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

Olympia Tsilochristou, MD[1,2], George du Toit, MB, BCh, FRCPCH[1,2,3], Peter H. Sayre, MD, PhD[4], Graham Roberts, FRCPCH, DM[5], Kaitie Lawson, MS[6], Michelle L. Sever, MSPH, PhD[6], Henry T. Bahnson, MPH[7], Suzana Radulovic , MD[1,2,3], Monica Basting, MA[1,2,3], Marshall Plaut, MD[8] and Gideon Lack , MB, BCh, FRCPCH[1],[2,3] for the Immune Tolerance Network Learning Early About Peanut Allergy study team

Affiliations:

[1] Peter Gorer Department of Immunobiology, School of Immunology & Microbial Sciences, King's College London, London, United Kingdom.

[2] Pediatric Allergy Group, Department of Women and Children's Health, School of Life Course Sciences, King's College London, London, United Kingdom.

[3] Children's Allergy Service, Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom.

[4] Division of Hematology-Oncology, Department of Medicine, University of California, San Francisco, Calif.

[5] University of Southampton and Southampton NIHR Biomedical Research Centre, Southampton, and the David Hide Centre, Isle of Wight, United Kingdom.

[6] Rho Federal Systems Division, Chapel Hill, NC.

[7] Immune Tolerance Network, San Francisco, Calif.

[8] National Institute of Allergy and Infectious Diseases, Bethesda, Md.

Corresponding author: Gideon Lack, School of Life Course Sciences and School of Immunology & Microbial Sciences at King's College London, and Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom. Electronic address: gideon.lack@kcl.ac.uk

Address reprint requests to Prof Gideon Lack:

Gideon Lack, Children's Allergy Service, 2nd Floor, Stairwell B, South Wing, Guy's and St Thomas' NHS Foundation Trust, Westminster Bridge Road, London SE1 7EH, United Kingdom. Email: gideon.lack@kcl.ac.uk

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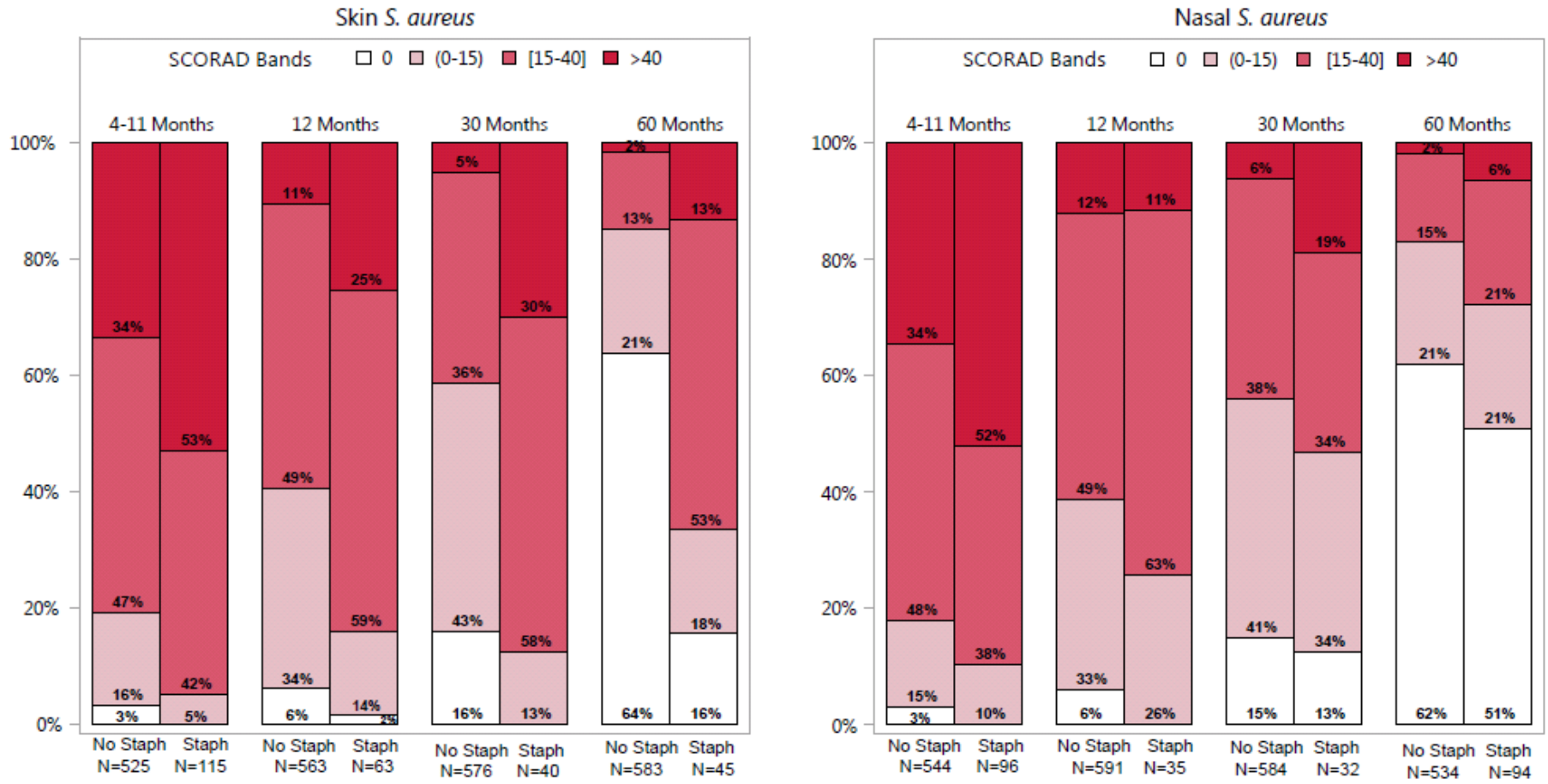
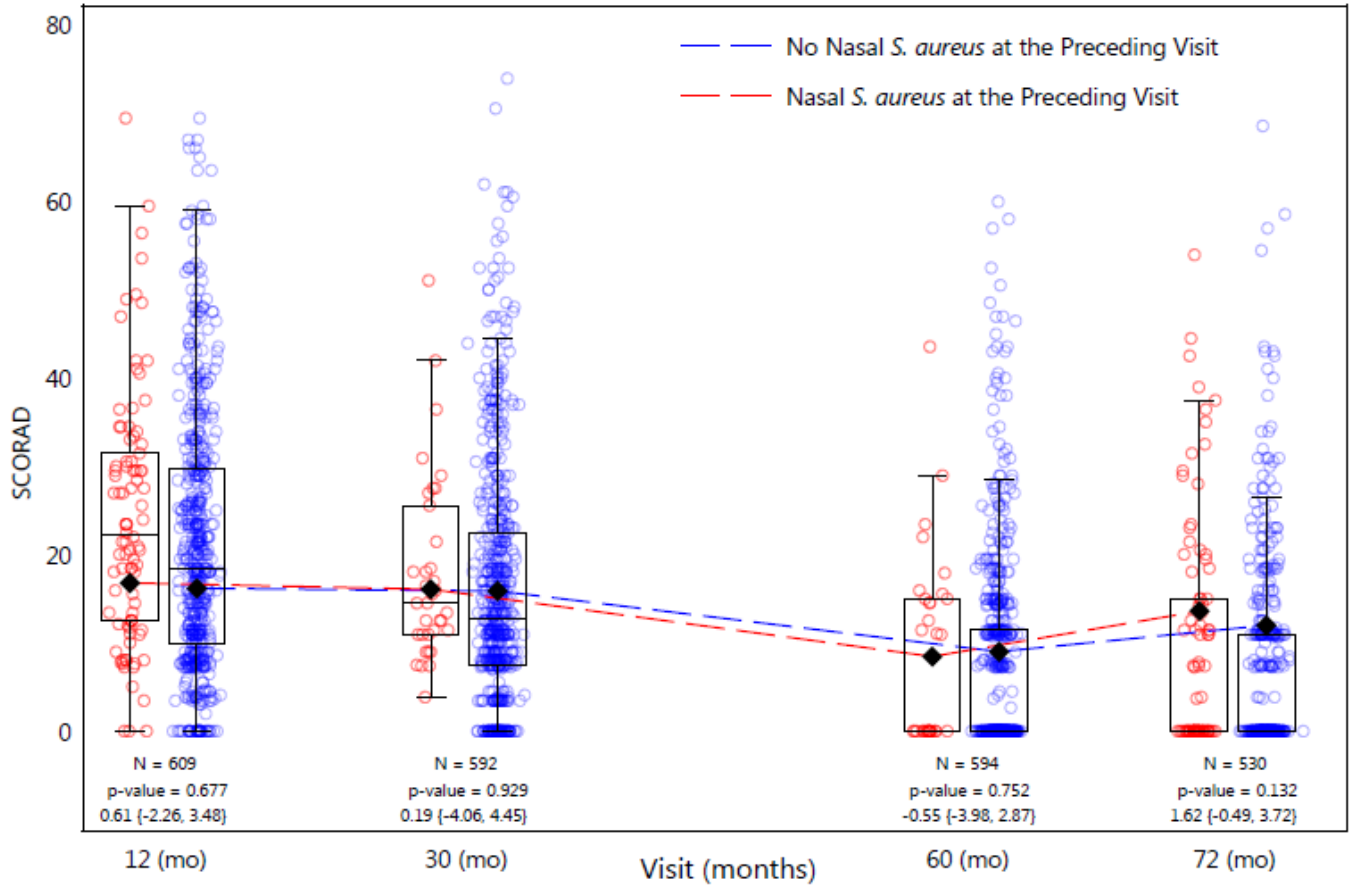
1. SUPPLEMENTARY FIGURES

