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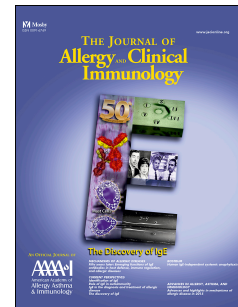
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Patterns of tree nut sensitisation and allergy in the first 6 years of life in a population-based cohort.

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Abstract:

Introduction: Longitudinal population-based data regarding tree nut allergy are limited.

Objectives: To determine population prevalence of tree nut allergy at age 6 years, and explore the relationship between egg and peanut allergy at age 1 year and development of tree nut allergy at age 6 years.

Methods: A population-based sample of 5,276 children were recruited at age one and followed up at age 6 years. At age 1 allergy to egg and peanut were determined by oral food challenge and parents reported their child's history of reaction to tree nuts. Challenge-confirmed tree nut allergy was assessed at age 6.

Results: At age 1, prevalence of parent-reported tree nut allergy was 0.1% (95% CI 0.04-0.2). Only 18.5% of infants had consumed tree nuts in the first year of life. At age 6, challenge-confirmed tree nut allergy prevalence was 3.3% (95%CI 2.8-4.0), with cashew the most common (2.7%, 95% CI 2.2-3.3).

Of children with peanut allergy only at age 1, 27% (95%CI 16.1-39.7) were tree nut allergic at age 6, compared with 14% (95%CI 10.4-17.9) of those with egg allergy only and 37% (95%CI 27.2-47.4) of those with both peanut and egg allergy.

Conclusions: Tree nut allergy is uncommon in the first year of life, likely due to limited tree nut consumption. At age 6 years, tree nut allergy prevalence is similar to peanut allergy prevalence. Over a third of children with both peanut and egg allergy in infancy have tree nut allergy at age 6. Understanding how to prevent tree nut allergy should be an urgent priority for future research.

Key words: food allergy, sensitisation, tree nut allergy, prevalence, population

Abbreviations:

OFC - oral food challenge

SPT - skin prick test

Clinical Implications

Up to 48% of those with food allergy at age 1 were found to be tree nut sensitised and more than a third of those tree nut sensitised had developed tree nut allergy at age 6 years.

Capsule Summary:

This study presents population prevalence of tree nut allergies in children and describes the relationship between early tree nut sensitisation and food allergy type, and development of tree nut allergy at age 6 years.

Introduction:

Tree nut allergies are usually life-long and together with peanut allergy are the most common cause of food-induced anaphylaxis and related fatalities^{1, 2}. Unlike peanut allergy, population-based data regarding tree nut allergy is limited.

We recently reported that 2.3% of 10-14 year old Australian children had clinic-confirmed tree nut allergy³, however prevalence estimates using challenge confirmation remain limited under the age of 10 years. To date, studies in younger children of challenge-confirmed food allergy outcomes have been limited to regions reporting very low overall rates of food allergy (less than 1%)⁴⁻⁶ or where low numbers of tree nut food challenges were performed⁷.

Development of tree nut allergy in childhood is also understudied with little understanding of the role of early tree nut sensitisation and food allergy type. The first presentation for children with food allergy is often via reactions to peanut or egg in infancy. Children with peanut allergy are thought to be at increased risk of tree nut allergies, with around 30% of paediatric patients presenting with peanut allergy reported to have allergies to tree nuts⁸⁻¹². To date, no study has explored this clinical observation at the population-level nor systematically assessed the association using protocolised challenges. The current clinical dilemma remains: what should be done regarding tree nut allergy testing and introduction advice for those with either peanut allergy or other forms of food allergy in infancy?

The objectives of this study were to estimate the population prevalence of clinic-confirmed tree nut allergies during the first 6 years of life and describe the patterns of co-existing allergies to peanut and other tree nuts. We also aimed to explore the relationship between food allergy at age 1 year and the subsequent development of tree nut allergy at age 6 years.

Methods:

The HealthNuts study is a population-representative, longitudinal study of 5276 children recruited at age 1 and followed up to age 6 years. The study methods have been described in detail previously¹³⁻¹⁵. To summarise, between 2007 and 2011, 5276 infants aged between 11-15 months were recruited from immunisation clinics around Melbourne, Australia. At recruitment all infants underwent skin prick testing (SPT) to egg, peanut and sesame and parents completed a questionnaire. The first half of the cohort additionally had SPT to shrimp, while the second half of the cohort had SPT to cow's milk¹³. SPTs were performed with single-tine lancets (Stallergenes, Antony, France) on the infant's back using allergens from ALK-Abello (Madrid, Spain), along with a positive control (10mg/ml histamine) and a negative control (saline). Wheal size was measured after 15 minutes and calculated by subtracting the negative control from the average of the longest diameter and the diameter perpendicular to it. Parent report of history of allergic reactions in the first year of life was determined via questionnaire.

All children who showed any reaction on SPT (wheal size ≥ 1 mm), as well as a random sample of those who were SPT negative, were invited to attend a study clinic at the Royal Children's Hospital for repeat SPT and OFC. At age 1 year, OFC were performed only for egg, peanut and sesame.

Those attending clinic had additional SPT performed for tree nuts (almond, cashew and hazelnut). No OFC were performed for tree nuts at age 1. For those who were SPT negative to tree nuts home introduction was advised. For those who were SPT positive avoidance was recommended.

Age 4 and 6 years follow up methods have been described previously¹⁴. To summarise, at age 4 years all participants were followed up via questionnaire and those who reported a new food allergy reaction, and those who had any food allergy at age 1 year, were invited for clinic assessment that included skin prick testing and oral food challenges.

At age 6 years the entire cohort (n=5276) were invited to participate in questionnaire and SPT assessment. Questionnaires were mailed to all participants capturing demographic details, history of food allergy and new food reactions, common allergen exposure information, history of asthma/wheeze and eczema. All participants were invited for an allergy/health assessment that included SPT to a predetermined panel of 8 foods (milk, egg, peanut, wheat, sesame, cashew, almond, and hazelnut) and was conducted either in the child's home or at the Royal Children's Hospital. Those with positive SPT (>1 mm) or parent-reported reactions to foods consistent with an IgE-mediated allergy were invited for a clinic appointment with a specialist allergy nurse and OFC were conducted when indicated by a standardised protocol (refer to supplementary figure 1). Those

who were sensitised to almond, cashew or hazelnut had additional tree nut SPT performed to all non-tolerated tree nuts at the second clinic visit including Brazil nut, macadamia, pecan, pistachio, and walnut. OFC were conducted as previously described¹⁶ and deemed positive if they met at least one of the following predefined criteria: (1) three or more concurrent non-contact urticaria lasting at least 5 minutes; (2) severe persistent vomiting; (3) peri-oral or peri-orbital angioedema; or (4) anaphylaxis (evidence of circulatory or respiratory involvement), within 2 hours of the last challenge dose in the presence of IgE sensitisation.

Definitions:

Age 1 year definitions

Sensitised tolerant to egg, peanut or sesame: SPT $\geq$ 2mm and negative OFC to that food.

Egg, peanut or sesame allergy: SPT: $\geq$ 2mm and positive OFC to that food.

