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Body mass index and physical activity in relation to asthma and atopic diseases in young adults $\overset{\backsim}{\sim}$

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Summarv **KEYWORDS** Background: Obesity has been shown to increase the risk of asthma and wheezing. Asthma: Conditioning exercise might decrease the asthma risk, and that could partly explain Wheezing; the association. The relation between obesity and allergic diseases is quite Atopic disease; conflicting. Body mass index; Methods: The association between body mass index (BMI) and physician-diagnosed Physical activity; asthma, allergic rhinitis or conjunctivitis, atopic dermatitis, and self-reported Obesity wheezing was investigated in a questionnaire study among 10667 Finnish first-year university students aged 18-25 years. Logistic regression was used to evaluate possible confounding by parental education, passive smoking at age 0-2, childhood residential environment, current and past smoking and leisure time physical activity index. Results: In men, there was a greater risk of asthma, but not wheezing with increasing BMI. Compared to those with BMI below 20, OR for male asthma was 1.98 (95% CI 1.11-3.52) in BMI category 20.0-22.4, 1.90 (95% CI 1.05-3.41) in BMI 22.5–24.9, and 3.5 (95% CI 1.63–7.64) in BMI≥27.5. Among women, the risks of asthma and wheezing were about two-fold among the overweight-obese subjects. Moderate leisure time physical activity was associated with lower risk of asthma in men (OR 0.62, 95% CI 0.62 (0.42–0.92), but not among women. The risk of allergic rhinoconjunctivitis and atopic dermatitis increased quite linearly with BMI among women but not men.

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Conclusions: Low leisure time physical activity seems not to explain the greater risk of asthma among obese men and women. The quite linear association between BMI and both allergic rhinoconjunctivitis and wheezing among women suggests the independent effect of body fat on atopic diseases. © 2006 Elsevier Ltd. All rights reserved.

Introduction

In cross-sectional studies, there is a positive association between increasing body mass index (BMI) or obesity in childhood¹⁻³ and adult⁴⁻⁷ asthma. In a few studies the association seems to be stronger²⁻⁴ in females. Among men in China⁸ and USA,⁷ the association between BMI and asthma was shown to be U-shaped, showing the greatest risk among the underweight and obese men. Further evidence on the association between obesity and asthma is gained in longitudinal studies that show a greater risk of adult-onset asthma among women who gain weight.^{9,10} Furthermore, weight reduction in obese people^{11,12} and obese women¹³ has been reported to improve lung function and asthma symptoms.

Obesity may be closely linked to other factors, such as physical activity, that might partly explain the relation between obesity and asthma. In fact, conditioning exercise compared to more sedentary lifestyle decreased the risk asthma among Finnish adult twins.^{14,15} Similarly, low physical activity in childhood was associated with the development of asthma in young adulthood.¹⁶ Asthmatics, on the other hand, seem to be as physically active as nonasthmatics measured by energy expenditure of leisure activities in a Canadian Population Health Survey 1994–95.¹⁵ Three studies reporting coexistence of obesity and asthma have precisely evaluated the impact of physical activity on asthma risk.^{7,9,15} In these studies, physical activity did not explain the association between obesity and asthma. Moreover, in the Finnish twin-study, conditioning exercise decreased the risk of adult onset asthma.15

The studies reporting the association between obesity and atopic disease or sensitization have given conflicting results. No association,² or a greater risk of allergic rhinitis^{1,18} or atopic dermatitis¹⁸ has been reported among obese children. Sensitization was more common among teenage obese girls in Taiwan¹⁸ and Australia¹⁹ and in a Finnish birth cohort study among adults aged 31 years,²⁰ but the risk of sensitization was equal in all BMI quartiles in children in the United States,¹ and among adults taking part in the European Community Respiratory Health Survey (ECRHS).²¹ We report the association between BMI in adulthood and physician-diagnosed asthma and self-reported wheezing among young men and women with relatively low BMI, after adjustment for confounding factors, such as leisure time physical activity and smoking. Correspondingly, the association between BMI and physician-diagnosed allergic rhinitis or conjunctivitis, and atopic dermatitis was investigated. We also report the independent impact of leisure time physical activity on atopic diseases.