Figure E1. Concurrent *S. aureus* Colonization and Eczema Severity



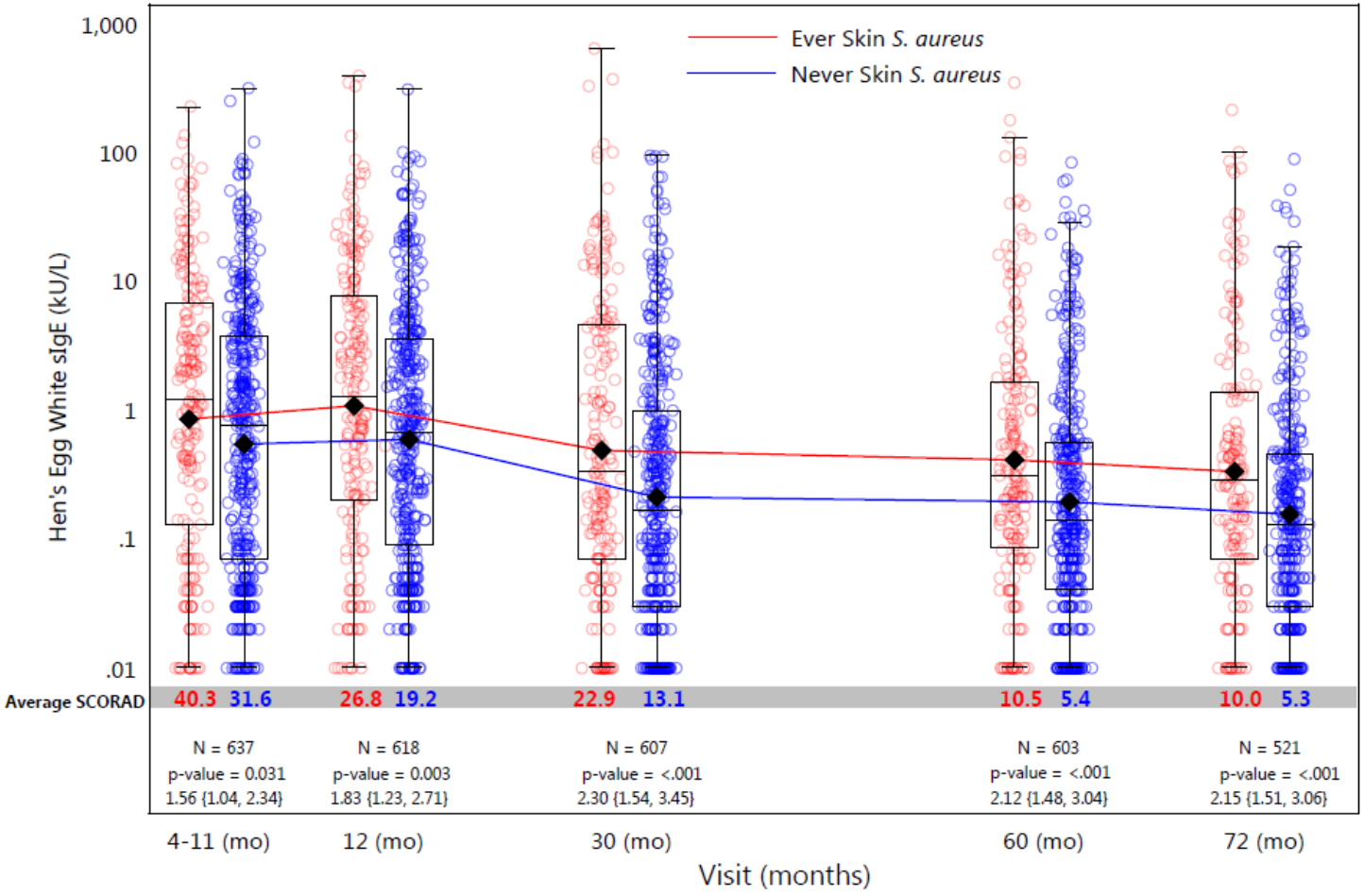
The percent of individuals with SCORAD assessments for eczema of 0, >0-15, ≥15-40 and >40 are shown at 4-11, 12, 30, and 60 months within those who do not have concurrent *S. aureus* (left bar of each pair) and those who do have concurrent *S. aureus* (right bar of each pair) in the intention to treat population. Skin *S. aureus* colonization is shown in the left panel while Nasal *S. aureus* colonization is shown in the right panel.

Figure E2. Eczema Severity by Nasal *S. aureus* Colonization at the Preceding Visit



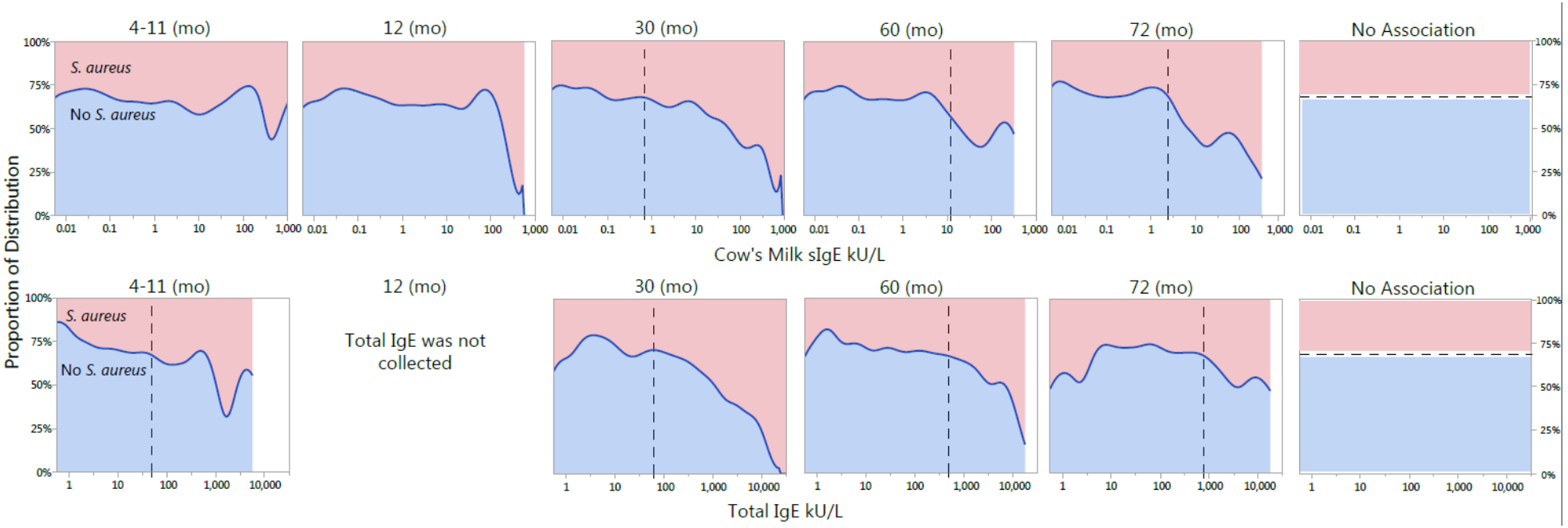
Data is presented for all participants who were in LEAP and LEAP-On with available SCORAD data for each study assessment time point divided into groups based on whether the subjects had nasal *S. aureus* at the previous visit (in red) or did not have nasal *S. aureus* at the previous visit (in blue). 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 each 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 mean 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 nasal *S. aureus* at the previous visit and those who did not have nasal *S. aureus* at the previous visit using a longitudinal 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 E3. Hen's Egg White sIgE Over Time by Skin *S. aureus* Colonization Status



