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

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Summary

Background Oral immunotherapy has attracted much interest as a potential treatment for food allergy, yet little is known about its long-term effects. We aimed to assess long-term outcomes in participants who completed a randomised, double-blind, placebo-controlled trial of combined probiotic and peanut oral immunotherapy (PPOIT), which was previously shown to induce desensitisation and 2-week sustained unresponsiveness.

Methods All participants who completed the PPOIT randomised trial were eligible to participate in this follow-up study 4 years after treatment cessation. Peanut intake and adverse reactions to peanut in the 4 years after treatment cessation were systematically documented with a structured questionnaire administered by allergy nurses. Additionally, participants were invited to undergo peanut skin prick tests, measurement of peanut sIgE and sIgG4 concentrations, and double-blind placebo-controlled peanut challenge to assess 8-week sustained unresponsiveness.

Findings 48 (86%) of 56 eligible participants were enrolled in the follow-up study. Mean time since stopping treatment was 4·2 years in both PPOIT (SD 0·6) and placebo (SD 0·7) participants. Participants from the PPOIT group were significantly more likely than those from the placebo group to have continued eating peanut (16 [67%] of 24 *vs* one [4%] of 24; absolute difference 63% [95% CI 42–83], p=0·001; number needed to treat 1·6 [95% CI 1·2–2·4]). Four PPOIT-treated participants and six placebo participants reported allergic reactions to peanut after intentional or accidental intake since stopping treatment, but none had anaphylaxis. PPOIT-treated participants had smaller wheals in peanut skin prick test (mean 8·1 mm [SD 7·7] *vs* 13·3 mm [7·6]; absolute difference –5·2 mm [95% CI –10·3 to 0·0]; age-adjusted and sex-adjusted p=0·035) and significantly higher peanut sIgG4:sIgE ratios than placebo participants (geometric mean 67·3 [95% CI 10·3–440·0] *vs* 5·2 [1·2–21·8]; p=0·031). Seven (58%) of 12 participants from the PPOIT group attained 8-week sustained unresponsiveness, compared with one (7%) of 15 participants from the placebo group (absolute difference 52% [95% CI 21–82), p=0·012; number needed to treat 1·9 [95% CI 1·2–4·8]).

Interpretation PPOIT provides long-lasting clinical benefit and persistent suppression of the allergic immune response to peanut.

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Introduction

The prevalence of food allergy has risen substantially in the past 20 years.[1–3] Whereas allergies to egg, milk, wheat, and soy generally resolve during childhood, nut and seafood allergies often persist throughout life. Management includes allergen avoidance and education in the emergency management of allergic reactions. The constant vigilance required to avoid allergens substantially impairs quality of life of both food-allergic children and their families.[4,5] The quality of life of children with food allergies is worse than that of children with diabetes.[6,7] Accidental exposure to food allergens is common, with an annual incidence of 12–15% in children with peanut allergy.[8,9] Although most allergic reactions to food can be managed successfully, fatalities still occur, with an incidence of roughly three per million person-years in children aged 0–19 years.[10] A curative treatment could potentially improve quality of life and prevent allergy-related deaths.

There is intense interest in oral immunotherapy as a food allergy treatment to induce desensitisation (defined as being able to tolerate the allergen while on treatment),[11–17] or sustained unresponsiveness (defined as being able to tolerate the allergen weeks or months after stopping treatment).[14,18,19] Results of randomised placebo-controlled trials suggest that oral immunotherapy can induce desensitisation in around two-thirds and sustained unresponsiveness of several weeks' duration in a small subset of children.[20] Whether desensitisation or sustained unresponsiveness persists long term after oral immunotherapy remains unknown.[21] Long-term effects after oral immunotherapy have been reported in participants with egg or milk allergies, for which natural resolution can be expected in a substantial proportion of people. In the only follow-up study[22] of a randomised trial of oral immunotherapy, no difference in frequency of egg ingestion was reported between people in the egg

Research in context

Evidence before this study

We searched PubMed with the medical subject heading terms "food allergy", "peanut allergy", "oral immunotherapy", and "long term" for articles published in English on or before May 12, 2017. We found four publications about long-term outcomes after cessation of oral immunotherapy, one in children with egg allergies and three in children with milk allergies. However, we identified no studies of similar outcomes in patients who received peanut oral immunotherapy. Unlike most allergies to milk or eggs, peanut allergies tend to persist into adulthood, so long-term outcomes after egg or milk oral immunotherapy cannot be generalised to peanut oral immunotherapy. Furthermore, we identified no studies in which challenge-proven sustained unresponsiveness was assessed years after stopping oral immunotherapy.

Added value of this study

Ours is the first report, to our knowledge, of long-term outcomes several years after cessation of peanut oral immunotherapy and the first study to incorporate challenge-proven sustained unresponsiveness as an outcome measure. Our findings show that combined probiotic and peanut oral immunotherapy provides long-lasting clinical benefit compared with placebo, with two-thirds of treated participants symptom free after peanut ingestion 4 years after completing treatment. The treatment resulted in symptom-free peanut ingestion, food-challenge-proven sustained unresponsiveness, and persistent suppression of the allergic immune response to peanut 4 years after treatment cessation.

Implications of all the available evidence

Long-term clinical benefits after standard oral immunotherapy remain uncertain. Combined administration of probiotic and peanut oral immunotherapy provided long-term clinical benefits, and the findings of this study are a step towards identification of an effective treatment to address the food allergy problem in developed countries.

oral immunotherapy group and those in the placebo group at least 12 months after treatment.