Methods

Study population

In 1995–96 a total of 10667 (75.0%) first-year Finnish university students younger than 25 years responded a questionnaire on asthma, allergic diseases, and possible risk factors. Of the subjects 4164 (39.0%) were men and 6503 (61.0%) women. The questionnaire was returned by 66.5% of men, and 81.8% of women. The mean age of the respondents was 20.9 years (sD+1.6 years), range 18–25 years.

Asthma, wheezing and atopic diseases

The lifetime occurrence of physician-diagnosed asthma, allergic rhinitis or allergic conjunctivitis, and atopic dermatitis were analyzed. In addition, episodic wheezing with shortness of breath, but not diagnosed asthma (referred to as wheezing) was investigated. The questionnaire was validated in a clinical sample.²² In that study, questions on physician diagnosed asthma yielded specificity of 99% when lung function tests and interview were used as golden standard. Even guestions on shortness of breath with wheezing, referred in this article to "wheezing", yielded specificity of 93%. Similarly, physician diagnosed allergic rhinitis and allergic conjunctivitis were highly specific, 93% and 98%, correspondingly, when compared to symptoms at interview and positive skin prick tests of specific lgE.²²

BMI and confounding factors

Reported BMI at the time of the questionnaire response was calculated by dividing weight in kilograms by the square of height in meters (kg/ m²). The population was divided in five BMI categories (BMI < 20, 20.0-22.4, 22.5-24.9. 25.0–27.4, \geq 27.5) were analyzed. The subjects in the two highest categories were regarded as overweight-obese subjects. Factors possibly related to BMI and asthma or atopic disorders were included as follows: gender, birth weight (<2500 g or \geq 2500 g), parental asthma or atopic disease, and parental education in three categories: basic education, non-university graduates and university graduates. Maternal smoking during pregnancy, passive smoking at age 0-2 years (a family member smoking daily indoors at home) and current smoking status were regarded. Current smokers were those who smoked daily, past smokers had stopped daily smoking at least for a half year earlier, and nonsmokers had never smoked daily. Number of older siblings in the childhood family was categorized from none to \geq four older siblings. Childhood residential environment was divided to farm environment at age 0–6 years and non-farm environment at age 0–6 years.

An index to measure leisure time physical activity during the past year was calculated from frequency, duration, and intensity of the physical activity during the last month.²³ Frequency of leisure time physical activity was graded as follows: 1 = less than once a month, 2 = once a month to once a week, 3 = 2-3 times in a week, 4 = daily or almost daily. Duration of physical activity yielded the grades 1 = less than half an hour, 2 = at least a half but less than 1h, 3 = at least one but less than 2h, 4 = more than 2h. Intensity of physical

Table 1 Association between body mass index (BMI, kg/m²) and background variables among Finnish university students aged 18–25 years.

Background variables	ВМІ						
	<20 N = 2643		22.5–24.9 <i>N</i> = 2486	25.0–27.4 <i>N</i> = 687	≥27.5 N = 297		
Male <i>n</i> = 4135	18.0	38.7	56.9	55.2	48.8	0.0001	
Female <i>n</i> = 6420	82.0	61.3	43.1	44.8	51.2		
Maternal smoking during pregnancy (%) $n = 9849$	5.4	5.2	5.2	7.4	7.5	0.113	
Birth weight $< 2500 \text{g}$ (%) $n = 9250$	10.0	8.3	8.0	5.9	7.4	0.009	
Passive smoking at age 0–2 (%)* $n = 10,475$	19.9	20.8	22.5	24.6	27.8	0.001	
Parental asthma or atopic disease (%) $n = 10,481$	42.8	40.3	39.8	40.5	38.8	0.167	
Parental education (%)							
Low <i>n</i> = 2992	26.5	28.3	31.4	34.2	33.5	0.0001	
Medium $n = 3792$	36.4	36.9	37.2	35.6	41.5		
High <i>n</i> = 3495	37.1	34.8	31.4	30.2	25.1		
Living on a farm at age 0–6 (%) $n = 10,142$	9.2	10.6	12.0	12.4	12.8	0.008	
Number of older siblings (%)							
One <i>n</i> = 3571	34.2	34.6	33.1	33.5	30.3	0.199	
Two <i>n</i> = 916	8.1	8.8	9.5	7.9	8.4		
Three $n = 254$	2.3	2.1	2.7	3.8	2.0		
\geq four $n = 141$	1.1	1.4	1.5	0.9	2.0		
Leisure time physical activity † (%)							
Low $n = 3036$	32.7	27.6	27.8	36.8	51.1	0.0001	
Moderate $n = 3175$	33.2	32.2	30.1	27.1	28.5		
Vigorous $n = 3858$	34.0	40.2	42.2	36.1	20.4		
Daily smoking (%)							
$Past^{\ddagger} n = 1589$	2.4	3.9	3.5	4.1	5.0	0.0001	
$Current^{\S} n = 364$	12.1	14.6	17.1	20.6	20.5		

*At least one family member smoking daily indoors in the home.

