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Immediate skin reactivity to histamine and to allergens in cohorts of 9-year-old schoolchildren studied 16 years apart

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Summary

Background Differing or increasing prevalence of positive allergen skin-prick tests observed in Europe could at least in part be explained by population changes in histamine skin reactivity. These changes would also alter the relationship between positive allergen skin-prick tests and serum IgE.
Objective To assess changes in histamine reactivity, allergen skin-prick tests and serum IgE in our geographical setting.
Methods We compared the outcome of two epidemiological surveys conducted 16 years apart in unselected 9-year-old schoolchildren (170 in 1983 and 176 in 1999) from a semi-rural region in central Italy. Outcome measures were skin-prick tests with two histamine concentrations (10 and 1 mg/mL) and 11 locally relevant allergens; serum total and specific IgE for positive allergens.
Results The two histamine concentrations induced significantly larger mean weal diameters in 1999 than in 1983 (10 mg/mL: 5.28 ± 0.82 mm vs. 3.25 ± 0.97 mm; $P < 0.001$). Whereas the prevalence of subjects with at least one positive allergen-induced weal reaction (≥ 3 mm) increased over the 16 years (from 15.3% in 1983 to 25.6% in 1999), the prevalence of positive skin-prick tests, expressed as the allergen/ histamine weal ratio, remained almost unchanged. A given allergen weal diameter yielded less total ($P < 0.05$ by Student's *t*-test for cumulative weals < 8 mm) and specific ($P < 0.01$ by Student's *t*-test for weals < 3 mm, $P < 0.05$ by Kruskal–Wallis test) serum IgE in 1999 than in 1983.
Conclusions Although the causes and mechanisms remain unclear, the increased histamine skin reactivity over time is associated with an increase in positive allergen skin-prick tests. In the presence of increased tissue and organ susceptibility to histamine, minute amounts of specific IgE could have important biological consequences.

Keywords skin allergen reactivity, skin histamine reactivity, skin-prick test/specific IgE relationship.
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Introduction

The prevalence of asthma, eczema, allergic rhinoconjunctivitis, and skin sensitization to allergens is on the increase in industrialized countries [1] and is higher in Western than in Eastern Europe [2–5], in urban than in rural areas [6, 7], and in populations whose average intake of fresh fruit is low [8]. Environmental conditions associated with Westernization facilitate allergic sensitization even in the short term, as suggested by the rapid rise in skin sensitization and allergic rhinoconjunctivitis in East Germany only 4 years after the German reunification [9]. The causes and mechanisms of this phenomenon remain largely unknown. Although some have attributed the enhanced reactivity to allergens to a higher propensity to develop specific IgE responses against common allergens [10], scarce evidence is available to confirm this trend [11]. Most investigators agree that the observed differences and rapid changes in atopic illnesses are at least in part independent or unexplained by variations in the occurrence of allergic sensitization [12, 13].

On the other hand, allergen-specific IgE antibodies are a necessary, but not exclusive, component of weal reaction to allergens, whose intensity also depends on non-specific factors. These factors include skin mast cell concentrations and their ability to release histamine and, most importantly, the responsiveness to histamine of the surrounding tissue and vascular components [14, 15].

Skin reactivity to histamine is usually tested as a positive control of allergen skin-prick tests (SPTs), but rarely analysed as a relevant outcome *per se*. Yet this variable comes under the influence of the changing environmental factors [16, 17], as do bronchial and nasal aspecific reactivity. Thus, an increasing trend of positive reactions to allergens could arise not only from an enhanced presence of allergen-specific IgE, but also from increased skin reactivity to histamine. To test this hypothesis, in 1983 and 1999 we studied two cohorts of unselected 9–13-year-old children living near Viterbo, Italy. Using the same methods in these two populations, we measured and compared skin weals produced by two different concentrations of histamine; skin weals produced by a panel

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of common allergens; and total and specific serum IgE concentrations and their relationship with the allergen-induced weals.

