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The Association of *S. aureus* colonization with Food Allergy Occurs Independent of Eczema Severity

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Abbreviations

CI – Confidence interval

LEAP Study – Learning Early About Peanut Allergy Study

LEAP-On Study – 12 month extension of LEAP Study: Persistence of Oral Tolerance to Peanut

OR – Odds Ratio

SCORAD – SCORing Atopic Dermatitis

S. aureus - *Staphylococcus aureus*

SEB – staphylococcal enterotoxin B

SPT – Skin prick test
sIgE – specific Immunoglobulin E

Conflict of Interest disclosure statement:

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Abstract

Background: *S. aureus* has been implicated in the pathophysiology of eczema, allergic rhinitis, asthma, and food allergy. *S. aureus* is a marker of more severe eczema which is a risk factor for food sensitization/allergy. It may therefore be that the association between *S. aureus* and food allergy in eczematous patients is related to eczema severity.

Objective: To investigate the association of *S. aureus* colonization with specific IgE (sIgE) production to common food allergens and allergies in early childhood independent of eczema severity. We additionally determined the association of *S. aureus* colonization with eczema severity and persistence.

Methods: In LEAP participants, eczema severity was assessed and skin/nasal swabs cultured for *S. aureus*. Sensitization was identified by sIgE. Peanut allergy was primarily determined by oral food challenge and persistent egg allergy by skin prick test.

Results: Skin *S. aureus* colonization was significantly associated with eczema severity across LEAP while at 12 and 60 months of age it was related to subsequent eczema deterioration. Skin *S. aureus* colonization at any time-point was associated with increased levels of hen's egg white and peanut sIgE, independent of eczema severity. Participants with *S. aureus* were more likely to have persistent egg allergy and peanut allergy at 60 and 72 months of age, independent of eczema severity. All but one of the 9 LEAP consumers who developed peanut allergy (9/312) were colonized at least once with *S. aureus*.

Conclusion: *S. aureus,* independent of eczema severity, is associated with food sensitization and allergy and may impair tolerance to foods. This could be an important consideration in future interventions aimed at inducing and maintaining tolerance to food allergens in eczematous infants.

Clinical Implications:

There may be a role for *S. aureus* eradication in interventions aimed at inducing and maintaining tolerance to foods in eczematous infants.

Capsule Summary:

S. aureus colonization, independent of eczema severity, is associated with hen's egg and peanut sensitization and allergy. *S. aureus* colonization may impair tolerance to foods.

10 Keywords:

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INTRODUCTION

There are many studies that implicate Staphylococcus aureus (*S. aureus*) in the pathophysiology of eczema and other atopic outcomes. Epicutaneous sensitization with staphylococcal enterotoxin B (SEB) elicits local cutaneous inflammation consistent with eczema in mice (1) and subjects with normal and atopic skin (2). Prospective population-based birth cohorts report that skin (3) or nasal (4) colonization by *S. aureus* precedes the clinical diagnosis of eczema in infancy. Patients with eczema are more likely to be colonized with *S. aureus* than healthy controls and disease severity is associated with *S. aureus* colonization on the lesional skin (5). Additionally, patients with allergic rhinitis are more frequently colonized with nasal *S. aureus* (6, 7) or sensitized to *S. aureus* enterotoxins (8) than healthy controls, and those that are *S. aureus* positive have more severe allergic rhinitis than the *S. aureus* negative (6, 7). Furthermore, *S. aureus* enterotoxins trigger airway inflammation and increased airway responsiveness (9) and SEB facilitates allergic sensitization in murine asthma models (10). Clinically, nasal *S. aureus* or serum IgE to *S. aureus* toxins is associated with wheeze and/or asthma in children and adults (11-13). Finally, the presence of *S. aureus* or IgE to *S. aureus* toxins is related to asthma severity (12-14), poor asthma control (15) and higher prevalence of aeroallergen sensitization (14). Therefore, there are indications that *S. aureus* is associated with the development and/or severity of these atopic outcomes.

Interestingly, *S. aureus* colonization has also been associated with food sensitization and allergy. Jones et al retrospectively analysed skin culture results from eczematous children, aged 0-18 years, and report that those with skin *S. aureus* had peanut, egg, and milk specific IgE (sIgE) levels that correlated to a greater than 95% positive predictive value of oral food challenge reactions to the respective allergen (16). As eczema and eczema severity are risk factors for food sensitization and allergy (17, 18) and *S. aureus* is a marker of more severe eczema, it may be that the association between *S. aureus* and food allergy in patients with eczema is related to eczema severity.

In the Learning Early About Peanut Allergy (LEAP) Study, we sequentially recorded eczema severity and tested for *S. aureus* colonization at 4 different time points in 640 children (19). This design provides a unique opportunity for the detailed investigation of the relationship between *S. aureus* and food allergy. In an exploratory secondary analysis, we aimed to investigate the association of *S. aureus* colonization with sIgE production to common food allergens and food allergy in early childhood independent of eczema severity. In addition, we sought to determine the association of *S. aureus* colonization with eczema severity and persistence.