Milk allergy: SPT $\geq$ 2mm and history of reaction consistent with IgE-mediated food allergy.

Parent-reported tree nut allergy: parent report of a reaction consistent with IgE-mediated food allergy to one or more tree nuts (any acute onset of skin rash, facial swelling, vomiting or breathing difficulties within 1 hour of food ingestion).

Tree nut sensitisation: SPT $\geq$ 3mm to almond, cashew or hazelnut.

Tree nut tolerance: history of tolerance on ingestion or negative SPT when undertaken.

Age 6 years definitions

Tree nut sensitisation: SPT $\geq$ 3mm to almond, Brazil nut, cashew, hazelnut, macadamia, pecan, pistachio or walnut.

Definite tree nut allergy: Any of the following: (1) positive OFC and IgE sensitised at 6 years; (2) history of objective reaction in the past 12 months consistent with HealthNuts OFC stopping criteria following definite exposure to the food of interest and evidence of IgE sensitization at 6 years; or (3) positive OFC at age 4 years and SPT $\geq$ 8mm at 6 years of age.

Probable tree nut allergy: Any of the following: (1) SPT $\geq$ 8mm but no age 4 OFC or recent reaction history, and no known tolerance; or (2) SPT 3-7mm at age 6 and one of the following a) positive OFC at age 4, b) history of objective reaction >12 months ago consistent with HealthNuts OFC stopping

criteria following definite exposure to the food of interest, or c) parent-report avoiding food due to allergy.

Tree nut tolerant: Any of the following (1) negative OFC; (2) SPT 0-2mm; (3) SPT 3-7mm and parent reported ingestion history (eaten >1 time since age 4); or (4) no reaction since age 4 and no parental report of avoiding.

Statistical Methods:

The prevalence of tree nut allergy was calculated among those that completed an allergy assessment at age 6 years (n=3232) and limited to the tree nuts included in the screening SPT panel (cashew, hazelnut and almond). Those who were SPT negative were deemed tree nut tolerant. To assess whether these estimates were influenced by characteristics that were associated with participation in allergy assessment at age 6 years, we adjusted for differences in risk factors between participants with and without missing data at age 6 years using the inverse probability weighting method described by Little and Rubin¹⁷. This reweighting was used to reflect the distribution of risk factors among those approached but did not participate versus those who underwent a full allergy assessment. The weights were the inverse of the predicted probability of participation obtained after fitting a logistic regression model including co-variate risk factors that were associated with completing an assessment, rather than questionnaire only or non-participation (socioeconomic status, family history of allergy, parent country of birth, and whether the child had challenge-confirmed food allergy or eczema at 1 year). This generated a propensity score for each participant¹⁷. As a sensitivity analysis tree nut allergy prevalence was calculated as the number of children with tree nut allergy (definite or probable tree nut allergy) to one or more tree nuts, expressed as a proportion of the entire HealthNuts cohort (n=5276). It was assumed that those with no SPT data and no known food allergy were tree nut tolerant. This provides the most conservative prevalence estimate.

All prevalence estimates are reported as the observed proportion with 95% confidence intervals calculated using the normal approximation to the binomial distribution.

The proportion of those with nut allergies (definite and probable allergy) with co-allergy to other tree nuts was calculated. As a sensitivity analysis this proportion has also been calculated with all those sensitised between 3-7mm that did not have an OFC performed included as allergic. Both calculations have been reported.

Ethics

191 Ethics approval was obtained for the HealthNuts study from the Victorian State Government Office
192 for Children (reference no. CDF/07/492), the Victorian State Government Department of Human
193 Services (reference no. 10/07), and the Royal Children's Hospital Human Research Ethics Committee
194 (reference no. 27047).

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Results:

HealthNuts study participation

An overview of the HealthNuts study is provided in Figure 1. A total of 5276 one-year-old infants participated in the HealthNuts study at age 1 year (74% participation). Of these, 924 were SPT positive to egg, peanut, sesame or shrimp/cow's milk, attended OFC clinic and had SPT to tree nuts (cashew, almond, hazelnut). An additional 193 negative controls attended clinic for SPT. At age 1 year, 530 participants were diagnosed with OFC-confirmed food allergy to egg, peanut or sesame.

At age 6 years, 84.4% of the cohort participated in follow up, with 61.3% (n=3232) completing both a questionnaire and allergy assessment including tree nut SPT and 23.1% (n=1222) completing a questionnaire only. Participants with a family history of allergy, and children with eczema or food allergy at age one, were more likely to participate in the age 6 follow up (Table 1).

Parent-reported tree nut allergy at age 1 year

At age 1 year, 6 parents reported a reaction to tree nut consistent with an IgE-mediated food reaction, representing an overall prevalence of 0.1 % (95% CI 0.04, 0.2) among the whole cohort of 5276 infants. The low prevalence of reactions may be due to the low consumption of tree nuts in the first year of life, with only 18.5% of parents reporting that their infant had consumed any tree nut by age 1 year.

Tree nut sensitisation at age 1 year among those with food allergy

Among those with challenge-confirmed food allergy at age 1 year, 31%, (95% CI 26.6, 34.7) were sensitised to one or more tree nuts. Tree nut sensitisation was less common in infants who were sensitised to one or more foods but not allergic (sensitised tolerant) (12% [95%CI 9.4, 16.6]) and in infants with no food sensitisation (5.2% [95% CI 2.7, 9.3]) (Table 2).

Tree nut sensitisation was more common in infants with both peanut and egg allergy (48.4% [95%CI 38, 58.9]) compared to infants with single egg or peanut allergies 23.6% (95% CI 19.7, 28.9) and 33.3% (95%CI 21.7, 46.7) respectively (Table 2).

Tree nut allergy at age 6 years

Tree nut allergy prevalence: At age 6 years, 234 children were sensitised and 154 children allergic to one or more tree nuts. Of those who had a skin prick test to tree nuts at age 6 years (n=3232), the observed prevalence of tree nut sensitisation was 7.3% (95%CI 6.4, 8.3) and tree nut allergy was 4.3% (95% CI 3.8, 5.2) (Supplementary Table 3). After re-weighting this estimate for differences in characteristics of individuals who did and did not participate in assessments at age 6 years, the

weighted prevalence of tree nut allergy was 3.3% (95%CI 2.8, 4.0). Cashew was the most common tree nut allergy (2.7%; 95% CI 2.2, 3.3) followed by hazelnut (0.9%, 95%CI 0.7, 1.3) then almond (0.3%, 95% CI 0.1, 0.5) (Figure 2).

All other individual tree nut allergies were diagnosed in less than 1.0% of participants (Pistachio n=50, Walnut n=28, Macadamia n=12, Pecan n=8, Brazil nut n=5) (Table 3).

Among the whole cohort of 5,276 children, the prevalence of tree nut sensitisation was 4.4% (95%CI 3.9, 5.0) and tree nut allergy was 3.1% (95%CI 2.6, 3.6). This estimate is likely to be conservative, since it assumes that all children who were lost to follow up were not tree nut allergic.

Collectively, tree nut allergy prevalence (3.3%) was similar to peanut allergy prevalence (2.8% 95% CI 2.4, 3.3).