No data for long-term outcomes after peanut oral immunotherapy have been published. The natural history of peanut allergy is different from that of egg or milk allergy, so long-term outcomes for egg or milk oral immunotherapy cannot be extrapolated to peanut oral immunotherapy. We previously reported that combined probiotic and peanut oral immunotherapy (PPOIT) was effective at inducing desensitisation and sustained unresponsiveness after 2 weeks of secondary allergen information in children with peanut allergy (number needed to treat 1·27 [95% CI 1·06–1·59]).[23] We selected the probiotic *Lactobacillus rhamnosus* CGMCC 1.3724 (NCC4007), which is genetically indistinguishable from *L rhamnosus* ATCC 53103, for use in PPOIT on the basis of its ability to induce regulatory T cells, antigen-specific IgA, and regulatory and T helper 1 cytokine responses.[24–28] We postulated that administration of this probiotic with peanut oral immunotherapy would support redirection of the peanut-specific allergic response towards tolerance by providing a tolerogenic milieu at the time of antigen uptake and processing by antigen-presenting cells. In this study, we aimed to examine whether the previously reported clinical and immunological benefits of PPOIT were maintained 4 years after treatment.

Methods

Study background

Participants who completed the PPOIT randomised trial (the parent study) were assessed 4 years after treatment cessation (between January, 2015, and April, 2016). The parent study design and outcomes have been reported previously.[23] Briefly, 62 children with peanut allergy were randomly assigned (1:1) to receive PPOIT (2×10^{10} colony-forming units of *L rhamnosus* CGMCC 1.3724 and 2 g of peanut protein) or placebo (two formulations of maltodextrin) once daily for 18 months. During the study, all participants and their parents were reminded at each scheduled study visit to avoid taking probiotics and to remain on a peanut elimination diet as stipulated in the study protocol. At 18 months, all participants underwent a double-blind, placebo-controlled food challenge to assess for desensitisation. Participants who passed the desensitisation food challenge completed a second challenge 2–6 weeks after discontinuation of study treatment to assess for sustained unresponsiveness.

Participants who passed the sustained unresponsiveness challenge at the end of the parent study were instructed to continue intake of peanut ad libitum (ie, as part of their normal diet, without specific instructions on ingestion frequency or amount). Participants who did not attain sustained unresponsiveness were instructed to continue with strict peanut avoidance. Participants probably did not continue to take the probiotic at treatment doses because *L rhamnosus* CGMCC 1.3724 is not known to the general public by that name, and participants were advised that the dose they were taking was equivalent to 20 tubs of yogurt each day. The parent study's primary outcome was the proportion of participants who attained sustained unresponsiveness.

Data collection and procedures

All participants who completed the parent study (n=56) were eligible for this follow-up study; consent was given at enrolment into the follow-up study (ie, 4 years after treatment). A structured peanut intake questionnaire and a validated food allergy quality-of-life questionnaire (Food

Allergy Quality of Life Questionnaire [FAQLQ]-parent form if participants were younger than 13 years, FAQLQ-teenager form if they were aged 13 years or older)[29] were administered to participants or their parents by allergy research nurses. The peanut intake questionnaire systematically recorded participants' peanut intake history, including the average amount ingested, ingestion frequency, and adverse reactions to peanuts after accidental or intentional ingestion since stopping study treatment. We also invited participants to give additional, optional consent for peanut skin prick tests, blood sample measurements of peanut specific IgE (sIgE) and specific IgG4 (sIgG4), and double-blind placebo-controlled food challenge (cumulative dose 4 g peanut protein after peanut elimination for 8 weeks; appendix) to assess for sustained unresponsiveness.

Peanut skin prick tests (peanut extract from Hollister-Stier, Spokane, WA, USA; Greer Pick from Greer Laboratories, Lenoir, NC, USA) were done by allergy research nurses. Serum concentrations of peanut sIgE and sIgG4 were measured by ImmunoCAP (Phadia AB, Uppsala, Sweden). The peanut sIgG4:sIgE ratio was calculated by converting peanut sIgG4 concentrations from μg/L to ng/mL and peanut sIgE concentrations from kU/L to ng/mL and then using the formula:[30]

$$\frac{\text{IgG4}}{\text{IgE} \times 2{\cdot}4}$$

Ethics approval for this study, including the study protocol and an a-priori statistical analysis plan, was obtained from the Royal Children's Hospital Human Research Ethics Committee (HREC 27086Q).

Statistical analysis

Allowing for 30% loss to follow-up, we calculated that a sample size of 19 participants in each intervention arm would provide 90% power to detect a difference between 12% sustained unresponsiveness in the placebo group (3% spontaneously outgrowing peanut allergy per year) and 60% sustained unresponsiveness in the PPOIT group. Clinical outcomes at 4-year follow-up were analysed by intention to treat. The effects of treatment were estimated in terms of absolute differences, numbers needed to treat, and risk ratios (RRs) with 95% CIs. We tested the hypothesis of no difference in the effect of treatment between intervention groups with the test of proportions with Fisher's exact tests (with double the one-tailed exact probability), and with unadjusted and adjusted (by age, sex, or time since treatment cessation) generalised linear models. We addressed missing data (ie, participants lost to follow-up) with two approaches: worst-case-scenario models for peanut ingestion outcomes and weighted inverse probability models for sustained unresponsiveness outcomes. Detailed description of these approaches and the formula for calculating reaction rates per 10 person-years are in the appendix. Peanut skin prick test wheal sizes were reported as mean (SD), and between-group comparisons were analysed by *t* test. Other immune markers, including peanut sIgE, sIgG4, and the sIgG4:sIgE ratio had skewed distributions, and were summarised as geometric means (95% CI) and assessed by regression models. Study data were gathered and managed with REDCap[31] electronic data-capture tools hosted at Murdoch Childrens Research Institute and analysed with Stata (version 14.2).

Role of the funding source

The study sponsors had no role in study design; data collection, analysis, or interpretation; or writing of the Article. K-CH, CA, and MLKT had access to all study data and final responsibility for the decision to submit for publication.