METHODS

Study population, design and procedures

This is a secondary analysis of LEAP and LEAP-On (20) outcomes that includes all participants recruited to these studies. Full study details have been previously published (19, 20). The LEAP Study enrolled infants aged ≥4 to <11 months with severe eczema and/or egg allergy. Participants were randomly assigned to avoid (LEAP avoiders) or consume peanut (LEAP consumers). Assessments were undertaken at baseline (age 4-11 months) visit and at age 12, 30 and 60 months. They included eczema clinical evaluation, acquisition and culture of skin and nasal swabs, food allergen SPT and sIgE as well as total IgE. The LEAP-On Study assessments were undertaken at 72 months of age, after 12 months of peanut avoidance in both groups. Concurrent and past medication use was recorded at all LEAP and LEAP-On study visits.

Clinical assessment of eczema severity

Eczema was clinically evaluated by a pediatric allergist at baseline, and at 12, 30, 60 and 72 months of age; eczema severity was determined according to the SCORAD (SCORing Atopic Dermatitis) index. Mild, moderate and severe eczema was defined as SCORAD values <15, >15-40, and >40 respectively. Persistent eczema was defined as eczema where the severity did not decrease over sequential time points.

Skin and nasal swabs and *S. aureus* assessment

Skin and nasal swabs were obtained at baseline, and at 12, 30, and 60 months of age. Samples were taken using sterile, cotton tipped transport swabs suitable for isolating aerobes and anaerobes. A skin swab was obtained from the most severe eczema lesion or - in the absence of eczema - the knee flexure. If the skin was dry, a drop of sterile water was placed on the skin prior to the swab being taken. The skin swab was then placed in medium. The nasal swab was inserted into one anterior nostril, and was then slowly withdrawn with a rotating motion and subsequently placed in medium (Amies Medium used for both samples). Swabs were incubated overnight and plated directly onto Columbia Blood Agar, CLED or MacConkey Agar (aerobic incubation) and Chocolate Agar (CO2). Sensitivity was reported using BSAC (British Society for Antimicrobial Chemotherapy) or via BioMerieux analyser Vitek2.

SPTs, sIgE and total IgE measurement

SPTs and allergen sIgEs were conducted at baseline, 12, 30, 60 and 72 months of age. Total IgE was measured at all visits except for 12 months. Test methodologies and SPT materials have been published previously (19-21).

Definitions of peanut allergy

Peanut allergy was determined by means of an oral peanut challenge at 60 and 72 months (20, 21). At 72 months, the allergic status of participants for whom the results of the oral peanut challenge were inconclusive or not available was determined as per the diagnostic algorithm published previously (21).

Definitions of egg allergy

At baseline, egg allergy was defined as an SPT ≥6 mm to raw hen's egg white and no history of previous egg tolerance, or an SPT ≥3 mm to pasteurized hen's egg white and allergic symptoms related to exposure to hen's egg. At 60 and 72 months of age we defined persistent egg allergy as SPT≥ 6mm to raw or pasteurized hen's egg in the participants diagnosed as egg allergic at baseline.

Statistical analysis

Statistical analyses were performed on all LEAP and LEAP-On Study participants for whom an outcome measurement was obtained. No imputation for missing data was conducted. Two separate repeated measures longitudinal models were used to assess if Skin or Nasal *S. aureus* (independent variable) was associated with concurrent eczema severity as assessed by SCORAD (dependent variable). Analogously another two separate repeated measures longitudinal models were used to assess if Skin or Nasal *S. aureus* at the immediately preceding visit was associated with eczema persistence. Average Peanut and Egg sIgE levels (dependent variables) were compared between those who ever had Skin *S. aureus* to those who never had Skin *S. aureus* (independent variable) via longitudinal repeated measures models (one for peanut and one for egg respectively) which also included a covariate for SCOARD. All repeated measures longitudinal models utilized an unstructured covariance structure to model the correlation among time points within each subject, treated time as categorical and also included covariates for time and the interaction between time and *S. aureus* colonization status. Bootstrap sampling of 1,000 replicates within each time point was utilized to assess where (or if) a divergence existed in the relative distribution of IgE production to Egg, Peanut and Milk sIgEs and Total IgE comparing those who ever had Skin *S. aureus* to those who never had Skin *S. aureus*. As peanut and egg allergy (independent variables) were only assessed at 60 and 72 months, four (peanut allergy at 60 and 72 months, egg allergy at 60 and 72 months) separate logistic regression models were constructed for each *S. aureus* colonization location (skin, nose, and combination of skin or nose – dependent variables). These logistic regression models included covariates for SCORAD (collected at 60 or 72 months respectively), LEAP treatment assignment, and the interaction between LEAP treatment assignment and *S. aureus* colonization status. As there were a small number of subjects with peanut allergy and complete separation occurred, the Firth penalized likelihood method was used only for the peanut allergy models. These were secondary analyses on study outcomes, and no adjustments have been made for multiple comparisons. All analyses were performed at the 0.05 level of significance using SAS software version 9.4 or JMP version 12. Datasets for the analyses are available through TrialShare, a public Web site managed by the Immune Tolerance Network (https://www.itntrialshare.org/LEAP_JACI_2019.url)

RESULTS

Participants

The characteristics of participants screened and enrolled in the LEAP and LEAP-On Studies have been previously published (19, 20).

Characteristics of *S. aureus* colonization in the LEAP Study with no differences noted in *S. aureus* colonization between intervention groups.