Co-allergy patterns among the tree nuts: Co-allergy patterns among those with any nut allergy are outlined in Table 3. Of the 154 children with any tree nut allergy at age 6 years, 42.9% (n=66) also had peanut allergy at age 6 years. Eighty-four (52.2%) were allergic to only one tree nut, 26.7% to two tree nuts, 12.4% three tree nuts and 8.7% more than three tree nuts. Of those with cashew allergy 36% had co-existing pistachio allergy and if all those with pistachio sensitisation between 3-7mm that did not have an OFC were deemed allergic this increased to 46%.

Tree nut co-allergy patterns for those with peanut allergy: Of the 147 children with peanut allergy at age 6 years, 45% also had one or more tree nut allergies. The most common tree nut co-allergy for those with peanut allergy at age 6 years was to cashew (36.7%) (Table 3).

Tree nut allergy at age 6 years among children with egg or peanut allergy in infancy

Of those with peanut allergy only at age 1, 27% were tree nut allergic at age 6 years compared to 14% of those with egg allergy only. A higher proportion (37%) of those with both peanut and egg allergy at age 1 year were tree nut allergic at 6 years (Figure 3).

Tree nut sensitisation at age 1 year and development of tree nut allergy at age 6 years

Of 168 children who were sensitised to cashew at age 1, 39% were cashew allergic and 35% were cashew tolerant at age 6 years (Table 4). Of those sensitised to almond at age 1 (n=87), 11% were almond allergic and 59% were almond tolerant, while of those sensitised to hazelnut (n=72), 19% were hazelnut allergic and 53% hazelnut tolerant at age 6 years

Discussion:

This is the first population-based longitudinal study to characterise food-challenge confirmed tree nut allergy in childhood. It is also the first population-based study to characterise the development of tree nut allergy among children with infantile egg and peanut allergy. We found the prevalence of tree nut allergy at age 6 years (3.3%) to be as common as peanut allergy (2.8%), with cashew the most common individual tree nut allergy (2.7%). At age one year, 41% of those with challenge-confirmed allergy to egg or peanut were already sensitised to one or more tree nuts and those with both allergies were at highest risk for tree nut sensitisation. Cashew was the most common tree nut sensitisation at age 1 year and around 40% of children sensitised to cashew at age 1 were allergic to cashew at 6 years of age. Almost half (48%) of the children with both peanut and egg allergy at 1 year of age had tree nut allergy at age 6 years.

The strengths of the HealthNuts study are the large population-representative sample, high participation fraction and good internal and external validity¹³. The age 6-year follow up had high cohort retention (>80%). Most tree nut allergy outcomes were clinically confirmed based on objective criteria, with outcomes based on predetermined objective stopping criteria, and the remainder determined by large SPT wheal sizes and history of reported objective adverse reactions consistent with IgE-mediated food allergies.

Limitations included use of open oral food challenges rather than double-blind-placebo controlled OFCs, although only objective criteria were used to define a positive challenge and nurses were blinded to SPT wheal size and history of previous reaction. Not all study participants had SPT or nut consumption data available at age 6 years. Almond, cashew and hazelnut had more complete screening and there is limited SPT and OFC for the additional tree nuts. Most of those deemed allergic to Brazil nut, macadamia, pecan, pistachio and walnut are based on high-level sensitisation (SPT>8mm) not OFC and many of those with mid-range sensitisation (3-7mm) did not have OFC performed. This limits the co-allergy patterns reported and is an important factor to consider for tree nut allergy studies in the future. In addition, there is also likely to be an allergic bias in participation and follow up at age 6. Therefore we have reported a range of more and less conservative observed prevalence estimates and a population prevalence estimate for tree nut allergy re-weighted for factors that were associated with participation at age 6 years.

There are few prevalence studies reporting challenge-confirmed outcomes for tree nut allergy. We recently undertook a systematic review of tree nut allergy prevalence internationally and found estimates ranged from 0.05%-4.9%. However there was significant study heterogeneity resulting from differences in study design and method of diagnosis. We found 7 studies in children reporting challenge confirmed tree nut outcomes ranging from 0-1.4%¹⁸. Most of these estimates come from

countries or regions with low overall food allergy prevalence⁴⁻⁶. Our SchoolNuts study of 10,000 10-14 year olds reported clinic defined tree nut allergy of 2.3%, utilising the same OFC protocols as the current HealthNuts study³. The slightly lower prevalence in the SchoolNuts study may reflect the older age group studied.

Estimates of allergy prevalence for individual tree nuts are also limited. Our systematic review found high regional variation with European studies reporting hazelnut as the most common tree nut allergy, largely due to the high rate of birch pollen allergy and its cross-reactivity with hazelnut. In the UK Brazil nut was reported as the most common tree nut allergy and walnut and cashew in the US. Here, we found cashew as the most common tree nut sensitisation at age 1 and allergy at age 6. Our Australian SchoolNuts study also reported cashew as the most common tree nut allergy in 10-14 year olds and the most common tree nut trigger for food-induced anaphylaxis³. The overall nut co-allergy prevalence reported in our study (45%) was higher than the population representative SchoolNuts study reporting 30% of 10-14 year olds with peanut allergy having one or more tree nut allergies and 30% of those with a tree nut allergy having one or more additional tree nut allergies. Several single centre allergy clinics have also reported similar rates of co-allergy to one or more tree nuts among peanut allergic children^{10, 11, 19, 20}, however Fleischer and colleagues reported a higher rate of co-existing peanut allergy (68%) among 190 children with tree nut allergy in 2005², which may indicate that children with multiple food allergies were over represented in their clinics.

Cashew-pistachio and walnut-pecan belong to the same botanical families (Anacardiaceae and Juglandaceae respectively) and have a reported high degree of serological cross reactivity^{8, 21} and co-allergy. The NutCracker study, a single centre, prospective cohort study of 83 children with tree nut allergy in Israel reported two thirds of those with walnut and cashew allergy were also allergic to pecan and pistachio respectively, while all those with pecan and pistachio allergy were allergic to walnut and cashew, respectively²². Andorf and colleagues, in a study of 60 selected multi-food allergic patients reported all those allergic to walnut had co-existing pecan allergy. They reported a uni-directionality of the co-allergies with only two thirds of those patients allergic to walnut and cashew allergic to pecan and pistachio respectively suggesting that some allergenic proteins are shared while others are unique to cashew and walnut resulting in mono-allergy²³. We found a lower proportion of those with cashew allergy having co-existing pistachio allergy. We did not find pecan-walnut co-allergy to be as common as other studies. The differences observed may be due to the limitations of tree nut screening and OFCs in our study as although a smaller study (n=87) the NutCracker study did complete OFC for all sensitised tree nuts which we were not able to achieve. The observed differences may also to be due to regional differences.

Data on rates of early tree nut sensitisation are limited. In 2005, Clark and Ewan reported that by 2 years of age 19% of those with peanut allergy were sensitised to one or more nuts. This was a single centre allergy cohort limited to 47 patients with peanut allergy only. We have reported a markedly higher rate of tree nut sensitisation among those with peanut allergy in our population cohort of 33.3%. We also report for the first time a high rate of tree nut sensitisation among all those with food allergy at age 1 year, not just peanut allergy. Consideration should be given to identifying this high-risk group of infants in the general population in order to activate allergy prevention strategies.