See Online for appendix

Results

48 (86%) of 56 eligible participants were enrolled, 24 from the PPOIT group and 24 from the placebo group (figure). Mean time from treatment cessation to entry into this study was 4·2 years (SD 0·6) in the PPOIT group and 4·2 years (SD 0·7) in the placebo group. Baseline characteristics were similar between groups (table 1). Of the four PPOIT-treated participants who declined to participate in the follow-up study (figure), one was peanut desensitised and three had sustained unresponsiveness at the end of the parent study. All four placebo-treated participants who declined to participate were peanut

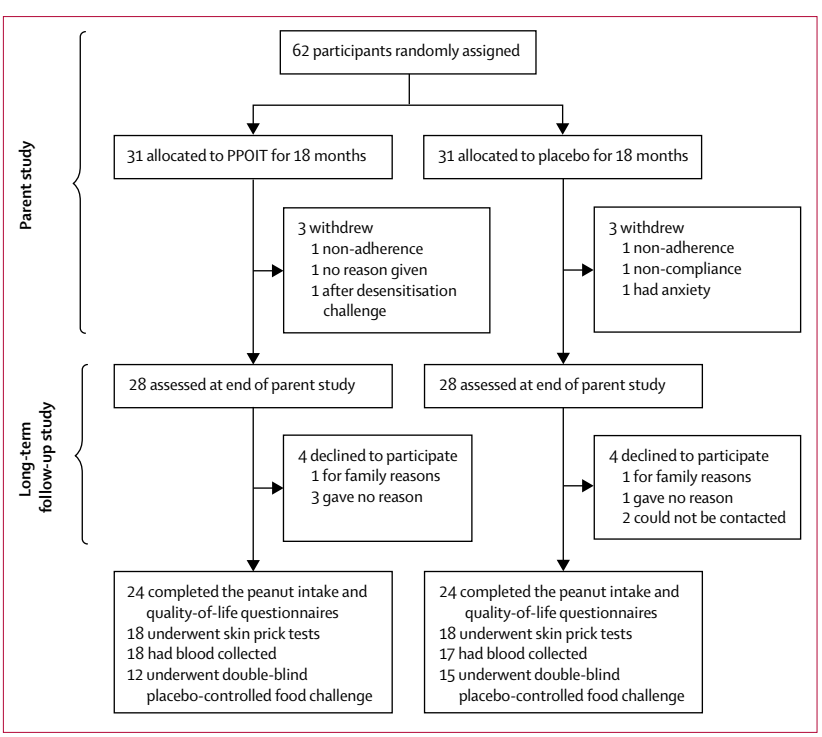

***Figure*: Participant flow**
PPOIT=probiotic and peanut oral immunotherapy.

	PPOIT group (n=24)	Placebo group (n=24)
At entry to parent study		
Age, years	12·1 (2·4)	11·7 (2·9)
Male sex	15 (63%)	16 (67%)
Had one or more siblings	22 (92%)	21 (88%)
Physician-diagnosed asthma	13 (54%)	9 (38%)
Physician-diagnosed eczema	19 (79%)	19 (79%)
At entry to 4-year follow-up study		
Time since commencing study treatment, years	5·8 (0·6)	5·8 (0·7)
Time since completing study treatment, years	4·2 (0·6)	4·2 (0·7)

Data are mean (SD) or n (%). PPOIT=probiotic and peanut oral immunotherapy.

Table 1: **Baseline characteristics**

	PPOIT group	Placebo group
Peanut ingestion		
Ingesting peanut	16/24 (67%)	1/24 (4%)
Not ingesting peanut	8/24 (33%)	23/24 (96%)
Sensitivity analysis		
Scenario 1*	16/28 (57%)	1/28 (4%)
Scenario 2†	16/28 (57%)	5/28 (18%)
Frequency of ingestion		
Never	8 (33%)	23 (96%)
Less than once a week	5 (21%)	0 (0%)
Once or twice a week	7 (29%)	0 (0%)
Three or more times a week	4 (17%)	1 (4%)
Amount of peanut ingested‡		
None	7 (30%)	23 (96%)
<2 g	4 (17%)	0 (0%)
2 g to <4 g	6 (26%)	1 (4%)
>4 g	6 (26%)	0 (0%)
≥2 g (ie, moderate-to-large amount)	12 (52%)	1 (4%)
Peanut skin prick test wheal size (mm)		
n	18	18
Mean (SD)	8·1 (7·7)	13·3 (7·6)
Median (IQR)	6·3 (1·0 to 14·0)	14·0 (9·0 to 18·5)

Data are n/N (%) or n (%), unless otherwise specified. PPOIT=probiotic and peanut oral immunotherapy. *All who declined to participate were recorded as not ingesting. †All participants in the PPOIT group who declined to participate were recorded as not ingesting; all placebo participants who declined to participate were recorded as ingesting. ‡n=23 for the PPOIT group because one participant did not provide a response about the amount of peanut they ingested.

Table 2: **Peanut ingestion and skin prick test**

allergic at the end of the parent study. We noted no between-group differences at entry to the parent study for participants who declined long-term follow-up, but significant differences in skin prick test results (p=0·001 at end of study; p=0·009 3 months later). All other demographic features were similar between groups for participants who declined follow-up.

16 (67%) of 24 participants in the PPOIT group were still eating peanut 4 years after stopping study treatment, compared with one (4%) of 24 in the placebo group (absolute difference 63% [95% CI 42–83], p=0·001; number needed to treat 1·6 [95% CI 1·2–2·4]; unadjusted RR 16·0 [95% CI 2·3–113·6]; table 2). Adjustment for age, sex, or time since treatment cessation as separate models resulted in RRs of 15·6–16·4 (p=0·006). 12 (52%) of 23 PPOIT-treated participants (one person did not provide information about peanut ingestion) were ingesting moderate-to-large amounts of peanut (ie, ≥2 g peanut protein) per ingestion episode, and 11 (46%) were ingesting peanut at least once a week (table 2), suggesting ingestion ad libitum. More participants in the PPOIT group were ingesting more than 2 g of peanut protein than in the placebo group (absolute difference 48% [95% CI 26–70], p=0·001; unadjusted RR 12·5 [95% CI 1·7–90·6]). The RR was 11·7 (1·5–90·6], p=0·019) after adjusting for age, sex, and time since completion of treatment as combined model.