Approximately half (48.8%) of the participants had some form of *S. aureus* colonization (32.2% skin and 32.3% nasal) on at least one LEAP study visit (Table 1), and the majority of these participants tested positive only once (Online Repository Table E1). The highest rates of colonization were recorded at 4-11 months of age (18% for skin and 15% for nose); these decreased up to 30 months of age with a small increase observed at 60 months of age (Table 1). With the exception of the results at 60 months, the skin was more commonly the sole colonized location compared to the nose (Table 1). No significant differences in terms of frequency and persistence in all forms of *S. aureus* colonization were noted between the LEAP avoiders and consumers (Online Repository Table E1). There was a small but significant association between *S. aureus* colonization in the nose and on the skin, but concordance at any particular time was slight (Online Repository Table E2).

Very few of the total *S. aureus* positive swab samples were identified as methicillin resistant [skin 7/263 (2.7%); nose 2/257 (0.8%)].

We additionally performed an exploratory analysis to investigate the relationship between skin *S. aureus* colonization at baseline and oral or topical antibiotic/steroid medication use at baseline. We did not find a statistically significant difference (p=0.695) in terms of skin *S. aureus* colonization when comparing subjects that were reported at baseline to have received these medications versus those that did not (data not shown).

S. aureus colonization affected eczema severity and resolution

I). Eczema Severity

S. aureus colonization was significantly associated with concurrent eczema severity (measured by SCORAD mean (SD) and SCORAD severity classification) across all study time points. Participants with skin *S. aureus* had higher SCORAD values compared to those who did not have skin *S. aureus* (Table 2). The majority of the subjects that were skin *S. aureus* colonized had concurrent moderate and severe eczema at all time points (Online Repository Figure E1). Those with nasal *S. aureus* colonization also had higher SCORAD values compared to those who did not have nasal *S. aureus*; however the association was less strong than that observed between skin *S. aureus* and eczema severity (Table 2).

II). Eczema persistence and deterioration

As previously published, eczema severity decreased over time, and there was no significant difference in eczema severity between the two LEAP intervention groups (21). Although SCORAD generally decreased over time, this was not the case for participants who were skin colonized with *S. aureus* at certain visits (Figure 1). Indeed, considering the 12-30 and 60-72

month time intervals, eczema significantly worsened in participants with immediately preceding skin *S. aureus* colonization relative to those without.
Preceding nasal *S. aureus* colonization was not associated with eczema persistence or deterioration (Online Repository Figure E2).

***S. aureus* colonization was associated with food sIgE and total IgE production**

Hen's egg white and peanut sIgE production at each LEAP and LEAP-On study visit was significantly associated with skin *S. aureus* positivity at any time point in the interval from baseline to 60 months (Online Repository Figure E3 and Figure 2 respectively). Importantly, these associations were corrected for eczema severity at each time point.

Notably, high levels of hen's egg white and peanut sIgE production at each visit were also associated with skin *S. aureus* positivity at any time point in the interval from baseline to 60 months ($p<0.05$) (Figure 3). In Figure 3, the divergence in the distribution at each time point demonstrates that high level hen's egg white and peanut sIgE values were disproportionately represented in those participants who were skin colonized with *S. aureus* compared to those who were not. For peanut sIgE, this association was most apparent at 30 months but remained subsequently. In contrast, the association for hen's egg white sIgE became stronger over time with *S. aureus* positive participants comprising over half of the upper tail of the relative distribution of sIgE despite only representing a third of the overall sample. Furthermore, we investigated the relationship between skin *S. aureus* and high level sIgE production to cow's milk, and found a similar relationship with that observed for egg white and peanut. Indeed, at 30, 60, and 72 months, high levels of cow's milk sIgE were associated with skin *S. aureus* colonization at any time point in the interval from baseline to 60 months (Online Repository Figure E4). Finally, high levels of total IgE at all assessments, were associated with any skin *S. aureus* positivity (Online Repository Figure E4).

In order to assess if the observed associations between *S. aureus* colonization and high sIgE production to foods were food specific or confounded by total IgE, we examined the correlation between total IgE and each of the three food sIgEs (cow's milk, egg white, and peanut). The three pairwise correlations between each food and total IgE were moderate and consistent over the 4 study visits (Online Repository Figure E5). Using multivariate logistic regression models, egg white and peanut sIgE levels at 60 months were significantly associated with skin *S. aureus* positivity after adjusting for total IgE at 60 months (Online Repository Figure E6). This association was less strong for cow's milk sIgE. In contrast, after adjustment with each food sIgE, total IgE levels were no longer significantly associated with skin *S. aureus* positivity (Online Repository Figure E6).

***S. aureus* colonization was related to persistence and development of food allergy**

I). Persistence of egg allergy

Of the 408 subjects with protocol defined egg allergy at baseline, 42.7% and 38.1% had persistent egg allergy at 60 and 72 months respectively.

Overall, participants that had skin and/or nasal *S. aureus* colonization in the interval from baseline to 60 months were 1.57 (95% CI, 1.02-2.42; p=0.042) times as likely to have persistent egg allergy at 60 months of age as opposed to those that did not (Table 3). This association was slightly stronger for nasal (OR 1.61; 95% CI, 1.03-2.52; p=0.036) as opposed to skin (OR 1.39; 95% CI, 0.88-2.19; p=0.160) *S. aureus* colonization. Skin *S. aureus* colonization prior to 72 months of age was the only colonization pattern significantly associated with the likelihood (OR 1.77; 95% CI, 1.09-2.89; p=0.022) of egg allergy persisting until that age. There was a non-significant trend for preceding nasal (OR 1.54; 95% CI, 0.95-2.49; p=0.079) as well as skin and/or nasal (OR 1.59; 95% CI, 0.99-2.55; p=0.055) colonization and egg allergy persisting at 72 months. When comparing the LEAP intervention groups, no association was noted between persistent egg allergy and *S. aureus* colonization. All odds ratios were corrected for eczema severity at 60 or 72 months accordingly (Table 3).

