Methods

Participants

Participants were mothers and their offspring from the Southampton Women's Survey.(1) Women aged 20-34 years were recruited during 1998-2002; those who became pregnant were followed through pregnancy and during their child's infancy and childhood. Women were interviewed and anthropometric measurements made at recruitment and, for those who became pregnant, at 11 and 34 weeks of pregnancy. Follow-up visits for all children occurred at 6, 12, 24 and 36 months. During 2006-2010 1529 children aged 6 years were invited for respiratory follow-up. Home-based questionnaire, skin test and spirometry data were collected from 940 children; 589 were lost to follow-up or declined participation. Participants were encouraged to attend the clinic for exhaled nitric oxide (eNO) testing, 596 did so but time and resources meant that not all participants attended. Maternal pre-pregnancy fat mass measurements were available for 927 women (98.6 %) and pre-pregnancy BMI measurements were available for 930 (98.9 %) (Figure 1). Parental consent was obtained and ethical approval was granted by the Southampton and South West Hampshire Local Research Ethics Committee (LREC Numbers 276/97, 307/97, 089/99, 06/Q1702/104).

Maternal anthropometry

Pre-pregnancy height and weight were measured using portable Seca 835 scales (Seca Ltd., Birmingham, UK) and Leicester stadiometer (Invicta Plastics Ltd., Leicester, UK). Biceps, triceps, suprailiac and subscapular skinfold thicknesses were measured with Harpenden 'John Bull' calipers (British Indicates Ltd., St Albans, UK). A standard protocol requiring three

readings within 10% was followed. BMI was calculated as weight (kg)/(height (m))^2. Fat mass was estimated from body weight and skinfolds using the Durnin and Womersley equation.(2) Analysis was limited to pre-pregnancy measurements as body composition equations are not validated in pregnancy.

Childhood anthropometry

Research nurses measured subscapular skinfold thicknesses at birth and 6 months. Subscapular adiposity change conditional upon initial measurements was calculated using regression to account for exact age and regression to the mean. BMI at 3 years was calculated from height and weight and BMI aged 6 years was calculated similarly and the values converted to age and gender specific z-scores using the Child Growth Foundation 1990 Reference (London, UK).

Atopy

Skin prick testing was conducted at age 6 years using cat, dog, house dust mite (*Dermatophagoides pteronyssinus*), egg, milk, grass and tree pollen allergens. A positive control of >3mm and negative control of 0mm was required. Any response ≥ 3 mm was considered evidence of atopy.

Childhood asthma and wheeze

Research nurses administered questions from the ISAAC core-questionnaire wheezing-module.(3) Mothers were asked whether their child had 'ever been diagnosed with asthma by a doctor' or had experienced 'any episodes of chestiness associated with wheezing or whistling in his/her chest since they were last seen?' Children who had ever been diagnosed with asthma and had experienced asthma symptoms or received asthma medication within the last year were considered to have current asthma; those reported to wheeze within the last year were considered to have current wheeze. Questionnaire data obtained at 6, 12, 24 and 36 months were combined with that from 6 years to define wheeze phenotypes based upon those of the Tuscon Children's Respiratory Study.(4)

Transient wheeze: Wheeze at 6, 12, 24 or 36 months but no wheeze or asthma treatment at 6 years.

Persistent wheeze: Wheeze at 6, 12, 24 or 36 months plus wheeze or asthma treatment at 6 years.

Late-onset wheeze: No wheeze at 6, 12, 24 or 36 months plus wheeze or asthma treatment at 6 years.

Very few children had late-onset wheeze so the persistent and late-onset wheeze groups were combined.

Lung function

Spirometry was measured using a portable Koko spirometer with incentive software (KoKo v4; PDS Instrumentation; Louisville, USA). Testing was conducted according to ATS

guidelines, (5) although to avoid discomfort, without nose-clips. Spirometry indices were standardized for height, gender and age using the growing lungs reference.(6)

Exhaled nitric oxide

Exhaled nitric oxide (eNO) was measured in children attending the clinic using a NIOX® chemiluminescence analyser (Aerocrine, Sweden). Measurement was according to ERS/ATS recommendations, during a controlled expiratory manoeuvre at 50 ml/sec.(7). A mean of three readings was calculated where possible. Values were normalized by inverse square root transformation then standardized. The sign was reversed so that high standardized scores represent high untransformed eNO values.

