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

3
4
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36
37 **Abbreviations**

38 CI – Confidence interval

39 LEAP Study – Learning Early About Peanut Allergy Study

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

41 OR – Odds Ratio

42 SCORAD – SCORing Atopic Dermatitis

43 *S. aureus* - *Staphylococcus aureus*

44 SEB – staphylococcal enterotoxin B

S. aureus and food allergy in LEAP/LEAP-On

45 SPT – Skin prick test
46 sIgE – specific Immunoglobulin E

47

48

49 **Conflict of Interest disclosure statement:**

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56 National Institute of Allergy and Infectious Diseases (NIAID, NIH) during the conduct of the
57 study.

58

59 **Abstract**

60

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

65

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

70

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

74

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

82

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

87

88

89

90 **Clinical Implications:**
91 There may be a role for *S. aureus* eradication in interventions aimed at inducing and
92 maintaining tolerance to foods in eczematous infants.

93
94

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

98
99

100 **10 Keywords:**
101 Food Sensitization. Food Allergy. Peanut Allergy. Egg allergy. Eczema. Atopic Dermatitis. *S.*
102 *aureus*. Prevention. LEAP. Microbiome

103
104

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109 **INTRODUCTION**

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

127

128 Interestingly, *S. aureus* colonization has also been associated with food sensitization and
129 allergy. Jones et al retrospectively analysed skin culture results from eczematous children, aged
130 0-18 years, and report that those with skin *S. aureus* had peanut, egg, and milk specific IgE
131 (sIgE) levels that correlated to a greater than 95% positive predictive value of oral food
132 challenge reactions to the respective allergen (16). As eczema and eczema severity are risk
133 factors for food sensitization and allergy (17, 18) and *S. aureus* is a marker of more severe
134 eczema, it may be that the association between *S. aureus* and food allergy in patients with
135 eczema is related to eczema severity.

136

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

144

145

146 **METHODS**

147 **Study population, design and procedures**

148 This is a secondary analysis of LEAP and LEAP-On (20) outcomes that includes all participants
149 recruited to these studies. Full study details have been previously published (19, 20). The LEAP
150 Study enrolled infants aged ≥ 4 to < 11 months with severe eczema and/or egg allergy.
151 Participants were randomly assigned to avoid (LEAP avoiders) or consume peanut (LEAP
152 consumers). Assessments were undertaken at baseline (age 4-11 months) visit and at age 12,
153 30 and 60 months. They included eczema clinical evaluation, acquisition and culture of skin and
154 nasal swabs, food allergen SPT and sIgE as well as total IgE. The LEAP-On Study
155 assessments were undertaken at 72 months of age, after 12 months of peanut avoidance in
156 both groups. Concurrent and past medication use was recorded at all LEAP and LEAP-On study
157 visits.

158 **Clinical assessment of eczema severity**

159 Eczema was clinically evaluated by a pediatric allergist at baseline, and at 12, 30, 60 and 72
160 months of age; eczema severity was determined according to the SCORAD (SCORing Atopic
161 Dermatitis) index. Mild, moderate and severe eczema was defined as SCORAD values < 15 ,
162 $> 15-40$, and > 40 respectively. Persistent eczema was defined as eczema where the severity did
163 not decrease over sequential time points.
164

165 **Skin and nasal swabs and *S. aureus* assessment**

166 Skin and nasal swabs were obtained at baseline, and at 12, 30, and 60 months of age. Samples
167 were taken using sterile, cotton tipped transport swabs suitable for isolating aerobes and
168 anaerobes. A skin swab was obtained from the most severe eczema lesion or - in the absence
169 of eczema - the knee flexure. If the skin was dry, a drop of sterile water was placed on the skin
170 prior to the swab being taken. The skin swab was then placed in medium. The nasal swab was
171 inserted into one anterior nostril, and was then slowly withdrawn with a rotating motion and
172 subsequently placed in medium (Amies Medium used for both samples). Swabs were incubated
173 overnight and plated directly onto Columbia Blood Agar, CLED or MacConkey Agar (aerobic
174 incubation) and Chocolate Agar (CO₂). Sensitivity was reported using BSAC (British Society for
175 Antimicrobial Chemotherapy) or via BioMerieux analyser Vitek2.
176

177 **SPTs, sIgE and total IgE measurement**

178 SPTs and allergen sIgEs were conducted at baseline, 12, 30, 60 and 72 months of age. Total
179 IgE was measured at all visits except for 12 months. Test methodologies and SPT materials
180 have been published previously (19-21).
181

182 **Definitions of peanut allergy**

183 Peanut allergy was determined by means of an oral peanut challenge at 60 and 72 months (20,
184 21). At 72 months, the allergic status of participants for whom the results of the oral peanut
185 challenge were inconclusive or not available was determined as per the diagnostic algorithm
186 published previously (21).
187

188 **Definitions of egg allergy**

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

195

196 **Statistical analysis**

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

226

227

228

229 RESULTS

230

231 Participants

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

234

235 Characteristics of *S. aureus* colonization in the LEAP Study with no differences noted in 236 *S. aureus* colonization between intervention groups.