II). Development of peanut allergy

Overall, participants that had skin and those that had nasal *S. aureus* colonization in the interval from baseline to 60 months were 2.94 (95% CI, 1.11, 7.76; p=0.029) and 2.41 (95% CI, 1.04, 5.59; p=0.04) times as likely to have a diagnosis of peanut allergy at 60 months respectively as opposed to those that were not colonized. In addition, any preceding form of *S. aureus* colonization was significantly associated with peanut allergy at 72 months of age. All odds ratios were corrected for eczema severity at 60 or 72 months accordingly (Table 4).

Within the peanut consumption group, subjects that were skin *S. aureus* colonized at any study point through LEAP were 7.13 (95% CI, 1.14, 44.47; p=0.035) and 3.87 (95% CI, 1.02, 14.65; p=0.047) times as likely to be diagnosed with peanut allergy at 60 and 72 months of age respectively compared with participants that were never skin *S. aureus* colonized (Table 4 and Figure 4). With regards to nasal or 'skin and/or nasal' colonization at both time points, this association was statistically significant only when it concerned nasal *S. aureus* and peanut allergy at 60 months of age (Table 4 and Online Repository Figures E7 & E8). These odds ratios are based on a small number of subjects who developed peanut allergy within the LEAP consumers group. Specifically, there were only 9 (6 by 60 months and an additional 3 by 72 months) LEAP consumers who did not have peanut allergy at baseline and were diagnosed with peanut allergy at 60 and/or 72 months. All but one of these 9 LEAP consumers (9/312) had *S. aureus* colonization at one or more time points (Online Repository Fig E9). The 6 LEAP consumers who were diagnosed with peanut allergy at both 60 and 72 months had all stopped consumption well before 60 months of age due to suspected allergic reactions following peanut consumption. In addition, there were 7 individuals in the consumption group who were allergic at baseline. Of these, 6 had some form of *S. aureus* colonization at some point during the study (data not shown). Within the avoidance group, there was no higher risk for peanut allergy at 60 or at 72 months in the subjects with any *S. aureus* colonization (Table 4).

The increased risk of peanut allergy at 60 or 72 months of age among the peanut avoiders compared to the consumers was less marked in those who had any *S. aureus* compared to those without *S. aureus* (Table 4, Panel B in Fig 4 and Online Repository Fig E7 & E8).

DISCUSSION

Previous findings that *S. aureus* colonization in eczema is associated with food sensitization and allergy (17, 18) may be confounded by eczema severity. In the LEAP and LEAP-On Studies we aimed to elucidate the relationship between *S. aureus* and food sensitization/allergy by correcting our analyses for eczema severity.

In the LEAP Study cohort, approximately half of the participants were found to be colonized by *S. aureus*. (Table 1 and Discussion in Online Repository). We demonstrate that skin colonization with *S. aureus* was related to eczema severity, persistence and deterioration. (Table 2, Fig 2 and Discussion in Online Repository).

In addition, we demonstrate that - even after correcting for eczema severity - hen's egg white and peanut sIgE values at each visit in LEAP and LEAP-On were significantly associated with skin *S. aureus* positivity at any LEAP study time point (Online Repository Fig E3 and Fig 2). This relationship was even stronger when we looked into high-level hen's egg white and peanut sIgE production (Fig 3). Similar findings are noted for cow's milk, where high level sIgE production to milk at 30, 60 and 72 months of age was related with any skin *S. aureus* colonization (Online Repository Figure E4). Together these data suggest that *S. aureus* is associated with hen's egg, peanut and cow's milk allergy.

Moreover, high levels of total IgE production were significantly associated with any skin *S. aureus* colonization (Online Repository Figure E4) which is consistent with literature reporting that *S. aureus* can promote a polyclonal IgE response [12]. In order to investigate whether sIgE to foods in subjects with *S. aureus* colonization is explained by total IgE production, we explored the relationship between total IgE levels and food sIgE levels to cow's milk, hen's egg white, and peanut and found a significant but moderate correlation (Online Repository Figure E5). Furthermore, we found that the association between egg white or peanut sIgE at 60 months and *S. aureus* colonization was not explained by total IgE (Online Repository Figure E6). However, the association between total IgE levels and skin *S. aureus* was not significant when we adjusted our analysis for each food sIgE (milk, egg white, peanut) (Online Repository Figure E6). Overall these results indicate that in our study population high polyclonal IgE production in the subjects with *S. aureus* colonization could only partly account for the association between skin *S. aureus* colonization and high levels of egg white and peanut sIgE.

Allergy to hen's egg typically resolves during early childhood (22). However, in LEAP and LEAP-On, 42.7% and 38.1% of the baseline egg allergic participants had persistent egg allergy at 60 and 72 months of age respectively. Our results demonstrate that any *S. aureus* positivity increased the odds of hen's egg allergy persisting at 60 (OR 1.57, p=0.042) or 72 (OR 1.59, p=0.055) months of age independent of eczema severity (Table 3) suggesting that *S. aureus* may prevent the acquisition of natural tolerance to hen's egg.