16 (80%) of 20 PPOIT-treated participants who achieved 2-week sustained unresponsivess at the end of the parent study were regularly ingesting peanuts at 4-year follow-up. Of the other four participants, one ceased all peanut intake shortly after the end of the parent study because they disliked the taste of peanut, two reported symptoms after peanut ingestion within 24 months of treatment cessation, and one maintained regular peanut ingestion for more than 1 year after stopping study treatment but then discontinued peanut intake for around 6 months because of disruptive social circumstances before notifying the study team. The latter three participants subsequently failed formal peanut challenges up to 3 years after stopping treatment and were instructed by the study team to discontinue peanut intake. The one participant in the placebo group who attained sustained unresponsiveness at the end of the parent study continued to ingest peanuts at 4-year follow-up, but did not undergo the 8-week food challenge. The other 23 placebo-treated participants were all peanut allergic at the end of the parent study and had been advised to avoid peanut. None intentionally ingested peanut to test their allergic status.

In the first sensitivity analysis, assuming that all participants who declined to participate in the follow-up study were abstaining from peanuts, the absolute difference between groups was 54% (95% CI 34–73, p=0·001), with an unadjusted RR of 16·0 (95% CI 2·2–114·6). The RR was 15·7 (2·1–117·8, p=0·008) after adjustment for for age, sex, and time since completion of treatment as a combined model. In the second sensitivity analysis, we assumed that all participants from the PPOIT group who declined follow-up had ceased peanut intake and that placebo-treated participants who declined follow-up had acquired the ability to eat peanut. The absolute difference between groups was 39% (95% CI 16–62, p=0·006), with an unadjusted RR of 3·2 (1·3 to 7·6) and an adjusted RR of 3·1 (1·3–7·7, p=0·013).

	PPOIT group (n=24)	Placebo group (n=24)
Patients who had one or more reactions	4 (17%)	6 (25%)
Reactions per patient		
0	20 (83%)	18 (75%)
1	2 (8%)	3 (13%)
2	1 (4%)	3 (13%)
7	1 (4%)	0 (0%)
Reactions by timepoint		
Total number of reactions	11	9
Within 3 months of stopping treatment	0 (0%)	0 (0%)
Between 3 and 12 months of stopping treatment	2 (18%)	1 (11%)
1–5 years after stopping treatment	9 (82%)	8 (89%)
Reactions by class*		
Anaphylaxis	0 (0%)	0 (0%)
Urticaria	0 (0%)	2 (22%)†
Urticaria plus abdominal pain with or without vomiting	0 (0%)	1 (11%)‡
Urticaria plus transient cough	7 (64%)‡	0 (0%)
Urticaria plus persistent cough	0 (0%)	0 (0%)
Oropharyngeal pruritus	2 (18%)‡	3 (33%)†
Abdominal pain with or without vomiting	2 (18%)†	1 (11%)‡
Abdominal pain plus transient cough plus oropharyngeal pruritis	0 (0%)	1 (11%)‡
Information not available	0 (0%)	1 (11%)‡
Total person-years followed up	99·8	102·0
Estimated number of events per 10 person-years	1·1	0·9

Data are n (%), unless otherwise specified. PPOIT=probiotic and peanut oral immunotherapy. *Two participants in the placebo group had more than one class of reaction. †Two participants. ‡One participant.

***Table 3*: Reported reactions from completion of study treatment to time of long-term follow-up study**

	PPOIT group	Placebo group	p value*
Peanut sIgE (kU/L)			
n	18	17	..
Median (IQR)	2·9 (0·7 to 8·8)	9·8 (1·6 to 100·0)	0·057
Geometric mean (95% CI)	2·8 (1·0 to 8·2)	10·7 (3·2 to 36·1)	0·089
Median difference in sIgE since time of entry to parent study (IQR)	–10·9 (–44·9 to –1·0)	–0·2 (–20·3 to 1·6)	0·086
Peanut sIgG4 (mg_A/L)			
n	15	14	..
Median (IQR)	0·7 (0·1 to 1·6)	0·2 (0·1 to 0·5)	0·150
Geometric mean (95% CI)	0·4 (0·1 to 1·5)	0·2 (0·1 to 0·5)†	0·409
Median difference in sIgG4 since time of entry to parent study (IQR)	0·3 (–0·1 to 1·4)	–0·1 (–0·3 to 0·0)	0·085
Peanut sIgG4:sIgE ratio			
n	15	14	..
Median (IQR)	91·4 (21·4 to 298·1)	2·4 (0·5 to 61·9)	0·018
Geometric mean (95% CI)	67·3 (10·3 to 440·0)	5·2 (1·2 to 21·8)†	0·031
Median difference in ratio since time of entry to parent study (IQR)	35·8 (–0·1 to 296·6)	–1·2 (–3·1 to 6·5)	0·013

PPOIT=probiotic and peanut oral immunotherapy. sIgE=specific IgE. sIgG4=specific IgG4. *The Wilcoxon rank sum (Mann-Whitney) test was applied for data expressed as medians (IQRs). The *t* test on the log scale was applied for data presented as geometric means (95% CIs). †n=13.

***Table 4*: Peanut sIgE and sIgG4 concentrations, and sIgG4:sIgE ratio 4 years after completion of study treatment**

Mean wheal size after skin prick test was similar in both groups before treatment (16·9 mm [SD 6·7] in the PPOIT group *vs* 16·9 mm [SD 6·9]).[23] At 4-year follow-up, wheals in PPOIT-treated participants (n=18) were smaller than those in placebo-treated participants (n=18; absolute difference –5·2 [95% CI –10·3 to 0·0]; unadjusted p=0·050; age-adjusted and sex-adjusted p=0·035).

