.81-1.60	.440		
zBMI	1.09	0.92-1.28	.322		

TG, Fasting serum triglycerides.

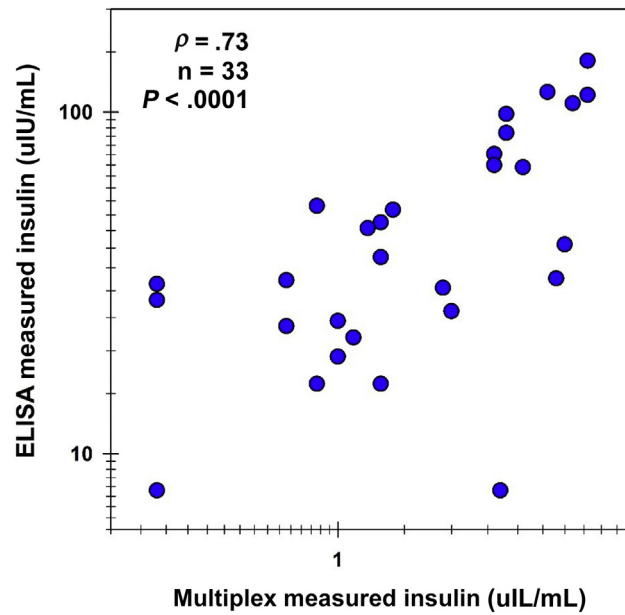
Model 1 includes all participants, model 2 is limited to those with age 8 BMI*, and models 3, 4, and 5 are limited to those with age 8 y BMI* and biomarkers of interest. All models adjusted for sex.

*BMI included as z scores based on UK growth charts; leptin, CRP, and TG were log₁₀ transformed and z scored.

TABLE E5. ALSPAC: Relation of insulin in adolescence to asthma in adolescence

Insulin designation (Age)	% with asthma at each age (n)					
	Age 14 y	<i>P</i>	Age 15 y	<i>P</i>	Age 17 y	<i>P</i>
Age 13 y insulin						
High quartile	23.2 (19 of 82)	.114	17.2 (16 of 93)	.206	17.9 (10 of 56)	.877
Low 3 quartiles	15.6 (42 of 227)		12 (33 of 274)		17 (36 of 212)	
Age 17 y insulin						
High quartile	—		—		21 (12 of 57)	.389
Low 3 quartiles	—		—		16.2 (32 of 198)	

P values calculated using χ^2 test. Quartiles of insulin calculated at each age using the same process as described in the Methods section for age 8 y.

**FIGURE E1.** TCRS: Correlation between Rules Based Medicine (multiplex) and ELISA data for insulin measurements ($n = 33$, $\rho = 0.73$).

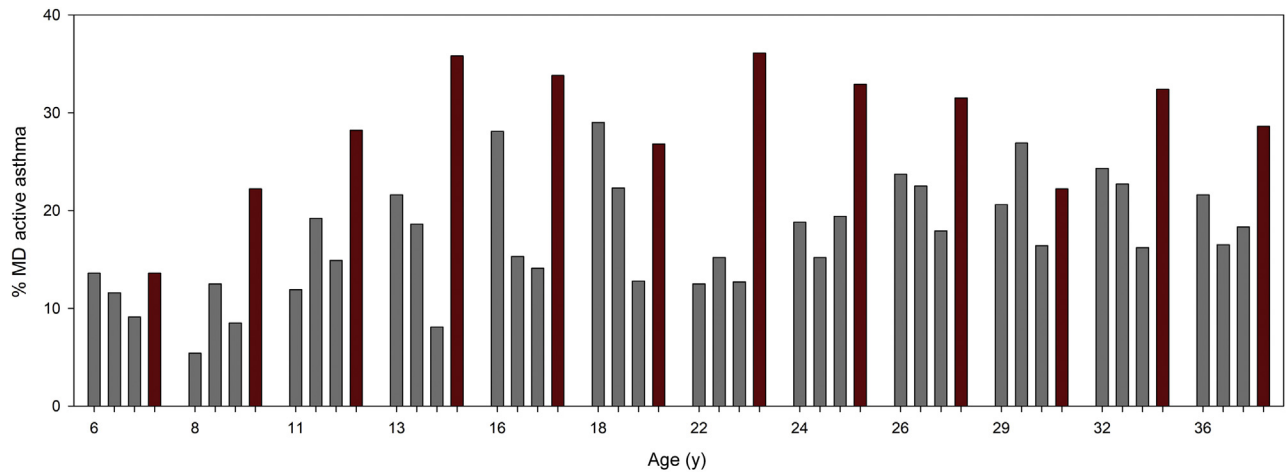


FIGURE E2. TCRS: Proportion of participants with asthma at each age, by insulin quartiles, in the TCRS cohort.

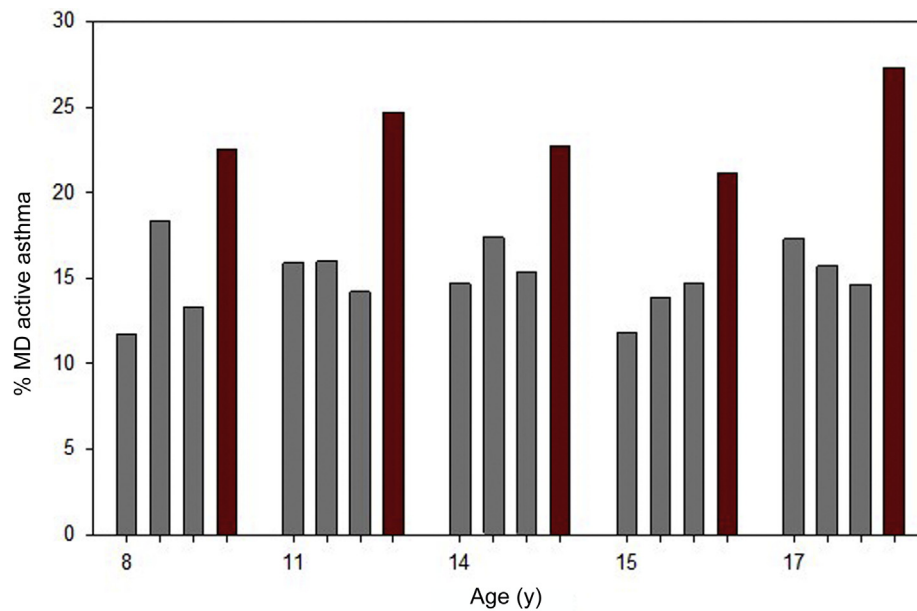


FIGURE E3. ALSPAC: Proportion of participants with asthma at each age, by insulin quartiles, in the ALSPAC cohort.

REFERENCE

- E1. Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, et al. Cohort profile: the 'children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2013;42:111-27.