Statistical methods

Relative risks for binary outcomes were modelled using poisson regression with robust variance; poisson regression being appropriate for common outcomes where odds ratios cannot be interpreted as relative risks.(8) Children with transient or persistent/late wheeze were compared to children who had never wheezed. As transient and persistent/late wheeze are mutually exclusive, members of each of these categories cannot be considered at risk of the opposite outcome. Linear regression was used for continuous outcomes. Relative risks and regression coefficients were expressed relative to 5 kg m^{-2}change in BMI or 10 kg change in fat mass. Analyses were also conducted considering Maternal BMI as a discrete variable to classify women according to the world health organisation scheme as either normal weight (BMI 18.5-24.99), overweight (BMI 25-29.99) or obese (BMI $\geq$ 30)(9) and

considering maternal weight gain in pregnancy according to institute of medicine categories. (10)

Potential confounders identified *a priori* were*;* maternal age, height, parity, education, socioeconomic status, smoking in pregnancy, asthma, eczema, rhinitis, and atopy and child's gender, birthweight, gestation, and age at last breastfeed. Potential confounders were tested for association with each respiratory outcome. Model 1 included all variables associated with each outcome but excluded birth weight, gestation, adiposity gain in the first 6 months of life and the child's BMI at age 6 years as they may lie on the causal pathway. Model 2 included birthweight and gestation if they were significantly associated with the outcomes and further analyses were conducted adjusting for all significantly associated confounders (including birthweight and gestation) and infant adiposity gain (birth-6 months) (Model 3) and child's BMI aged 6 (Model 4).

Based upon effect sizes of approaching 5% seen in previous studies we estimated approaching 100% power to detect an increase in risk of this magnitude per kg maternal fat mass and of 80-100% per BMI unit dependent upon physiological outcome. Bonferroni correction was considered over-conservative as the analyses were designed *a priori* to test a limited number of hypotheses and not all the tests were independent(11). We focused our interest on results with P-values ≤ 0.025 and considered consistency of the findings in our interpretation. Stata® 11 (Stata Corp., College Station, TX) was used for all analyses.

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Table S1 Relationship between maternal weight gain in pregnancy and wheeze and atopy phenotypes at age 6 years

	Ever wheeze			Ever asthma			Current wheeze			Current asthma		
Institute of Medicine Weight gain in pregnancy category	RR	(95% CI)	P-value	RR	(95% CI)	P-value	RR	(95% CI)	P-value	RR	(95% CI)	P-value
Acceptable	Reference											
Inadequate	0.96	(0.82-1.13)	*0.65*	0.97	(0.62-1.51)	*0.89*	0.69	(0.40-1.20)	*0.19*	0.95	(0.55-1.64)	*0.85*
Excessive	0.96	(0.84-1.09)	*0.49*	0.88	(0.61-1.27)	*0.49*	0.81	(0.55-1.21)	*0.31*	0.86	(0.55-1.35)	*0.51*
	Transient wheeze			Persistent wheeze			Atopy					
	RR	(95% CI)	P-value	RR	(95% CI)	P-value	RR	(95% CI)	P-value			
Acceptable	Reference											
Inadequate	1.00	(0.82-1.22)	*0.97*	0.76	(0.51-1.15)	*0.19*	0.97	(0.64-1.47)	*0.89*			
Excessive	0.97	(0.82-1.15)	*0.72*	0.85	(0.62-1.16)	*0.30*	1.07	(0.77-1.48)	*0.69*			

Adjusted for potential confounders significantly associated with the outcome, excluding child's birthweight, gestation.

Ever wheeze-maternal education, asthma and rhinitis, child's gender and age last breastfed; Ever asthma- maternal education, asthma and smoking in pregnancy, and child's gender; Current wheeze-maternal education, asthma and atopy; Current asthma-maternal education and asthma; Transient wheeze-maternal height, parity, asthma and rhinitis, child's gender and age last breastfed; Persistent wheeze-maternal education, asthma and rhinitis and child's gender; Atopy-maternal social class, asthma and atopy, and child's gender.

