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Stress and anxiety effects on positive skin test responses in young adults with allergic rhinitis

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ABSTRACT

Background: Anxiety and psychological stress affect allergy-related immune function. How these relations influence the evaluations of patients with allergic rhinitis is unknown.

Objective: To examine whether anxiety and stress exposure affect skin prick test (SPT) responses to common allergens for which patients with atopy showed no prior positive SPT response.

Methods: Patients with allergic rhinitis, evidenced by clinical history and SPT results, were admitted twice to a hospital research unit for 4 hours. In a crossover design, SPT wheals were assessed before and after the Trier Social Stress Test and then the following morning; for comparison, SPT wheals were assessed before and after a laboratory session without a stressor. Analyses focused on wheal responses for common allergens that tested negative (wheal size <3 mm larger than saline) from SPTs performed at multiple baseline assessments.

Results: After the Trier Social Stress Test, more anxious patients with atopy had a higher incidence of positive SPT reactions to antigens that previously tested negative. Anxiety was unrelated to positive SPT incidence under nonstressful conditions. Based on clinical symptom reports, newly positive SPT reactions after the stressor were apparently corrections of previously false-negative SPT reactions. The SPT wheal responses for allergens previously testing negative were enhanced after a stressor. Histamine (positive control) or saline (negative control) SPT responses were not affected.

Conclusion: A laboratory stressor affected allergen SPT responses in more anxious patients with allergic rhinitis. In addition to clinical history, assessment of anxiety and current stress at the time of the SPT may provide valuable information about a patient's allergic status and aid in clinical decision making.

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Introduction

The association between anxiety and allergic disorders, including allergic rhinitis (AR), is well documented.[1–3] Allergic individuals show higher levels of anxiety symptoms compared with nonallergic individuals,[3,4] and clinical anxiety disorders are over-represented in AR populations.[1,2] Anxiety symptoms include rumination and worry about stressful events, which exacerbate and prolong negative emotional arousal and physiologic stress activation.[5] Biopsychosocial models of allergic disease underscore stress-related endocrine and immune alterations in the exacerbation of clinical symptoms.[6,7] Indeed, psychological stress and negative emotions amplify immune-mediated clinical symptoms in individuals with allergic disorders.[8–10]

The authors also observed an important role for anxiety in allergy-related immune function. In a study of individuals with AR, anxiety enhanced the impact of stress on allergen-induced histamine release in response to skin prick tests (SPTs).[11] Skin prick testing is a major diagnostic tool in the clinic and can serve to confirm whether patient symptomatology is due to allergy.[12,13] Findings from the authors' original study were based on an

examination of wheal responses to antigens for which individuals met diagnostic criteria for allergy, that is, they reported a symptom history consistent with allergy and showed clinically positive SPT responses at baseline. In the present examination, it was determined whether these findings translated to clinical implications. In the original study, a subset of individuals who had negative SPT responses to particular allergens at baseline, when retested after a laboratory stressor, showed a positive response to at least 1 of these allergens. For the present analysis, the authors examined in these individuals whether anxiety in combination with stress exposure increased the incidence of positive SPT responses to allergens previously testing negative. Subsequently, participants' self-reported clinical history of allergies were used to determine how to interpret potential stress-related alterations in SPT responses; such findings may suggest 2 possibilities. First, anxiety and stress in susceptible individuals could increase the risk for acute allergic responses (ie, mast cell−derived histamine release) after allergen exposure. Second, in individuals without clinical symptoms in response to specific allergens, stress-related enhancement of positive SPT responses to these allergens may have implications for stress and anxiety affecting the validity and reliability of the SPT. The authors also examined, similar to original analyses, whether anxiety modulated the impact of stress on the magnitude of wheal responses to common allergens but extended this analysis to allergens that previously tested negative in the sample.