Methods

Study populations

The study, initiated in 1983 (hereafter referred to as the '1983 study'), has been described in detail elsewhere [18–20]. In brief, the survey was completed between October 1983 and January 1984 on a target population of 179 9-year-old schoolchildren, representing all the fourth-grade pupils attending elementary schools in a district adjoining three small towns near Viterbo (central Italy). The study was completed by 170/179 (95.0%) participants (83 males), whose parents gave informed consent. All children underwent allergen SPTs with 11 locally relevant allergens.

A total of 160 children had blood samples drawn for IgE testing.

From November 1998 to March 1999 (1999 study), we conducted a similar survey on all the fourth-grade pupils attending the same schools. The target population consisted of 207 9-year-old schoolchildren and was completed by 176 participants (85.0%) (86 boys) whose parents gave their informed consent for blood sampling. All children underwent allergen SPTs with the original allergen panel. The studied population was drawn from small towns without undue pollution, with some families engaged in agriculture, and without influxes of new families to the area. There were no differences in the mean distribution of height and weight of the children in the two studies.

The studies were approved by the Ethics Committee of the Paediatric Clinic.

Allergen skin-prick tests

In the 1983 study, allergens were tested with the following panel: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria tenuis*, *Aspergillus fumigatus*, *Poa pratensis*, *Artemisia vulgaris*, *Parietaria officinalis*, *Olea europea*, cat dander, milk albumin, and egg white. Allergens came from a single lot (Lofarma Allergeni S.p.A., Milan, Italy). SPTs with two histamine chloride concentrations (10, 1 mg/mL) were performed after the allergen prick tests on the lower volar side of the left forearm. Prick sites were at least 2 cm apart; disposable allergy prickers with a 1 mm tip were used. At 10 min after the last skin prick [21, 22], the mean diameter (the mean of the maximum diameter and its perpendicular) of the histamine and then of the allergens-induced weals was measured with a transparent Plexiglass ruler and recorded.

The Pepys [23] method for allergen SPTs was used throughout the study: instructions were to jab the pricker with moderate force through the drops at an angle of 45° to the skin and gently lift. All SPTs were performed by four experienced physicians. In pilot studies conducted before the study, each field operator performed four identical tests with the 10 mg/ml histamine solution on a group of six to eight adult volunteers. At 10 min after the SPT, the weal diameters for each reaction were measured and recorded and the mean value was calculated. The mean intra-operator's co-efficient of variation was 8.5%–20%. All SPTs were performed in the morning.

In the 1999 survey, histamine and allergen SPTs were performed following an identical procedure during the morning. Allergens were purchased from the same source as in 1983 (Lofarma Allergeni S.p.A., Milan, Italy) and the pricking device was identical to that used in the 1983 study. Allergen SPTs were performed by two experienced technicians. In pilot studies, the mean intra-operator's coefficient of variation for histamine 10 mg/mL was 7–14%.

Total and specific IgE assays

In the 1983 study, serum samples from venous blood were stored in aliquots at $-70\,^{\circ}C$ until tested. Aliquots of all sera were tested for total IgE (but not for specific IgE) in 1983 by the immuno-CAP method (Pharmacia, Uppsala, Sweden). In 1992, to test the quality of the sera, we tested all samples again for total IgE concentrations by the same method used in 1983 and found no significant differences in geometric mean value and in 10°, 50°, and 90° percentiles (data not shown). We also measured specific IgE by the immuno-CAP method (Pharmacia, Uppsala, Sweden) using 1983 serum samples.

In the 1999 survey, serum samples were also stored in aliquots at $-70\,^{\circ}C$ until tested. One month after collection, aliquots were tested for total and specific IgE by the immuno-CAP method (Pharmacia, Uppsala, Sweden).

On both occasions we assayed specific IgE, only in sera from subjects with a positive (mean weal diameter (3 mm) or doubtful allergen SPT reaction (mean weal diameter (1 mm) and only against the corresponding allergen. We avoided IgE testing for samples from subjects with negative SPT reactions based on a separate study (unpublished data) yielding nondetectable specific IgE (<0.35 kU/mL) in all subjects with a negative SPT reaction for the same airborne allergen.