Table S2 Relationship between maternal weight gain in pregnancy and wheeze and atopy phenotypes at age 6 years

	Ever wheeze			Ever asthma			Current wheeze			Current asthma		
Institute of Medicine Weight gain in pregnancy category	RR	(95% CI)	P-value	RR	(95% CI)	P-value	RR	(95% CI)	P-value	RR	(95% CI)	P-value
Acceptable	Reference											
Inadequate	0.98	(0.83-1.16)	*0.83*	1.15	(0.71-1.84)	*0.57*	0.80	(0.46-1.40)	*0.44*	1.24	(0.70-2.22)	*0.46*
Excessive	0.96	(0.84-1.09)	*0.54*	0.99	(0.67-1.46)	*0.96*	0.83	(0.55-1.26)	*0.38*	1.05	(0.64-1.71)	*0.85*
	Transient wheeze			Persistent wheeze			Atopy					
	RR	(95% CI)	P-value	RR	(95% CI)	P-value	RR	(95% CI)	P-value			
Acceptable	Reference											
Inadequate	0.99	(0.80-1.22)	*0.90*	0.91	(0.60-1.39)	*0.67*	0.93	(0.59-1.46)	*0.75*			
Excessive	0.95	(0.80-1.12)	*0.54*	0.94	(0.67-1.31)	*0.71*	1.05	(0.64-1.71)	*0.85*			

	Ever wheeze	Ever asthma	Current wheeze	Current asthma

Adjusted for infant adiposity gain between birth and 6 months and potential confounders significantly associated with the outcome (listed below) including child's birthweight and gestation.

Ever wheeze-maternal education, asthma and rhinitis, child's gender, age last breastfed and gestation; Ever asthma- maternal education, asthma, smoking in pregnancy and parity, child's gender and gestation; Current wheeze-maternal education, asthma and atopy, child's gender and gestation; Current asthma-maternal education and asthma, and child's gestation; Transient wheeze-maternal height, parity, asthma and rhinitis, child's gender, age last breastfed and gestation; Persistent wheeze-maternal education, asthma and rhinitis, child's gender and gestation; Atopy-maternal social class, asthma and atopy, and child's gender.

Table S3 Relationship between maternal pre-pregnancy BMI and nitric oxide and lung function in the offspring at age 6 years

	Unadjusted analysis				Model 1				Model 2			
	Beta	(95% CI)	P-value	n	Beta	(95% CI)	P-value	n	Beta	(95% CI)	P-value	n
FEV_1 z-score	0.0606	(-0.0067, 0.1278)	*0.08*	795	0.0541	(-0.0122, 0.1203)	*0.11*	795	0.0329	(-0.0347, 0.1006)	*0.34*	787
FVC z-score	0.0699	(-0.0069, 0.1468)	*0.07*	795	0.0564	(-0.0218, 0.1345)	*0.16*	767	0.0564	(-0.0218, 0.1345)	*0.16*	767
$FEF_{25-75\%}$ z-score	0.0396	(-0.0451, 0.1242)	*0.36*	795	0.0436	(-0.0410, 0.1281)	*0.31*	795	0.0318	(-0.0529, 0.1166)	*0.46*	795
FEV_1/FVC z-score	-0.0132	(-0.0885, 0.0621)	*0.73*	795	-0.0111	(-0.0879, 0.0656)	*0.78*	767	-0.0111	(-0.0879, 0.0656)	*0.78*	767
Exhaled nitric oxide	-0.0501	(-0.1490, 0.0487)	*0.32*	484	-0.051	(-0.1480, 0.0460)	*0.30*	478	-0.0510	(-0.1480, 0.0460)	*0.30*	478
					Model 3				Model 4			
					Beta	(95% CI)	P-value	n	Beta	(95% CI)	P-value	n
FEV_1 z-score					0.0245	(-0.0456, 0.0946)	*0.49*	724	0.0219	(-0.0482, 0.0921)	*0.54*	787
FVC z-score					0.0565	(-0.0238, 0.1367)	*0.17*	715	0.0274	(-0.0542, 0.1089)	*0.51*	767
$FEF_{25-75\%}$ z-score					0.0240	(-0.0638, 0.1119)	*0.59*	727	0.0437	(-0.0452, 0.1327)	*0.33*	795
FEV_1/FVC z-score					-0.0257	(-0.1041, 0.0528)	*0.52*	715	0.0087	(-0.0720, 0.0893)	*0.83*	767
Exhaled nitric oxide					-0.0604	(-0.1618, 0.0409)	*0.24*	435	-0.0483	(-0.1482, 0.0517)	*0.34*	478