This study has found that at 6 years of age collectively tree nut allergy is almost as high as peanut allergy, with cashew the most common tree nut allergy. Up to half of those with egg and peanut allergy can be sensitised to tree nuts as early as one year of age, therefore tree nut SPT screening has the potential to impose a significant burden on allergy clinics to confirm allergy status for each individual tree nut. Evidence that tree nut allergy can be prevented might be required before making recommendations to identify and target children at high risk of tree nut allergy early in life.

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Figure Legend

Figure 1. Overview of HealthNuts study participation, age 1 and 6 years

Figure 2. Tree nut sensitisation and allergy prevalence at age 6 years

Figure 3. Age 6 tree nut allergy (cashew, hazelnut, almond) by type of food allergy at age 1.

Table Legend

Table 1. Demographic and clinical characteristics of HealthNuts study cohort by participation status at age 6 years.

Table 2. Co-sensitisation to tree nuts at age 1 year among the HealthNuts clinic cohort, stratified by food allergy status at age 1 year

Table 3. Peanut and tree nut co-allergy patterns at age 6 years.

Table 4. Patterns of tree nut sensitisation status at age 1 and sensitisation and allergy status at age 6 years

Table 1: Demographic and clinical characteristics of the HealthNuts study cohort by participation status at age 6 years

	Assessment cohort (n=3232) %	Questionnaire only (n=1222) %	Did not participate (n=822) %	#p-value
Gender (male)	52	50	48	0.187
SEIFA*				
Quintile 1 (least disadvantaged)	18.8	20.3	25.3	
Quintile 2	20.0	20.3	19.2	
Quintile 3	21.9	20.3	18.2	
Quintile 4	19.7	19.5	17.8	
Quintile 5	19.6	19.4	19.4	0.003
Parent country of birth				
Both Australian	61.8	59.8	49.4	
One East Asian	4.4	4.5	4.1	
Both East Asian	5.8	5.0	9.8	
Other	28.0	30.7	36.6	<0.001
Mode of Delivery				
Vaginal	66.4	67.9	66.1	
Caesarean	33.6	32.1	33.9	0.601
Premature birth	6.2	6.0	5.4	0.470
Any siblings	49.9	49.1	50.1	0.450
Family history of any allergy	72	66	63	<0.001
Family history of food allergy	12	10	9	0.007
Family history of asthma	33	27	29	<0.001
Family history of eczema	32	28	26	0.001
Eczema diagnosis by age 1 year	29	21	24	<0.001
Wheeze by age 1 year	18	15	21	0.010
Any food allergy at age 1	13	7	6	<0.001

*socio-economic status was assigned on the basis of home postcode by using socio-economic indexes for areas (SEIFA) measures, derived from the 2006 Australian census, which accessed relative socio-economic advantage/disadvantage, economic resources (income, assets and expenditure) and educational and occupational characteristics .

χ^2 p-value refers to any difference between columns 1, 2 and 3

Table 2: Co-sensitisation to tree nuts at age 1 year among the HealthNuts clinic cohort (n=1117), stratified by food allergy status

Age 1 Food Allergy status	Cashew, almond or hazelnut sensitised % (95%CI)	Cashew sensitised % (95%CI)	Almond sensitised % (95%CI)	Hazelnut sensitised % (95%CI)
No food allergy/not sensitised (n=193)	5.2 (2.7, 9.3)	3.9 (1.7, 7.4)	1.5 (0.3, 4.1)	0.5 (0.1, 2.7)
Sensitised tolerant ^a (n=384)	12.0 (9.4, 16.6)	9.4 (6.36, 12.9)	3.6 (1.9, 6.1)	3.3 (1.7, 5.7)
Egg allergy only (n=347)	23.6 (19.7, 28.9)	17.7 (13.8, 22.0)	8.9 (6.2, 12.4)	7.5 (5, 10.8)
Peanut allergy only (n=60)	33.3 (21.7, 46.7)	23.3 (13.4, 36.1)	13.3 (5.9, 24.6)	8.3 (2.7, 18.4)
Peanut and egg allergy ^b (n=96)	48.4 (38, 58.9)	38.9 (29.1, 49.5)	24.2 (16.0, 34.1)	20 (12.5, 29.5)
Any other allergies ^c (n=37)	44.4 (27.9, 61.9)	38.9 (23.1, 56.5)	30.5 (16.3, 48.1)	25 (12.1, 42.2)

(a) sensitised tolerant = SPT $\geq$ 2mm to egg, peanut or sesame and negative OFC to that food

(b) Allergic to both peanut and egg, irrespective of other food allergies

(c) All other allergies –single milk=8, single sesame=5, milk or sesame with either egg or peanut=23

Table 3: Peanut and tree nut co-allergy patterns at age 6 years

	Coexistin g Peanut Allergy (%)	Coexistin g Almond allergy (%)	Coexistin g Brazil Allergy (%)	Coexistin g Cashew Allergy (%)	Coexistin g Hazelnut Allergy (%)	Coexisting Macadam ia Allergy (%)	Coexistin g Pecan Allergy (%)	Coexistin g Pistachio Allergy (%)	Coexistin g Walnut Allergy (%)
Peanut Allergic (n=147)		6.1-14.2	0.7-6.8	36.7-40.8	19.1-21.8	3.4-7.5	2.7-7.5	13.6-18.4	7.5-11.6
Almond Allergic (n=17)	42.8-60		14.3-25.7	78.5-80.0	57.0-64.3	7.1-20.0	14.3-22.9	34.3-35.7	20.0-28.6
Brazil Nut Allergic (n=5)	20.0-50	40.0-45.0		60.0-85.0	55.0-60.0	40.0-55.0	20.0-50.0	75.0-80.0	60.0-60.0
Cashew Allergic (n=121)	35.5-44.8	8.9-20.9	2.4-12.7		22.6-32.1	5.6-12.7	3.2-12.7	36.3-46.2	14.5-19.4
Hazelnut Allergic (n=44)	44.6-54.2	19.1-33.9	6.4-18.6	59.6-72.9		14.9-22.0	10.6-25.4	25.5-37.2	19.1-25.4
Macadamia Allergic (n=12)	25.0-47.8	8.3-30.4	16.6-47.8	58.3-74.0	56.5-58.3		16.6-56.5	50.0-73.9	65.2-66.6
Pecan Allergic (n=8)	25.0-40.7	25.0-29.6	12.5-37.0	50.0-63.0	55.6-62.5	25.0-48.1		37.5-59.2	50.0-77.8
Pistachio Allergic (n=50)	30.0-40.3	10.0-17.9	8.0-22.4	90.0-92.5	24.0-32.8	12.0-25.4	6.0-23.9		30.0-38.8
Walnut Allergic (n=28)	28.5-41.5	14.3-17.1	10.7-29.3	63.4-64.3	32.1-36.6	28.6-36.6	14.3-51.2	53.6-63.4	

* Screening for tree nut allergy varied for the various tree nuts. Hazelnut, almond and cashew SPT was performed for all study participants. The full tree nut SPT panel was only performed for those sensitised to either almond, cashew or hazelnut. OFC were limited for some tree nuts therefore, figures presented include those that had OFC and probable allergy as the lower figure, and as a sensitivity analysis those that had OFC and probable food allergy PLUS those sensitised at 3-7mm and no OFC performed included as the upper percentage.