Statistical analysis

Student's *t*-test for independent samples was used to compare the mean histamine-weal diameters and the Kruskal–Wallis test was used to compare their distribution. The χ^2 test was used to compare frequencies of sensitization to allergens between 1983 and 1999, and Fisher's exact test was used when appropriate. Student's *t*-test for independent samples was used to compare geometric mean concentrations of total and specific IgE and the Kruskal–Wallis test was used to compare the distribution of atopic sensitization in relation to total and specific IgE. All data were analysed with SPSS version 9.0 (SPSS Inc., Chicago, IL, USA).

Results

The mean weal diameter induced by histamine at a concentration of 10 mg/mL was significantly higher in 1999 than in 1983 (5.28 ± 0.82 vs. 3.25 ± 0.97 mm; $P < 0.001$) (Fig. 1a). Similarly, the mean weal diameter to histamine 1 mg/mL was significantly higher in 1999 than in 1983 (3.51 ± 0.68 vs. 2.21 ± 1.06 mm; $P < 0.001$) (Fig. 1b).

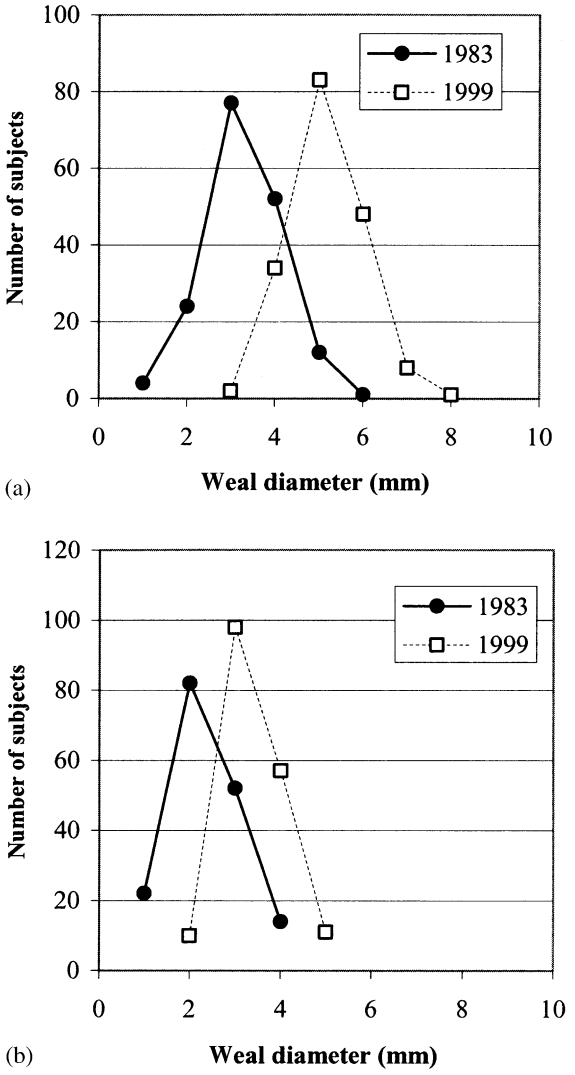

Fig. 1. Distribution of weal reaction to histamine 10 mg/mL (a) and 1 mg/mL (b) in 1983 and 1999. For both concentrations, the curves for 1999 were significantly shifted to the right ($P<0.001$ by the Kruskal–Wallis test).