Regression coefficient represents change in outcome per 5 kg m^{-2}unit of maternal pre-pregnancy BMI.

Model 1 Adjusted for potential confounders significantly associated with the outcome, excluding child's birthweight and gestation.

FEV_1-child's gender; FVC-maternal smoking in pregnancy and rhinitis, and child's gender; $FEF_{25-75\%}$-maternal parity and child's gender; FEV_1/FVC-maternal rhinitis, eczema, smoking in pregnancy and parity; eNO-maternal height, asthma and eczema.

Model 2 adjusted for potential confounders significantly associated with the outcome, including child's birthweight and gestation.

FEV_1-maternal height, and child's gender and birthweight; FVC-maternal smoking in pregnancy and rhinitis, and child's gender; $FEF_{25-75\%}$-maternal parity and child's gender and gestation; FEV_1/FVC-maternal rhinitis, eczema, smoking in pregnancy and parity; eNO-maternal height, asthma and eczema.

Model 3 adjusted for increase in adiposity between birth and 6 months and potential confounders significantly associated with the outcome including child's birthweight and gestation (listed in Model 2).

Model 4 adjusted for child's BMI at age 6 years and potential confounders significantly associated with the outcome including child's birthweight and gestation (listed in Model 2).

Table S4 Relationship between infant adiposity gain and wheeze and atopy phenotypes at age 6 years

	Unadjusted analysis				Model 1				Model 2			
Outcome	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Ever wheeze	1.05	(1.00, 1.11)	*0.07*	853	1.03	(0.98, 1.08)	*0.25*	829	1.03	(0.98, 1.09)	*0.22*	829
Ever asthma	1.15	(1.00, 1.32)	*0.05*	856	1.09	(0.94, 1.26)	*0.25*	834	1.10	(0.95, 1.27)	*0.22*	834
Current wheeze	1.06	(0.92, 1.23)	*0.41*	856	1.04	(0.88, 1.22)	*0.66*	750	1.05	(0.89, 1.24)	*0.55*	750
Current asthma	1.14	(0.97, 1.34)	*0.12*	856	1.09	(0.93, 1.29)	*0.29*	847	1.10	(0.93, 1.30)	*0.26*	847
Transient wheeze	1.04	(0.98, 1.12)	*0.21*	716	1.03	(0.96, 1.10)	*0.40*	693	1.03	(0.96, 1.10)	*0.38*	693
Persistent wheeze	1.16	(1.02, 1.33)	*0.02*	491	1.14	(1.00, 1.29)	*0.05*	484	1.14	(1.01, 1.30)	*0.04*	484
Atopy	0.95	(0.82, 1.10)	*0.50*	633	1.01	(0.87, 1.18)	*0.87*	551	1.01	(0.87, 1.18)	*0.87*	551

	Model 3				Model 4			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Ever wheeze	1.02	(0.96, 1.08)	*0.51*	785	1.02	(0.97, 1.08)	*0.39*	827
Ever asthma	1.04	(0.89, 1.22)	*0.61*	787	1.08	(0.93, 1.26)	*0.31*	833
Current wheeze	1.06	(0.88, 1.27)	*0.53*	712	1.06	(0.89, 1.27)	*0.49*	748
Current asthma	1.07	(0.89, 1.29)	*0.45*	800	1.11	(0.93, 1.33)	*0.25*	845
Transient wheeze	1.02	(0.94, 1.09)	*0.69*	656	1.01	(0.95, 1.09)	*0.69*	691
Persistent wheeze	1.10	(0.96, 1.27)	*0.16*	461	1.14	(1.00, 1.30)	*0.05*	484
Atopy	1.00	(0.84, 1.18)	*0.97*	525	1.01	(0.86, 1.18)	*0.91*	551

Relative risk represents change in outcome per SD of infant adiposity gain between birth and 6 months.