Table 4: Patterns of tree nut sensitisation status at age 1 and sensitisation and allergy status at age 6 years (n=5276)

Tree nut type	Age 1 tree nut sensitisation status	Age 6 tree nut outcome				
		Allergic n (%)	Tolerant n (%)	Missing allergy outcome		
				3-8mm SPT and no OFC n (%)	No SPT and unknown tolerance n (%)	Missing n (%)
Cashew	Positive N=168	66 (39.3)	59 (35.1)	6 (3.6)	2 (1.2)	35 (20.8)
	3-7mm (n=110)	26 (19.0)	49 (44.5)	2 (1.8)	2 (1.8)	31 (28.2)
	≥8mm (n=58)	40 (70.7)	10 (17.2)	4 (7.0)	0	4 (7.0)
	Negative, <3mm (n= 947)	38 (4.0)	633 (66.8)	6 (0.6)	7.0 (0.7)	263 (27.8)
	Tree nut SPT not done [#] (n=4161)	17 (0.4)	2358 (56.7)	1 (0.02)	39 (1.0)	1746 (42.0)
Almond	Positive (n=87)	10 (11.5)	51 (58.6)	6 (6.9)	1 (1.1)	19 (21.8)
	3-7mm (n=80)	7 (8.8)	49 (61.2)	5 (6.2)	1 (1.2)	17 (21.2)
	≥8mm (n=7)	2 (28.6)	2 (28.6)	1 (14.3)	0	2 (28.6)
	Negative, <3mm (n=1030)	4 (0.4)	733 (71.0)	8 (0.8)	6 (0.6)	279 (27.1)
	Tree nut SPT not done [#] (n= 4159)	3 (0.07)	2376 (57.1)	4 (0.1)	29 (0.7)	1747 (42.0)
Hazelnut	Positive N=72	14 (19.4)	38 (52.8)	3 (4.2)	1 (1.4)	16 (22.0)
	3-7mm (n=61)	7 (11.5)	35 (57.4)	3 (5.0)	1 (1.6)	15 (24.6)
	≥8mm (n=11)	7 (63.6)	3 (27.3)	0	0	1 (10.0)
	Negative, <3mm (n=1044)	24 (2.3)	719 (68.9)	10 (1.0)	8 (0.8)	283 (27.0)
	Tree nut SPT not done [#] (n=4160)	6 (0.1)	2370 (57.0)	2 (0.1)	35 (0.8)	1747 (42.0)

[#] Not sensitised to screening foods (egg, peanut, sesame, shrimp/cow's milk) and therefore did not attend allergy clinic for additional tree nut SPT at 1 year of age.

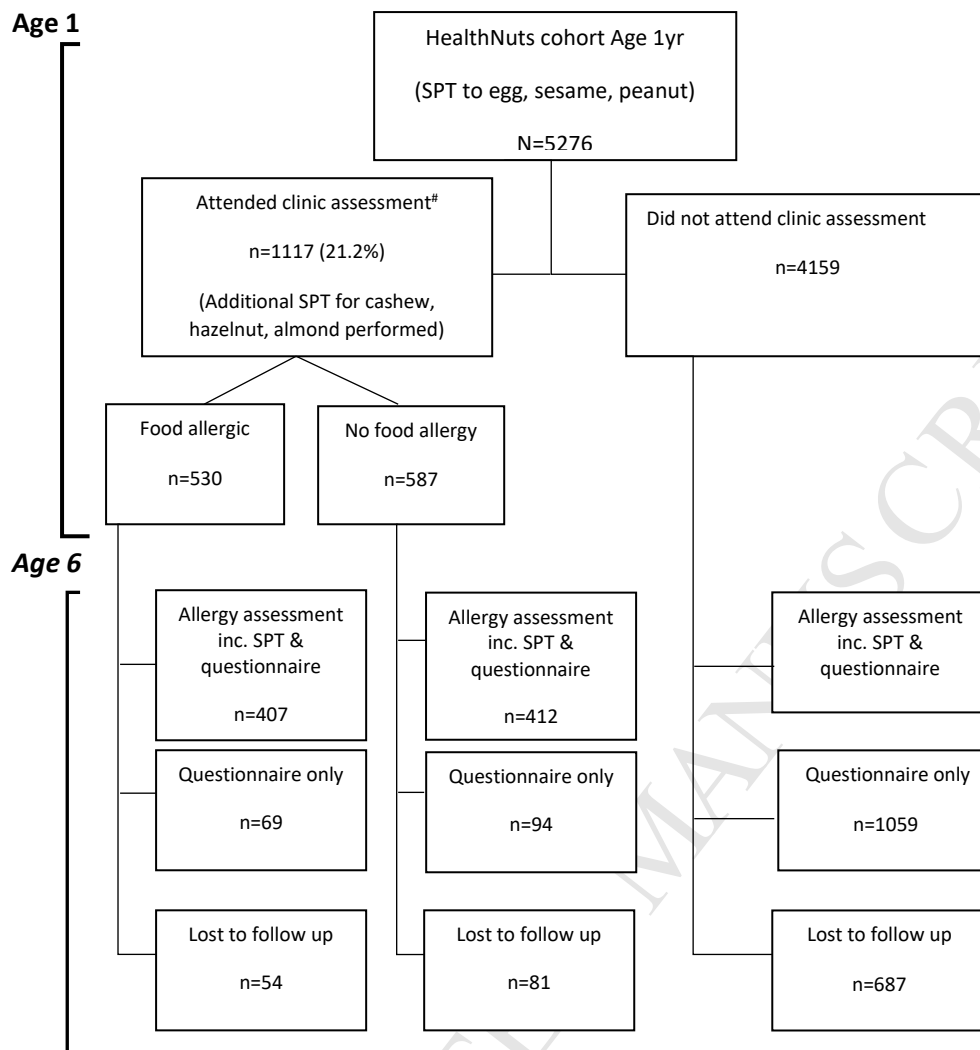
Figure 1: Overview of HealthNuts study participation, age 1 and 6 years