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

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

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

255

256 *S. aureus* colonization affected eczema severity and resolution

257 I). Eczema Severity

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

266

267 II). Eczema persistence and deterioration

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

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

276
277

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

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

283

284 Notably, high levels of hen's egg white and peanut sIgE production at each visit were also
285 associated with skin *S. aureus* positivity at any time point in the interval from baseline to 60
286 months ($p < 0.05$) (Figure 3). In Figure 3, the divergence in the distribution at each time point
287 demonstrates that high level hen's egg white and peanut sIgE values were disproportionately
288 represented in those participants who were skin colonized with *S. aureus* compared to those
289 who were not. For peanut sIgE, this association was most apparent at 30 months but remained
290 subsequently. In contrast, the association for hen's egg white sIgE became stronger over time
291 with *S. aureus* positive participants comprising over half of the upper tail of the relative
292 distribution of sIgE despite only representing a third of the overall sample. Furthermore, we
293 investigated the relationship between skin *S. aureus* and high level sIgE production to cow's
294 milk, and found a similar relationship with that observed for egg white and peanut. Indeed, at
295 30, 60, and 72 months, high levels of cow's milk sIgE were associated with skin *S. aureus*
296 colonization at any time point in the interval from baseline to 60 months (Online Repository
297 Figure E4). Finally, high levels of total IgE at all assessments, were associated with any skin *S.*
298 *aureus* positivity (Online Repository Figure E4).

299

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

310

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

312 I). Persistence of egg allergy

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

315 Overall, participants that had skin and/or nasal *S. aureus* colonization in the interval from
316 baseline to 60 months were 1.57 (95% CI, 1.02-2.42; p=0.042) times as likely to have persistent
317 egg allergy at 60 months of age as opposed to those that did not (Table 3). This association
318 was slightly stronger for nasal (OR 1.61; 95% CI, 1.03-2.52; p=0.036) as opposed to skin (OR
319 1.39; 95% CI, 0.88-2.19; p=0.160) *S. aureus* colonization. Skin *S. aureus* colonization prior to
320 72 months of age was the only colonization pattern significantly associated with the likelihood
321 (OR 1.77; 95% CI, 1.09-2.89; p=0.022) of egg allergy persisting until that age. There was a non-
322 significant trend for preceding nasal (OR 1.54; 95% CI, 0.95-2.49; p=0.079) as well as skin
323 and/or nasal (OR 1.59; 95% CI, 0.99-2.55; p=0.055) colonization and egg allergy persisting at
324 72 months. When comparing the LEAP intervention groups, no association was noted between
325 persistent egg allergy and *S. aureus* colonization. All odds ratios were corrected for eczema
326 severity at 60 or 72 months accordingly (Table 3).

327

328 II). Development of peanut allergy

329 Overall, participants that had skin and those that had nasal *S. aureus* colonization in the interval
330 from baseline to 60 months were 2.94 (95% CI, 1.11, 7.76; p=0.029) and 2.41 (95% CI, 1.04,
331 5.59; p=0.04) times as likely to have a diagnosis of peanut allergy at 60 months respectively as
332 opposed to those that were not colonized. In addition, any preceding form of *S. aureus*
333 colonization was significantly associated with peanut allergy at 72 months of age. All odds ratios
334 were corrected for eczema severity at 60 or 72 months accordingly (Table 4).

335

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

354

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

358

359 **DISCUSSION**

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

364
365 In the LEAP Study cohort, approximately half of the participants were found to be colonized by
366 *S. aureus*. (Table 1 and Discussion in Online Repository). We demonstrate that skin
367 colonization with *S. aureus* was related to eczema severity, persistence and deterioration.
368 (Table 2, Fig 2 and Discussion in Online Repository).

