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Supplementary appendix

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Supplement to: Hsiao K-C, Ponsonby A-L, Axelrad C, Pitkin S, Tang MLK, on behalf of the PPOIT Study Team. Long-term clinical and immunological effects of probiotic and peanut oral immunotherapy after treatment cessation: 4-year follow-up of a randomised, double-blind, placebo-controlled trial. *Lancet Child Adolesc Health* 2017; published online Aug 15. http://dx.doi.org/10.1016/S2352-4642(17)30041-X.

SUPPLEMENTARY MATERIAL

Title: Long-term clinical and immunological effects of Probiotic and Peanut Oral Immunotherapy (PPOIT) post-treatment cessation: Four-year follow-up of a randomized double-blind placebo-controlled trial

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METHOD

DBPCFC procedure

DBPCFC were performed by allergy research nurses under medical supervision. Two sets of containers (A and B) were prepared by the Royal Children's Hospital (RCH) Clinical Trials Pharmacy. One container (either A or B) was the placebo (maltodextrin, brown food coloring and peanut essence) and the other (B or A, respectively) was the peanut protein (12% defatted peanut flour, 50% peanut protein; Byrd Mill, Ashland, Va). The RCH Clinical Trials Pharmacy randomly assigned placebo or peanut to part A for each participant DBPCFC using a random number table provided by the study statistician. Individual challenge doses were prepared by RCH Nutritional Services. The doctor and nurse administering the challenge remained blinded to the contents of each part of the challenge and to the participant's pre-challenge peanut SPT wheal size. Doses were given at 15-minute intervals according to the challenge-dosing schedule shown in Supplementary table 5, identical to the DBPCFC protocol used in the parent study[1]. Participants were observed for 2 hours after the last challenge dose. Pre-determined challenge stopping criteria (Supplementary table 6) were used to ensure consistency in interpretation of reaction symptoms and signs. DBPCFC was classified as 'passed' if both parts of the challenge were passed. DBPCFC was classified as 'failed' if the participant experienced objective symptoms during the peanut but not placebo component of the DBPCFC. DBPCFC was classified as inconclusive if the participant experienced objective symptoms during the placebo challenge – no cases of inconclusive challenge occurred.

Statistical analysis

The number of reactions on peanut ingestion per 10 person-years in each intervention group was estimated using the formula: number of reactions x 10 / (number of participants followed-up x mean number of years since completion of treatment).

We addressed missing data (participants lost-to-follow-up) using two approaches. For the outcome of peanut ingestion, we estimated the RR in two worst-case scenarios. In the first scenario, we assumed all participants who declined follow-up were abstaining from peanut ingestion. In the second scenario, we assumed all PPOIT participants who declined follow-up had ceased peanut intake and all placebo-treated participants who declined follow-up had acquired the ability to eat peanut. For the DBPCFC-based SU outcome, we employed inverse probability models, weighted to correct for attrition after parent study end as follows: the weight for each subject was the inverse of the probability of the subject undergoing the DBPCFC at four year follow-up, among those that completed the parent study (and therefore eligible for long-term follow-up, n=56). These weights to correct for non-response accounted for participant's sex, age at entry to parent study, history of doctor diagnosed eczema at entry to parent study, history of doctor diagnosed asthma at entry to parent study and peanut allergy status (achieved SU vs. did not achieve SU) at end-of-parent-study.

Supplementary Table 1. Details of individual reactions since completion of study treatment