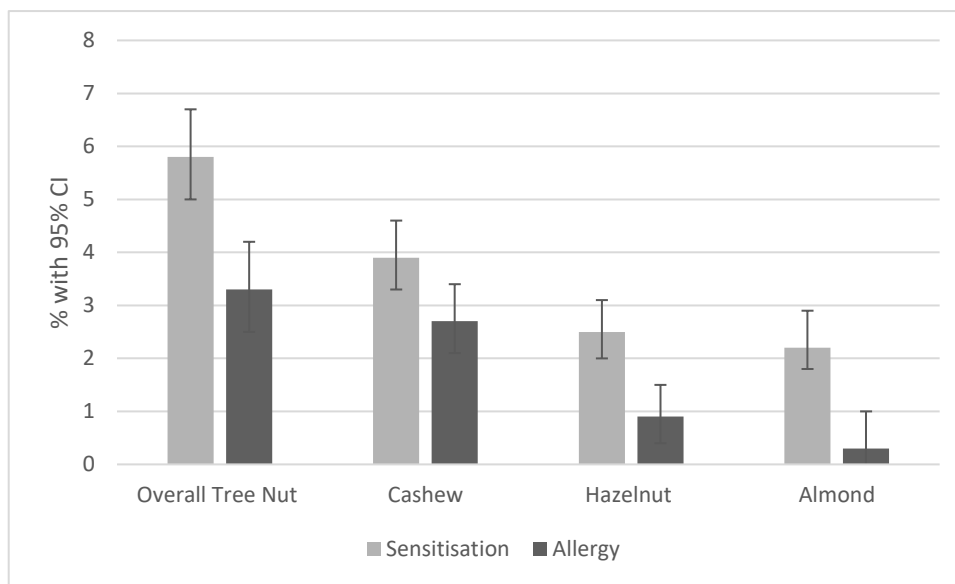
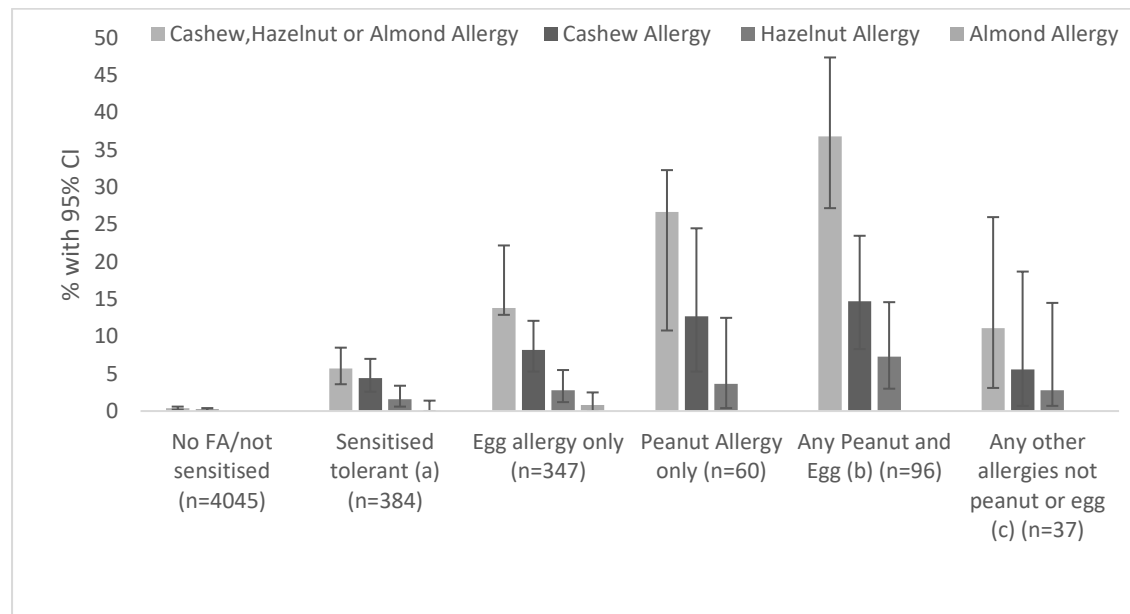
Figure 2: Tree nut sensitisation and allergy prevalence at age 6 years*

Figure 3: Development of tree nut allergy at age 6 years, stratified by type of food allergy at age 1 year (n=4969)



Supplementary Table 1. Oral food challenge protocol for tree nut challenges in the HealthNuts study

1 level 5ml teaspoon	Average total weight in 1 level teaspoon (grams)	Average total mg nut protein in 1 level teaspoon
Almond meal	4.0	1200
Hazelnut meal	4.0	600
Crushed walnut	2-2.5	550
Crushed cashew	2.5-3.0	700
Macadamia	3.0	300
Pecan	2.5	250
Pistachio	3.0	600

Supplementary Table 3: Sensitisation and allergy to almond, cashew and hazelnut at age 6 years using different definitions

	Any (Almond, Cashew, Hazelnut)				Almond				Cashew				Hazelnut			
	Sens	Prob [#]	Def*	Overall	Sens	Prob [#]	Def*	Overall	Sens	Prob [#]	Def*	Overall	Sens	Prob [#]	Def*	Overall
	(≥3mm)	(n=69)	(n=92)	(n=139)	(≥3mm)	(n=10)	(n=7)	(n=17)	(≥3mm)	(n=51)	(n=70)	(n=121)	(≥3mm)	(n=23)	(n=21)	(n=43)
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Assessment Group ¹ (Observed Prevalence) (n=3232)	7.3 (6.4,8.3)	2.7 (1.7,2.7)	2.3 (1.8,2.9)	4.3 (3.8,5.2)	2.8 (2.2,3.4)	0.3 (0.1,0.6)	0.2 (0.1,0.4)	0.5 (0.3,0.8)	5.0 (4.2,5.8)	1.6 (1.2,2.1)	2.2 (1.7,2.8)	3.8 (3.2,4.6)	3.3 (2.8,4.1)	0.7 (0.4,1.0)	0.7 (0.4,1.0)	1.3 (1.0,1.8)
Assessment Group ² (Weighted prevalence) (n=3232)	5.8 (5, 6.7)			3.3 (2.8,4.0)	2.2 (1.8,2.9)			0.3 (0.1,0.5)	3.9 (3.3,4.6)			2.7 (2.2,3.3)	2.5 (2.0,3.1)			0.9 (0.7,1.3)
Whole cohort ² (n=5276)		1.3 (1.0,1.7)	1.4 (1.1,1.8)	2.6 (2.3,3.2)		0.2 (0.1,0.3)	0.1 (0.04,0.2)	0.3 (0.2,0.5)		1.0 (0.7,1.3)	1.4 (1.1,1.7)	2.4 (2.0,2.8)		0.4 (0.3,0.7)	0.5 (0.3,0.7)	0.9 (0.7,1.2)

1. Assessment group (Observed Prevalence)-Those children who had an age 6 allergy/health assessment completed which included SPT to 8 foods (milk, egg, peanut, wheat, sesame, cashew, hazelnut, and almond). Those with negative SPT to all three tree nuts were deemed tree nut tolerant.

2. Assessment group (Weighted Prevalence) - Those children who had an age 6 allergy/health assessment completed which included SPT to 8 foods (milk, egg, peanut, wheat, sesame, cashew, hazelnut, and almond). Those with negative SPT to all three tree nuts were deemed tree nut tolerant. Prevalence estimates were calculated by weighting the proportion of the participants who had a full clinic assessment at age 6 years using sampling weights equal to the inverse probability of the family participating in the study at age 1 year.

3. Whole cohort- All children who participated in the HealthNuts study at age 1 year. Those who did not participate in age 6 follow up or had unknown tree nut exposure at age 6 were assumed to be tree nut tolerant (n=1975).

Probable food allergy- Any of the following: (1) SPT≥8mm but no age 4 OFC or recent reaction history, and no known tolerance; (2) SPT 3-7mm at age 6 and positive OFC at age 4, (3) evidence of IgE sensitization at 6 year with history of objective reaction consistent with HealthNuts OFC stopping criteria following definite exposure to the food of interest and evidence of IgE sensitization or (4) SPT 3-7mm and parent-report avoiding food due to allergy.