[†]Calculated from frequency, duration, and intensity of leisure time physical activity during past month.

[‡]Stopped daily smoking at least half a year earlier.

[§]Smoking daily.

activity was graded: 1 = equivalent of walking, 2 = between walking and jogging, 3 = equivalent of jogging, 4 = equivalent of running. Thereafter, the product of frequency, duration and intensity was calculated. Finally, three leisure time physical activity categories of about equal size of participants were formed: low = 1–8, moderate = 9–23, and vigorous \geq 24. For example, subjects exercising 2–3 times per week, for at least half an hour with mean intensity corresponding to alteration of walking and jogging, were regarded as moderate exercisers (index 12).^{15,23} Vigorous exercisers where,e.g. subjects exercising 2–3 times per week, at least 1 h per time at the intensity corresponding to at least jogging (index 27).

Statistical analysis

Association between BMI and background factors possible related BMI (Table 1), and asthma or allergic diseases were analyzed by χ^2 -tests. Background factors significantly related to BMI were used in the multiple logistic regression analyses.

Odds ratios (OR) with 95% confidence intervals (CIs) for diagnosed asthma, allergic rhinitis or conjunctivitis, atopic dermatitis and self-reported wheezing were calculated for five successive BMI categories and the lowest BMI category formed the comparison group. In multiple regression model adjustment was made for sex, birth weight, parental education, passive smoking at age 0-2, living on a farm at age 0–6, current smoking status. and leisure time physical activity. The risk of disease was calculated separately for men and women (Table 2). P-values below 0.05 or 95% CIs not including 1.00 were regarded as significant. Due to relatively low number of responses to question on birth weight, it was not included in the final regression analyses shown in the tables. However, in the preliminary analyses, birth weight did not change the associations.

In the additional analyses, the prevalence and adjusted risk of asthma and wheezing was calculated for men and women with low, moderate and vigorous leisure time physical activity during the last month (Table 3).

Table 2 Adjusted^{*} odds ratios (OR) for asthma, wheezing, allergic rhinitis or conjunctivitis in five body mass index (BMI) categories in men and women aged 18–25 years.

Disease	ВМІ	Men				Wome	Women			
		N	Prevalence %	Adj. OR [†]	95% CI	N	Prevalence %	Adj. OR [†]	95% CI	
Asthma	<20	472	3.2	1.00		2161	3.7	1.00		
	20.0-22.4	1712	5.5	1.98	1.11-3.52	2717	4.4	1.20	0.89–1.61	
	22.5–24.9	1407	5.3	1.90	1.05–3.41	1065	3.7	0.98	0.66–1.47	
	25.0-27.4	377	3.4	1.06	0.47-2.37	308	6.5	1.86	1.10-3.14	
	≥27.5	142	9.9	3.53	1.63–7.64	150	7.3	2.08	1.05-4.1	
Wheezing [‡]	<20	475	6.1	1.00		2168	6.4	1.00		
	20.0-22.4	1721	4.5	0.70	0.45-1.03	2721	7.7	1.24	0.99–1.56	
	22.5–24.9	1415	6.0	0.99	0.63-1.55	1071	7.7	1.24	0.93–1.67	
	25.0-27.4	379	9.0	1.55	0.91-2.63	308	11.0	1.74	1.15-2.64	
	≥27.5	145	6.9	1.00	0.46–2.18	152	12.5	2.22	1.32-3.74	
Allergic	<20	475	21.7	1.00		2167	21.4	1.00		
rhinitis or conjunctivitis	20.0-22.4	1719	24.2	1.14	0.89–1.46	2719	21.0	1.00	0.87–1.15	
	22.5–24.9	1415	22.9	1.10	0.85-1.42	1071	22.5	1.12	0.93–1.34	
	25.0-27.4	379	21.6	1.02	0.73–1.44	308	27.9	1.47	1.11-1.9	
	≥27.5	145	26.2	1.31	0.85–2.04	152	27.0	1.47	1.00-2.1	
Atopic	<20	468	12.8	1.00		2149	19.7	1.00		
dermatitis	20.0-22.4	1707	15.5	1.26	0.92-1.72	2709	22.5	1.21	1.05-1.40	
	22.5–24.9	1398	14.7	1.17	0.85-1.61	1065	20.6	1.02	0.85–1.24	
	25.0–27.4	375	15.7	1.34	0.89-2.00	305	25.2	1.41	1.06-1.88	
	≥27.5	145	17.2	1.46	0.86-2.46	151	22.5	1.17	0.78–1.77	