Methods

Participants

The participants (10 men and 18 women, mean age 24.73 years, SD 4.35 years, range 18−33 years) were recruited using advertisements seeking healthy individuals 18 to 40 years old who had a history of seasonal nasal allergies (hay fever; for details, see Kiecolt-Glaser et al[11]). Most participants were white, non-Hispanic (n = 22), 4 were African American, and 2 were Asian. Most (n = 26) had at least some college education. Exclusion criteria included active allergy immunotherapy, cardiovascular medications (statins, β blockers, etc), psychotropic medications, excessive alcohol use, smoking, asthma or other illnesses with immunologic or endocrinologic components other than allergy, or medications with obvious consequences for these systems or for allergies. The recent use of some allergy medications was restricted in accord with recommendations designed to optimize SPT results.[14,15] Participants were asked to refrain from the use of vitamin C supplements for at least 24 hours before all study sessions.

A screening visit included allergen SPTs to 9 common allergens and questionnaires. Subjects were selected for participation in the study based on clinical history and SPT results. To be eligible, participants had to meet SPT criteria for sensitivity to house dust mite (der p 1) owing to research objectives for the primary study[11] (details of the SPT battery and criteria are detailed under "Measurements," "Skin Prick Allergy Testing"). This study was approved by The Ohio State University biomedical research review committee and all participants provided written informed consent to participate.

Study Sessions: General Clinical Research Center

Two General Clinical Research Center laboratory sessions were scheduled at least 2 weeks apart (mean 38.72 days, SD 45.74 days). The 2 visits followed the same timeline, differing only in the stressor/control condition randomization for that visit. On arrival a heparin well was placed in 1 arm for subsequent serial blood draws. Then, the participant ate a standardized breakfast (after fasting since midnight) and completed questionnaires. Next, the participant sat quietly (or listened to soothing classical music) for a 30-minute relaxation period, after which a nurse obtained blood and saliva samples from the participant. Then, the nurse applied the pretask SPTs, which were followed by the Trier Social Stress Test or a control task, a post-task SPT performed on the opposite arm to the pretask SPT and blood and saliva sampling. Participants returned for a 1.5-hour follow-up General Clinical Research Center visit on the subsequent morning to undergo a final SPT.

Thus, each of these two 4-hour laboratory appointments included 2 SPTs using alternate arms (pretask and post-task) and a third SPT at a 1.5-hour appointment the following morning.

Stressor task

The Trier Social Stress Test, a well-validated laboratory stressor,[16] was used to induce an acute stress condition. Saliva samples for cortisol (a primary stress hormone) were obtained throughout the procedure (see Kiecolt-Glaser et al[11] for relevant findings). In brief, participants were escorted to a room with a microphone stand and video camera and a seated "audience" panel of 2 to 3 individuals and informed that they would make a speech and perform mental arithmetic in front of the panel. For the speech, the participants were told to imagine that they had applied for a position and were about to be interviewed by the selection committee; they had 10 minutes to prepare a speech about why they would be best for the job, 5 minutes to deliver the speech, followed by a 5-minute mental arithmetic task, subtracting serially the number 13 from 1,022 as fast and as accurately as possible They were told that at least 1 member of the panel was trained in behavioral observation and would rate their speech's content and style. Participants also were told that they would watch the videotape of their speech, without anyone else present, to review the performance on which the committee would base their evaluation.[17]

Then, participants were taken to another room for their speech preparation. They returned to the first room and performed the speech and serial subtraction task. After participants watched their speech and arithmetic task on videotape in private, a nurse performed the post-task SPT.

Control task

The control task served as the contrast condition for the Trier Social Stress Test in this crossover design study. Participants silently read a magazine section for 10 minutes before reading the same material out loud while being audiotaped. Afterward they listened to an audiotape, without anyone else present, of someone else reading the material. They were told that they were being asked to read aloud and listen to the audiotape to control for the effects of speaking and listening related to other experimental tasks; the authors emphasized that their performance was not being evaluated.

