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TITLE PAGE

Sequential hypoallergenic boiled peanut and roasted peanut oral immunotherapy

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Oral immunotherapy (OIT) using roasted peanut flour can effectively desensitize peanut-allergic children [1] but is considered not to be ready for clinical practice [2] due to high rates (≥45%) of adverse events (AEs) [3] [4]. This necessitates medically supervised up-dosing in hospital and limits the number of patients that can be treated. In 2001 Beyer et. al proposed that the prevalence of peanut allergy in China was lower than that of the Western world because peanuts consumed in China were boiled, not roasted [5]. They demonstrated that boiling peanuts for 20 minutes reduced IgE binding in vitro when compared to roasted peanut. We have subsequently shown that extended boiling progressively reduced peanut IgE binding to 12.5% at 2 hours and to 5.3% at 12 hours compared to raw peanut while still retaining T cell reactivity [6]. Further, inhibition ELISAs demonstrated that boiled peanuts have restricted ability (2-h ~70%, 12-h ~50%) to block the binding of patient IgE to raw peanut [6] suggesting boiled peanuts possess an incomplete repertoire of epitopes. This indicates that boiled peanuts alone are unlikely to expose a patient to the full spectrum of peanut epitopes and will therefore require a roasted peanut phase following the initial boiled peanut therapy. We hypothesize that AEs can be reduced by commencing OIT with hypoallergenic boiled peanut. Here we describe a pilot study that aims to characterize the incidence of AEs and successful desensitization in mild/moderate peanut allergic children using hypoallergenic 2-hour boiled peanut prior to roasted peanut OIT. Due to the home-based updosing procedure, a cautious approach was adopted which excluded severely allergic children.

The study was an open, non-randomised and non-controlled intervention trial. A consecutive sample of children matching the inclusion criteria attending the first author's allergy clinic at AllergySA, South Australia, were enrolled. Sample size was determined by the number of oral peanut challenges (OFC) that could be arranged in hospital over a 1 year study period. Inclusion criteria were a recent history of peanut allergy (mild to moderate reactions), skin prick test (SPT) 7-14 mm mean wheal diameter, and a positive OFC. Exclusion criteria were a history of severe anaphylaxis and/or SPT>14 mm. All OFCs to confirm allergic status were performed at Flinders Medical Centre, South Australia which involved administration of increasing doses of peanut butter, starting at 17.5 mg of peanut protein, given every 20 minutes until a definite and objective reaction was observed.

The 12-month protocol comprised a sequential 7-month initial phase of 2-hour boiled raw peanuts and a 5-month second phase of roasted peanuts. This pilot study used peanuts boiled for 2-hours as it was considered to be an appropriate balance between sufficient hypoallergenicity while still retaining allergens to commence desensitization. Boiled peanuts were prepared by boiling for 2 hours and then dehydrating for 24 hours as previously described [6]. Boiled and roasted peanut phases followed the same schedule for the first 18 weeks. Dosing began with 250 mg peanut daily (peanuts contain approximately 25% peanut protein), and frequency was increased weekly over the first three weeks from once a day to three times a day. From 4^{th} to 6^{th} week the dose was increased each week to 500 mg peanut three times a day. From 7^{th} to 9^{th} week it was increased to 1g peanut three times a day. From 10^{th} to 18^{th} week it was increased to 10 g peanuts per day in 3 divided doses, which became the maintenance dose.

Skin prick tests were performed using commercial raw peanut extract, and serum peanut-specific IgE and IgG_4 were measured by ImmunoCAP as previously described [6]. Skin prick test wheal sizes and serum antibody titres were recorded at baseline and completion of each OIT phase. Differences at each time-point were evaluated using Wilcoxon signed ranks test. Statistical comparisons were undertaken using Stata 14 (Stata Corp, College Station, Texas). All tests were 2-sided with an alpha level of 0.05. Precautionary measures included (1) not to ingest peanuts with empty stomach, (2) avoid exercise 2-hours before and after peanut ingestion, (3) temporary pause of OIT during sickness (up to 1 week). On resuming OIT, the "restarting" dose was set at half the previously tolerated amount and gradually raised back to original dose over 1-2 weeks.Ethics approval was obtained from Southern Adelaide Clinical Human Research Ethics Committee (approval number 473.13). The trial was registered with Australian New Zealand Clinical Registry (Trial ID ACTRN12614000919617).