*Adjusted for parental education, passive smoking at age 0–2, living on a farm at age 0–6, current and past smoking, and leisure time physical activity.

[†]Significant associations marked by bold.

[‡]Diagnosed asthma excluded.

Disease	BMI	N men/women	Leisure time physical activity				
			Low men/women	Moderate men/women	Vigorous men/women		
Asthma	<20	435/2058	3.2/2.7	4.6/2.7	2.0/5.3		
prevalence (%)	20.0-22.4	1644/2600	6.1/4.9	3.5/4.0	6.3/4.4		
	22.5-24.9	1339/1019	7.8/7.4	4.1/6.7	4.5/4.8		
	25.0-27.4	357/301	-/7.4	3.4/6.7	5.7/4.8		
	≥27.5	132/138	14.5/7.2	8.1/7.3	5.0/7.1		
Adjusted* OR		Men	1.00	0.62 (0.42–0.92) [†]	0.77 (0.56–1.07		
for asthma (95% CI)		Women	1.00	0.77 (0.56–1.07)	1.19 (0.88–1.60		
Wheezing [‡]	<20	438/2065	6.4/6.5	6.8/5.9	6.0/6.5		
prevalence (%)	20.0-22.4	1653/2604	4.2/8.2	4.7/7.7	4.3/7.6		
	22.5-24.9	1347/1025	7.1/7.2	7.5/8.5	5.3/7.1		
	25.0-27.4	359/301	10.6/10.7	8.9/9.0	8.6/12.7		
	≥27.5	135/139	3.6/13.10	8.1/9.8	11.9/14.3		
Adjusted* OR		Men	1.00	1.21 (0.85–1.73)	0.98 (0.70-1.37		
for wheezing (95% CI)		Women	1.00	0.91 (0.72–1.15)	0.92 (0.73–1.16		

Table 3 Asthma and wheezing in men and women in three leisure time physical activity categories (low, moderate, vigorous) according to BMI. Adjusted* odds ratios (OR) for asthma and wheezing among subjects with low, moderate or vigorous leisure time physical activity.

*Adjusted by BMI, parental education, passive smoking at age 0–2, living on a farm at age 0–6, and current and past smoking. $^{\dagger}P = 0.017$.

[‡]Diagnosed asthma excluded.

Results

Of women aged 18–25 years 33.8% (2168) had BMIvalue below 20, 42.4% (2727) from 20.0 to 22.4, 16.7% (1071) from 22.5 to 24.9, 4.8% (308) from 25.0 to 27.4, and 2.4% (152) had BMI \ge 27.5. Of men 11.5% (475) had BMI below 20, 41.6% (1721) from 20.0 to 22.4, 34.2% (1415) from 22.5 to 24.9, 9.2% (379) from 25.0 to 27.4, and 3.5% (145) had BMI \ge 27.5.

There was a significant association between BMI in young adulthood and sex, birth weight, passive smoking at age 0–2, current smoking status, parental education, childhood residential environment (farm vs. non-farm), and leisure time physical activity during the past year, and birth weight (Table 1). In general, passive smoking at age 0-2 and current daily smoking were related to higher BMI, such as was living on a farm at age 0–6. In the BMI category below 20 low, moderate and vigorous leisure time physical activity were evenly distributed, but half of the subjects with $BMI \ge 27.5$ belonged to the category of low physical activity, and 20.4% to vigorous activity. BMI in adulthood was not related to maternal smoking during pregnancy, parental asthma or atopic disease, or number of older siblings in the childhood family (Table 1).