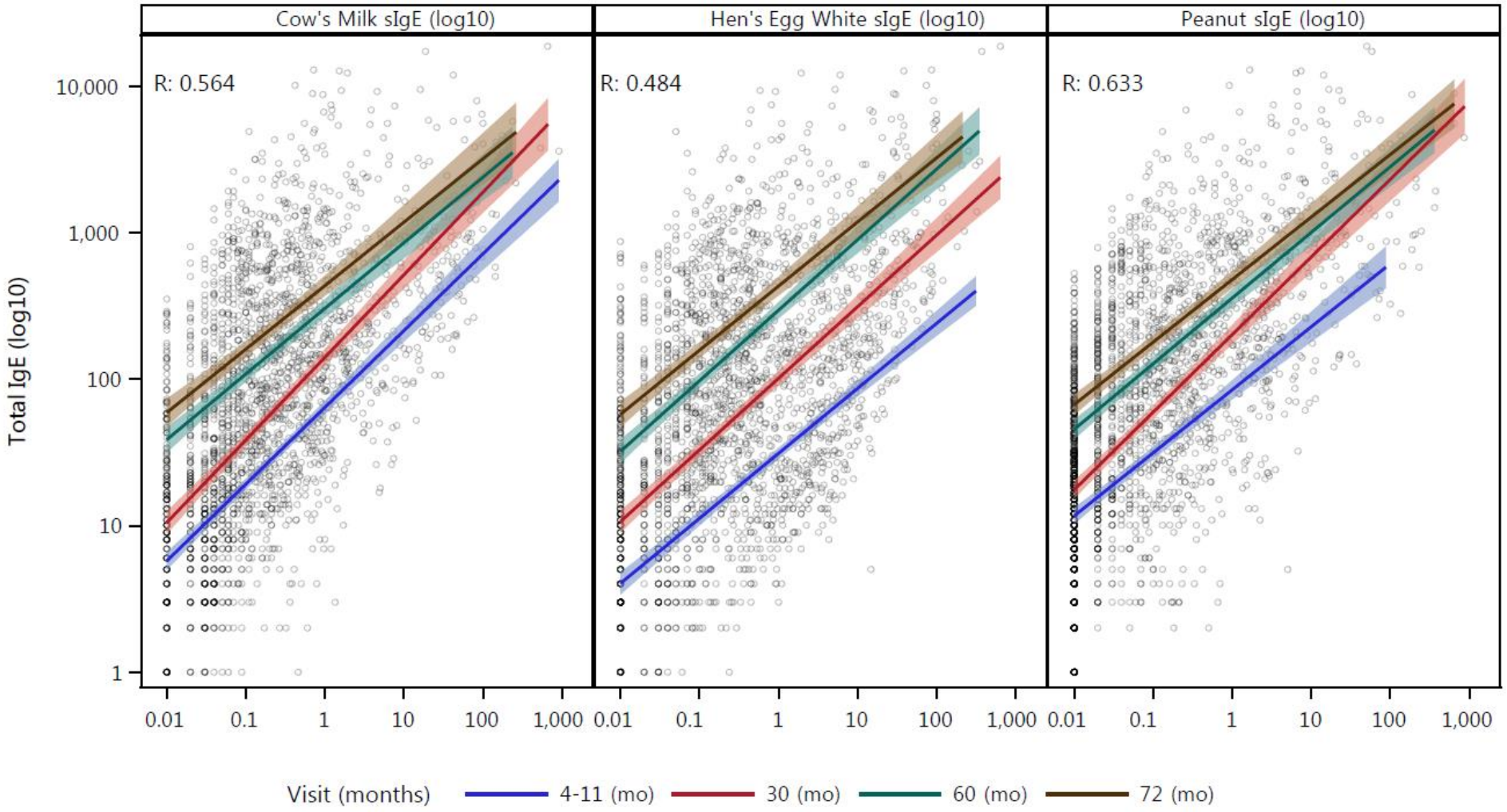
Data is presented for all participants who were in LEAP and LEAP-On with available Hen's Egg White Specific IgE data for each study assessment time point divided into groups based on whether subjects ever had skin *S. aureus* from baseline (4-11 mo) to 60 months (in red) or never had skin *S. aureus* from baseline (4-11 mo) 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 each 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 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* 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 E4. Relative Distribution of Cow's Milk sIgE and Total IgE Over Time by Skin *S. aureus* Colonization