Overall, the prevalence of subjects with at least one weal reaction of 3 mm or more against the 11 allergens tested in both surveys increased from 26/170 (15.3%) in 1983 to 45/176 (25.6%) in 1999 ($P<0.05$) (Table 1). All the allergens tested, except *Alternaria tenuis*, elicited a positive reaction more frequently in 1999 than in 1983; this difference was significant for *Dermatophagoides pteronyssinus* and *farinae* and for grass pollen. Polysensitized subjects were significantly more frequent in 1999 than in 1983 (31/176, 17.6% vs. 12/170, 7.1%; $P<0.005$); as were those with a cumulative weal reaction >10 mm (17/176, 9.7%, vs. 6/170, 3.5%; $P<0.05$) (Table 1). SPTs changes between 1983 and 1999 were not significantly influenced by gender or parental smoking.

To account for variations in skin reactivity to histamine, the results of allergen SPTs were also expressed as the ratio between allergen and histamine weals. Using a cut-off value of 0.5 and the 10 mg/mL histamine concentration as the reference, we still found a higher prevalence of subjects with at least one positive reaction in 1999, but only two of the differences were significant (Table 2).

Geometric mean values of total IgE were slightly lower in 1999 than in 1983 (52.0 ± 3.9 vs. 66.7 ± 4.3 kU/L; $P=0.106$). Plotting classes of cumulative weal diameter of allergen SPTs against the geometric mean of serum total IgE on a log scale, we found, as expected [24] that the prevalence rate of atopy was related to concentrations of total IgE in both surveys. But for any level of atopic sensitization less than 8 mm (the highest class of atopy), the level of total IgE was significantly higher in 1999 than in 1983 ($P<0.05$ by Student's *t*-test) (Fig. 2).

When we evaluated the relationship between the diameter of the allergen skin weal and the concentration of serum-specific IgE against that allergen in both surveys, we found that for weals smaller than 3 mm, the mean concentration of specific IgE (log kU/L) was lower in 1999 than in 1983 ($P<0.01$). The same nonsignificant trend was observed for weals $\geqslant3$ mm. The Kruskal–Wallis test on the whole set of data gave a $P<0.05$ (Fig. 3).

Discussion

Comparing the results of two surveys conducted 16 years apart in non-selected 9-year-old schoolchildren from central Italy, we showed that histamine skin reactivity increased in our setting during the past two decades. The prevalence of positive allergen SPTs also increased. Moreover, the relationship between positive allergen SPTs and total and specific IgE was substantially weaker in 1999 than in 1983.

When in a given population skin reactivity to histamine increases i.e. the histamine intradermally injected or released by mastocytes after an allergen–IgE interaction generates larger weals – we envisage two major consequences. Our study verified both. First, the prevalence of positive allergen SPTs increases because the effect of histamine on the end-organs in the skin increases whatever the amount of serum IgE. Second, the presence of this 'non-specific' component in the weals elicited by allergen SPTs produces a discrepancy between allergen SPT results and serum IgE: i.e. any given skin reaction is associated with lower concentrations of serum total or specific IgE.

Epidemiological results, especially those achieved 16 years apart, could be variously biased. To avoid bias, our group has monitored the population under study every 3–4 years since 1983, always using the same procedures and well-trained field operators [16, 17]. During this period, histamine-induced weals progressively increased [16]. In this paper, we report only the results of the first and last surveys in which we measured total and specific serum IgE. As we tested two different histamine concentrations in both studies, we avoided duplicate histamine pricking. The fact that the differences in allergen skin-prick tests between 1983 and 1999 substantially diminished when we expressed allergen skin tests as the ratio between allergen and histamine weals proves that the weal increases observed in 1999 were not random reading errors of the field operators. Although no precise data were available, a change in allergen potency from 1983 to 1999 large enough to explain the changes we witnessed in SPTs (a doubling weal diameter requires a ten-fold increase in the allergen concentration) has been excluded by the manufacturer (Lofarma, Milano, Italy, personal communication).