In the LEAP Study, peanut consumption was successful in preventing peanut allergy at 60 months of age. Interestingly, LEAP consumers with *S. aureus* skin colonization were 7.13 (p=0.035) and 3.87 (p=0.047) times more likely to develop peanut allergy primarily confirmed by

peanut challenge at 60 or 72 months of age respectively (Table 4, Fig 4). Whilst these associations are based on only 9 (6 by 60 months and an additional 3 by 72 months) LEAP consumers who did not have peanut allergy at baseline and were diagnosed with peanut allergy at 60 and/or 72 months, it is worth noting that all but one of these participants were colonized with *S. aureus* at one or more LEAP visits (Online Repository Fig E9). The 6 subjects that developed peanut allergy by 60 months of age had all stopped consuming peanut well before 60 months of age. It could therefore be argued that the reason for failing to acquire oral tolerance was inadequate consumption rather than the immunological effect of S. aureus. However, these 6 subjects stopped eating peanut during the course of the study because of symptoms during consumption that strongly suggested peanut allergy. This indicates that the reduced duration of peanut consumption was the consequence of an accelerated development of peanut allergy. More specifically, there are two possible explanations for the development of peanut allergy despite previous peanut consumption in these subjects: A) they developed an accelerated form of peanut allergy potentiated by S. aureus, and/or B) S. aureus may have inhibited tolerance mechanisms related to peanut consumption. The fact that *S. aureus* was associated with a higher risk of peanut allergy among peanut consumers but not avoiders (Table 4, Panel B in Fig 4 and Online Repository Fig E7 & E8) further suggests that peanut consumption was less effective in the prevention of peanut allergy among participants with *S. aureus* compared to those with no *S. aureus.*

S. aureus has been implicated in the development and severity of atopic diseases such as eczema, allergic rhinitis and asthma. With regards to food allergy, an epidemiological clinical study indicates an association between skin *S. aureus* and milk, egg or peanut allergy in children with eczema (16). There are murine studies that support a biological explanation between *S. aureus* and food allergy. Indeed, SEB co-applied on the skin with ovalbumin or peanut extract increases the systemic production of ovalbumin sIgE (23) and enhances peanut specific $CD4^+$ Th2 responses on subsequent exposure to peanut extract alone (24) respectively. Additionally, SEB administered orally with antigen (ovalbumin or peanut) results in highly Th2 polarized immune responses to the antigen, while subsequent oral challenge with the respective antigen triggers anaphylaxis (25). In all three studies, the antigen specific immune responses were not observed with SEB or the antigen alone suggesting that *S. aureus* might be acting as adjuvant. Our results show an association between skin *S. aureus* and high sIgE production to hen's egg white, peanut and cow's milk as well as to high total IgE levels. However, we demonstrated that the relationship between *S. aureus* and sIgE production to egg white and peanut was primarily explained by the corresponding food allergen sIgE and not total IgE levels. *S. aureus* has been associated with more severe forms of atopic diseases, and our data extend these observations in food allergy.

Study strengths include the longitudinal design of the LEAP Study with detailed clinical assessments and colonization results obtained at four scheduled study intervals. As our results are corrected for eczema severity, we are able to confirm that the association between *S. aureus* carriage and egg/peanut sIgE production or allergy occurred independent of eczema severity.

There are limitations to the colonization results reported as use was made of less sensitive bacteriological culture techniques and not DNA-based testing. Nevertheless, cultures allow for the detection of live microorganisms and not remnant, nonviable genetic material from prior infection. As we did not genotype the isolated strains, it is not possible to match organisms over time and between skin and nasal swabs. Swabs were collected on only 4 occasions in LEAP and were not collected in LEAP-On. Diagnostic food challenges were undertaken to peanut but not hen's egg. A major limitation is related to the interpretation of the association between *S. aureus* and peanut allergy in the consumers, which, although significant, is based on the very small numbers of LEAP consumers who became peanut allergic as it is reflected in the wide confidence intervals around the odds ratios. Larger numbers of participants who become peanut allergic - despite being fed peanut in infancy/early childhood - would be required to assess if these findings do indeed demonstrate that *S. aureus* colonization interferes with oral tolerance induction. Finally, even after adjusting for eczema severity, we cannot rule out that the observed association between colonization and food allergy could be due to other confounding factors.

S. aureus has been implicated in the development and severity of atopic diseases namely eczema, allergic rhinitis and asthma; our findings extend these observations to the development of food allergy, independent of eczema severity. The role of *S. aureus* as a potential environmental factor should be considered in future interventions aimed at inducing and maintaining tolerance to food allergens in eczematous infants. Further prospective longitudinal studies measuring *S. aureus* with more advanced techniques and interventional studies eradicating *S. aureus* in early infancy will help elucidate its role in the development of eczema or food allergy.