20 (83%) of 24 PPOIT-treated participants reported no allergic reactions after peanut ingestion since stopping treatment (table 3). 11 reactions occurred after intentional peanut ingestion in four participants who attained 2-week sustained unresponsiveness in the parent study (table 3). Six placebo-treated participants reported nine allergic reactions after accidental ingestion of peanut (table 3). In both groups, all reactions were minor, none were anaphylaxis, and none necessitated adrenaline treatment (appendix). The number of reactions to peanut was 1·1 per 10 person-years in the PPOIT group and 0·9 per 10 person-years in the placebo group.

4 years after treatment cessation, PPOIT-treated participants had numerically lower peanut sIgE concentrations (geometric mean 2·8 kU/L [95% CI 1·0–8·2] *vs* 10·7 kU/L [95% CI 3·2–36·1]; p=0·089) and higher peanut sIgG4 concentrations (geometric mean 0·4 mg_A/L [95% CI 0·1–1·5] *vs* 0·2 mg_A/L [95% CI 0·1–0·5]; p=0·409) than placebo-treated participants, although these differences were not significant (table 4). Peanut sIgG4:sIgE ratios were significantly higher in PPOIT-treated than in placebo-treated participants (table 4).

27 participants (12 from the PPOIT and 15 from the placebo group) consented to double-blind placebo-controlled food challenge to assess 8-week sustained unresponsiveness. In the placebo group, participants who consented to food challenge were younger than those who declined (p=0·02; appendix), but no other differences—specifically, in peanut ingestion frequency, amount of peanut ingested, peanut skin prick test wheal size, peanut sIgE and sIgG4 concentrations, and status of peanut intake (ingesting or not ingesting) at the time of follow-up—were noted between participants who consented to food challenge and those who did not (appendix). Furthermore, among PPOIT-treated participants, those who achieved 2-week sustained unresponsiveness and maintained regular peanut ingestion afterwards, those who initially achieved sustained unresponsiveness but then discontinued peanut ingestion before the follow-up study, and those who did not achieve 2-week sustained unresponsiveness at the end of the parent study were equally distributed

	PPOIT group (n=12)	Placebo group (n=15)
8-week sustained unresponsiveness at 4-year follow-up	7 (58%)	1 (7%)*
Achieved sustained unresponsiveness at end of parent study	10	0
Achieved sustained unresponsiveness at end of parent study and maintained it at 4-year follow-up	7	0

Data are n (%) or n. PPOIT=probiotic and peanut oral immunotherapy.
*One participant was on azathioprine at the time of food challenge; they were not ingesting peanut, had a peanut skin prick test result of 13 mm and a peanut specific IgE concentration of 5·43 kU/L, but they passed the 4 g food challenge.

Table 5: **Long-term peanut challenge outcomes in participants who underwent sustained unresponsiveness food challenge after 8 weeks' peanut elimination**

between the participants who consented to food challenge and the participants who declined.

8-week sustained unresponsiveness as shown by double-blind placebo-controlled food challenge was significantly more common in the PPOIT group than in the placebo group (seven [58%] of 12 *vs* one [7%] of 15; absolute difference 52% [95% CI 21–82], p=0·012; unadjusted RR 8·8 [95% CI 1·2–64·0]; table 5). The RR was 8·7–9·9 (p=0·023–0·036) after adjustment for age or time since completion of treatment as separate models; the number needed to treat was 1·9 (95% CI 1·2–4·8). Seven (70%) of ten PPOIT-treated participants who had achieved 2-week sustained unresponsiveness at the end of the parent study showed challenge-proven 8-week sustained unresponsiveness 4 years after stopping treatment. The one placebo-treated participant who passed the follow-up food challenge was taking azathioprine for severe eczema (commenced 4 months before the follow-up study) and had a peanut skin prick test wheal size of 13 mm at the time of the challenge. Exclusion of this participant's outcomes strengthened the effect of PPOIT treatment on 8-week sustained unresponsiveness (p=0·002). The median cumulative doses tolerated during food challenge were 4000 mg (IQR 2438–4000) in the PPOIT group and 938 mg (188–1938) in the placebo group (Mann-Whitney test p=0·003).

The sensitivity analysis to account for non-participation, including backweighting for factors that could relate to the likelihood of failing the food challenge (ie, participant's sex, age at entry to parent study, history of doctor-diagnosed eczema or asthma at entry to parent study, and peanut allergy status [achieved sustained unresponsiveness *vs* did not achieve sustained unresponsiveness] at the end of the parent study) showed a beneficial effect for PPOIT as measured by challenge-proven 8-week sustained unresponsiveness (adjusted RR 10·0 [95% CI 1·3–78·5], p=0·029).

Characteristics of the five PPOIT-treated participants who did not have sustained unresponsiveness after food challenge are in the appendix. Two were peanut allergic (ie, did not attain 2-week sustained unresponsivess after the parent study), one attained sustained unresponsiveness at the end of the parent study but became resensitised 26 months after treatment cessation, and two reported regular peanut ingestion without reactions before the follow-up study, but reacted during food challenge at a cumulative dose of 4 g peanut protein. Two participants in the PPOIT group and four in the placebo group developed anaphylaxis during food challenge and required intramuscular adrenaline (appendix). Quality-of-life questionnaires were also completed as part of this study; findings will be reported separately.