Table 1. Skin sensitization among Italian schoolchildren in 1983 and 1999

	1983 170 subjects		1999 176 subjects		Ratio	$\chi 2$
	n	%	*n*	%	%1999/%1983	*P*
Prevalence of sensitization to allergens						
Dermatophagoides pteronyssinus	15	8.8	34	19.3	2.2	<0.01
Dermatophagoides farinae	12	7.1	29	16.5	2.3	<0.01
Alternaria tenuis	1	0.6	1	0.6	1.0	NS
Poa pratensis	4	2.4	20	11.4	4.8	<0.005
Cat	2	1.2	9	5.1	4.3	NS
Parietaria officinalis	6	3.5	10	5.7	1.6	NS
Olea europea	5	2.9	6	3.4	1.2	NS
Any of the above allergens	26	15.3	45	25.6	1.7	<0.05
Polysensitized subjects	12	7.1	31	17.6	2.5	<0.005
Cumulative wheal diameter (seven allergens)						
>5 mm	14	8.2	28	15.9	1.9	<0.05
>10 mm	6	3.5	17	9.7	2.7	<0.05

Positive allergen skin prick test defined as a mean weal diameter $\geqslant 3$ mm. NS = not significant.

Table 2. Skin sensitization among Italian school children in 1983 and 1999

	1983 170 subjects		1999 176 subjects		Ratio	χ^2
	n	%	*n*	%	%1999/%1983	*P*
Prevalence of sensitization to allergens						
Dermatophagoides pteronyssinus	25	14.7	35	19.9	1.4	NS
Dermatophagoides farinae	21	12.4	31	17.6	1.4	NS
Alternaria tenuis	4	2.4	3	1.7	0.7	NS
Poa pratensis	7	4.1	21	11.9	2.9	<0.05
Cat	2	1.2	10	5.7	4.8	<0.05
Parietaria officinalis	11	6.5	12	6.8	1.1	NS
Olea oeuropea	9	5.3	7	4.0	0.8	NS
Any of the above allergens	41	24.1	51	29.0	1.2	NS
Polysensitized children	23	13.5	33	18.8	1.4	NS

Positive allergen skin prick test defined as a weal of allergen/weal of histamine (10 mg/mL) $\geqslant 0.5$. NS = not significant.

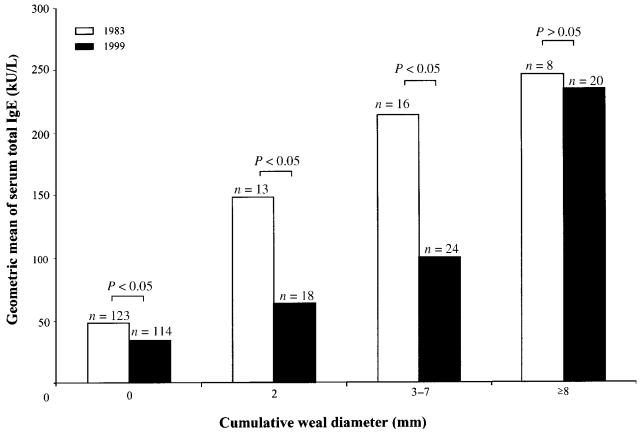

Fig 2. Geometric mean concentration of serum total IgE (log kU/L) by levels of atopy (classes of cumulative weal diameter elicited by 11 allergens). For each class of weal diameter less than 8 mm, concentrations of total IgE were lower in 1999 than in 1983 ($P<0.05$ by Student's *t*-test).

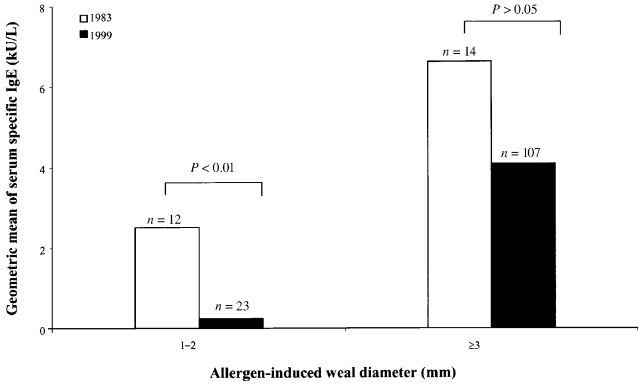

Fig 3. Geometric mean concentration of specific IgE (log kU/L) according to weal reaction to the same allergen in 1999 and in 1983. For a given weal diameter, the concentrations of total IgE were lower in 1999 than in 1983 ($P<0.05$ by the Kruskal–Wallis test). Student's *t*-test was significant only for the class of allergen weal diameter 1–2 mm.