Measurements

State anxiety

The 20-item State Anxiety scale of the Spielberger State-Trait Anxiety Scale is a widely used, reliable, and valid measurement of the current state of anxiety, with excellent norms.[18] Participants report how they feel at the moment, with adjectives such as calm, tense, at ease, and upset rated on a scale from 1 to 4. The scale was administered at the beginning of each 4-hour session, before the interaction tasks were introduced. The correlation (r) between the 2 was 0.85 (P < .0001); thus, the mean of the 2 administrations was used as the summary anxiety measurement for each participant.

Skin prick allergy testing

Skin prick testing[12,13] was used to assess allergic status to a battery of 9 allergens: house dust mite (Dermatophagoides pteronyssinus [der p 1]), North American dust mite (Dermatophagoides farinae), ragweed, mold mix, weed mix, tree mix, grass mix, cat

dander, and sagebrush. All participants met SPT criteria for sensitivity to der p 1 in accord with the research objectives for the primary study.[11]

The battery was performed on the volar aspect of the 2 forearms (alternating between tests) using the DermaPIK SPT system.[19] Histamine sulfate 6 mg/mL served as the positive control,[14] and glycerinated saline was the negative control.[20] The SPTs were applied using a DermaPIK and read 20 minutes later by measuring the largest diameter of the wheal and flare (millimeters). SPT data are expressed as the difference between wheal size produced by specific allergens and the concurrent saline control. The nurses who performed and read the tests were blind to the participant's assigned condition for the day.

A wheal at least 3 mm larger than the concurrent saline control provided evidence of an allergen-specific IgE response[14] and was considered a "positive" SPT reaction. Participants were considered nonsensitized for a given antigen if a wheal less than 3 mm larger than the concurrent saline control (considered a "negative" SPT result) was evident from SPTs performed at screening and the pre-task assessment at the 2 visits (ie, at all 3 baseline testing time points). A "positive" SPT count variable was calculated as the total number of SPT wheals at least 3 mm larger than the concurrent saline control immediately or the day after the stress or nonstress tasks for allergens that previously tested negative at the multiple baseline assessments. In addition, mean wheal diameters were calculated across all allergens for which participants tested negative (wheal <3 mm larger than concurrent saline) at the 3 baseline assessments.

Self-reported allergies and comparison with SPT results

Participants self-reported whether they had an allergy to a series of 7 allergen categories (dust; weed, tree, or grass; cat; mold; ragweed; food) that represented 8 of the 9 allergens used in the SPT panel, in addition to 5 other allergens not used in the panel (dog; pollen; animal; feather; food). Specifically, participants who responded "yes" to having nasal symptoms (sneezing, itching, runny nose/postnasal drainage, and/or nasal congestion) after exposure to environments rich with the specific allergen (such as outdoor exposure during a known pollen season in the area of Columbus, Ohio) were considered allergic to that allergen.

Using these self-reports, the authors determined the extent to which the positive post-task SPT reactions were falsely positive or the conversion of a previously false-negative SPT reaction. A positive post-task SPT reaction was considered falsely positive if a participant self-reported no clinical symptoms after exposure to the allergen that was negative at baseline but then positive at post-task SPT assessments. If the participant's clinical history was consistent with specific allergen sensitivity, the positive SPT reaction at post-task was considered a conversion of a previous false-negative SPT result at baseline.