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LEAP & LEAP-On Study Team

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Display Legends

Table 1. Skin and Nasal *S. aureus* Colonization Prevalence Over Time in LEAP

	4-11 (mo)	12 (mo)	30 (mo)	60 (mo)	Ever Colonized 4-11(mo) – 60(mo)
Skin *S. aureus*					
N	640	626	618	630	640
S. aureus	115 (18.0%)	63 (10.1%)	40 (6.5%)	45 (7.1%)	206 (32.2%)
No *S. aureus*	525 (82.0%)	563 (89.9%)	578 (93.5%)	585 (92.9%)	434 (67.8%)
Nasal *S. aureus*					
N	640	626	618	630	640
S. aureus	96 (15.0%)	35 (5.6%)	32 (5.2%)	94 (14.9%)	207 (32.3%)
No *S. aureus*	544 (85.0%)	591 (94.4%)	586 (94.8%)	536 (85.1%)	433 (67.7%)
Skin and/or Nasal *S. aureus*					
N	640	626	618	630	640
S. aureus	166 (25.9%)	87 (13.9%)	66 (10.7%)	125 (19.8%)	312 (48.8%)
No *S. aureus*	474 (74.1%)	539 (86.1%)	552 (89.3%)	505 (80.2%)	328 (51.3%)
Skin and Nasal *S. aureus* Combination					
N	640	626	618	630	
Nasal Only	51 (8.0%)	24 (3.8%)	26 (4.2%)	80 (12.7%)	
Skin Only	70 (10.9%)	52 (8.3%)	34 (5.5%)	31 (4.9%)	
Skin and Nasal	45 (7.0%)	11 (1.8%)	6 (1.0%)	14 (2.2%)	
Neither	474 (74.1%)	539 (86.1%)	552 (89.3%)	505 (80.2%)	

The prevalence of skin, nasal, skin or nasal, and the combination of skin and nasal *S. aureus* colonization for all subjects enrolled in LEAP at baseline (4-11 months), 12 months, 30 months, and 60 months are shown. If a subject has at least one instance of *S. aureus* colonization at any of the 4 LEAP visits (4-11 mo to 60 mo) then that subject is summarized as '*S. aureus*' in the 'Ever Colonized' column. Analogously, if a subject

never has *S. aureus* at any of the 4 LEAP visits (4-11 mo to 60 mo) then that subject is summarized as 'No *S. aureus*' in the 'Ever Colonized' column. This definition of 'Ever Colonized' is utilized in subsequent analyses.

Table 2. Concurrent Skin and Nasal *S. aureus* Colonization and Eczema Severity

	Skin *S. aureus*											
	4-11 (mo)			12 (mo)			30 (mo)			60 (mo)		
	No *S. aureus*	*S. aureus*	p-value	No *S. aureus*	*S. aureus*	p-value	No *S. aureus*	*S. aureus*	p-value	No *S. aureus*	*S. aureus*	p-value
SCORAD			<.001			<.001			<.001			<.001
N	525	115		563	63		576	40		583	45	
Mean (SD)	32.6 (18.5)	42.3 (18.6)		20.5 (14.1)	31.6 (16.5)		15.1 (12.9)	33.1 (16.8)		5.9 (9.9)	22.1 (15.3)	
LS Means (SE)	33.1 (0.8)	40.1 (1.6)		21.0 (0.6)	27.5 (1.5)		15.4 (0.5)	28.4 (1.8)		6.3 (0.4)	17.1 (1.3)	
Diff LS Means (*S. aureus* - No *S. aureus*)			6.9 (3.6, 10.2)			6.5 (3.3, 9.6)			13.0 (9.4, 16.6)			10.8 (8.1, 13.5)
	Nasal *S. aureus*											
	4-11 (mo)			12 (mo)			30 (mo)			60 (mo)		
	No *S. aureus*	*S. aureus*	p-value	No *S. aureus*	*S. aureus*	p-value	No *S. aureus*	*S. aureus*	p-value	No *S. aureus*	*S. aureus*	p-value
SCORAD			0.009			0.015			0.024			0.005
N	544	96		591	35		584	32		534	94	
Mean (SD)	33.5 (18.9)	39.6 (17.8)		21.4 (14.7)	26.5 (14.1)		16.0 (13.6)	21.7 (17.5)		6.5 (10.3)	10.6 (14.6)	
LS Means (SE)	33.7 (0.8)	38.5 (1.7)		21.4 (0.6)	26.4 (2.0)		16.0 (0.6)	20.6 (2.0)		6.6 (0.5)	9.5 (1.0)	
Diff LS Means (*S. aureus* - No *S. aureus*)			4.8 (1.2, 8.3)			5.0 (1.0, 9.1)			4.6 (0.6, 8.7)			2.9 (0.9, 4.9)

Data is presented for Eczema severity defined by SCORAD for all participants who were in LEAP with available data for each time point divided into groups based on whether a subject had *S. aureus* at the concurrent visit or did not have *S. aureus* at the concurrent visit. P-values are from a longitudinal repeated measures model comparing the difference in least squares means in SCORAD between subjects without *S. aureus* colonization to those with *S. aureus* colonization.