369
370 In addition, we demonstrate that - even after correcting for eczema severity - hen's egg white
371 and peanut sIgE values at each visit in LEAP and LEAP-On were significantly associated with
372 skin *S. aureus* positivity at any LEAP study time point (Online Repository Fig E3 and Fig 2). This
373 relationship was even stronger when we looked into high-level hen's egg white and peanut sIgE
374 production (Fig 3). Similar findings are noted for cow's milk, where high level sIgE production to
375 milk at 30, 60 and 72 months of age was related with any skin *S. aureus* colonization (Online
376 Repository Figure E4). Together these data suggest that *S. aureus* is associated with hen's egg,
377 peanut and cow's milk allergy.

378
379 Moreover, high levels of total IgE production were significantly associated with any skin *S.*
380 *aureus* colonization (Online Repository Figure E4) which is consistent with literature reporting
381 that *S. aureus* can promote a polyclonal IgE response [12]. In order to investigate whether sIgE
382 to foods in subjects with *S. aureus* colonization is explained by total IgE production, we explored
383 the relationship between total IgE levels and food sIgE levels to cow's milk, hen's egg white,
384 and peanut and found a significant but moderate correlation (Online Repository Figure E5).
385 Furthermore, we found that the association between egg white or peanut sIgE at 60 months and
386 *S. aureus* colonization was not explained by total IgE (Online Repository Figure E6). However,
387 the association between total IgE levels and skin *S. aureus* was not significant when we
388 adjusted our analysis for each food sIgE (milk, egg white, peanut) (Online Repository Figure
389 E6). Overall these results indicate that in our study population high polyclonal IgE production in
390 the subjects with *S. aureus* colonization could only partly account for the association between
391 skin *S. aureus* colonization and high levels of egg white and peanut sIgE.

392
393 Allergy to hen's egg typically resolves during early childhood (22). However, in LEAP and LEAP-
394 On, 42.7% and 38.1% of the baseline egg allergic participants had persistent egg allergy at 60
395 and 72 months of age respectively. Our results demonstrate that any *S. aureus* positivity
396 increased the odds of hen's egg allergy persisting at 60 (OR 1.57, p=0.042) or 72 (OR 1.59,
397 p=0.055) months of age independent of eczema severity (Table 3) suggesting that *S. aureus*
398 may prevent the acquisition of natural tolerance to hen's egg.

399
400 In the LEAP Study, peanut consumption was successful in preventing peanut allergy at 60
401 months of age. Interestingly, LEAP consumers with *S. aureus* skin colonization were 7.13
402 (p=0.035) and 3.87 (p=0.047) times more likely to develop peanut allergy primarily confirmed by

403 peanut challenge at 60 or 72 months of age respectively (Table 4, Fig 4). Whilst these
404 associations are based on only 9 (6 by 60 months and an additional 3 by 72 months) LEAP
405 consumers who did not have peanut allergy at baseline and were diagnosed with peanut allergy
406 at 60 and/or 72 months, it is worth noting that all but one of these participants were colonized
407 with *S. aureus* at one or more LEAP visits (Online Repository Fig E9). The 6 subjects that
408 developed peanut allergy by 60 months of age had all stopped consuming peanut well before 60
409 months of age. It could therefore be argued that the reason for failing to acquire oral tolerance
410 was inadequate consumption rather than the immunological effect of *S. aureus*. However, these
411 6 subjects stopped eating peanut during the course of the study because of symptoms during
412 consumption that strongly suggested peanut allergy. This indicates that the reduced duration of
413 peanut consumption was the consequence of an accelerated development of peanut allergy.
414 More specifically, there are two possible explanations for the development of peanut allergy
415 despite previous peanut consumption in these subjects: A) they developed an accelerated form
416 of peanut allergy potentiated by *S. aureus*, and/or B) *S. aureus* may have inhibited tolerance
417 mechanisms related to peanut consumption. The fact that *S. aureus* was associated with a
418 higher risk of peanut allergy among peanut consumers but not avoiders (Table 4, Panel B in Fig
419 4 and Online Repository Fig E7 & E8) further suggests that peanut consumption was less
420 effective in the prevention of peanut allergy among participants with *S. aureus* compared to
421 those with no *S. aureus*.