Discussion

PPOIT was associated with long-lasting peanut tolerance 4 years after stopping treatment. Two-thirds of PPOIT-treated participants were able to continue regular peanut ingestion, and more than half were ingesting moderate-to-large amounts of peanut on a regular basis, compared with only one (4%) of 24 placebo-treated participants. Allergic reactions from intentional peanut ingestion were uncommon and all reactions were mild, suggesting that those who achieved PPOIT-induced sustained unresponsiveness can safely continue peanut ingestion. However, larger studies and meta-analyses of long-term safety outcomes are needed. 58% of PPOIT-treated participants who completed a double-blind placebo-controlled food challenge 4 years after stopping treatment attained 8-week sustained unresponsiveness, and 70% of participants who had attained sustained unresponsiveness at the end of the parent study maintained this status.

Strengths of our study include the multidimensional assessment of long-term efficacy, the standardised follow-up protocol, the high rate of participation (86%) with equal numbers from PPOIT and placebo groups, and the administration of double-blind, placebo-controlled food challenge after an extended 8-week period of secondary peanut elimination, with similar levels of participation from both groups. Furthermore, the inclusion of placebo-treated participants in this follow-up study allowed detection of effects attributable to the natural resolution of peanut allergy over time. In our study, excluding the participant who was taking a systemic immunosuppressant at the time of food challenge, none of the placebo-treated participants who were allergic to peanuts at the end of the parent study acquired an ability to ingest peanut or passed the food challenge 4 years later.

The major limitation of our study is low participation in the food challenge at 4-year follow-up. However, to our knowledge, this study is the first peanut oral immunotherapy study in which persistence of sustained unresponsivenss by double-blind, placebo-controlled food challenge years after treatment cessation was assessed. Furthermore, prolonged secondary peanut elimination for 8 weeks allowed a high level of confidence in outcome. We noted no differences in

clinical and immunological characteristics (including age at follow-up, peanut ingestion frequency, amount of peanut ingested, sustained unresponsiveness *vs* allergy status at end of parent study, peanut skin prick test wheal size, and sIgE and sIgG4 concentrations) between those who participated in the challenge and those who did not, suggesting that our findings are probably representative of the total sample of the parent study. Furthermore, we used inverse probability weighting to account for the potential effect of participants lost to follow-up, and a sensitivity analysis accounting for non-response, which still showed a favourable effect of PPOIT treatment 4 years after treatment. Nonetheless, we acknowledge that some caution is needed when generalising our challenge findings to those who declined to participate in the food challenge.

Other limitations include the retrospective collection of data for peanut ingestion and reactions, although this approach has been used in other oral immunotherapy follow-up studies,[32,33] and the small sample size, which was similar to that in other published oral immunotherapy follow-up studies[14,15,17–19,22] and reflects the proof-of-concept nature of the parent study. Generalisability of our findings might be partly limited by the single-centre study design. We also acknowledge the limitations of the parent study design, which we have previously discussed.[23] Briefly, the absence of a probiotic only group and an oral immunotherapy only group limit our ability to delineate the individual contributions of the two constituents of PPOIT. We note, however, that PPOIT did not modify skin prick test wheal sizes for other food allergens,[23] suggesting that probiotic alone probably did not produce the noted beneficial effects. Future randomised controlled trials comparing allergen oral immunotherapy, a combination of a probiotic and allergen oral immunotherapy, and placebo are needed to establish whether the probiotic acts synergistically with oral immunotherapy. The parent study also did not include a double-blind, placebo-controlled food challenge at study entry to confirm peanut allergy, and instead relied on objective reaction to peanut and 95% positive predictive value thresholds for sIgE concentrations and skin prick tests, in line with other oral immunotherapy trials at the time. However, randomisation would distribute any misclassifications evenly between groups, and placebo participants had a low frequency of sustained unresponsiveness, suggesting that misclassification of peanut allergy was unlikely.

Participants who achieved 2-week sustained unresponsiveness after 18 months of study treatment were instructed to incorporate peanut into their normal diet ad libitum, without predefined ingestion frequency or amounts, which is generally the advice given to patients who have naturally outgrown their food allergy. Consistent with the general view, we do not consider ad libitum peanut ingestion to be the same as continuing active peanut oral immunotherapy. The proportion of PPOIT-treated participants who were ingesting peanuts 4 years after treatment compares favourably with those from other post-treatment follow-up studies of milk and egg oral immunotherapy.[32,33] 80% of participants who attained 2-week sustained unresponsiveness after PPOIT treatment were ingesting peanut ad libitum 4 years after treatment, with 60% tolerating moderate-to-large amounts (>2 g peanut protein). Few studies of sustained unresponsiveness years after cessation of oral imunotherapy are available for comparison, but the proportion of patients achieving sustained unresponsiveness at 4 years is more than double that achieved immediately after oral immunotherapy alone in most studies, suggesting that addition of the probiotic could enhance the tolerance-inducing capacity of oral immunotherapy.

An important finding was that reactions to intentional peanut ingestion were uncommon in those who had attained sustained unresponsiveness at the end of the parent study and who had been instructed to ingest peanut freely. The frequency and severity of reactions in PPOIT-treated participants were similar to those after accidental ingestion in placebo-treated participants, suggesting that PPOIT-induced sustained unresponsiveness offers an equivalent safety profile to peanut avoidance. Furthermore, PPOIT-treated participants who maintained their sustained unresponsivess status did not report any allergic reactions to peanut in the preceding 4 years. By comparison, 24 (75%) of 32 children who were desensitised after milk oral immunotherapy reported reactions in the preceeding 12 months when assessed 4–5 years after treatment cessation, with anaphylaxis in six (25%).[32] These findings suggest that sustained unresponsiveness is a preferred outcome for individuals with food allergy. Further studies are needed to establish the safety of continuing peanut ingestion in patients who initially achieve sustained unresponsiveness but subsequently have symptoms after ingestion. Investigation of long-term quality of life in people who achieve sustained unresponsiveness or desensitisation compared with those who do not achieve such outcomes is also important.