Data Analysis

All data were analyzed using SPSS 19 (SPSS, Inc, Chicago, Illinois); tests were 2-tailed with α set at 0.05. A generalized linear model was used to examine whether stress and anxiety influenced the total number (count) of post-task positive SPT reactions to allergens for which participants previously tested negative. The dependent variable was the total number of positive SPT reactions; the independent variables were visit (stress vs control) and baseline anxiety (noted as "anxiety"). This model, using Poisson distributions, accounted for the non-normally distributed count data. The percentage of positive post-task SPT reactions that were falsely positive or corrections of false-negative reactions was calculated to inform the interpretation of new positive SPT reactions. To examine whether stress and anxiety more generally affected wheal size to negative SPT reactions at baseline, a mixed model was fit that
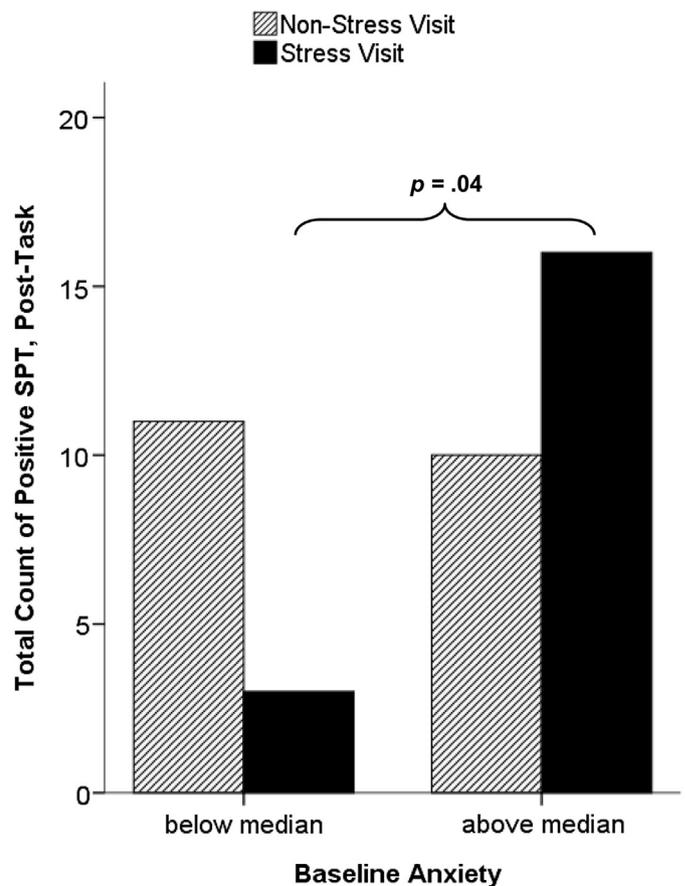

Figure 1. Total number of skin prick test (SPT) responses that tested negative at multiple baselines (screening and each clinical research center visit) but tested positive after the task-by-stress condition and anxiety category.

accounted for the correlation in measurements from the same participant across each time point. The dependent variable in the model was the mean wheal size across all allergens for which the participant tested negative at screening and pretask assessments. Independent variables in the mixed model included visit (stress vs control), time, baseline anxiety (noted as "anxiety"), and their interactions. Sex and the baseline wheal measurement served as covariates (see Kiecolt-Glaser et al[11]). Wheal size data were logarithmically transformed to achieve normality. Separate post hoc models probing significant interactions from the full mixed and generalized linear models were Bonferroni adjusted to decrease family-wise error. Effects of visit order (stress vs control) were not apparent for any outcomes (see Kiecolt-Glaser et al[11]); thus, order was not included in the final models.