422
423 *S. aureus* has been implicated in the development and severity of atopic diseases such as
424 eczema, allergic rhinitis and asthma. With regards to food allergy, an epidemiological clinical
425 study indicates an association between skin *S. aureus* and milk, egg or peanut allergy in
426 children with eczema (16). There are murine studies that support a biological explanation
427 between *S. aureus* and food allergy. Indeed, SEB co-applied on the skin with ovalbumin or
428 peanut extract increases the systemic production of ovalbumin sIgE (23) and enhances peanut
429 specific CD4⁺ Th2 responses on subsequent exposure to peanut extract alone (24) respectively.
430 Additionally, SEB administered orally with antigen (ovalbumin or peanut) results in highly Th2
431 polarized immune responses to the antigen, while subsequent oral challenge with the respective
432 antigen triggers anaphylaxis (25). In all three studies, the antigen specific immune responses
433 were not observed with SEB or the antigen alone suggesting that *S. aureus* might be acting as
434 adjuvant. Our results show an association between skin *S. aureus* and high sIgE production to
435 hen's egg white, peanut and cow's milk as well as to high total IgE levels. However, we
436 demonstrated that the relationship between *S. aureus* and sIgE production to egg white and
437 peanut was primarily explained by the corresponding food allergen sIgE and not total IgE levels.
438 *S. aureus* has been associated with more severe forms of atopic diseases, and our data extend
439 these observations in food allergy.

440
441 Study strengths include the longitudinal design of the LEAP Study with detailed clinical
442 assessments and colonization results obtained at four scheduled study intervals. As our results
443 are corrected for eczema severity, we are able to confirm that the association between *S.*
444 *aureus* carriage and egg/peanut sIgE production or allergy occurred independent of eczema
445 severity.

446

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

461
462 *S. aureus* has been implicated in the development and severity of atopic diseases namely
463 eczema, allergic rhinitis and asthma; our findings extend these observations to the development
464 of food allergy, independent of eczema severity. The role of *S. aureus* as a potential
465 environmental factor should be considered in future interventions aimed at inducing and
466 maintaining tolerance to food allergens in eczematous infants. Further prospective longitudinal
467 studies measuring *S. aureus* with more advanced techniques and interventional studies
468 eradicating *S. aureus* in early infancy will help elucidate its role in the development of eczema or
469 food allergy.

470
471
472

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493
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506
507

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509

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- 586

587 **Display Legends**

588

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

590

	4-11 (mo)	12 (mo)	30 (mo)	60 (mo)	Ever Colonized 4-11(mo) – 60(mo)
Skin <i>S. aureus</i>					
N	640	626	618	630	640
<i>S. aureus</i>	115 (18.0%)	63 (10.1%)	40 (6.5%)	45 (7.1%)	206 (32.2%)
No <i>S. aureus</i>	525 (82.0%)	563 (89.9%)	578 (93.5%)	585 (92.9%)	434 (67.8%)
Nasal <i>S. aureus</i>					
N	640	626	618	630	640
<i>S. aureus</i>	96 (15.0%)	35 (5.6%)	32 (5.2%)	94 (14.9%)	207 (32.3%)
No <i>S. aureus</i>	544 (85.0%)	591 (94.4%)	586 (94.8%)	536 (85.1%)	433 (67.7%)
Skin and/or Nasal <i>S. aureus</i>					
N	640	626	618	630	640
<i>S. aureus</i>	166 (25.9%)	87 (13.9%)	66 (10.7%)	125 (19.8%)	312 (48.8%)
No <i>S. aureus</i>	474 (74.1%)	539 (86.1%)	552 (89.3%)	505 (80.2%)	328 (51.3%)
Skin and Nasal <i>S. aureus</i> 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%)	

591

592

593 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
594 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
595 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

S. aureus and food allergy in LEAP/LEAP-On

596 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'
597 column. This definition of 'Ever Colonized' is utilized in subsequent analyses.
598

599 **Table 2.** Concurrent Skin and Nasal *S. aureus* Colonization and Eczema Severity
600

	Skin <i>S. aureus</i>											
	4-11 (mo)			12 (mo)			30 (mo)			60 (mo)		
	No <i>S. aureus</i>	<i>S. aureus</i>	p-value	No <i>S. aureus</i>	<i>S. aureus</i>	p-value	No <i>S. aureus</i>	<i>S. aureus</i>	p-value	No <i>S. aureus</i>	<i>S. aureus</i>	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 (<i>S. aureus</i> - No <i>S. aureus</i>)			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 <i>S. aureus</i>											
	4-11 (mo)			12 (mo)			30 (mo)			60 (mo)		
	No <i>S. aureus</i>	<i>S. aureus</i>	p-value	No <i>S. aureus</i>	<i>S. aureus</i>	p-value	No <i>S. aureus</i>	<i>S. aureus</i>	p-value	No <i>S. aureus</i>	<i>S. aureus</i>	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 (<i>S. aureus</i> - No <i>S. aureus</i>)			4.8 (1.2, 8.3)			5.0 (1.0, 9.1)			4.6 (0.6, 8.7)			2.9 (0.9, 4.9)