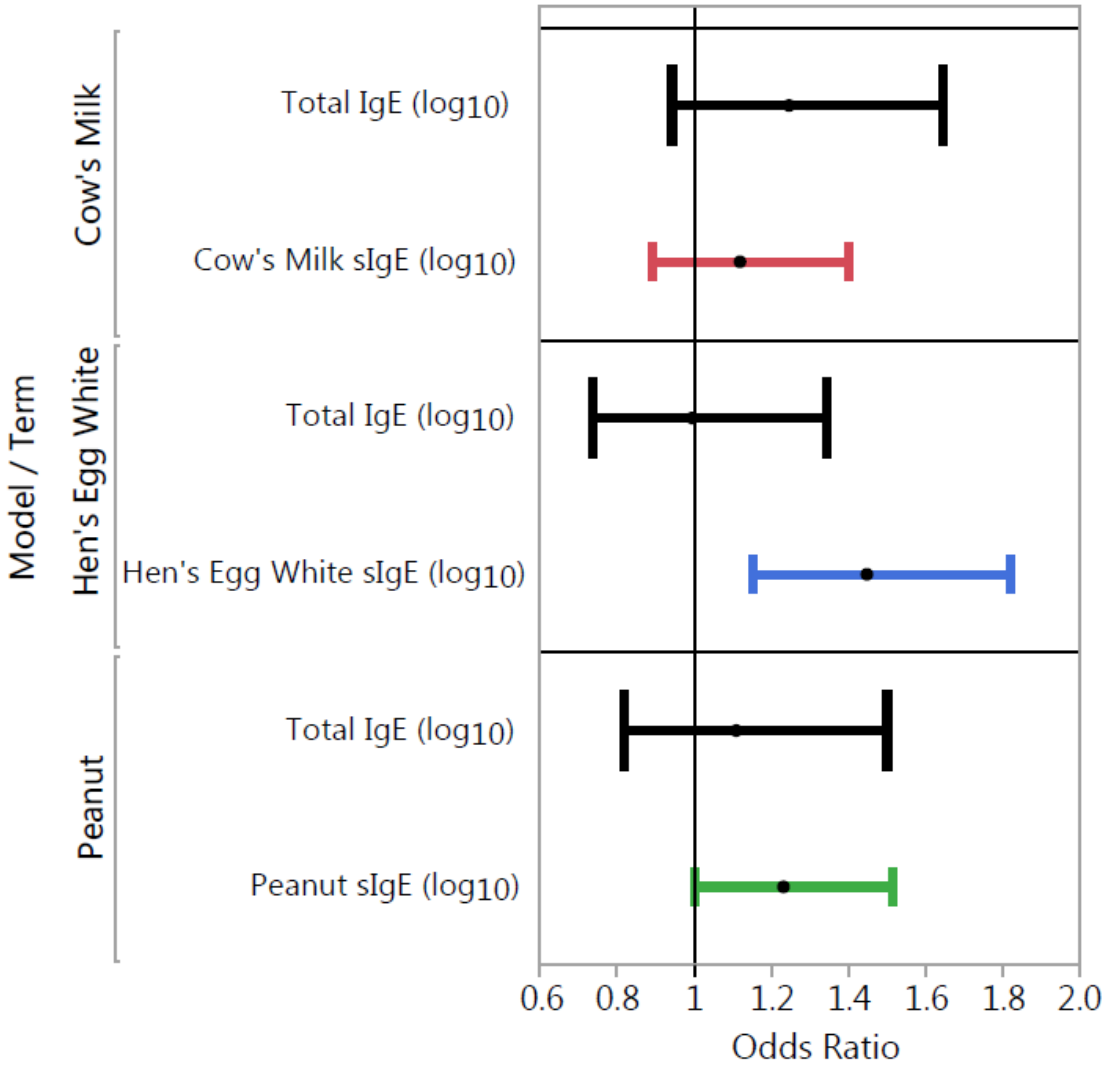
These figures show the relative distribution of milk-specific IgE and Total IgE between those who ever have skin *S. aureus* (shown in red) from 4-11 months to 60 months and those who never have skin *S. aureus* (shown in blue). The vertical reference lines indicate where the distribution begins to significantly 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 IgE. 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 IgE levels would look like.

Figure E5. Correlation Between Total IgE and Food Specific IgEs



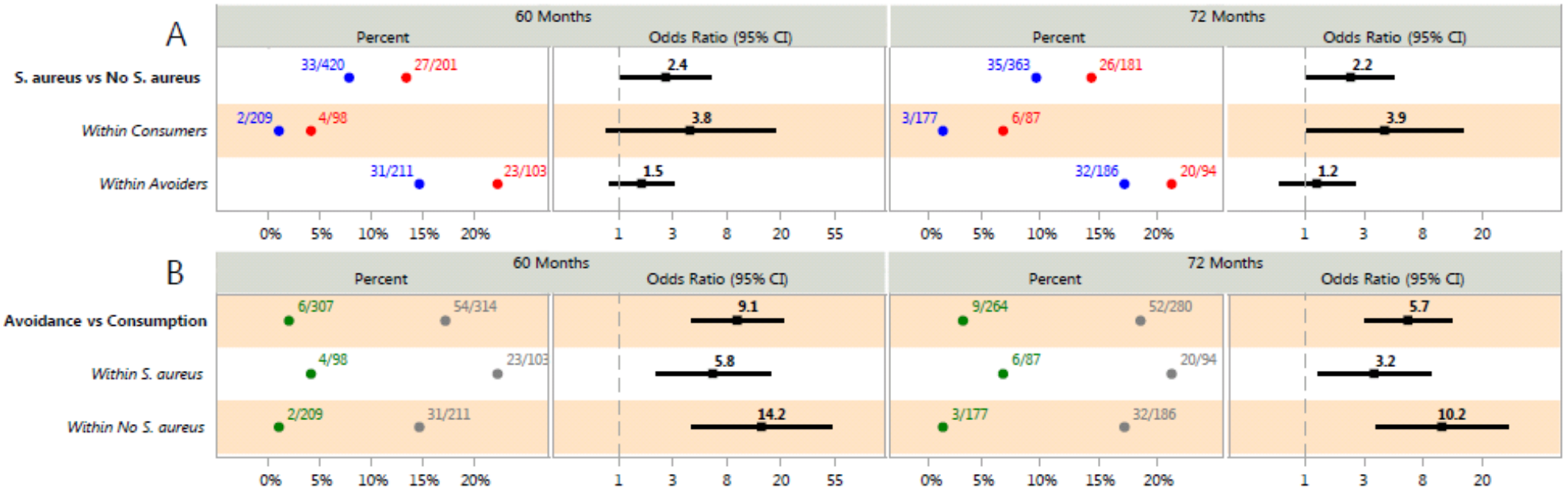
Data is presented for all participants who were in LEAP and LEAP-On with available Milk sIgE, Egg sIgE, Peanut sIgE, and Total IgE. Data at all available time points are included in each panel. Linear regression lines and 95% confidence intervals between Total IgE and food specific IgEs are displayed for each assessment time 4-11 (in blue), 30 (in red), 60 (in green), and 72 months (in brown). Total IgE was not collected at 12 months; therefore the 12 months data is not reported here. Pearson correlations between Total IgE and food specific IgEs averaged over all time points are displayed in each panel.