Results

Descriptive Statistics

More than half the participants (n = 17, 60.7%) had at least 1 positive post-task SPT response (wheal ≥3 mm larger than saline control immediately for post-task or day 2 SPTs) at the stress or nonstress visit for an antigen that tested negative at baseline (ie, at screening and 2 pretask assessments). Across these 17 participants, there were 40 SPTs that tested positive post-task after previously testing negative at baseline (mean 2.38, SD 1.78, range 1–6). Eight of the 10 men (80%) and 9 of the 18 women (50%) had at least 1 positive SPT post-task response, proportions that were not significantly different (χ^2 = 2.43, P = .23). Mean Spielberger State-Trait Anxiety Scale[18] anxiety scores did not statistically differ between
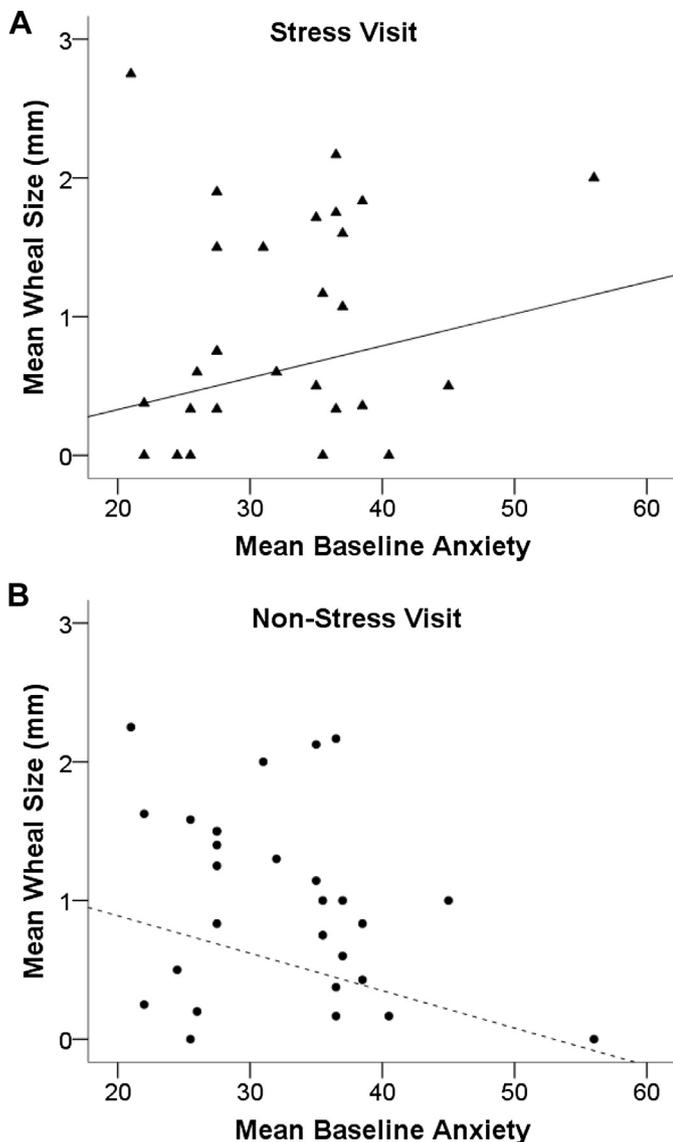
Figure 2. (A) Stress and (B) nonstress mean skin prick test wheal responses (allergen minus saline) for all allergens that tested negative at baseline assessments as a function of anxiety (*lines* represent associations between anxiety and wheal response derived from regression models).

participants who showed any positive post-task SPT responses to a previously identified negative SPT response (mean 33.59, SD 8.75) and those with no new post-task SPT conversions (mean 30.82, SD 6.09, $t_{26} = -0.91$, $P = .37$). Further, participants' mean anxiety scores (mean 32.5, SD 7.81) were not associated with the total number of previously negative SPT responses that tested positive after the stress or nonstress tasks ($r = 0.09$, $P = .66$).

Using self-reported allergies and positive SPT incident counts, it was determined that most incidents were conversions of false-negative responses at baseline: across participants, 15 of 20 allergens (75%) that were positive at post-task, but tested negative at baseline, were allergens to which participants reported being allergic (sagebrush was not included because participants were not asked to self-report an allergy to sagebrush).

Anxiety and Stress Influences on Positive SPT Responses to Allergens Testing Negative at Baseline

Results of the generalized linear model supported a combined role for anxiety and stress on the total count of positive post-task

SPT reactions (shown by a significant visit-by-anxiety interaction; $\chi^2_1 = 4.10$, $P = .043$). After exposure to the stressor, participants with higher baseline anxiety had a higher incidence of positive SPT reactions to allergens testing negative at baseline compared with participants with lower anxiety (adjusted $P = .032$). Anxiety was not associated with the number of positive post-task SPT reactions at any SPT assessment during the nonstress visit (adjusted $P = .512$). Figure 1 depicts these findings, using anxiety categories of high (above median anxiety score) and low (below median anxiety score) for illustration. The significant difference noted in Figure 1 between high and low anxious participants ($P = .04$) was derived from a Mann-Whitney U test comparing total new positive SPT reactions across post-task and day 2 between the stress and non-stress visits.