There were 15 children who satisfied the inclusion criteria. One child immediately withdrew from the study at parental request after receiving adrenaline following the OFC. 14 children commenced biphasic peanut OIT. The age, sex and need for adrenaline at OFC for each participant are shown in Table 1. Maximum eliciting doses at OFC ranged from 200 mg to 2500 mg peanut butter. Two more children withdrew during the boiled-peanut phase. One child withdrew because of refusal to ingest more than 250 mg boiled peanut per dose (despite absence of allergic symptoms). A second child withdrew because of social issues impacting on treatment adherence. The remaining 12 children all completed ingestion of boiled peanuts and advanced to roasted peanuts. At the end of full OIT, 11 reached the target dose of 10 roasted peanuts daily and continued on this dose until OFC. One child stopped while eating 8 roasted peanuts per day, after being infected with Dengue Fever. At the end of the pilot study, 11 children were witnessed to ingest 10 roasted peanuts at the first author's office without reaction within 4 weeks of completion of OIT.

All AEs are documented in Table 1. During the boiled peanut phase, three out of 12 children experienced mild AEs but proceeded to complete the OIT. One child reported 3 episodes of mild upper lip angioedema after eating ¼ boiled-peanut in the first 3 days with no medication required. A second child experienced two isolated episodes of urticaria, which responded to oral antihistamine. A third child experienced recurrent itchy mouth, mild abdominal discomfort and lip swelling while ingesting 250 mg boiled-peanut, but all symptoms resolved when the dose was reduced to 60 mg of boiled-peanut and then gradually increased back to 250 mg. No medication was required. During the roasted-peanut phase two AEs were reported. One child experienced mild abdominal pain with a brief emesis while travelling home after ingestion of first dose of 250 mg roasted-peanut, and was treated with a single dose of oral antihistamine. A second child reported a brief sensation of oral swelling that was not evident visually on day 3 after taking 250 mg roasted-peanut, with no treatment required.

The boiled treatment phase was associated with a 41% reduction in SPT wheal diameter and an increase of 9.5 fold in IgG_4 levels. The roasted peanut phase further reduced the SPT wheal diameter by 28%. These changes were statistically significant (Table 1 and Figure 1). While IgG_4 increased following the roasted peanut phase this was not statistically significant. No significant change was observed in peanut-specific IgE following either phase.

This study is the first to utilize boiled peanuts within a structured desensitization regimen prior to oral desensitization using roasted peanuts. Turner et. al have previously reported in a research letter [7] a case series of 4 pediatric patients with either confirmed or presumed peanut allergy where 3 were treated with boiled peanuts and achieved variable tolerance. Boiling preparation varied from 2-16 hours at initiation. One patient described was treated with boiled peanuts and "over the course of 2 years she was transitioned to daily raw peanut". Two further patients were reported to have only received boiled peanut, with a fourth untreated. Raw peanut OFCs to confirm allergy were performed on only 2 patients, with a further patient tolerating a boiled peanut OFC. Post treatment raw peanut OFC was only performed on 1 patient. The lack of a consistent treatment regimen and description of adverse events prevents the assessment of the efficacy and safety of boiled nut treatment in that study. Our study differs in that all patients had OFCs to confirm allergy and a standardized regimen of boiled peanut desensitization was utilized, followed by a standardized sequential roasted peanut desensitization phase. In addition, Turner et. al made conclusions regarding post treatment-related changes to peanut specific IgE-reactivity by comparison to an unrelated control, rather than comparing pre and post treatment as we have done in this study.