Figure E6. Forest Plot of Skin *S. aureus* Colonization with Food Specific IgE and Total IgE



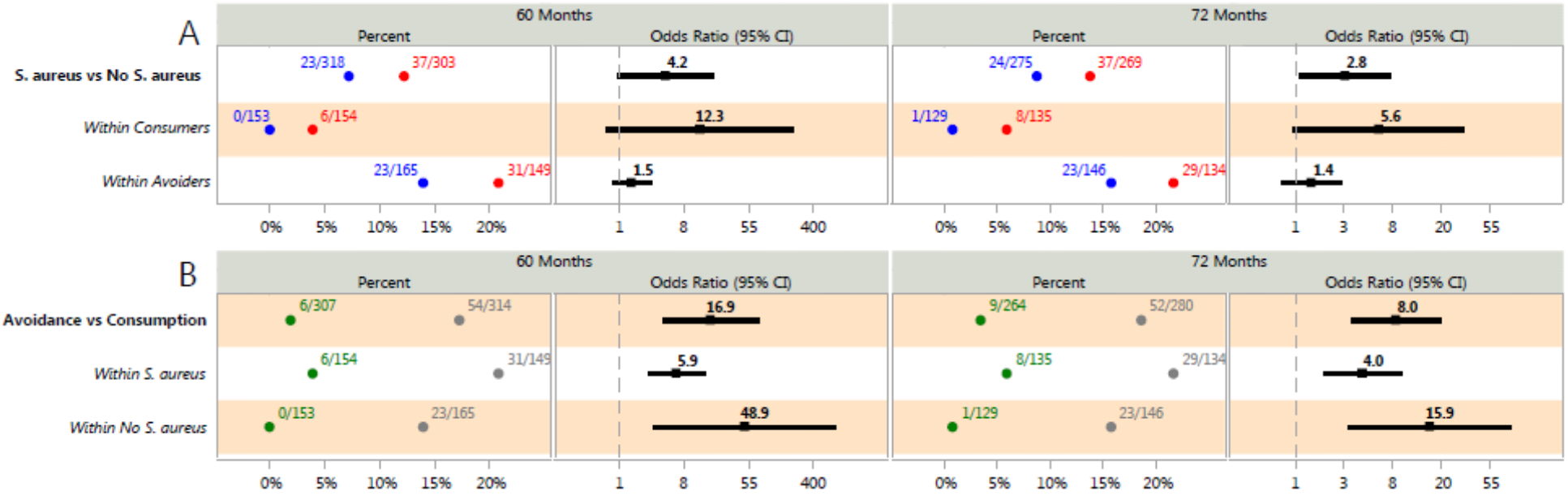
This forest plot shows odds ratios and 95% confidence intervals from 3 multivariate logistic regression models. The outcome of each model is skin *S. aureus* colonization at any time point and the predictors are Total IgE and each food specific IgE at 60 months individually in each model. The odds ratios for a 1 log unit change in each predictor are plotted showing a positive association with food specific IgE and skin *S. aureus.*

Figure E7. Peanut Allergy in Relation to Nasal *S. aureus* Colonization and Treatment Assignment



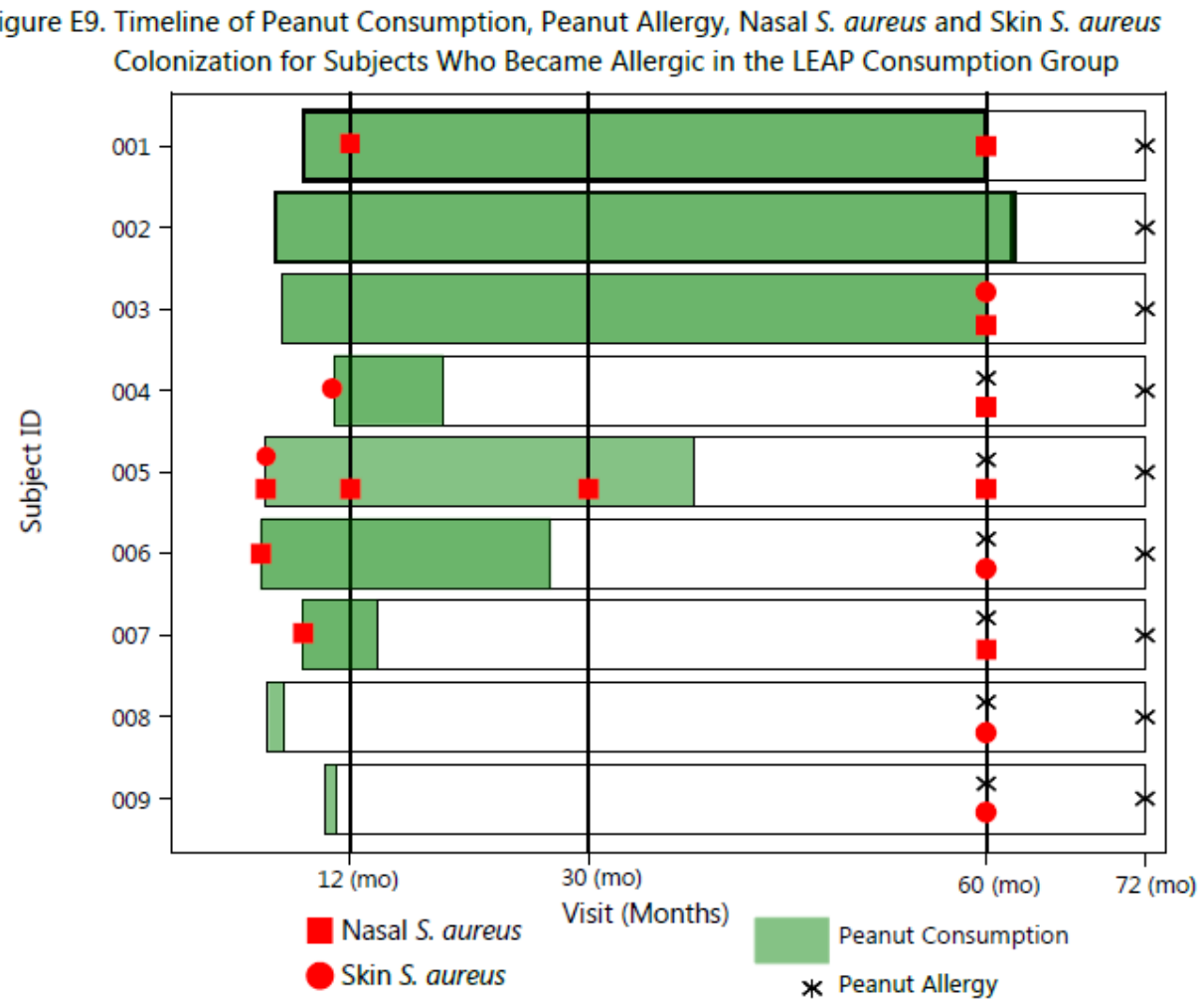
Percentages (from raw data), odds ratios, and 95% confidence intervals from multiple multivariate logistic regression models using the Firth penalized likelihood method are displayed. One model was fit for the 60 month data (outcome of interest being peanut allergy as assessed by oral food challenge 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 at 72 months). Predictors of interest included nasal *S. aureus* colonization status adjusted for SCORAD (at 60 and 72 months respectively), LEAP treatment assignment, and the interaction between nasal *S. aureus* status and treatment assignment. Panel A of the plot summarizes the relationship between peanut allergy and nasal *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 denominators refer to the number of subjects with nasal *S. aureus* (in red) and those without nasal *S. aureus* (blue). Panel B of the plot summarizes the relationship between peanut allergy and peanut consumption (overall, within those with nasal *S. aureus*, within those without nasal *S. aureus*). In the 'Percent' panel, the numerators refer to the number of subjects with peanut allergy while the denominators refer 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.