601
602 Data is presented for Eczema severity defined by SCORAD for all participants who were in LEAP with available data for each time point divided
603 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
604 longitudinal repeated measures model comparing the difference in least squares means in SCORAD between subjects without *S. aureus*
605 colonization to those with *S. aureus* colonization.
606

607 **Table 3.** Persistent Egg Allergy in Relation to *S. aureus* Colonization and Treatment Assignment
 608

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

609
 610 This table displays the odds ratios, 95% confidence intervals, and p-values from multiple multivariate logistic regression models. One set of models was
 611 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
 612 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
 613 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
 614 SCORAD (at 60 and 72 months respectively), LEAP treatment assignment, and the interaction between *S. aureus* status and treatment assignment. Those
 615 who do not have protocol-defined egg allergy at baseline are not included in this analysis.

616
S. aureus and food allergy in LEAP/LEAP-On

617 **Table 4.** Peanut Allergy in Relation to *S. aureus* Colonization and Treatment Assignment
 618

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

619
 620 This table displays the odds ratios, 95% confidence intervals, and p-values from multiple multivariate logistic regression models using the Firth penalized
 621 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),
 622 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
 623 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),
 624 LEAP treatment assignment, and the interaction between *S. aureus* status and treatment assignment. Infants randomly assigned to consumption underwent a
 625 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
 626 of subjects with peanut allergy (especially in the Peanut Consumption arm) contribute to these analyses.

S. aureus and food allergy in LEAP/LEAP-On

627 **Figure 1.** Eczema Severity by Skin *S. aureus* Colonization at the Preceding Visit
628 Data is presented for all participants who were in LEAP and LEAP-On with available SCORAD
629 data for each study assessment time point divided into groups based on whether subjects had skin
630 *S. aureus* at the previous visit (in red) or did not have skin *S. aureus* at the previous visit (in
631 blue). Black diamonds represent model predicted means, boxes represent 25th and 75th centiles,
632 error bars represent 2.5th and 97.5th centiles, and the middle line of the box represents the
633 median. The total number of subjects contributing to the analysis at each time point, p-values,
634 mean differences and 95% confidence intervals around that difference directly above each
635 assessment time point refer to the least squares mean difference (*S. aureus* – no *S. aureus*) and
636 p-value comparison between those who had skin *S. aureus* at the previous visit and those who
637 did not have skin *S. aureus* at the previous visit using a longitudinal repeated measures model
638 adjusted for SCORAD at the previous visit, time, *S. aureus* status at the previous visit, and the
639 interaction between *S. aureus* status at the previous visit and time.

640
641 **Figure 2.** Peanut sIgE Over Time by Skin *S. aureus* Colonization Status
642 Data is presented for all participants who were in LEAP and LEAP-On with available Peanut
643 Specific IgE data for each study assessment time point divided into groups based on whether
644 subjects ever had skin *S. aureus* from baseline to 60 months (in red) or never had skin *S. aureus*
645 from baseline to 60 months (in blue). Black diamonds represent model predicted means, boxes
646 represent 25th and 75th centiles, error bars represent 2.5th and 97.5th centiles, and the middle line
647 of the box represented the median. The total number of subjects contributing to the analysis at
648 each time point, p-values, mean differences and 95% confidence intervals around that mean
649 difference directly above each assessment time point refer to the comparison between those who
650 never have *S. aureus* and those who ever have *S. aureus* groups using a longitudinal repeated
651 measures model adjusted for SCORAD, time, *S. aureus* status, and the interaction between *S.*
652 *aureus* status and time. Average SCORAD values at each time point are annotated directly
653 below the box plots for those who ever had skin *S. aureus* (red) and those who never had skin
654 *S. aureus* (blue).

655
656 **Figure 3.** Relative Distribution of Hen's Egg White and Peanut sIgE Over Time by Skin *S.*
657 *aureus* Colonization Status
658 These figures show the relative distribution of hen's egg white-specific IgE and peanut-specific
659 IgE between those who ever have skin *S. aureus* (shown in red) from 4-11 months to 60 months
660 and those who never have skin *S. aureus* (shown in blue). The vertical reference lines indicate
661 where the distribution begins to significantly differ ($p < 0.05$) between the two groups using
662 bootstrap sampling of 1000 replicates of the upper percentiles indicating that those with *S.*
663 *aureus* colonization are over represented in the higher end of the distribution of sIgE (which is
664 more indicative of allergy).

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

668

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