Group	Individual	idno	Months since completion of study treatment	Regular peanut ingestion prior to reaction	Typical amount ingested prior to reaction	Cofactors (Exercise / Intercurrent viral illness / Prolonged avoidance)	Reaction eliciting amount	Summary of symptoms	Current status
Placebo	1	105	16	N	Nil	Nil	Smear	Urticaria	Avoiding peanut
Placebo	2	215	11	N	Nil	Nil	Smear	Oropharyngeal pruritis	Avoiding peanut
Placebo	2	215	45	N	Nil	Nil	Smear	Oropharyngeal pruritis	Avoiding peanut
Placebo	3	221	40	N	Nil	Nil	Smear	Urticaria	Avoiding peanut
Placebo	4	405	56	N	Nil	Nil	Smear	Abdominal pain	Avoiding peanut
Placebo	5	415	46	N	Nil	Nil	Smear	Abdominal pain, occasional cough, oropharyngeal pruritis	Avoiding peanut
Placebo	5	415	55	N	Nil	Nil	Smear	Oropharyngeal pruritis	Avoiding peanut
Placebo	6	429	29	N	Nil	Nil	Smear	*	Avoiding peanut
Placebo	6	429	35	N	Nil	Nil	Smear	Urticaria, abdominal pain, vomit	Avoiding peanut
PPOIT	7	223	5	Y	Large amount (approx 15 peanuts)	Illness	Large amount (approx 15 peanuts)	Hives, transient cough	Still ingesting peanut; large amount (approx 4g or more)
PPOIT	7	223	16	Y	Large amount (approx 15 peanuts)	Illness	Large amount (approx 15 peanuts)	Hives, transient cough	Still ingesting peanut; large amount (approx 4g or more)
PPOIT	7	223	22	Y	Large amount (approx 15 peanuts)	Illness	Large amount (approx 15 peanuts)	Hives, transient cough	Still ingesting peanut; large amount (approx 4g or more)
PPOIT	7	223	28	Y	Large amount (approx 15 peanuts)	Illness	Large amount (approx 15 peanuts)	Hives, transient cough	Still ingesting peanut; large amount (approx 4g or more)
PPOIT	7	223	34	Y	Large amount (approx 15 peanuts)	Illness	Large amount (approx 15 peanuts)	Hives, transient cough	Still ingesting peanut; large amount (approx 4g or more)
PPOIT	7	223	42	Y	Large amount (approx 15 peanuts)	Illness	Large amount (approx 15 peanuts)	Hives, transient cough	Still ingesting peanut; large amount (approx 4g or more)
PPOIT	7	223	45	Y	Large amount (approx 15 peanuts)	Illness	Large amount (approx 15 peanuts)	Hives, transient cough	Still ingesting peanut; large amount (approx 4g or more)
PPOIT	8	401	34	Y	Unsure*	Unsure*	Unsure*	Oropharyngeal pruritis	Still ingesting peanut; moderate amount (up to approx 4g)
PPOIT	8	401	34	Y	Unsure*	Unsure*	Unsure*	Oropharyngeal pruritis	Still ingesting peanut; moderate amount (up to approx 4g)
PPOIT	9	409	21	N	Small amount (< 8 peanuts)	Prolonged avoidance (approx. 4 weeks)	Small amount (satay chicken)	Abdominal pain	Avoiding peanut
PPOIT	10	431	9	N	Small amount (< 8 peanuts)	Prolonged avoidance (approx. 6 weeks)	Small amount (a few peanuts)	Abdominal pain, vomit	Avoiding peanut

Supplementary Table 2. Clinical and immunologic characteristics of subjects who consented to 8-week SU DBCPFC and those who did not