Interpretation of Poststressor Positive SPT Reactions using Clinical History

Then, the authors explored how to interpret the stress-related enhancement of positive SPT reactions in the more anxious participants with atopy using their self-reported clinical history of allergies. In these participants, 8 of 10 SPT results (80%) that were positive at post-stress task but tested negative at baseline were allergens to which participants reported being allergic, suggesting that the stress task primarily converted the false-negative SPT reactions[21] observed at screening and the 2 pretask assessments in the more anxious participants with atopy.

Anxiety, Stress Condition, and Wheal Responses to Allergens Testing Negative at Baseline

Then, the authors examined whether stress and anxiety influenced average wheal size across all allergens to which participants tested negative at baseline. From the mixed model, there was support for the combined effect of anxiety and stress exposure on SPT reactions (indicated by a significant anxiety-by-visit interaction; $F_{1,59} = 6.93$, $P = .011$). Specifically, after stressor exposure, higher anxiety was associated with larger mean wheals for the allergens testing negative at baseline, although this association was nonsignificant (adjusted $P = .08$; Fig 2A). At the nonstress visit, higher anxiety was associated with smaller post-task mean wheals, although this association was nonsignificant (adjusted $P = .10$; Fig 2B). The participant with the highest mean anxiety score had 1 outlying wheal response to an allergen for which he tested negative at baseline (sagebrush); when that response was excluded from the calculation of his average wheal responses, this participant had no apparent influence on model fit and so was included in the final models.

To determine whether the effects of stress and anxiety were specific to allergen SPT reactions, a separate mixed effect model tested stress and anxiety effects on histamine (positive control) and saline (negative control) SPT responses. There were no significant effects of stressor exposure, anxiety, or their interactions for either SPT control (saline, $P = .26$; histamine, $P = .60$).

Discussion

The role of stress in the disease activity of patients with various allergic illnesses has long been recognized. Stress also appears to have an immediate influence on allergic symptoms, possibly by affecting mast cell activity. As such, patients' stress levels at the time of an SPT could have important clinical implications in interpreting these mast cell–mediated SPT results. The present study addressed this issue by examining whether the sensitivity and/or specificity of SPT is affected by stress exposure and anxiety levels. Across the stress visit, higher anxiety was associated with higher total counts of positive SPT reactions after the stress task. Anxiety had no apparent influence on SPT thresholds at the nonstress visit,

suggesting that anxiety and stress in combination can lead to SPT reactions that are interpreted as positive test results in participants with atopy. The fundamental question raised by these findings is whether the newly positive SPT results represent increased sensitivity to stressful situations or represent a false-positive result that would be important for clinicians to anticipate when interpreting these results for clinical action (ie, avoidance/environmental control measures, allergen-specific immunotherapy prescriptions). The preliminary data in this pilot trial suggest that in more anxious individuals, SPT results are more likely to correspond with their reported clinical allergy history when these individuals have recently experienced significant psychological stress. These findings underscore implications for accuracy in the clinical identification of specific allergic sensitivity. Future studies are needed that more directly evaluate associations among stress and anxiety, SPT responses, and individuals' day-to-day clinical symptom levels.

Skin prick testing is characterized by 2 stages of skin responses to antigen—an immediate reaction occurring within the first 20 minutes after allergen exposure and a so-called late-phase reaction that manifests 3 to 24 hours later with no additional allergen exposure.[21] The immediate reaction to allergen is due to IgE-mediated activation of mast cells that release histamine, resulting in the characteristic wheal and flair skin reaction. In their initial examination,[22] the authors found that anxiety increased immediate wheal responses to SPT-positive allergens after stressor exposure.[11] In the present analysis, in more anxious participants with atopy, SPT wheal responses to previously identified "nonallergens" were enhanced after a laboratory stressor. Together, these findings suggest that more anxious individuals with atopy in stressful environments have alterations in immune function that enhance histamine release to allergens, including allergens that show low immunogenicity when tested under nonstressful conditions. Translated to clinical practice, information about anxiety and stress exposure may allow the clinician to help patients identify symptom "triggers" and consider stress management approaches in the therapeutic plans for these patients.