Model 1 Adjusted for potential confounders significantly associated with the outcome, excluding child's birthweight, gestation.

Ever wheeze-maternal education, asthma and rhinitis, child's gender and age last breastfed; Ever asthma- maternal education, asthma and smoking in pregnancy, and child's gender; Current wheeze-maternal education, asthma and atopy; Current asthma-maternal education and asthma; Transient wheeze-maternal height, parity, asthma and rhinitis, child's gender and age last breastfed; Persistent wheeze-maternal education, asthma and rhinitis and child's gender; Atopy-maternal social class, asthma and atopy, and child's gender.

Model 2 adjusted for potential confounders significantly associated with the outcome, including child's birthweight, gestation.

Ever wheeze-maternal education, asthma and rhinitis, child's gender, age last breastfed and gestation; Ever asthma- maternal education, asthma, smoking in pregnancy and parity, child's gender and gestation; Current wheeze-maternal education, asthma and atopy, child's gender and gestation; Current asthma-maternal education and asthma, and child's gestation; Transient wheeze-maternal height, parity, asthma and rhinitis, child's gender, age last breastfed and gestation; Persistent wheeze-maternal education, asthma and rhinitis, child's gender and gestation; Atopy-maternal social class, asthma and atopy, and child's gender.

Model 3 adjusted for child's BMI at age 3 years and potential confounders significantly associated with the outcome (listed in Model 2) including child's birthweight and gestation.

Model 4 adjusted for child's BMI at age 6 years and potential confounders significantly associated with the outcome including child's birthweight and gestation (listed in Model 2).

Table S5 Relationship between infant adiposity gain and nitric oxide and lung function in the offspring at age 6 years

	Unadjusted analysis				Model 1				Model 2			
	Beta	(95% CI)	P-value	n	Beta	(95% CI)	P-value	n	Beta	(95% CI)	P-value	n
FEV_1 z-score	-0.0059	(-0.0701, 0.0584)	*0.86*	736	-0.0149	(-0.0783, 0.0484)	*0.64*	736	-0.0165	(-0.0800, 0.0471)	*0.61*	728
FVC z-score	0.0471	(-0.0273, 0.1216)	*0.21*	736	0.042	(-0.0332, 0.1173)	*0.27*	723	0.02	(-0.0575, 0.0976)	*0.61*	732
FEF25-75% z-score	-0.0924	(-0.1728, -0.0119)	*0.02*	736	-0.0956	(-0.1759, -0.0152)	*0.02*	736	-0.0973	(-0.1777, -0.0168)	*0.02*	736
FEV_1/FVC z-score	-0.0827	(-0.1541, -0.0113)	*0.02*	736	-0.0807	(-0.1533, -0.0081)	*0.03*	723	-0.0807	(-0.1533, -0.0081)	*0.03*	723
Exhaled nitric oxide	-0.0238	(-0.1167, 0.0691)	*0.61*	445	-0.0394	(-0.1307, 0.0518)	*0.39*	438	-0.0394	(-0.1307, 0.0518)	*0.39*	438

Model 3				Model 4			
Beta	(95% CI)	P-value	n	Beta	(95% CI)	P-value	n
-0.0253	(-0.0951, 0.0444)	*0.47*	685	-0.0303	(-0.0967, 0.0362)	*0.37*	728
0.0025	(-0.0760, 0.0810)	*0.95*	689	0.02	(-0.0575, 0.0976)	*0.61*	732
-0.0836	(-0.1715, 0.0042)	*0.06*	693	-0.0983	(-0.1823, -0.0143)	*0.02*	736
-0.058	(-0.1367, 0.0207)	*0.15*	680	-0.0706	(-0.1462, 0.0049)	*0.07*	723
-0.0462	(-0.1460, 0.0536)	*0.36*	420	-0.0272	(-0.1223, 0.0680)	*0.57*	438

Relative risk represents change in outcome per SD of infant adiposity gain between birth and 6 months.

Models 1 - 4 adjusted for confounders as described in Table S4