Table 3. Persistent Egg Allergy in Relation to *S. aureus* Colonization and Treatment Assignment

***S. aureus* Colonization (Baseline to 60 Months)**	**LEAP N=363**			**LEAP-On N=318**		
	Odds Ratio	**95% CI**	**p-value**	**Odds Ratio**	**95% CI**	**p-value**
Overall (*S. aureus* vs No *S. aureus*)						
Skin *S. aureus*	1.39	{0.88, 2.19}	0.160	1.77	{1.09, 2.89}	0.022
Nasal *S. aureus*	1.61	{1.03, 2.52}	0.036	1.54	{0.95, 2.49}	0.079
Skin and/or Nasal *S. aureus*	1.57	{1.02, 2.42}	0.042	1.59	{0.99, 2.55}	0.055
Within Peanut Consumption Group (*S. aureus* vs No *S. aureus*)						
Skin *S. aureus*	1.37	{0.73, 2.58}	0.326	1.68	{0.85, 3.35}	0.139
Nasal *S. aureus*	1.42	{0.76, 2.67}	0.276	1.65	{0.83, 3.26}	0.154
Skin and/or Nasal *S. aureus*	1.65	{0.89, 3.03}	0.108	1.88	{0.96, 3.70}	0.066
Within Peanut Avoidance Group (*S. aureus* vs No *S. aureus*)						
Skin *S. aureus*	1.39	{0.74, 2.64}	0.300	1.86	{0.95, 3.67}	0.072
Nasal *S. aureus*	1.83	{0.98, 3.43}	0.059	1.44	{0.73, 2.86}	0.295
Skin and/or Nasal *S. aureus*	1.49	{0.81, 2.73}	0.196	1.34	{0.69, 2.58}	0.385
Within Those With *S. aureus* (Avoidance vs. Consumption)						
Skin *S. aureus*	0.88	{0.44, 1.77}	0.717	0.94	{0.44, 1.99}	0.869
Nasal *S. aureus*	1.02	{0.49, 2.09}	0.955	0.81	{0.37, 1.77}	0.600
Skin and/or Nasal *S. aureus*	0.85	{0.47, 1.52}	0.573	0.78	{0.41, 1.47}	0.440
Within Those Without *S. aureus* (Avoidance vs. Consumption)						
No Skin *S. aureus*	0.86	{0.51, 1.47}	0.583	0.85	{0.47, 1.54}	0.587
No Nasal *S. aureus*	0.79	{0.47, 1.34}	0.386	0.93	{0.52, 1.66}	0.799
No Skin and/or Nasal *S. aureus*	0.93	{0.50, 1.74}	0.829	1.09	{0.55, 2.18}	0.797

This table displays the odds ratios, 95% confidence intervals, and p-values from multiple multivariate logistic regression models. One set of models was fit for the 60 month data (outcome of interest being persistent egg allergy as assessed by raw and pasteurized egg skin prick test wheal cut-offs at 60 months), and another set of models was fit for the 72 month data (outcome of interest being persistent egg allergy as assessed by raw and pasteurized egg skin prick test wheal cut-offs at 72 months) with *S. aureus* colonization status (one model each for skin, nasal, and skin and/or nasal) adjusted for SCORAD (at 60 and 72 months respectively), LEAP treatment assignment, and the interaction between *S. aureus* status and treatment assignment. Those who do not have protocol-defined egg allergy at baseline are not included in this analysis.

Table 4. Peanut Allergy in Relation to *S. aureus* Colonization and Treatment Assignment

***S. aureus* Colonization (Baseline to 60 Months)**	**LEAP N=619**			**LEAP-On N=538**		
	Odds Ratio	**95% CI**	**p-value**	**Odds Ratio**	**95% CI**	**p-value**
Overall (*S. aureus* vs No *S. aureus*)						
Skin *S. aureus*	2.94	{1.11, 7.76}	0.029	2.19	{1.04, 4.61}	0.039
Nasal *S. aureus*	2.41	{1.04, 5.59}	0.040	2.18	{1.05, 4.56}	0.037
Skin and/or Nasal *S. aureus*	4.24	{0.97, 18.59}	0.055	2.78	{1.09, 7.07}	0.031
Within Peanut Consumption Group (*S. aureus* vs No *S. aureus*)						
Skin *S. aureus*	7.13	{1.14, 44.47}	0.035	3.87	{1.02, 14.65}	0.047
Nasal *S. aureus*	3.78	{0.79, 18.11}	0.096	3.88	{1.03, 14.61}	0.045
Skin and/or Nasal *S. aureus*	12.26	{0.68, 220.56}	0.089	5.57	{0.96, 32.26}	0.055
Within Peanut Avoidance Group (*S. aureus* vs No *S. aureus*)						
Skin *S. aureus*	1.21	{0.65, 2.25}	0.545	1.24	{0.65, 2.37}	0.508
Nasal *S. aureus*	1.54	{0.84, 2.82}	0.162	1.23	{0.65, 2.32}	0.519
Skin and/or Nasal *S. aureus*	1.47	{0.81, 2.67}	0.208	1.39	{0.75, 2.58}	0.293
Within Those With *S. aureus* (Avoidance vs. Consumption)						
Skin *S. aureus*	4.29	{1.60, 11.51}	0.004	3.27	{1.27, 8.43}	0.014
Nasal *S. aureus*	5.78	{2.01, 16.65}	0.001	3.23	{1.25, 8.34}	0.015
Skin and/or Nasal *S. aureus*	5.86	{2.43, 14.14}	<0.001	3.97	{1.77, 8.95}	0.001
Within Those Without *S. aureus* (Avoidance vs. Consumption)						
No Skin *S. aureus*	25.26	{4.86, 131.35}	<0.001	10.18	{3.31, 31.35}	<0.001
No Nasal *S. aureus*	14.19	{3.86, 52.21}	<0.001	10.19	{3.31, 31.33}	<0.001
No Skin and/or Nasal *S. aureus*	48.89	{2.93, 815.20}	0.007	15.90	{2.98, 84.66}	0.001