The cellular and molecular mechanisms linking anxiety and stress to enhanced allergen-induced histamine release remain to be identified. Increased sensitivity of mast cells to antigen-induced activation remains an intriguing possibility. For instance, mast cells can be activated in response to stress,[23] which may help explain, at least in part, observed relations between anxiety and psychological stress and enhanced atopic symptoms.[24] The authors' observations do not support increased nonspecific mast cell degranulation or increased skin responsiveness to histamine as primary mechanisms for these results. There were no significant differences caused by instrinsic histamine release by glycerinated saline (negative control) or skin responsiveness to a standard dose of histamine (positive control). Further, the number of new positive post-task SPT results did not correlate with anxiety scores, and none of the participants in this study had all previously negative allergens turn positive after the stress task, regardless of anxiety level. It is more likely that these participants had allergen-specific IgE production, but not in sufficient quantity to cause a positive baseline SPT reaction. With higher anxiety and the stress associated with the laboratory task, it is plausible that mast cell sensitivity increased enough to result in a positive SPT reaction. Indeed, this could be a contributing mechanism to the well-known clinical phenomenon of priming whereby lower allergen concentrations trigger mast cell activity after an initial response.[25]

The combination of environmental stressors and anxiety may be particularly important to understanding these mechanisms. In less anxious participants, there was a trend toward decreased post-task SPT positive responses at the stress compared with the nonstress visit (Fig 1), and under less stressful conditions, higher anxiety was associated with smaller post-task mean wheals from SPTs (Fig 2).

These findings may point to conditions under which physiologic arousal, especially catecholamine release, might be optimal for regulating allergic responses. For example, β_2-adrenergic receptor−mediated suppression of mast cell activity has been reported,[26] and AR symptoms can be alleviated after moderate intensity exercise, which increases catecholamine levels.[27] Under the most stressful conditions, however, exacerbated or dysregulated physiologic responses might inadequately regulate IgE production, upregulate mast cell sensitivity, and increase vulnerability in the most anxious individuals with atopy experiencing stressful events.

The present findings require replication with a larger sample. Further, this sample was predominately white, non-Hispanic, young, and college educated. Additional studies are needed to determine whether the present findings generalize to other demographic groups. Moreover, the analysis of self-reported allergy should be considered preliminary because all allergens used in the SPT battery were not assessed in the self-reported clinical history. Future studies should include a more extensive clinical history assessment that parallels the SPT to determine the extent to which stress and anxiety influence the sensitivity of the SPT. In addition, although the SPT is a widely used standard, studies evaluating the influence of stress and anxiety on other allergy testing approaches, such as intradermal testing or nasal challenge, would help strengthen the clinical relevance of the present findings.

Allergy skin testing is a major tool used by allergists to confirm the clinical diagnosis of atopy. The test has high sensitivity and specificity and results from SPTs are used to devise specific treatment strategies.[12] The present results suggest that, in anxious patients with atopy, whether specific allergens test positively or negatively in the clinic may be influenced by a patient's recent stress levels. Further, it does not appear that the change from negative to positive status for a given allergen was a function of repeated testing. Under nonstressful conditions, anxiety was unrelated to whether a negative allergen later tested positive. This implies that repeated skin prick testing within a short period can be performed without concerns about reliability and validity. More generally, these data suggest possible mechanisms linking high stress levels, in individuals who are more anxious, to increased symptoms in allergic conditions such as rhinitis, asthma, atopic dermatitis, and urticaria/angioedema.[28,29] In contrast, these data also suggest that inquiry into acute stress (ie, exercise or experiencing an acutely stressful event) exposure immediately before an SPT may be important for assessing the sensitivity of the SPT, particularly when these tests fail to confirm strong clinical suspicions of allergen sensitivity in a particular patient. Assessment of current anxiety and stress at the time of skin prick testing and clinical history may provide valuable information about the allergic status of the patient and aid in clinical decision making.

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