Our data indicate that children with peanut allergy can be desensitized to roasted peanut with few adverse events by using hypoallergenic peanut prior to roasted peanut OIT. The apparent protective effect of boiled peanut in reducing adverse reactions to subsequent roasted peanut OIT has potential clinical significance. The finding that boiled peanut OIT reduces SPT wheal size to raw peanut extract and increases production

of peanut-specific IgG_4 is also novel. This indicates that the boiled peanuts are immunologically active and provides a biological basis for the clinical observations. Desensitization to the ingestion of 8-10 roasted peanuts occurred in 12/14 children who commenced the biphasic OIT. This is considerably more successful than current data pertaining to roasted peanut OIT alone. The biphasic regimen used in our study avoided any hospital-based supervision. This contrasts with current best practice for roasted peanut OIT which mandates hospital supervision. This observation suggests, within study limitations, the potential for greater cost effectiveness compared to stand-alone roasted peanut OIT.

There are limitations to our data however, and our findings should be interpreted with caution. The initial OFC was not double-blinded, and children with more severely reactive SPTs or anaphylaxis history were excluded. Furthermore, the study was not randomized with a control group. Hence selection bias cannot be excluded and no comment can be made regarding efficacy in more severely allergic children. Until these limitations are addressed in future clinical trials e.g. {ref: https://clinicaltrials.gov/ct2/show/NCT02149719 and trial ID: ACTRN12617000803392}, the role of boiled peanut OIT in clinical practice requires clarification. We suggest that adequate characterization and quality control of the range and nature of allergens induced by boiling will be important in clinical trials. In summary, the combination of graded dose hypoallergenic boiled peanuts followed by a similar regimen using roasted peanuts has the potential to optimize the safety and efficacy of peanut OIT.

Study I.D.	Symptoms at OFC	Ara h 2 (kU/L)	Skin Prick Test (mm)			Peanut-Specific IgE (kU/L)			Peanut-Specific IgG_4 (mg_A/l)		
			BL	BP	RP	BL	BP	RP	BL	BP	RP
1[#]	a,b,c	5.67	14	6	2	8.2	9.1	3.42	0.45	13.2	43.9
2**[#]	a,g	0.56	14	15	12	0.41	0.69	0.59	0.01	0.01	0.04
3*	a,c,d	1.05	11	5	4	4.2	4.1	2.2	0.16	2.34	3.53
4	a,c	3.61	13	9	5	19	19	12	0.16	0.18	0.49
5*[#]	a,b,c,f	>100	11	7	4	>100	>100	>100	1.98	11.4	21.7
6	a,b	0.56	7	6	NA	0.37	0.35	NA	0.05	0.08	NA
7*	a	60.4	14	8	5	>100	>100	>100	1.24	11.4	9.24
8[#]	a,d,b,f	11.1	12	7	5	11	12	5.32	0.2	1.52	0.84
9[#]	b,f	0.53	14	7	4	0.65	NA	0.46	0.13	NA	0.45
10**[#]	b,c,e	4.01	8	5	6	11	8.8	10	0.3	0.38	1.4
11[#]	a,b,c,f	9.81	12	6	4	12	27	13	0.15	3.41	23.7
12	a,b,c	0.75	10	8	5	2.4	4.8	5.1	0.16	1.18	9.3
13[W]	a,b,e	0.68	9	NA	NA	2.6	NA	NA	0.16	NA	NA
14[W#]	a,b,f	64.7	14	NA	NA	94	NA	NA	0.7	NA	NA
Median		3.81	12	7	5	9.6	9.1	5.32	0.16	1.52	3.53

Legend
OFC: clinical signs and symptoms during OFC, Ara h 2: Ara h 2-specific IgE at enrolment, [#] given epinephrine following OFC, * AE while ingesting boiled peanut, ** AE while ingesting roasted peanut, [W] withdrawn while eating boiled peanuts, NA: not available.
a: itchy palate and throat, urticaria, lip, eyelid or face swelling, b: persistent abdominal pain with or without vomiting, c: angioedema of tongue, chest or throat tightness, d: tachycardia, signs of dyspnea, e: persistent coughing, f: looking pale or unwell, becoming drowsy or complaining of dizziness, sweating, g: not responding to oral antihistamine and steroid.