This table displays the odds ratios, 95% confidence intervals, and p-values from multiple multivariate logistic regression models using the Firth penalized likelihood method. One set of models was fit for the 60 month data (outcome of interest being peanut allergy as assessed by oral food challenge at 60 months), and another set of models was fit for the 72 month data (outcome of interest being peanut allergy as assessed by oral food challenge at 72 months). Predictors of interest included *S. aureus* colonization status (one model each for skin, nasal, and skin and/or nasal) adjusted for SCORAD (at 60 and 72 months respectively), LEAP treatment assignment, and the interaction between *S. aureus* status and treatment assignment. Infants randomly assigned to consumption underwent a baseline, open-label food challenge; the 7 subjects who reacted to that challenge are not included in this analysis. Interpret results with caution as a small number of subjects with peanut allergy (especially in the Peanut Consumption arm) contribute to these analyses.

Figure 1. Eczema Severity by Skin *S. aureus* Colonization at the Preceding Visit

Data is presented for all participants who were in LEAP and LEAP-On with available SCORAD data for each study assessment time point divided into groups based on whether subjects had skin *S. aureus* at the previous visit (in red) or did not have skin *S. aureus* at the previous visit (in blue). Black diamonds represent model predicted means, boxes represent 25th and 75th centiles, error bars represent 2.5th and 97.5th centiles, and the middle line of the box represents the median. The total number of subjects contributing to the analysis at each time point, p-values, mean differences and 95% confidence intervals around that difference directly above each assessment time point refer to the least squares mean difference (*S. aureus* – no *S. aureus*) and p-value comparison between those who had skin *S. aureus* at the previous visit and those who did not have skin *S. aureus* at the previous visit using a longitudinal repeated measures model adjusted for SCORAD at the previous visit, time, *S. aureus* status at the previous visit, and the interaction between *S. aureus* status at the previous visit and time.

Figure 2. Peanut sIgE Over Time by Skin *S. aureus* Colonization Status

Data is presented for all participants who were in LEAP and LEAP-On with available Peanut Specific IgE data for each study assessment time point divided into groups based on whether subjects ever had skin *S. aureus* from baseline to 60 months (in red) or never had skin *S. aureus* from baseline to 60 months (in blue). Black diamonds represent model predicted means, boxes represent 25th and 75th centiles, error bars represent 2.5th and 97.5th centiles, and the middle line of the box represented the median. The total number of subjects contributing to the analysis at each time point, p-values, mean differences and 95% confidence intervals around that mean difference directly above each assessment time point refer to the comparison between those who never have *S. aureus* and those who ever have *S. aureus* groups using a longitudinal repeated measures model adjusted for SCORAD, time, *S. aureus* status, and the interaction between *S. aureus* status and time. Average SCORAD values at each time point are annotated directly below the box plots for those who ever had skin *S. aureus* (red) and those who never had skin *S. aureus* (blue).

Figure 3. Relative Distribution of Hen's Egg White and Peanut sIgE Over Time by Skin *S. aureus* Colonization Status

These figures show the relative distribution of hen's egg white-specific IgE and peanut-specific IgE between those who ever have skin *S. aureus* (shown in red) from 4-11 months to 60 months and those who never have skin *S. aureus* (shown in blue). The vertical reference lines indicate where the distribution begins to significantly differ ($p < 0.05$) between the two groups using bootstrap sampling of 1000 replicates of the upper percentiles indicating that those with *S. aureus* colonization are over represented in the higher end of the distribution of sIgE (which is more indicative of allergy).

A reference panel is included to illustrate the 67.8% of the trial participants who never had skin *S. aureus* and the 32.2% who ever had skin *S. aureus* and what a pattern with no association of skin *S. aureus* with sIgE levels would look like.

Figure 4. Peanut Allergy in Relation to Skin *S. aureus* Colonization and Treatment Assignment
Percents (from raw data), odds ratios and 95% confidence intervals from multiple multivariate logistic regression models using the Firth penalized likelihood method are displayed. One model was fit for the 60 month data (outcome of interest being peanut allergy as assessed by oral food challenge at 60 months), and another model was fit for the 72 month data (outcome of interest being peanut allergy as assessed by oral food challenge or the relevant diagnostic algorithm at 72 months). Predictors of interest included skin *S. aureus* colonization status adjusted for SCORAD (at 60 and 72 months respectively), LEAP treatment assignment, and the interaction between skin *S. aureus* status and treatment assignment. Panel A for the plot summarize the relationship between peanut allergy and skin *S. aureus* colonization status (overall, within consumers, and within avoiders). In the 'Percent' panel, the numerators refer to the number of subjects with peanut allergy while the denominator refers to the number of subjects with skin *S. aureus* (in red) and those without skin *S. aureus* (blue). Panel B of the plot summarize the relationship between peanut allergy and peanut consumption (overall, within those with skin *S. aureus*, within those without skin *S. aureus*). In the 'Percent' panel, the numerators refer to the number of subjects with peanut allergy while the denominator refers to the number of subjects in the avoidance group (in grey) and those in the consumption group (green). Interpret results with caution as a small number of subjects with peanut allergy (especially in the Peanut Consumption arm) contribute to these analyses.