Figure E8. Peanut Allergy in Relation to Skin and/or Nasal *S. aureus* Colonization and Treatment Assignment



Percentages (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 at 72 months). Predictors of interest included skin and/or nasal *S. aureus* colonization status adjusted for SCORAD (at 60 and 72 months respectively), LEAP treatment assignment, and the interaction between skin and/or nasal *S. aureus* status and treatment assignment. Panel A of the plot summarizes the relationship between peanut allergy and skin and/or nasal *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 denominators refer to the number of subjects with skin and/or nasal *S. aureus* (in red) and those without skin and/or nasal *S. aureus* (blue). Panel B of the plot summarizes the relationship between peanut allergy and peanut consumption (overall, within those with skin and/or nasal *S. aureus*, within those without skin and/or nasal *S. aureus*). In the 'Percent' panel, the numerators refer to the number of subjects with peanut allergy while the denominators refer 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.

Figure E9. Timeline of Peanut Consumption, Peanut Allergy, Nasal *S. aureus* and Skin *S. aureus* Colonization for Subjects Who Became Allergic in the LEAP Consumption Group



The 9 (6 by 60 months and an additional 3 by 72 months) LEAP Consumers who became allergic are displayed. This timeline illustrates the length of time each subject consumed peanut (in green), when they tested positive for nasal *S. aureus* (red box), when they tested positive for skin *S. aureus* (red circle), and diagnosis of peanut allergy at the end of LEAP and/or LEAP-On (black asterisk). Subjects 001 to 003 had a negative peanut challenge at 60 months but a positive one at 72 months. Subjects 004 to 009 stopped peanut consumption due to allergic reactions following peanut consumption as opposed to subjects 001 to 003 who consumed peanut until 60 months as per the LEAP protocol. All the subjects in this plot, aside from Subject 002, had skin and/or nasal *S. aureus* at some point during LEAP (4-11 to 60 months of age).

2. SUPPLEMENTARY TABLES

Table E1. Prevalence and Persistence of Skin and Nasal *S. aureus* Colonization by LEAP Treatment Groups

	***S. aureus* Skin Swab Results**			***S. aureus* Nasal Swab Results**			***S. aureus* Skin or Nasal Swab Results**		
	Peanut Avoidance	**Peanut Consumption**	**p-value**	**Peanut Avoidance**	**Peanut Consumption**	**p-value**	**Peanut Avoidance**	**Peanut Consumption**	**p-value**
4-11 (mo)			0.690			0.204			0.530
N	321	319		321	319		321	319	
No *S. aureus*	260 (81.0%)	265 (83.1%)		278 (86.6%)	266 (83.4%)		240 (74.8%)	234 (73.4%)	
S. aureus	61 (19.0%)	54 (16.9%)		43 (13.4%)	53 (16.6%)		81 (25.2%)	85 (26.6%)	
12 (mo)			0.507			0.357			0.856
N	315	311		315	311		315	311	
No *S. aureus*	281 (89.2%)	282 (90.7%)		300 (95.2%)	291 (93.6%)		272 (86.3%)	267 (85.9%)	
S. aureus	34 (10.8%)	29 (9.3%)		15 (4.8%)	20 (6.4%)		43 (13.7%)	44 (14.1%)	
30 (mo)			0.803			0.249			0.640
N	310	308		310	308		310	308	
No *S. aureus*	290 (93.5%)	288 (93.5%)		291 (93.9%)	295 (95.8%)		276 (89.0%)	276 (89.6%)	
S. aureus	20 (6.5%)	20 (6.5%)		19 (6.1%)	13 (4.2%)		34 (11.0%)	32 (10.4%)	
60 (mo)			0.827			0.526			0.676
N	316	314		316	314		316	314	
No *S. aureus*	292 (92.4%)	293 (93.3%)		271 (85.8%)	265 (84.4%)		254 (80.4%)	251 (79.9%)	
S. aureus	24 (7.6%)	21 (6.7%)		45 (14.2%)	49 (15.6%)		62 (19.6%)	63 (20.1%)	
Ever Colonized (4-11 mo to 60 mo)			0.650			0.976			0.478
N	321	319		321	319		321	319	
No *S. aureus*	215 (67.0%)	219 (68.7%)		217 (67.6%)	216 (67.7%)		169 (52.6%)	159 (49.8%)	
S. aureus	106 (33.0%)	100 (31.3%)		104 (32.4%)	103 (32.3%)		152 (47.4%)	160 (50.2%)	
Number of Visits			0.409			0.395			0.801
N	321	319		321	319		321	319	
0	215 (67.0%)	219 (68.7%)		217 (67.6%)	216 (67.7%)		169 (52.6%)	159 (49.8%)	
1	82 (25.5%)	79 (24.8%)		87 (27.1%)	74 (23.2%)		101 (31.5%)	106 (33.2%)	
2	16 (5.0%)	18 (5.6%)		16 (5.0%)	27 (8.5%)		35 (10.9%)	45 (14.1%)	
3	7 (2.2%)	3 (0.9%)		1 (0.3%)	1 (0.3%)		15 (4.7%)	8 (2.5%)	
4	1 (0.3%)	0 (0.0%)		0 (0.0%)	1 (0.3%)		1 (0.3%)	1 (0.3%)	

Note: P-values for Number of Visits are based on Cochran-Armitage trend tests. P-values for Ever Colonized are based on Chi-Squared tests. All other p-values are based on three separate longitudinal models (skin, nasal, and the combination of skin or nasal *S. aureus*) with the outcome of interest being *S. aureus* colonization status adjusted for LEAP treatment assignement, SCORAD, time, and the interaction between LEAP treatment assignment and time.