Reactions to accidental peanut ingestion in placebo participants were infrequent and reaction severity tended to be mild. This finding contrasts with published reports of retrospective studies,[34–36] which describe accidental reactions in 50% of children with peanut allergy within 1–2 years, and 75% within 3–5 years, with roughly 50% of these reactions involving the respiratory system. In both the parent study and this follow-up study, we systematically gathered information about reactions and calculated the number and severity of reactions over a fixed period in all participants. This procedure probably increased the accuracy of information gathered but could also have modified participant behaviour, leading to improved vigilance and early management of allergic reactions. During the parent study, allergy nurses reviewed the symptoms and emergency management of

allergic reactions with each participant or their parents, or both, at each study visit (every 2 weeks for at least 8 months, then every month for up to 10 months). This intensive education probably provided trial participants and their caretakers with increased knowledge, confidence, and ability to avoid allergens and recognise and treat allergic symptoms, which could have led to fewer and less severe reactions. This intensive education is the most likely explanation for the low frequency of accidental peanut ingestion and mild reactions reported by participants in the placebo group, and consistent with the common observation that participation in a clinical trial leads to improved outcomes for placebo participants, highlighting the importance of including a placebo group. However, another plausible explanation is that published data for rates and severity of accidental reactions might not be generalisable to the wider population or to our select study population.

We did not establish whether the three PPOIT-treated participants who attained 2-week sustained unresponsiveness at the end of the parent study but did not achieve 8-week sustained unresponsivess at 4 years had become resensitised or had never attained true sustained unresponsiveness in the first place. Perhaps these participants would not have passed an 8-week sustained unresponsiveness food challenge at the end of the parent study despite passing the 2-week challenge, because the likelihood of passing a sustained unresponsiveness food challenge reduces with increasing periods of secondary allergen elimination.[19,37] Alternatively, initial sustained unresponsiveness might have been lost during the subsequent 4 years, a possibility that is supported by the finding that reactions to peanut occurred more than 12 months after stopping treatment despite continued peanut intake. Future studies of 8-week sustained unresponsiveness immediately after treatment and again years later will clarify this issue.

Probable reasons for participants to decline to participate in the placebo-controlled double-blind food challenge include potential inconvenience, risk of severe allergic reaction, and perceived risk of losing the ability to ingest peanut. Regular intake is known to maintain a desensitised state. Thus, many PPOIT-treated participants who had attained sustained unresponsiveness at the end of the parent study and were ingesting peanut declined to participate in the follow-up study food challenge (data not shown). Conversely, some participants welcomed the opportunity to undergo a food challenge to clarify whether they had maintained sustained unresponsiveness or were only desensitised. We suggest that sustained unresponsiveness as confirmed by placebo-controlled, double-blind food challenge should be used to monitor long-term clinical effectiveness in oral immunotherapy trials.

Although our results suggest long-lasting modulation of the peanut-specific immune response with reduction in typical allergy markers and increased sIgG4, these changes have also been reported with desensitisation in the absence of tolerance,[18] emphasising the need to identify accurate and reliable biomarkers to distinguish desensitisation from sustained unresponsiveness.

To conclude, our results suggest that PPOIT is effective at inducing long-term sustained unresponsiveness that persists for up to 4 years after completing treatment and is safe. Furthermore, the finding that sustained unresponsiveness was maintained without the need to follow a regular prespecified ingestion schedule provides a compelling argument that PPOIT-induced immune tolerance. Ours is the first study to show prolonged 8-week sustained unresponsiveness several years after treatment has ceased, and suggests the possibility that tolerance is a realistic target for food allergy treatments. In a future study, microbial composition of stool samples will be analysed to examine effects of PPOIT therapy on the gut microbiome. Furthermore, a three-group, multicentre randomised controlled trial (ACTRN12616000322437) of PPOIT versus peanut oral immunotherapy versus placebo is underway to address the important and as-yet unanswered question of whether the addition of a probiotic confers greater benefit than oral immunotherapy alone.

Contributors

K-CH contributed to study conception and design; managed the study; did the clinical procedures, mechanistic work, and statistical analysis; drafted the Article; and contributed to the final version. A-LP contributed to study design and conduct, statistical analysis, data interpretation, and writing of the final report. CA and SP did the clinical procedures, and contributed to and reviewed the final report. MLKT conceived the parent PPOIT randomised trial, developed the follow-up study protocol, was responsible for oversight of study conduct and data analyses, and contributed to writing of the Article as senior author.

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Declaration of interests

K-CH reports grants from Murdoch Childrens Research Institute and the Australian Food Allergy Foundation. A-LP reports grants from Murdoch Childrens Research Institute and the Australian Food Allergy Foundation (previously the Ilhan Food Allergy Foundation), and personal fees from the National Health and Medical Research Council. MLKT reports grants from the Australian Food Allergy Foundation and Murdoch Childrens Research Institute, and personal fees from Nestlé Nutrition Institute, Danone Nutricia, GLG Consulting, Deerfield Consulting, Bayer, Prota Therapeutics, and Wiley. She also has a licensed patent method for inducing tolerance. CA and SP declare no competing interests.

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References

1 Osborne NJ, Koplin JJ, Martin PE, et al. Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. *J Allergy Clin Immunol* 2011; **127:** 668–76.

2 Prescott S, Pawankar R, Allen K, et al. A global survey of changing patterns of food allergy burden in children. *World Allergy Organ J* 2013; **6:** 21.

3 Sicherer SH, Sampson HA. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol* 2014; **133:** 291–307.

4 Van der Velde JL, Flokstra-de Blok BM, de Groot H, et al. Food allergy-related quality of life after double-blind, placebo-controlled food challenges in adults, adolescents, and children. *J Allergy Clin Immunol* 2012; **130:** 1136–43.