Table 2 shows the occurrence, adjusted odds ratios for asthma and atopic diseases among men and women in different BMI categories. In men, increasing BMI was most clearly positively associated with asthma, but not significantly with wheezing, allergic rhinitis or conjunctivitis and atopic dermatitis. Among men the risk of asthma was nearly two-fold in BMI categories 20.0–22.4 and 22.5–24.9, and in BMI category \geq 27.5 the risk was 3.5-fold (Table 2).

In women, the risk of asthma was about two-fold among overweight-obese subjects (BMI 25.0–27.5 and \geq 27.5). Correspondingly, there was a significant positive association between overweightobesity and wheezing and allergic rhinitis or conjunctivitis (Table 2). The association between increasing BMI and atopic dermatitis was not as linear, but reached significance in BMI categories 20.0–22.4 (OR 1.21) and 25.0–27.4 (OR 1.41). No interaction effect of obesity and sex on asthma was found (*P*-value for interaction 0.093).

In all multiple logistic regression analyses, adjustment by leisure time physical activity during past month (low, moderate, vigorous) did not change the association between the diseases and BMI (Table 2). Table 3 shows the occurrence of asthma and wheezing in five BMI categories according to leisure time physical activity. In general, the prevalence of asthma was highest among obese men and women regardless of leisure time exercising activity. Wheezing, on the other hand, increased quite linearly with increasing BMI among women in all exercise categories.

In adjusted models, the risk of asthma was smaller among men exercising moderately or vigorously, reaching significance among men with moderate exercise (OR 0.62, 95% CI 0.42–0.92) (Table 3). In women moderate exercise did not show significant protective effect on asthma. Wheezing (without asthma) was not significantly related to exercise either in men or women after adjustment for BMI and the other confounding factors.

Discussion

Asthma, wheezing and BMI

In the present study, among young adults with relatively low BMI values, we found that the risk of asthma was increased among overweight-obese women and among normal-weighted and obese men when BMI below 20 was used as comparison category. In men, the association between BMI and asthma was not as clearly U-shaped as in a guite recent study in USA.⁷ Most studies, on the other hand, have reported a greater risk of asthma among women⁴ or girls,² or exclusively among females.⁹ We found a greater risk of wheezing among overweight-obese women but not men, which might be partly explained by the under-diagnosis of asthma among female adolescent girls.²⁴ In the present study, BMI and sex had no interaction effect on asthma, either. However, a great number of subjects with very low BMI-values, different from general population, suggest that the findings should be cautiously generalized to the all adult ages. Among the present young adult population, misclassification of asthma and COPD, possibly explaining the greater occurrence of obstructive disease among the leanest subjects, seems to be improbable.

Obesity might affect asthma by mechanical effects and possible easier perception of symptoms, such as dyspnoea and wheezing. It was recently shown that increasing BMI was related to lower lung volumes, and subsequently airway narrowing, at least in men.²⁵ Studies using objective markers of asthma, such as bronchial hyperreactivity (BHR) have given conflicting results. BHR was not increased among Australian obese adults⁵

or children¹⁹ suggesting that diagnosis of asthma is given more easily among obese subjects. On the other hand, BHR was increased among teenage obese girls in Taiwan,¹⁸ among men with increasing BMI taking part in ERCHS,²⁶ and in the leanest and obese men.²⁷ The conflicting data on BMI and BHR, possibly reflect the fact that, BHR as a feature of asthma is confounded also by other factors, such as atopy and sex.

In cross-sectional studies, overweight-obesity could affect asthma and wheezing by the fact that subjects with asthma symptoms have adopted more sedentary lifestyle and thus gain more weight. The hypothesis has been opposed by few studies that have used precise measures of physical activity. Chen et al. reported that leisure time physical activity measured by energy expenditure was not lower among asthmatic than non-asthmatic subjects.^{17,18} Beckett et al.⁹ reported that low physical activity was not responsible for the increased risk of incident asthma among women that gained weight, and neither did the degree of physical activity have an independent impact on asthma. Similarly, we found that the association of BMI and asthma was not affected by a three-graded index formed of grades of frequency, duration and intensity of leisure time physical activity.

In our study, moderate leisure time physical was related to smaller risk of asthma in men. Similarly, among Finnish adult twins, the risk of asthma¹⁴ and onset of asthma in adulthood¹⁵ were lower among men but not women, having conditioning exercise. In elite athletes asthma seems to be more common than in general population.²⁸ We did not, however, find such an association between vigorous leisure time activity and asthma in our student population.