***Definite food allergy**- Any of the following: (1) positive OFC and IgE sensitized at 6 years; (2) history of recent objective reaction in the past 12 months consistent with HealthNuts OFC stopping criteria following definite exposure to the food of interest and evidence of IgE sensitization at 6 years; or (3) positive OFC at age 4 years and SPT ≥8mm at 6 year

^B Overall any tree nut allergy- Numbers do not add to total of probable and definite food allergy as some participants had multiple nut allergies

Supplementary Table 4: Co-sensitisation to tree nuts at age 1 year (<2mm cut-off) among the HealthNuts clinic cohort (n=1117), stratified by food allergy status

Age 1 Food Allergy status	Cashew, almond or hazelnut sensitised % (95%CI)	Cashew sensitised % (95%CI)	Almond sensitised % (95%CI)	Hazelnut sensitised % (95%CI)
No food allergy/not sensitised (n=193)	7.5 (4.0,11.9)	5.7 (2.8, 10.0)	2.0 (0.5,5.2)	1.0 (0.1,3.7)
Sensitised tolerant ^a (n=384)	18.8 (15.0,23.0)	12.7 (9.6, 16.5)	5.2 (3.2,7.9)	7.0 (4.7,10.1)
Egg allergy only (n=347)	31.0 (26.2,36.3)	24.9 (20.4,29.8)	14.4 (10.9,18.5)	12.6 (9.3,16.6)
Peanut allergy only (n=60)	43.3 (30.6, 56.8)	30.0 (12.1, 34.2)	21.7 (12.1,34.2)	11.7 (4.8,22.5))
Peanut and egg allergy ^b (n=96)	52.0 (45.0,68.4)	44.2 (32.8, 55.9)	25.9 (16.7,37.2)	23.4 (14.5, 34.4)
Any other allergies not peanut or egg ^c (n=37)	59.0 (45.0,72.4)	53.0 (39.6,67.4)	46.0 (32.6,60.4)	40.7 (27.5, 55.0)

(a) sensitised tolerant = SPT $\geq$ 2mm to egg, peanut or sesame and negative OFC to that food

(b) Allergic to both peanut and egg, irrespective of other food allergies

(c) All other allergies –single milk=8, single sesame=5, milk or sesame with either egg or peanut=23

Supplementary Table 2. Summary of SPT, OFC and allergy outcomes for additional tree nuts

Tree nut type (number of SPT performed)	Not Sensitised ($<3\text{mm}$)	Sensitised $\geq 3\text{mm}$		
		3-7mm and OFC	3-7mm no OFC	$\geq 8\text{mm}$
Brazil nut (n=90)	70	0	15 15=missing	5 5= probable allergy
Macadamia (n=101)	75	0	11 3= allergic, recent reaction 8=missing	15 14= probable allergy 1=tolerant
Pecan (n=107)	80	0	19 1= allergic, recent reaction 1=tolerant 17=missing	6 6= probable allergy
Pistachio (n=116)	43	5 3=positive 1=negative 1=inconclusive	19 6=tolerant 13=missing	49 4=tolerant 45= probable allergy
Walnut (n=111)	66	8 5=positive 2=negative 1=inconclusive	17 3=tolerant 14=missing	20 3=allergic age 4 17= probable allergy

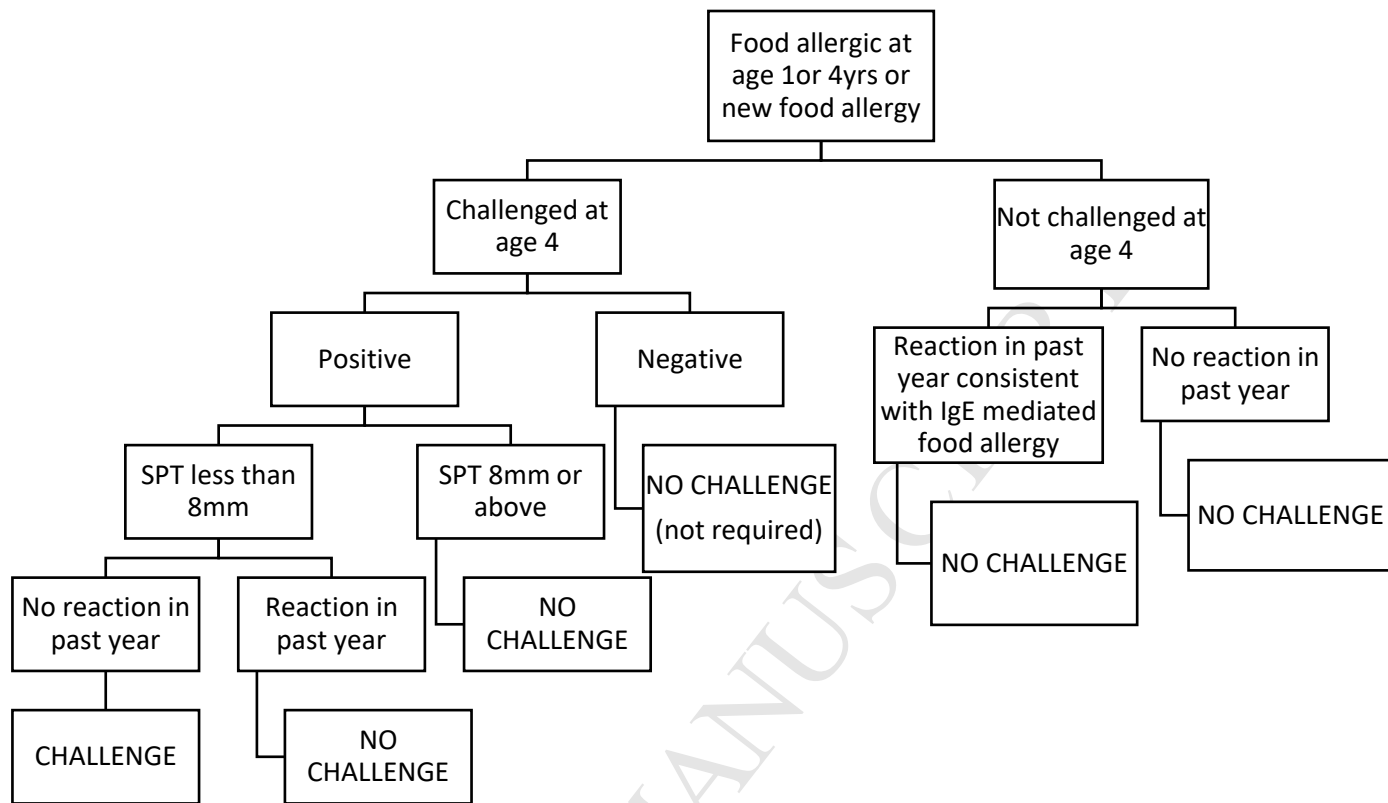
Definite allergy: Any of the following: (1) positive OFC and IgE sensitised at 6 years; (2) history of objective reaction in the past 12 months consistent with HealthNuts OFC stopping criteria following definite exposure to the food of interest and evidence of IgE sensitization at 6 years ; or (3) positive OFC at age 4 years and SPT $\geq 8\text{mm}$ at 6 years of age .