Support for our epidemiological findings comes from various European reports describing differences in weal histamine dimensions in populations that also differed in the prevalence of positive allergen skin-prick tests. A group of Swedish researchers [2, 7] reported that the mean weal produced by histamine 10 mg/mL was 4.8 mm in Estonian children (prevalence of at least one positive allergen SPT, 8.1–14.3%), 5.5 mm in Polish children (prevalence of at least one positive allergen SPT, 13.7%), and 6.2 mm in Swedish children (prevalence of at least one positive allergen SPT, 24–35%). In a longitudinal study conducted only 4–5 years apart in Estonian children, histamine weals (10 mg/mL) increased from 4.5 ± 0.4 to 5.9 ± 0.2 mm (+31%) [25]. Children in East Germany also had smaller histamine weals than those in West Germany (von Mutius, personal communication). Although in each circumstance these differences were attributed to minor technical differences in the field procedures, they are only slightly smaller than the differences in histamine weals that we observed over a comparatively long time span.

Allergen SPTs results strongly reflect the influence of many factors other than antigen IgE antibody reactions, including gender [26], total serum IgE concentration [24, 27], and unknown or nonspecified factors [28]. These and other non-IgE-linked factors could increase skin reactivity to histamine through many different mechanisms: among them, heightened H_1-receptor number or sensitivity, increased H_1-induced production of mediators [29], increased action of factors that normally enhance IgE dependent mediator release [30], or increased sensitivity of sensory nerve endings that after H_1 receptor stimulation release (through an antidromic axon reflex) several mediators, including substance P. Substance P produces a dose-related weal response in human skin [31, 32]. A newly identified histamine receptor, the H_4 receptor, may be closely involved in the regulation of immune function and could have clinical relevance in allergy and asthma [33]. Also, endogenous opioid peptides can induce cutaneous mast cell degranulation [34].

Whatever the mechanisms that increase histamine-induced skin weals, our findings may have clinical importance: many allergen skin reactions previously read as borderline or negative will now be considered positive. This does not mean that we are measuring "false"-positive skin reactions, but it does imply that skin hyper-reactivity is less completely IgE dependent. For clinical purposes, this hyper-reactivity (of the skin and other organs) presumably remains as important as before. Higher skin reactivity to histamine could therefore explain the increased prevalence of children with positive allergen SPTs to common allergens in our 1999 study and the increased prevalence of positive allergen SPTs in populations living with a Western lifestyle, even in the absence of an increase in tissue concentrations of specific IgE.

Several environmental factors known to be associated with a higher prevalence of allergenic sensitization (including urban residence, specific dietary patterns, and western lifestyle) could act, at least in part, by increasing histamine skin reactivity more than by increasing IgE production.

Hence we believe future epidemiological studies should regard skin histamine reactivity not merely as a positive control in allergen skin testing, but as a measure with an independent, intrinsic biological meaning that may help in interpreting the overall set of experimental data.

Trends in allergic disease may in part reflect factors other than IgE-mediated reactions. Clinical symptoms and severity of allergic asthma, rhinitis, or eczema depend not only on the production and tissue concentration of IgE but also on the biological effects produced by the mediators released by the allergen-IgE encounter. In a subject whose end-target organs (including the skin, nose, and lungs) are highly susceptible to histamine or other related mediators, antigen stimulation could have important biological consequences even in the presence of a minute amount of specific IgE.

Future research should establish whether the apparent changes in the biological pattern of old diseases (e.g. the clinical consequences on asthma of the increased reactivity to histamine in the population) should lead us to reappraise the place of certain drugs, e.g. antihistamines [35], in the treatment of these diseases.

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