Table 1: Boiled-to-roasted OIT reduces SPT wheal size and increases psIgG4 with unchanged psIgE. SPT wheal size, psIgE and psIgG4 were measured at baseline (BL), post-boiled-peanut OIT (BP) and post-roasted-peanut OIT (RP). *AE during boiled peanut phase, **AE during roasted peanut phase; no participant had both. Both participants with psIgE >100 had AE during boiled peanut phase but not roasted peanut phase.

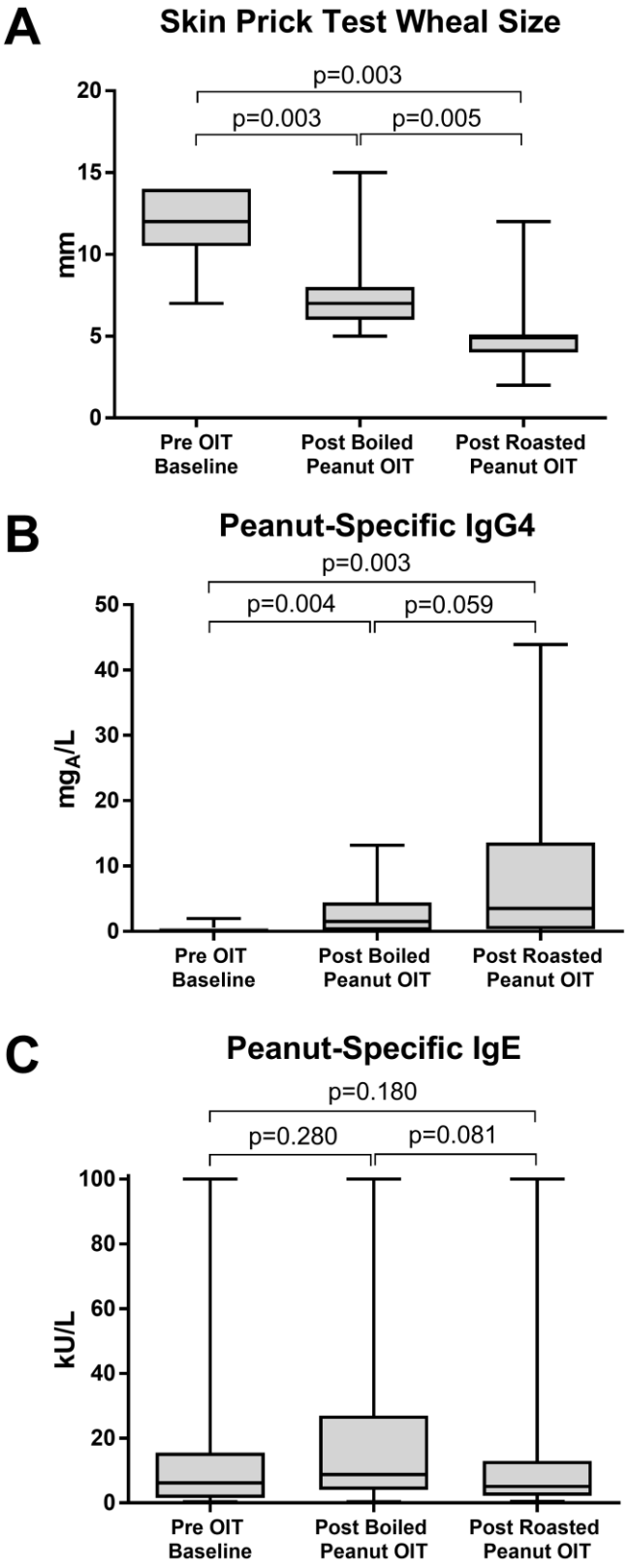

Figure 1: Boiled-to-roasted OIT leads to statistically significant decrease in SPT wheal size (A), statistically significant increase in IgG4 (B) but no significant change in psIgE (C) at the end of each phase.

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