Probable allergy: Any of the following: (1) SPT $\geq 8\text{mm}$ but no age 4 OFC or recent reaction history, and no known tolerance; or (2) SPT 3-7mm at age 6 and one of the following a) positive OFC at age 4 , b) history of objective reaction >12 months ago consistent with HealthNuts OFC stopping criteria following definite exposure to the food of interest, or c) parent-report avoiding food due to allergy.

Tolerant: Any of the following (1) negative OFC (n=23); (2) SPT 3-7mm and parent reported ingestion history (eaten >1 time since age 4); or (3) not sensitised and no reaction since age 4; (4) no parental report of avoiding and parent reported ingestion history (eaten >1 time since age 4).

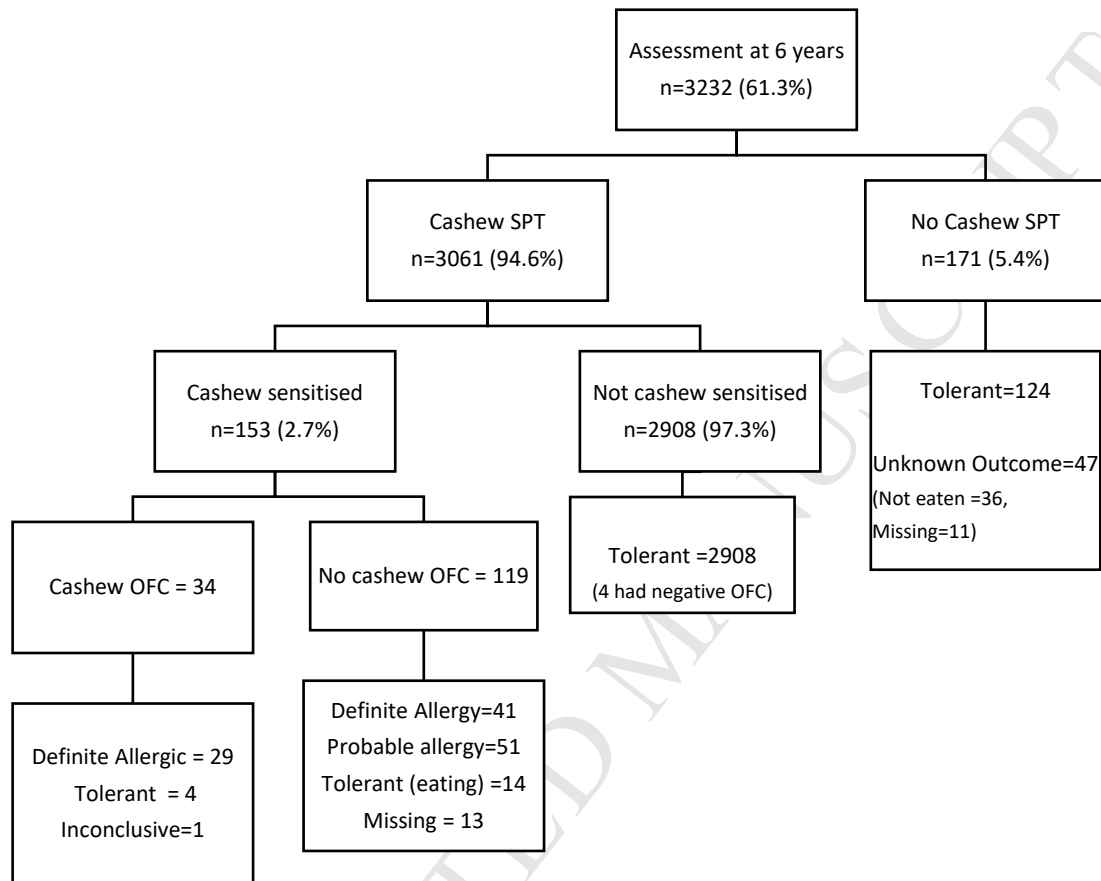
Missing: sensitised, no OFC and unknown ingestion history of specified tree nut

Supplementary Figure 1. Flow chart of Age 6 years HealthNuts challenge criteria protocol

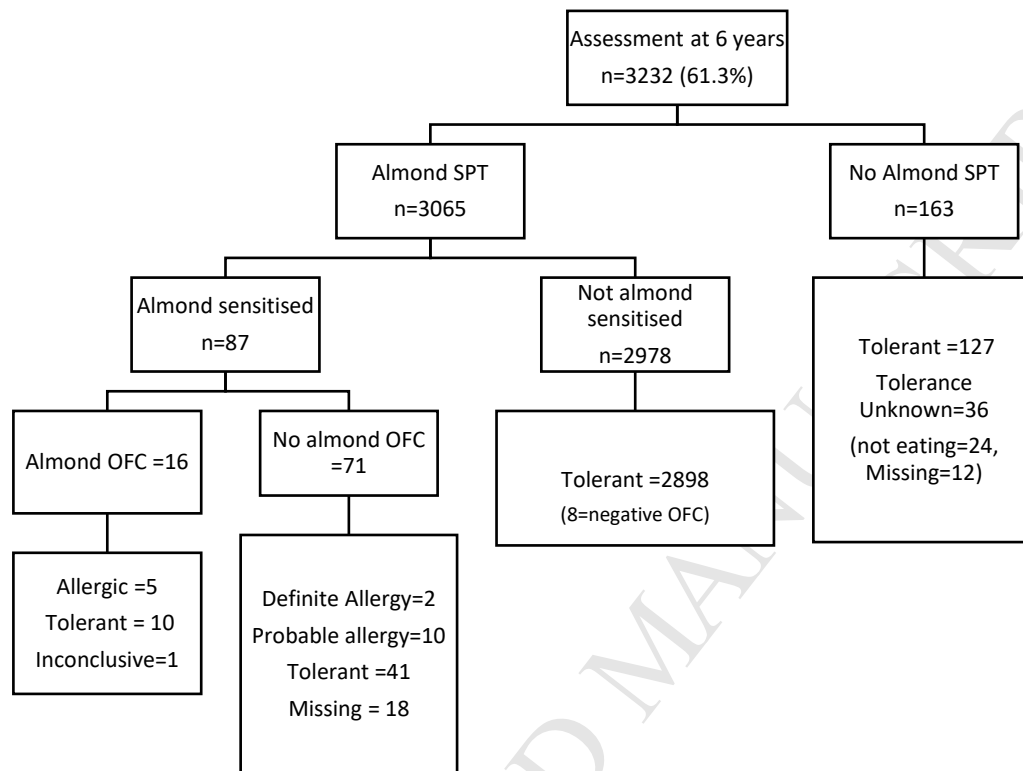


Supplementary Figure 2a. Summary of SPT and OFC outcomes for tree nuts (almond, cashew, hazelnut) at age 6 years

Supplementary Figure 2a Cashew



Supplementary Figure 2b Almond



Supplementary Figure 2 c. Hazelnut