	PPOIT			**Placebo**		
Underwent 8-week SU DBPCFC at 4 year follow-up	Yes (n=12)	No (n=12)		Yes (n=15)	No (n=9)	
Age at time of 4 year follow-up study			p-value*			p-value*
Mean (SD), n	11·1 (2·4), 12	13·0 (2·2), 12	0·072	**10·1 (2·2), 15**	**14·4 (1·5), 9**	**0·016**
Baseline characteristics, n (%)						
% Male	7 (58)	8 (67)	0·674	8 (53)	8 (89)	0·099
% Passed 2-week SU DBPCFC at end of parent study	10 (83)	10 (83)	1·000	0 (0)	1 (11)	N/A
% Ingesting peanut, any frequency	8 (67)	8 (67)	1·000	0 (0)	1 (11)	N/A
% Not ingesting peanut	4 (33)	4 (33)	1·000	15 (100)	8 (89)	N/A
% Ingesting moderate to large amount of peanut	6 (50)	6 (55)#	0·827	0 (0)	1 (11)	N/A
Frequency of peanut ingestion, n (%)						
Nil	4 (33)	4 (33)		15 (100)	8 (89)	
Less than once a week	3 (25)	2 (17)		0 (0)	0 (0)	
1-2 times a week	3 (25)	4 (33)		0 (0)	0 (0)	
3 or more times a week	2 (17)	2 (17)		0 (0)	1 (11)	
Typical amount of peanut ingested, n (%)						
Avoiding peanut	4 (33)	3 (27)		15 (100)	8 (89)	
Small amount (< 2 g)	2 (17)	2 (18)		0 (0)	0 (0)	
Moderate amount (2 g to <4 g)	3 (25)	3 (27)		0 (0)	1 (11)	
Large amount (4 g or more)	3 (25)	3 (27)		0 (0)	0 (0)	
Peanut SPT weal size; Mean (SD), n			p-value**			p-value**
At baseline, mm	15·0 (8·1), 12	18·9 (4·5), 12	0·152	16·7 (7·2), 15	17·2 (6·8), 9	0·876
At completion of study treatment, mm	4·7 (5·2), 12	4·0 (3·5), 12	0·733	13·5 (4·4), 15	13·9 (7·8), 9	0·870
At 3 months after completion of study treatment, mm	4·9 (5·4), 12	3·7 (4·3), 12	0·547	15·1 (6·3), 15	12·3 (5·1), 9	0·273
At 4 year follow-up, mm	6·6 (6·9), 12	11·2 (8·9), 6	0·249	12·9 (6·0), 15	15·2 (15·3), 3	0·656
Immune indices; Median (IQR), n			p-value***			p-value***
Peanut sIgE at baseline, kU/L	9·3 (0·6, 64·4), 12	12·0 (1·8, 131·0), 12	0·583	14·0 (3·1, 51·9), 15	1·9 (0·8, 11·4), 9	0·180
Peanut sIgG4 at baseline, mgA/L	0·4 (0·1, 0·7), 12	0·7 (0·1, 1·1), 12	0·418	0·3 (0·2, 0·8), 15	0·2 (0·2, 0·7), 9	0·612

Peanut sIgE at completion of study treatment, kU/L	2·6 (0·8, 35·7), 12	3·1 (1·1, 33·6), 12	0·863	28·6 (1·9, 72·1), 15	1·4 (0·8, 23·1), 9	0·144
Peanut sIgG4 at completion of study treatment, mgA/L	2·1 (1·3, 28·4), 12	8·1 (2·2, 43·4), 12	0·453	0·2 (0·2, 0·5), 15	0·2 (0·1, 0·6), 9	0·591
Peanut sIgE at 3 months after completion of study treatment, kU/L	3·0 (0·5, 31·9), 12	3·3 (1·4, 30·8), 12	0·863	33·7 (1·8, 155·0), 15	2·0 (1·6, 17·7), 9	0·325
Peanut sIgG4 at 3 months after completion of study treatment, mgA/L	2·4 (1·1, 8·8), 12	3·8 (1·2, 28·0), 12	0·525	0·3 (0·2, 0·6), 15	0·7 (0·4, 1·7), 9	0·189
Peanut sIgE at 4 year follow-up, kU/L	2·4 (0·8, 14·7), 12	8·3 (0·5, 8·8), 6	0·574	20·7 (5·4, 100·0), 14	0·9 (0·0, 100·0), 3	0·251
Peanut sIgG4 at 4 year follow-up, mgA/L	0·8 (0·3, 1·6), 10	0·4 (0·0, 1·1), 5	0·358	0·2 (0·1, 0·5), 13	0·3 (0·3, 0·3), 1	0·710

 # one of the participants did not provide a response to amount of peanut ingesting

*Logistic regression, **t test, ***Mann-Whitney test

Supplementary Table 3: Characteristics participants who reacted at 8w-SU-DBPCFC despite previous regular ingestion and/or passed 2w-SU-DBPCFC