Our data clearly showed that the prevalence of shortness with wheezing, without diagnosis of asthma, increased concomitantly with increasing BMI regardless of leisure time physical activity (Table 3). Based on that finding, confounding effect of BMI on reported wheezing is evident not only in obese, but also normal-weighted subjects, especially women.

Allergic rhinitis or conjunctivitis and BMI

We found astonishingly linear association between increasing BMI and physician-diagnosed allergic rhinitis or conjunctivitis, most clearly in women. The association was not as evident in atopic dermatitis. However, the risk of atopic dermatitis was significantly greater among normal-weighted (BMI 20.0–22.5) and over-weighted women (BMI 25.0–27.4) compared to the leanest subjects (BMI < 20).

Among European 18-44-year-old adults²¹ and Australian 17-73-year-old adults,¹⁹ neither the risk of nasal allergies, nor sensitization were related to obesity. On the other hand, among obese Taiwanese girls¹⁸ and obese Finnish²⁰ and Danish adults²⁹ atopic sensitization was more common than in lean subjects. Differences in age range and the reliability of questionnaire-based diagnoses might be responsible for the reported controversial results on nasal allergies. In the present study, physiciandiagnosed allergic rhinitis or conjunctivitis was shown to be highly specific and the positive predictive value was high, e.g. in most cases subjects who reported allergic rhinoconjunctivitis also had symptoms and positive skin prick test results or specific IgE to airborne allergens.²² Furthermore, atopic dermatitis, at least in adults, is more heterogeneous disease than allergic rhinoconjunctivitis as regards to IgE-mediated sensitization. Therefore, such a linear association as between BMI and atopic dermatitis would be unexpected.

Body fat and its relation to atopic diseases and asthma

BMI as a measure of body fat is dependent on age, sex and ethnicity.³⁰ Women have higher total body fat during the whole lifespan, and in both genders body fat increases with age. In the present study, the age range of the subjects was narrow (18–25 years) and women and men were analyzed separately to exclude possible confounding by these factors.

Body fat may affect asthma and allergic disease by enhancing immune response. Leptin, a satiety hormone produced by adipocytes, is increased among obese people, and among women compared to men. The overall leptin response is to stimulate a Th1 cytokine profile³¹ counteracting asthma and atopy. Leptin resistance among obese³² might lead to opposite Th2 cytokine profile. Moreover, leptin enhances production of proinflammatory mediators, such as TNF α and IL-6 from macrophages,³³ which in turn increase IL-4 and IL-5 production under antigen exposure. Recently, in ovalbumin sensitized mice, serum leptin was increased during allergic reactions in the airways.³⁴ The higher serum leptin also correlated with stronger IgE response and airway responsiveness.³⁴ In children, levels of serum leptin were higher only in asthmatic boys in all BMI-levels,³⁵ suggesting the inflammatory role of leptin in childhood asthma, more common in boys.

Especially in obese women, not only leptin, but also oestrogen hormone, might affect immune response by enhancing Th2 cytokine profile.³⁶ In fact, an increased risk of asthma has been reported in some studies among women having oestrogen replacement therapy.³⁷ Similarly, early onset of puberty favours the persistence of asthma symptoms during adolescence.³⁸ Moreover, in a recent case control study, there was a stronger association between BMI and asthma among women with early menarche.³⁹

In addition, shared genes or shared risk factors, for obesity and atopic disease⁴⁰ might explain the burden of both conditions. In the present study, shared risk factors (Table 1), such as birth weight,⁶ parental education, childhood environment (farm, non-farm), leisure time physical activity and current and past smoking were adjusted for, but the association did not change.

In conclusion, a linear association between increasing BMI and allergic rhinoconjunctivitis was found in women. It would be interesting to find out whether the association could be explained by the interplay of leptin and oestrogen hormone on allergic inflammation. The link between obesity and asthma is evidently more heterogeneous, and therefore such a linear association between body fat and asthma could not be seen. Hormonal, genetic and mechanical factors might be responsible for the complexity of the association between BMI and asthma.

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Ethics Approval

The study has been approved in the Ethics Committee of University of Turku and Turku University Hospital, Finland in December 1994.

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