5 Wassenberg J, Cochard MM, Dunngalvin A, et al. Parent perceived quality of life is age-dependent in children with food allergy. *Pediatr Allergy Immunol* 2012; **23:** 412–19.

6 Flokstra-de Blok BM, Dubois AE, Vlieg-Boerstra BJ, et al. Health-related quality of life of food allergic patients: comparison with the general population and other diseases. *Allergy* 2010; **65:** 238–44.

7 Avery NJ, King RM, Knight S, Hourihane JO. Assessment of quality of life in children with peanut allergy. *Pediatr Allergy Immunol* 2003; **14:** 378–82.

8 Davis KL, Mikita CP. Accidental ingestions in children with peanut allergy. *Pediatrics* 2007; **120:** S117–18.

9 Cherkaoui S, Ben-Shoshan M, Alizadehfar R, et al. Accidental exposures to peanut in a large cohort of Canadian children with peanut allergy. *Clin Transl Allergy* 2015; **5:** 16.

10 Umasunthar T, Leonardi-Bee J, Hodes M, et al. Incidence of fatal food anaphylaxis in people with food allergy: a systematic review and meta-analysis. *Clin Exper Allergy* 2013; **43:** 1333–41.

11 Dello Iacono I, Tripodi S, Calvani M, Panetta V, Verga MC, Miceli Sopo S. Specific oral tolerance induction with raw hen's egg in children with very severe egg allergy: a randomized controlled trial. *Pediatr Allergy Immunol* 2013; **24:** 66–74.

12 Meglio P, Giampietro PG, Carello R, Gabriele I, Avitabile S, Galli E. Oral food desensitization in children with IgE-mediated hen's egg allergy: a new protocol with raw hen's egg. *Pediatr Allergy Immunol* 2013; **24:** 75–83.

13 Yeung JP, Kloda LA, McDevitt J, Ben-Shoshan M, Alizadehfar R. Oral immunotherapy for milk allergy. *Cochrane Database Syst Rev* 2012; **11:** CD009542.

14 Burks AW, Jones SM, Wood RA, et al. Oral immunotherapy for treatment of egg allergy in children. *N Engl J Med* 2012; **367:** 233–43.

15 Varshney P, Jones SM, Scurlock AM, et al. A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response. *J Allergy Clin Immunol* 2011; **127:** 654–60.

16 Vickery BP, Pons L, Kulis M, Steele P, Jones SM, Burks AW. Individualized IgE-based dosing of egg oral immunotherapy and the development of tolerance. *Ann Allergy Asthma Immunol* 2010; **105:** 444–50.

17 Skripak JM, Nash SD, Rowley H, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 2008; **122:** 1154–60.

18 Vickery BP, Scurlock AM, Kulis M, et al. Sustained unresponsiveness to peanut in subjects who have completed peanut oral immunotherapy. *J Allergy Clin Immunol* 2014; **133:** 468–75.

19 Syed A, Garcia MA, Lyu SC, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol* 2014; **133:** 500–10.

20 Tang MLK, Hsiao K-C. An update on oral immunotherapy for the treatment of food allergy. *Paediatr Child Health* 2016; **26:** 304–09.

21 Greenhawt MJ. STOPping peanut allergy: the saga of food oral immunotherapy. *Lancet* 2014; **383:** 1272–74.

22 Jones SM, Burks AW, Keet C, et al. Long-term treatment with egg oral immunotherapy enhances sustained unresponsiveness that persists after cessation of therapy. *J Allergy Clin Immunol* 2016; **137:** 1117–27.

23 Tang ML, Ponsonby AL, Orsini F, et al. Administration of a probiotic with peanut oral immunotherapy: a randomized trial. *J Allergy Clin Immunol* 2015; **135:** 737–44.

24 Miettinen M, Matikainen S, Vuopio-Varkila J, et al. Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. *Infection Immunity* 1998; **66:** 6058–62.

25 Pessi T, Sütas Y, Hurme M, Isolauri E. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clin Exper Allergy* 2000; **30:** 1804–08.

26 Pohjavuori E, Viljanen M, Korpela R, et al. *Lactobacillus* GG effect in increasing IFN-γ production in infants with cow's milk allergy. *J Allergy Clin Immunol* 2004; **114:** 131–36.

27 Rautava S, Arvilommi H, Isolauri E. Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. *Pediatr Res* 2006; **60:** 221–24.

28 Rautava S, Kalliomaki M, Isolauri E. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol* 2002; **109:** 119–21.

29 DunnGalvin A, de BlokFlokstra BM, Burks AW, Dubois AE, Hourihane JO. Food allergy QoL questionnaire for children aged 0–12 years: content, construct, and cross-cultural validity. *Clin Exp Allergy* 2008; **38:** 977–86.

30 Du Toit G, Roberts G, Sayre PH, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy: correction. *N Engl J Med* 2016; **375:** 398.

31 Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Informatics* 2009; **42:** 377–81.

32 Keet CA, Seopaul S, Knorr S, Narisety S, Skripak J, Wood RA. Long-term follow-up of oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 2013; **132:** 737–39.

33 Paassilta M, Salmivesi S, Maki T, Helminen M, Korppi M. Children who were treated with oral immunotherapy for cows' milk allergy showed long-term desensitisation seven years later. *Acta Paediatr* 2016; **105:** 215–19.

34 Bock SA, Atkins FM. The natural history of peanut allergy. *J Allergy Clin Immunol* 1989; **83:** 900–04.

35 Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992; **327:** 380–84.

36 Skolnick HS, Conover-Walker MK, Koerner CB, Sampson HA, Burks W, Wood RA. The natural history of peanut allergy. *J Allergy Clin Immunol* 2001; **107:** 367–74.

37 Keet CA, Frischmeyer-Guerrerio PA, Thyagarajan A, et al. The safety and efficacy of sublingual and oral immunotherapy for milk allergy. *J Allergy Clin Immunol* 2012; **129:** 448–55.