ID number	228	416	209	101	406
Age at 8-week SU DBPCFC, y	8·5	14·2	11.0	12·5	14.0
Frequency of peanut ingestion	1-2 times a week	Less than once a week	Not ingesting peanut	Not ingesting peanut	Not ingesting peanut
Typical amount of peanut ingestion	Moderate amount (2g to less than 4g)	Moderate amount (2g to less than 4g)	Nil	Nil	Nil
Longest gap without peanut, prior to 8 week SU DBPCFC	Months	Months	N/A	N/A	N/A
Cumulative dose tolerated in DBPCFC (mg)	2937·5	2937·5	187·5	1937·5	1937·5
Reaction eliciting cumulative dose (mg)	4000·0	4000·0	437·5	2937·5	2937·5
DPBCFC reaction signs	Lower respiratory (wheeze), cutaneous, orapharyngeal	Gastrointestinal (vomited), oropharyngeal	Gastrointestinal, cutaneous, oropharyngeal, rhinoconjunctival	Gastrointestinal, cutaneous, rhinoconjunctival	Lower respiratory (wheeze), cutaneous, orapharyngeal
DPBCFC treatment	Oral antihistamine, IM adrenaline (2 doses)	None required	Oral antihistamine	None required	Oral antihistamine, IM adrenaline (2 doses)
Peanut SPT, mm	11·5	7·5	17	12·5	19·5
Peanut sIgE, kU/L	24·7	24·6	100.0	2·1	2·6

Supplementary Table 4. Reactions during 8-week SU DBPCFC

	PPOIT	Placebo
DBPCFC reaction symptoms [as % of all undergoing DBPCFC]		
Any cutaneous, n (%)	4 (33)	8 (53)
Any gastrointestinal, n (%)	3 (25)	12 (80)
Any rhinoconjunctival, n (%)	2 (17)	11 (73)
Any oropharyngeal, n (%)	4 (33)	9 (60)
Any lower respiratory, n (%)	2 (17)	4 (27)
Any cardiovascular, n (%)	0 (0)	0 (0)
DBPCFC related anaphylaxis, n (%)	2 (17)	4 (27)
Patients needing any IM adrenaline, n (%)	2 (17)	4 (27)
Patients needing overnight admission, n (%)	2 (17)	0 (0)

Supplementary Table 5. DBPCFC dosing schedule

		Food Challenge A	
1000	Dose 1	62.5mg	
1015	Dose 2	125mg	
1030	Dose 3	250mg	
1045	Dose 4	500mg	
1100	Dose 5	1000mg	
1115	Dose 6	1000mg	
1130	Dose 7	1062.5 mg	Spirometry
Observed for 2 hour, if no allergic symptoms develop continue to food challenge B			
		Food Challenge B	
1330	Dose 1	62.5mg	
1345	Dose 2	125mg	
1400	Dose 3	250mg	
1415	Dose 4	500mg	
1430	Dose 5	1000mg	
1445	Dose 6	1000mg	
1500	Dose 7	1062.5mg	Spirometry
Observe for 2 hours if no allergic symptoms develop discharge home			

Supplementary Table 6: DBPCFC challenge stopping criteria

Standardised cessation criteria for a positive OFC result = ANY of the following objective signs occurring within TWO (2) hours of ingestion:

- 3 or more concurrent non-contact urticaria persisting for at least 5 minutes[1];
- perioral, periorbital[1] or facial angioedema;
- vomiting (excluding gag reflex)[1] and/or diarrhoea;
- persistent cough (i.e. not just intermittent and transient throat clearing), wheeze (either audible (without stethoscope) or on auscultation with stethoscope), change in voice, stridor, difficulty breathing, collapse[1], hypotension (ViCTOR chart)[2];
- long bursts of sneezing, [3]

Standardise criteria to delay the next scheduled dose by 15 minutes:

- persistent throat tightness/pain[3] (subjective)
- severe abdominal pain (subjective) and/or notably distressed due to GI symptoms with decreased activity[3]
- mild subjective cardiovascular response (weak, dizzy) <u>without</u> evidence of hypotension or tachycardia[2,3]

Objective signs that might not necessitate cessation of challenge or delaying of a dose:

- occasional scratching[3]
- [3]
- a few areas of faint erythema[3]
- rare bursts (of sneezing), occasional sniffing[3]
- complaints of nausea or abdominal pain, itchy mouth/throat

Staff required to proceed with a T9 DBPCFC

PPOIT Study Nurse physically present in immediate vicinity of challenge area; and

PPOIT Study Doctor physically present in immediate vicinity of challenge area; and

PPOIT Chief Investigator (CI) or Principal Investigator (PI) physically in Melbourne and contactable by phone

References

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