Table E2. Agreement Between Skin and Nasal *S. aureus* Colonization Over Time

	4-11 (mo) Nasal *S. aureus*				12 (mo) Nasal *S. aureus*				30 (mo) Nasal *S. aureus*				60 (mo) Nasal *S. aureus*				Ever Colonized (4-11 mo to 60 mo) Nasal *S. aureus*			
	No *S. aureus* (N=544)	*S. aureus* (N=96)	Statistic 95% CI	p-value	No *S. aureus* (N=591)	*S. aureus* (N=35)	Statistic 95% CI	p-value	No *S. aureus* (N=586)	*S. aureus* (N=32)	Statistic 95% CI	p-value	No *S. aureus* (N=536)	*S. aureus* (N=94)	Statistic 95% CI	p-value	No *S. aureus* (N=433)	*S. aureus* (N=207)	Statistic 95% CI	p-value
Skin *S. aureus*			0.31	<0.001			0.16	<0.001			0.12	0.004			0.12	0.002			0.25	<0.001
No *S. aureus*	474 (87.1%)	51 (53.1%)	(0.22, 0.41)		539 (91.2%)	24 (68.6%)	(0.05, 0.28)		552 (94.2%)	26 (81.3%)	(0.00, 0.24)		505 (94.2%)	80 (85.1%)	(0.02, 0.21)		328 (75.8%)	106 (51.2%)	(0.17, 0.32)	
S. aureus	70 (12.9%)	45 (46.9%)			52 (8.8%)	11 (31.4%)			34 (5.8%)	6 (18.8%)			31 (5.8%)	14 (14.9%)			105 (24.2%)	101 (48.8%)		

Note: P-values are based on Kappa Statistics. The Kappa Statistics ranges from 0-1 (1 indicating perfect agreement and 0 indicating no agreement).

3. SUPPLEMENTARY TO THE TEXT

Severe eczema was an inclusion criterion for LEAP enrolment; indeed 89.2% of children had protocol defined severe eczema at recruitment (1). Overall, approximately half of all LEAP participants had some form of *S. aureus* colonization in the interval from baseline to 60 months of age (Table 1) and usually at only one of the four time points (Online Repository Table E1). The highest rates of colonization were recorded at the baseline visit, with 18% and 15% for the skin and nose respectively. That these colonization rates are lower than previously reported in patients with eczema (2), may be because swabs were only collected at 4 study time points which did not necessarily coincide with occasions when eczema might have been flaring, while *S. aureus* colonization is known to be transient. With the exception of the results at 60 months of age, the skin was more commonly the sole colonized location compared to the nose while concomitant nasal and skin colonization was the least common colonization pattern observed. Nasal carriage has been acknowledged as a risk factor for skin *S. aureus* re-colonization (3) and our findings of relatively low nasal colonization rates in the LEAP cohort may thus explain why the majority of participants only tested positive on one occasion. Notably and reassuringly, there was a low prevalence of methicillin-resistant *S. aureus* skin and nasal swab samples which also explains the non-persistent colonization pattern of the LEAP subjects. Despite the low concomitant skin and nasal colonization rates, our results do indicate a weak association between this concomitant *S. aureus* carriage at all study time points.

Meanwhile, although antibiotic treatment usually reduces *S. aureus* colonization and steroids or combined steroid/antibiotic formulations (4) appear to have a similar result, we found no difference in baseline skin *S. aureus* colonization amongst the subjects that were reported at baseline to have received these medications versus those that did not. Consequently, we did not proceed with an investigation of the relationship between *S. aureus* colonization and antibiotic/steroid use at later LEAP study visits.

The association between skin *S. aureus* and concomitant eczema severity was highly significant at all LEAP time points as opposed to that with nasal *S. aureus* whichwas weaker . The association between skin *S. aureus* and eczema severity is consistent with the report of a recent systematic review (2) which was unable to identify an association between nasal carriage and eczema severity. As LEAP participants with skin *S. aureus* had more severe eczema, we sought to investigate the causality of this relationship through a longitudinal model examining the correlation between immediately preceding skin *S. aureus* positivity and eczema severity. Interestingly, we found that the immediately preceding skin *S. aureus* colonization altered the pattern of eczema resolution observed from 12 to 30 months and 60 to 72 months of age. In particular, subjects found to be skin colonized at 12 months of age, and similarly at 60 months of age, were represented in Figure 1 with increasing disease trajectories due to higher mean SCORAD values at the subsequent follow-up visits. This was not the case for the participants without skin *S. aureus* carriage, reflected also by the fact that overall eczema severity was consistently reduced throughout LEAP and LEAP-On. Literature reports that *S. aureus* and/or SEB may cause, exacerbate and sustain the inflammatory process in eczema and can thus support our results (2,5, 6). Finally, according to the latent eczema classes recently described on the basis of two birth cohorts by Paternoster et al, the most prevalent class is the early-onset-early-resolving eczema phenotype characterized by a decline to 10% of eczema (defined as the reported presence of a rash consistent with eczema) prevalence by 6-7 years (7). Further to this, in our study skin *S. aureus* could predict the subsequent change of the early-onset-early-resolution eczema phenotype to a more persistent and worsening phenotype.

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